Optimizing Pyroxasulfone Efficacy on Wild Oat (*Avena fatua* L.)

by

Amy Rose Mangin

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Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Herbicide resistance in wild oat to current herbicidal mechanisms of action is widespread across western Canada. Pyroxasulfone is a soil-applied very long chain fatty acid inhibitor (Group 15/K_3) that has recently been registered in Canada and may become a future tool in managing this resistance, but control of wild oats by pyroxasulfone is inconsistent across various cropping systems. Trials were conducted in controlled conditions to investigate the influence of wild oat seed depth, site of pyroxasulfone interception in wild oat seedling and the downward movement of pyroxasulfone in the soil. Field experiments were then conducted to determine influences and interactions of seed depth, tillage and application timing on control of wild oat by pyroxasulfone. Additionally, resistance screening was used to examine resistance patterns of Canadian wild oat populations to pyroxasulfone and sulfentrazone. It was determined that the pyroxasulfone efficacy on wild oat is influenced greatly by position of the seed in the soil profile relative to the concentrated herbicide layer. Deep-seeded wild oats may be able to avoid herbicidal injury because the location of effective site of herbicide/seedling interception is below the concentrated herbicide layer in the soil. The position of the seed in the soil profile and the soil conditions interacted to influence the control of wild oat by pyroxasulfone in the field. Resistance to pyroxasulfone and sulfentrazone was found in a Canadian wild oat population previously selected for resistance to ACCase-, ALS- and fatty acid biosynthesis inhibitors, which may potentially limit pyroxasulfone and sulfentrazone use in managing herbicide-resistant populations.

“How can something seem so plausible at the time and so idiotic in retrospect?”

-- Calvin / Bill Watterson

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Contribution of Authors

The contributions made by the candidate and co-authors to the completion of this thesis are described below. Chapter three of this thesis is co-authored by the candidate, Dr. Linda Hall, Dr. Hugh Beckie and Dr. Jeff Schoenau. The candidate was responsible for conduction of the greenhouse and field trials, statistical analysis and preparation of the manuscript. Dr. Jeff Schoenau and his lab group at the University of Saskatchewan conducted the canola shoot bioassay for the leaching study. Chapter four was authors by the same individuals as above. The candidate was responsible for trial conduction, statistical analysis and manuscript preparation. Breanne Tidemann assisted with trial location and site preparation at Lacombe, AB and all co-authors contributed to experimental design. Chapter 5 was co-authored by the candidate, Dr. Linda Hall and Dr. Hugh Beckie. The candidate was responsible for trial conduction, data analysis and manuscript preparation. Dr. Hugh Beckie supplied resistant wild oat biotypes for this work. Statistical support for all chapters was provided by Jamie Polzien, Department of Renewable Resources, University of Alberta. Technical support for work in Chapters three, four and five was provided by Judy Irving, Lisa Raatz, Elise Martin, Keith Topinka and numerous graduate students in the Weed Science program. Dr. Linda Hall was the graduate student supervisor and worked closely with the candidate on all manuscript writing, experimental design and statistical analysis throughout the program.

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List of Abbreviations

ACCase	Acetyl-CoA Carboxylase
ac	Acre
ae	Acid equivalent
ai	Active ingrediant
ALS	Acetolactate synthase
ANOVA	Analysis of variance
C	Celsius
cm	Centimeter
DAT	Days after treatment
EC	Emulsifiable concentrate
g	Gram
GDD	Growing Degree-Day
ha	Hectare
HRAC	Herbicide Resistance Action Committee
kg	Kilogram
kPa	Kilopascal
L	Litre
LCFA	Long chain fatty acid
LD_{50}	Lethal dose that decreases survival by 50%
m	Metre
mm	Millimetre

mmol	Millimole
mg	Milligram
mL	Millilitre
MPa	Mega pascals
oz	Ounces
PPO	Protoporphyrinogen oxidase
R	Resistant
s	Second
S	Susceptible
SE	Standard error
SOM	Soil organic matter
SN	Solution
v	Version
VLCFA	Very long chain fatty acid
WDG	Water dispersible granule
WSSA	Weed Science Society of America

Chapter One: Introduction

1.1. Background

Wild oat (*Avena fatua* L.) is the most economically detrimental weed in western Canada and has been selected for resistance by the repeated use of the same or similar herbicides (Beckie et al. 2012). Resistance to the common herbicidal groups ACCase-, ALS- and fatty acid synthesis inhibitors, have been reported in over 44% of the fields across the Prairie Provinces (Beckie et al. 2013). Additionally, wild oat populations with cross-resistance to a number of herbicides by a single resistance mechanism, as well as multiple resistance by two or more resistance mechanisms to these three herbicide groups has been reported (Beckie et al. 2013). Annual cropping systems in Western Canada rely heavily on herbicides for wild oat control (Harker et al. 2016); this, paired with increased incidence of resistant populations, has created the need for integrated weed management tools and new herbicidal mechanisms of action for wild oat control.

Pyroxasulfone is soil-applied, pre-emergence, very long chain fatty acid (VLCFA) biosynthesis inhibitor that has only recently been used in western Canada and is classified as a Group 15 (WSSA) or K_3 (HRAC). Pyroxasulfone's primary site of inhibition is long chain fatty acid elongase and inhibition of multiple steps in the elongation pathway has been described (Tanetani et al. 2011; Tanetani et al. 2009). The decrease in VLCFAs produced is thought to be directly lethal to the plant, as it will affect the formation of cell membranes and waxy cuticle components in developing plant tissues (Babczinski et al. 2012; Trenkamp et al. 2004).

Pyroxasulfone resistance has not yet been reported in any weed species worldwide (Heap 2016) but rapid selection has been demonstrated experimentally in annual ryegrass (*Lolium rigidum*) (Busi et al. 2012). Low-dose selection of a multi resistant annual ryegrass population by pyroxasulfone exhibited increased levels of resistance in 3 generations. When examining cross-resistance patterns of this resistance Busi and Powles (2013) observed a corresponding shift in resistance to triallate and prosulfocarb indicating cross-resistance from a single mechanism.

Pyroxasulfone's general weed control spectrum includes annual grasses and small-seeded broadleaves, with acceptable crop tolerance in corn, soybeans, field pea and wheat (Tanetani et al. 2011; Tanetani et al. 2013). Tidemann et al. (2014) reported control of wild oat by pyroxasulfone in Alberta and Saskatchewan, but currently wild oat is not included for control on the product labels containing pyroxasulfone (Focus, Zidua, Anthem, Fierce).

Similar to other soil-applied herbicides, pyroxasulfone interacts with edaphic and environmental factors to control weeds. Soil organic matter, pH, structure, moisture and residue cover can all affect the efficacy of a soil-applied herbicide depending on its particular physical-chemical properties (Chauhan et al. 2006). Soil moisture is required for activity, as most herbicides must enter the soil-water solution phase before being available for uptake by a plant (Walker 1971). Generally, herbicides with low water solubility, such as pyroxasulfone (3.49 mg L^{-1} @ 20°C) will strongly bind to the soil. Herbicide adsorption to the soil will influence its availability for uptake by the plant as well as movement within the soil profile. A strong positive correlation was reported between increasing soil organic matter content and

pyroxasulfone binding (Szmigielski et al. 2014; Westra 2012). With increased soil binding there is decreased vertical movement of herbicides in the soil profile as they must be in the soil-water solution for movement in the soil. Westra (2012) reported >90% of pyroxasulfone remained in the top 7.5 cm of the soil profile during the growing season and that soil type was more important than moisture in determining movement within the soil profile. Pyroxasulfone's reported half-life across Canadian Prairie soils ranged widely, from 16-69 days (Szmigielski et al. 2014).

Pyroxasulfone is a pre-emergent herbicide, which allows for a large application window. Fall application can be beneficial to control winter annuals and early emerging spring annuals, and provides flexibility in application timing for producers; however, fall-applied herbicides have longer to dissipate in the soil before the growing season. Alternatively efficacies of spring-applied herbicides have potential to provide longer in-season control of emerging weeds but efficacy could be reduced with insufficient spring rainfall. Therefore, application-timing effects of pre-emergent herbicides are difficult to predict.

Due to soil factors influencing pyroxasulfone's availability and dissipation, any field operation that affects the soil will also affect pyroxasulfone's efficacy. There has been a shift towards reduced or zero tillage from intensive tillage in western Canada. This shift has led to soil with increased soil organic matter (Arshad et al. 1999; Franzluebbers and Arshad 1996; Janzen et al. 1998; Woods 1989), improved soil structure (Karlen et al. 1994), reduced soil drying (Arshad et al. 1999; Karlen et al. 1994) and increased microbial activity (House and Brust 1989). Generally, soil-applied herbicides are less available in reduce-tillage systems because of

increased soil binding and dissipation, but they can also leach into the soil profile easier with greater water infiltration through macro-pore channels (Chauhan et al. 2006; Locke and Bryson 1997). Mahoney et al. (2014) reported pyroxasulfone efficacy on velvetleaf (*Abutilon theophrasti*), pigweed (*Amaranthus retroflexus*; *Amaranthus hybridus*), common ragweed (*Ambrosia artemisiifolis*) and lambs quarters (*Chenipodium album*) to be greatly reduced in zero-tillage compared to conventional-tillage fields.

Tillage operations can also redistribute weed seeds within the soil profile (Pareja et al. 1985; Starica et al. 1990; Yenish et al. 1992; Yenish et al. 1996). The vertical position of seed in the soil will influence its germination and emergence (Mohler 1993), as well as the portion of a weed seedling that intercepts a soil-applied herbicide. In direct-seeding systems, the weed seed bank will remain on or near the soil surface while tillage operations have the potential to redistribute seeds to the depth of tillage (Cousens and Moss 1990; Starica et al. 1990). In studies conducted in Manitoba, wild oat emerged from twice the depth in tilled fields compared to untilled fields (du Croix Sissons et al. 2009). Wild oat can emerge from up to 20 cm in the soil profile, due to a large seed size and the ability to elongate its first internode (mesocotyl elongation) (Beckie et al. 2012b). Populations of wild oat have been reported to increase with intensive tillage practices (Medd 1990), which may be contributed to an increased wild oat seed bank persistence as burial depth increases, and tillage stimulating germination (Banting 1966; Peltzer and Matson 2002; Wilson and Cussans 1975).

The large number of factors influencing the efficacy of a soil-applied herbicide, such as pyroxasulfone, makes it difficult to predict the control of a single weed species across tillage

systems. To increase our ability to make inferences beyond pyroxasulfone alone, we included a comparative herbicide, triallate, which is similarly soil-applied and has activity on wild oat. As a result the following research objectives were established to isolate individual factors that may be affecting efficacy of the control of wild oat:

1.2. Research Objectives

1.2.1. Determine effect of wild oat seed depth on control by pyroxasulfone and triallate.

Knowledge of the differences in control of wild oat by pyroxasulfone and triallate when seeds are located at various depths in the soil profile is required to determine the influence of weed seed and herbicide placement in the soil for control. This research objective was investigated in Chapter 3 to address the following hypotheses:

- Placement of pyroxasulfone in soil close to the surface will improve efficacy when wild oats are located at shallow depth.
- Seed depth will have similar effects on efficacy of pyroxasulfone and triallate

1.2.2. Determine the effective site of interception of pyroxasulfone and wild oat seedlings for control to take place.

In order to understand the implications of weed seed bank depth, it was necessary to reveal the effective site of interception for the wild oat seedling and the herbicidal layer in the soil. This information was not available in the literature. This objective was investigated in Chapter 3 and the following hypothesis was made:

- Control of wild oat by pyroxasulfone is dependent on the site of seedling and herbicide interception.

1.2.3. Quantify vertical movement of pyroxasulfone in the soil with a simulated rainfall event.

Because pyroxasulfone has limited water solubility, we hypothesized that pyroxasulfone would remain at or near the soil surface. To investigate the vertical position of pyroxasulfone in soil and as influenced by a rainfall event, we conducted experiments outlined in Chapter 3. The following hypothesis was made:

- Pyroxasulfone will remain relatively close to the soil surface after application, even after a rainfall event.

1.2.4. Determine pyroxasulfone and triallate control of shallow- and deep-seeded wild oats in tilled and untilled soil.

To examine the interactions of tillage and weed seed depth, field trials were conducted in the Edmonton area. Tilled and untilled soils have numerous soil and microclimatic differences between them that can potentially influence efficacy of a soil-applied herbicide. Pyroxasulfone and triallate efficacy on wild oat seeded either shallow or deep, in tilled and untilled field soils, was examined to isolate these potential influencing factors. This objective was examined in Chapter 3 and the following hypothesis was made:

- Pyroxasulfone and triallate will have increased control on shallow-seeded wild oat typical of a no-till system compared to deep-seeded wild oat that occur under tillage.

1.2.5. Determined wild oat vertical distribution in the soil with and without tillage.

The vertical position of weed seeds in the soil profile will influence what part of the emerging seedling contacts a soil-applied herbicide; therefore we require information about how tillage is vertically distributing wild oat seeds in the soil in order to predict how emerging seedlings will intercept pyroxasulfone in various tillage systems. This objective was examined in Chapter 4 and the following hypothesis was made:

- Wild oat seeds will be distributed deeper in the soil profile with tillage than without.

1.2.6. Determine the efficacy of pyroxasulfone applied in the fall and spring in tilled and untilled soil for wild oat control.

Fall verses spring application can influence dissipation and degradation as well as water available for activation. Application timing of pyroxasulfone may not affect efficacy similarly in tilled and untilled soil. Moisture and temperature difference, in tilled and untilled soil may influence availability of pyroxasulfone, as well as wild oat emergence patterns. The effectiveness of pyroxasulfone on tilled and untilled soil for wild oat control when applied in the fall and the spring is discussed in Chapter 4. The following hypotheses were made:

- Fall applications will have better control on early emerging wild oat.

- Increased soil drying in tilled soil will reduce pyroxasulfone efficacy and wild oat emergence.

1.2.7. Determine if triallate-resistant Canadian wild oat populations exhibit cross-resistance to pyroxasulfone and the PPO-inhibitor sulfentrazone.

Populations of wild oat in western Canada exist that have already been selected for resistance to the soil-applied herbicide triallate. If triallate-resistant populations are cross-resistance to pyroxasulfone, the utility of pyroxasulfone for control of herbicide-resistant populations could be limited. This led to the development of the final objective of this thesis investigated in Chapter 5 where the following hypotheses was tested:

- Increased levels of endogenous gibberellins in triallate-resistant wild oat will confer cross-resistance to pyroxasulfone.

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Chapter Two: Literature Review

2.1. Wild Oats

Wild oat (*Avena fatua L.*) is the most economically important weed in western Canada, with over $500 million dollars spent on its control each year (Leeson et al. 2006). It is classified as a secondary noxious weed under the Canadian federal legislation and a noxious weed in British Columbia, Quebec, Manitoba and Saskatchewan. Wild oat populations are found in all Canadian provinces and all US states except the southeastern states of Arkansas, Georgia, and North and South Carolina (Beckie et al. 2012a). Over 50% of the cultivated fields in the Canadian Prairies have wild oat infestations, as it is the second most abundant grassy weed after green foxtail (*Setaria viridis* L.).

2.1.1. Lifecycle and Reproduction

Wild oat is an annual weed species, and in western Canada can begin emergence in April and potentially continue through until the end of June with one generation per year (Beckie et al. 2012a). There are a number of physiological and environmental factors that affect wild oat emergence. Wild oat can exhibit primary and secondary dormancy, with primary dormancy being overcome by a warm, dry period of after-ripening (Myers et al. 1997). Environmental conditions such as air temperature (Sawhney and Naylor 1980) and soil moisture (Sawhney and Naylor 1982) can also influence the release of dormancy in wild oat.

Similar conditions influence wild oat germination and emergence. Optimum temperature reported for wild oat emergence ranges from 10-21°C, with decreased emergence when temperature exceeds 27°C (Banting 1962; Friesen and Shebeski 1961; Sharma et al. 1976). Optimum soil moisture for wild oat germination is 50 – 75% of field capacity (Sharma et al. 1976), and germination of wild oat was not limited until water potential in the soil reached -1200 kPa (Fernandez-Quinantilla et al. 1990). Hsiao and Simpson (1971) reported that under low water potential, white, red, blue, and far red light inhibit wild oat germination, while in high water potential, wild oats will germinate in white light, compared to seeds in red, blue and far red light.

Martinson et al. (2007) investigated emergence models for wild oat in Minnesota and North Dakota and found soil growing degree-days (GDD) and hydrothermal time to be accurate predictors of wild oat emergence. A base temperature of 1°C and a base water potential of 20.6 MPa were reported acceptable for the prediction of wild oat emergence. Hydrothermal time provides a slightly more reliable emergence estimate than soil GDD. Across four site-years, 100% wild oat emergence was observed in June at approximately 600 soil GDDs and 600 hydrothermal time (Martinson et al. 2007). Bullied et al. (2003) reported that by late June, 100% of the wild oats of that year had emerged at 850 GDD.

A typical wild oat plant will produce up to 150 seeds, but under low competition with increased tillering, one plant may produce up to 500 seeds (Sharma et al. 1977). Wild oats shed their seeds as they mature during the growing season. Shirtliffe and Ents (2005b) reported 100,

80, 60, 40, 20, and 10% of wild oat seeds remaining on the plant at 1300, 1500, 1550, 1600, 1675, and 1800 GDDs (base 0) after spring emergence.

Weed seed dispersal is influenced by both natural (primary dispersal) and agricultural operations (secondary dispersal) (Barroso et al. 2006). Dispersal of seeds from a parent plant is dependent on several factors, including height and distance of the seed source, concentration of the seed source, dispersability of the seed, and the intensity of the dispersing agents (e.g. wind) (Harper 1977). Barroso et al. (2006) reported a maximum natural dispersal of 1.5 m from the parent plant. Secondary dispersal of 2-3 m by tillage was reported, with a much larger distance observed if tillage was performed on a slope in the downward direction (Barroso et al. 2006). Shirtliffe and Entz (2005a) reported wild oat seed moved up to 145 m after a combine harvester pass through a dense wild oat patch, but was reduced to 45 m when chaff was collected behind the combine harvester.

2.1.2. Wild Oats and Tillage

There has been a large shift towards reduced-tillage practices in agricultural cropland of Canada. Cutting fuel and labor costs (Frye 1984), ability for earlier seeding, as well as numerous soil benefits such as decreased soil erosion (Cogo et al. 1984), increased soil moisture (Blevins et al. 1983; Griffith et al. 1986), increased soil organic matter (Arshad et al. 1999; Blevins et al. 1983; Franzluebbers and Arshad 1996; Janzen et al. 1998; Woods 1989), improved soil structure (Karlen et al. 1994), decreased soil drying (Arshad et al. 1999; Karlen et al. 1994) and increased microbial activity (House and Brust 1989) have led to this transition. Even with these benefits of reduced tillage, some producers continue using conventional-tillage practices to prepare an

optimal seedbed for planting, control problematic weeds with limited herbicide options, as well as to help manage large amounts of crop residue.

Tillage can have a large impact on weed seed survival, germination and emergence due to vertical redistribution of weed seeds in the soil profile (Mohler 1993). In reduced or zero-tillage systems, the majority of weed seeds remain on or near the soil surface. Pareja et al. (1985) reported 85% of the weed seed bank to be in the upper 5 cm with reduced tillage, and Yenish et al. (1992; 1996) reported 60 and 90% of weed seeds in the top 1 and 2 cm of the soil profile, respectively. Alternatively, in intensive-tillage systems, only 51% of the weed seed bank was reported in the top 4 cm (Starica et al. 1990) and 30% in the top 2 cm of the soil profile (Yenish et al. 1992).

The effect of weed seed placement in the soil profile on germination and emergence will differ depending on the weed species. Wild oat can be recruited from depths up to 20 cm; this ability is attributed to its large seed size and ability to elongate its first internode (mesocotyl elongation) (Sharma and Van den Born 1978). Tanveer et al. (2010) investigated emergence of wild oat seeds buried at various depths and reported 100% emergence from the 3-cm depth, 80% from the 5-cm depth and 40% from the depth of 10 cm. Froud-Williams et al. (1984) reported no difference in emergence of wild oat when seeded at 2.5 cm compared to 7 cm from the soil surface in sandy loam soil. Similar wild oat emergence was reported from depths of 0.5 to 15 cm in Western Australia, with a slight decrease in emergence when emerging from 17.5 cm below the soil surface (Patterson et al. 1976). A survey of cropped fields across western Canada reported wild oat were recruited from 1.5 cm deeper in the soil profile in fields under

conventional-tillage practices than fields under reduced or zero tillage (du Croix Sissons et al. 2000). Although wild oats have the ability to emerge from considerable depths in the soil profile, there are additional interactions between soil properties and microclimatic variations in conventional- and zero-tillage soils that can also influence wild oat germination and emergence.

Wild oat seed bank persistence has been reported to increase with burial depth because the supply of moisture and oxygen tends to be more favorable for germination at shallow depths in the soil profile (Banting 1966; Thurston 1961; Wilson and Cussans 1975). Banting (1966) and Thurston (1961) reported persistence of wild oat to be greatest when buried 5 to 15 cm from the soil surface compared with the 0 to 5 cm depth. In zero-tillage, seeds on or near the soil surface and seed bank persistence may be reduced due to predation, physiological decay, fatal germination and exposure to environmental conditions (Buhler et al. 1997; Miller and Nalewaja 1990). Gallandt et al. (2004) reported no differences in microbial degradation of wild oat seeds located in no-till and conventional-tillage soils.

Cultivation can alleviate dormancy and encourage germination and emergence of wild oat by increasing the diffusion of oxygen, temperature fluctuations and seed to soil contact (Mohler 1993). Wild oat emergence has been reported to increase with tillage operations (Medd 1990; Peltzer and Matson 2002). Tillage may also influence the emergence timing of wild oat during the growing season. Bullied et al. (2003) reported earlier emergence in reduced-tillage systems, where 50% of the wild oats of that year had emerged at 460 GDD, compared to 553 GDD in intensive tillage.

Increasing crop residue will be present on the soil surface as tillage practices are reduced, which may influence the germination and emergence of weeds by affecting the environment surrounding the seed. Delayed weed emergence has been reported due to decreased soil thermal time and decreased light penetration with increasing soil cover (Dyer 1995). The effect of residue on germination and emergence of weed seeds is very difficult to estimate as it is controlled by many factors including residue type, residue quantity, residue position, allelopathy, weed species, weed seed depth in soil, soil type, and the environmental conditions, as well as their interactions (Buhler 1995).

2.1.3. Herbicidal Control of Wild Oat

Wild oat infestation can cause substantial yield reductions in annual cropping systems, which contributes to the importance of management. A wild oat density of 118 plants m^{-2} in spring wheat showed a 39% yield loss in Saskatchewan (Kirkland 1993). O'Donovan et al (1985) predicted 10 wild oat plants m^{-2} would reduce spring wheat yield by 3% if wild oat were one leaf stage behind wheat, by 6% when they where at the same stage and by 10% if the wheat was one leaf stage behind wild oat. In barley, a very competitive crop, a wild oat density of 170 plants m^{-2} resulted in a 45% yield reduction when wild oats emerged 6 days before seeding, 25% yield reduction when barley and wild oats emerged on the same day and a 5% yield reduction when wild oats emerged 6 days after crop emergence (O'Donovan et al. 1985). Row crops such as soybeans, also experience significant yield losses from wild oat populations. A study in North Dakota reported a 6 to 51% yield reduction in soybeans as wild oat populations increased from 1 to 30 plants 1 m row^{-1} (Rathmann and Miller 1981). Control of wild oats in

summer annual cropping systems is heavily reliant on herbicidal control. Harker et al (2016) reported an 709 kg ha^{-1} increase in wild oat biomass and a 32 plants m^{-2} increase in wild oat density when wild oat herbicide was removed in only 2 years of a 5-year annual crop rotation compared to an annual application of a wild oat herbicide. The only annual cash crop grown in western Canada with no in-crop wild oat herbicide option is tame oats due to the closeness of these two species.

The most beneficial timing of weed removal in crops will vary, for example, removal of weeds in spring canola by the 4 leaf stage is suggested to minimize yield loss (Harker et al. 1999, 2004, 2008; Martin et al. 2001), while wild oat removal in field pea was recommended by as early as 2 weeks after crop emergence (Harker et al. 2001). No significant relationship was observed between wild oat removal timing and barley yield (Stougaard et al. 1997). Yield loss models for wild oat can aid in the herbicide application decision, and include weed density and/or time of weed emergence relative to the crop (O'Donovan et al. 2005). By being able to estimate crop loss with a particular wild oat infestation, producers can determine if a herbicide application will cost less than the potential value of lost yield on a field and make the economically smart decision.

Herbicides can be used pre-emergence and in-crop for control of wild oats. The first wild oat herbicides, introduced in the 1960's, were the soil-applied, fatty acid inhibitor triallate, and a post emergent herbicide 4-Chloro-2-butynyl-N-(3-chlorophenyl) carbamate (Barban) (Beckie et al. 2012a). In the 1970's and 80's the introduction of the highly selective ACCase inhibitors (Group 1) and ALS inhibitors (Group 2) shifted use patterns away from soil-applied chemistries

(Beckie et al. 2013). Repeated use of similar mechanisms of action for control of wild oat has led to herbicide-resistant populations across Canada. The concern raised by resistance was partially alleviated by the introduction of genetically modified crops that provided additional herbicide modes of action for in-crop application including glyphosate (group 9) and glufosinate (group10) in canola, corn, and soybeans. A survey of the Canadian Prairies from 2007-2011 reported 44% of all surveyed fields contained populations resistant to either group 1, 2 or 8 herbicides (Beckie et al. 2013). With the high occurrence of herbicide resistance in wild oats, future control options may be limited.

2.1.4. Herbicide Resistance of Wild Oats

In response to repeated herbicide use in the last 40 years, wild oats have been selected for herbicide resistance in over 40% of the fields in Western Canada (Beckie et al. 2013). Wild oat resistant to one or more herbicides are found in 15 different countries; Argentina, Australia, Belgium, Brazil, Canada, Chile, France, Germany, Iran, Mexico, Poland, South Africa, Turkey, United Kingdom and United States of America (Heap 2016). A survey from 2007-2011 indicated 44% of cropped fields in Manitoba, Saskatchewan and Alberta had wild oat biotypes resistant to fatty acid inhibitors, ACCase inhibitors and ALS inhibitors (Beckie et al. 2013). Resistance to ACCase inhibitors, ALS Inhibitors and fatty acid biosynthesis inhibitors were reported in 41, 12 and 8% of all surveyed field, respectively. Inter-group herbicide resistance reported was ACCase and ALS inhibitors (8%), ACCase and fatty acid biosynthesis inhibitors (5%), ALS and fatty acid biosynthesis inhibitors (2%) and ACCase, ALS and fatty acid synthesis inhibitors (3%) (Beckie et al. 2013).

O’Donovan (1994) observed wild oat populations resistant to triallate, a soil-applied fatty acid biosynthesis inhibitor (group 8/N), were cross-resistant to difenzoquat, a foliar-applied cell elongation inhibitor (Group 8/Z) (O’Donovan et al. 1994). Resistance to herbicides with two different sites of action can either be conferred by a single resistance mechanism (non-target site resistance), or multiple separate mechanisms such as two or more target site mutations or a target site mutation and non-target site resistance such as enhanced metabolism (Beckie and Tardif 2012). The mechanism of resistance to both triallate and difenzoquat is speculated to be due to enhanced levels of endogenous gibberellins, which would result in resistance by rapid germination and shoot growth allowing for avoidance of herbicidal effects (O'Donovan et al. 1999; Rashid et al. 1998). Alternatively, two separate mechanism of resistance have also been reported to confer resistance to triallate and difenzoquat, decreased metabolic activation for triallate resistance and difenzoquat exclusion from the cytoplasm and being irreversible bound to the cell wall (Kern et al. 1996; Kern and Dyer 1998).

Beckie et al. (2012b) investigated the mechanisms of resistance to ACCase and ALS inhibitors in 16 wild oat populations from across western Canada. Molecular analysis revealed seven target site mutations conferring resistance to ACCase inhibitors (Ile1781Leu, Trp2027Cys, Asp2078Gly, Trp1999Cys, Ile2041Asn, Cys2088Arg, and Gly2096Ser substitutions) and two target site mutations conferring resistance to ALS inhibitors (Ser653Thr and Ser653Asn substitutions). Enhanced metabolism by P450 monoxygenase was also examined using a known P450 monoxygenase inhibitor (malathion) in 5 wild oat populations. When malathion was applied before herbicide application all resistant populations showed increased herbicidal

effects compared to no malathion treatment, indicating that the P450 monoxygenases complex is contributing to herbicidal resistance in these populations (Beckie et al. 2012b). Dose-response experiments of two wild oat populations in Montana that were resistant to ACCase and ALS inhibitors and difenzoquat, revealed additional resistance to the photosystem-I inhibitor paraquat (group 22/D), as well as triallate (Keith et al. 2015). Molecular analysis did not reveal any known ALS and ACCase target-site mutations in these populations but a malathion treatment partially reversed resistance to flucarbazone, imazamethabenz, difenzoquot and pinoxaden, suggesting the involvement of P450 monoxygenase conferring resistance in these populations (Keith et al. 2015).

Additionally, wild oat resistance has been reported to the group 3/K_1 (microtubule inhibitors) herbicide propyzamide in the United States and the group 25/Z (antimicrotubule mitotic disrupter) herbicide flamprop-methyl in Canada, Australia and the United Kingdom (Heap 2016). The mechanisms of resistance for these cases have not been investigated.

2.2. Soil-Applied Herbicides For Wild Oat Control

Soil-applied herbicides offer a number of benefits compared to foliar-applied herbicides, including application flexibility and residual control. In western Canada, soil-applied herbicides can be applied in either the fall before freeze-up or before emergence in the spring.

Depending on herbicide physical and chemical properties, mechanical incorporation may be recommended after application to aid in herbicide placement and to decrease losses

through volatilization and photodegradation, therefore increasing persistence in the soil (Curran et al. 1992; Locke and Bryson 1997). Mickelson et al. (2001) reported decreased herbicide losses through leaching and run-off of metolachlor when incorporated after application compared to when it was sprayed on untilled soil or soil that was tilled previous to application. Deep incorporation may dilute the herbicide applied and decrease herbicide efficacy, For example Knake et al. (1967) reported decreasing control of green foxtail as the depth of incorporation increased. Incorporation by irrigation or rainfall after the application of soil-applied herbicide can also greatly influence efficacy. Water incorporation can influence herbicides in a number of ways; it can move herbicides into the soil to reduce surface losses, it can move herbicide deeper into the soil profile, influencing the position at which it contacts the germinating seed or emerging weed seedling and it also will create moist conditions in the soil allowing for absorption of the herbicide by the seedlings (Stickler et al. 1969; Walker and Roberts 1975).

Increased soil cover by crop residue in reduced-tillage fields can also have a significant influence on efficacy of soil-applied herbicides (Chauhan et al. 2006; Locke and Bryson 1997). Residues can intercept 15-80% of the herbicide being applied, making it more susceptible to volatilization and photodegradation losses and also directly reduces the amount of herbicide contacting the soil surface (Banks and Robinson 1982; Buhler 1995; Grover et al. 1997; Isensee and Sadeghi 1994). Additionally, herbicides formulation can influence how they interact with residue on the soil surface. Granule-applied alachlor, cynazine and metolachlor were reported to have increased efficacy in dry years than their liquid formulations, and it is thought to be due to the liquid formulations being adsorbed by the surface residue (Johnson et al. 1989). The

degree of herbicide by residue interaction in a cropping system is difficult to evaluate due to the variable distribution of residue over a field and because it is influenced by many factors including the herbicide's chemical and physical properties as well as residue type.

The number of registered soil-applied herbicides for control of wild oat are limited, and include two group 8 herbicides triallate and EPTC and two group 3 herbicides, trifluralin and ethafluralin (2015 Crop Protection Guide).

2.2.1. Pyroxasulfone

The widespread resistance to many of the common mechanisms of action used for wild oat control has led to significant target weed-focused research. Pyroxasulfone is a soil-applied pre-emergent herbicide that inhibits the biosynthesis of very long chain fatty acids and has been classified as a group 15 (WSSA) and K3 (HARC). Triallate, is a soil-applied group 8 fatty acid biosynthesis inhibitors that was first introduced in the 1960's for wild oat control before more effective herbicides where released. Once Group 1/A and Group 2/B grass herbicides were introduced, the use of long chain fatty acid (LCFA) inhibitors decreased and this decreased further with the introduction of herbicide-resistant Roundup Ready crops in 1996. Even though triallate use patterns have decreased significantly, with the prevalence of Group 1 and Group 2-resistant wild oat, its use is being actively promoted (Gowan). We compare pyroxasulfone to triallate, as they have similar physical chemical properties (Table 1), to discuss advantage and limitations of pyroxasulfone use in western Canadian cropping systems.

2.2.2.1. Applications and uses. Both pyroxasulfone and triallate are soil-applied pre emergent herbicides with relatively long application windows. They can be applied in the spring before or

shortly after planting as well as in the fall before freeze-up (Fuerst 1987; Tanetani et al. 2011; Westra 2012). Tidemann et al. (2014) found that applying pyroxasulfone in the spring or the fall did not consistently impact the control of wild oats or cleavers (*Galium aparine*).

Weed establishment is prevented after seed germination, with growth inhibited in both the root and the shoot, but germination is not affected (Fuerst 1987). Incorporation of triallate is recommended after application to place the herbicide in a position to optimize plant uptake in the soil (Fuerst 1987; Westra 2012). Pyroxasulfone has activity on a wide variety of weeds, which include many small-seeded broadleaves and grasses (Tanetani et al. 2011), while triallate is used primarily for the control of wild oats. Weeds controlled and potential crops with tolerance are listed in Table 2.

2.2.2.2. Physical-chemical properties. Herbicide physical-chemical properties are the main influencing factors for determining activity in plants, soil and the environment. A list of triallate (thiocarbamate) and pyroxasulfone (isoxazoline) compound descriptions and physical-chemical properties can be found in Table 3-1. The main physical-chemical properties that are of interest when looking at herbicidal characteristics include pKa, log K_{ow}, and K_{oc}. pKa, the pH at which 50% of the compound is dissociated (dissociation constant), is an important factor when considering uptake and translocation in the plant. Pyroxasulfone and triallate do not have pKa values assigned to them because they do not have dissociable ions in an accessible pH range (Senseman 2007; Szmigielski et al. 2014). This suggests they are not affected by 'acid trapping' that facilitates phloem movement for some herbicides. K_{ow} (octanol-water partition co-efficient) describes the solubility of a compound, the lower the K_{ow} the more soluble it is in water. The

related property, sorption coefficient (K_{oc}) predicts the ratio of herbicide associated with the carbon fraction in the soil. Both triallate and pyroxasulfone have similar physical-chemical K_{ow} and K_{oc} values that indicate moderate to low water solubility and strong soil adsorption.

2.2.2.3. Uptake and translocation. Adsorption from the soil can take place through any plant tissue that contacts the herbicide solution including root, seed, or shoot tissue. Herbicides must be solubilized in water in order for them to be available for uptake by diffusion, the major driving force. Triallate has been shown to absorb through both the root and the shoot, but most herbicidal activity is when absorbed through the shoot, in particular the coleoptile node (Fuerst 1987). Parker (1963) reported that the site of adsorption for wild oat and the most susceptible area to be 10-15mm above the coleoptile node. The uptake pattern of pyroxasulfone has not been examined in detail, but it was suggested that it is absorbed primarily through the shoot of germinating seedlings with some uptake through the roots (Westra 2012).

Once in the plant, herbicides can be translocated in the phloem and/or xylem or not translocated at all. Weak acid trapping is common method required for uptake and translocation in phloem, but because neither triallate nor pyroxasulfone have a dissociable ion this method of uptake or translocation in unlikely (Senseman 2007). It was reported that triallate moves in both the xylem and the phloem (Fuerst 1987). Movement in the phloem was based on an osmotic pressure gradient generated by sucrose uptake at the source and degradation in sink tissues. Therefore, weak acid herbicides generally move from source tissues to sink tissues, which leads to activity first seen at apical meristems and new tissues. The main function of the xylem is to transport of water as well as a few nutrients from the soil up to the

rest of the plant in a one-directional flow. Those herbicides not acid-trapped and water-soluble can be transported in the xylem and if so, activity is first seen near leaf edges.

2.2.2.4. Site of action. Long-chain fatty acid synthesis occurs in the plastid as a result of a four-step reaction that is synthesized by the membrane-bound multienzyme acyl-CoA elongase containing 4 individual enzymes. Synthesis begins with the condensation of an acyl-CoA primer (16C) with malonyl-CoA to form 3-ketoacyl-CoA via 3-ketoacyl-CoA synthase (Babczinski et al. 2012; Trenkamp et al. 2004). Next there is a reduction to 3-hydroxyacyl-CoA (via 3-ketoacyl-CoA reductase), and a dehydration reaction by 3-hydroxyacyl-CoA dehydratase to result in 2-enoyl-CoA. Finally, a longer acyl-CoA is produced by second reduction via 2,3-*trans*-enoyl-CoA reductase (Babczinski et al. 2012). Very long chain fatty acids of greater than 18 carbon atoms are formed in the endoplasmic reticulum by a microsomal elongation system (Tanetani et al. 2009). Steric acid (C18:0 fatty acid) is the primary substrate and is formed in the endoplasmic reticulum from palmitic acid (C16:0 fatty acid), which is supplied by the plastids (Tanetani et al. 2009).

The primary site of inhibition for pyroxasulfone is long chain fatty acid elongase (Babczinski et al. 2012). Elongation steps from C18:0 to C20:0, C20:0 to C22:0, C26:0 and C26:0 to C28:0 have shown the largest inhibition by pyroxasulfone (Tanetani et al. 2011). Inhibition from C18:0 to C20:0 will affect stearic acid elongation in both endoplasmic reticulum and plastid; elongation steps are catalyzed by VLCFA elongase (Tanetani et al. 2011). Thiocarbamates such as triallate are thought to inhibit a variety of processes including lipid,

isoprenoid and flavonoid biosynthesis and synthesis of gibberellins, but the primary mode of action is still thought to be the disruption of metabolism of coenzyme A (Fuerst 1987).

When triallate first enters the plant, it is rapidly oxidized to sulfoxide, which allows it to act similar to other group 8 herbicides such as chloroacetamide and have alkylating activity (Fuerst 1987). These compounds will bind covalently to biomolecules that contains a sulfhydryl group; this ability has been correlated with herbicidal activity and may explain the similar mode of action between these two chemical families (Fuerst 1987). Alkylation can take place at many different locations, with the primary detrimental location not yet identified. Potential sites of alkylation include lipoic acid, acyl carrier proteins and coenzyme A (Fuerst 1987). Lipoic acid is an oxidation coenzyme in glycolysis and the tricarboxylic acid cycle. Acyl carrier proteins act as an anchor where acyl groups are esterified in the fatty acid cycle. Lastly, coenzyme A is a carrier of acyl groups during metabolic acylation and de-acyltion reactions (Fuerst 1987).

When biosynthesis of long chain fatty acids is inhibited there are a number of detrimental effects that can injure the plant. Long chain fatty acids act as components of wax, suberin and cutin, which are used in the leaf cuticle, storage lipids in seeds and as various components of membranes throughout the plant (Trenkamp et al. 2004). The decrease in long chain fatty acid's produced is believed to be the lethal effect of both triallate and pyroxasulfone.

2.2.2.5. Selectivity. Long chain fatty acid inhibitors have the ability to be selective in a variety of different ways. Triallate undergoes sulfoxidation and is metabolized quickly after entering the plant by glutathione conjugation or homoglutathion conjugation (Fuerst 1987; Westra 2012).

This conjugation can occur with the help of the enzyme glutathione S-transferase or independently (Fuerst 1987). Conjugates are formed very quickly, with the initial compounds half-life being only a few hours; the resulting conjugates are non-toxic to plants, indicating the herbicidal compounds are effective at very low doses (Fuerst 1987). The role of metabolism in selectively of plants to triallate is currently unclear (Fuerst 1987). Selectivity of triallate between wild oats and wheat/barley is primarily contributed by plant morphology differences between these species (Fuerst 1987).

Pyroxasulfone metabolism is due to glutathione conjugation of the isoxazoline ring by the cleavage of the methylene-sulfonyl linkage (Tanetani et al. 2013). When looking at metabolism between wheat and annual ryegrass, there were no differences in the route of metabolism between the two species, but an increased rate of metabolism was seen in wheat (Tanetani et al. 2013). Pyroxasulfone inhibited VLCFA elongase in rice, Italian ryegrass, wheat, corn and soybean, but a lower inhibitory potency was reported in the tolerant species wheat, corn and soybean compared to the susceptible rice and Italian ryegrass (Tanetani et al. 2011). This difference in VLCFA inhibition is a second mechanism of selectivity to pyroxasulfone observed in plants.

2.2.2.6. Soil interactions. Adsorption of soil-applied herbicides to soil surfaces is an important quality that determines availability of herbicides. Adsorption can influence herbicide distribution, movement and fate within the soil. Herbicide molecules can bind to the soil in one of two ways; chemical binding, which is an ionic or covalent bond with soil particles, or physical binding, which is weaker than a chemical bond but can be effective over larger distances.

Properties of the herbicide molecule itself have a large effect on how it will bind to the soil. Soil colloids are negatively charged, therefore if the molecule is cationic it will bind easier than if it is anionic. As previously discussed, K_d, K_{oc}, and K_{ow} of herbicides describe the adsorption of an herbicide. Normally herbicide adsorption to soil (K_{oc}) is positively correlated with its K_{ow} and negatively correlated with water solubility (Carter 2000). Westra (2012) reported the K_{oc} values of pyroxasulfone and s-metolachlor, another group 15 herbicide, and found that s-metolachlor bonded stronger than pyroxasulfone, which was the opposite to what was expected after examining their water solubility's (Westra 2012). Pyroxasulfone sorption coefficient indicates that it should be the most available in the soil water and available for uptake compared to other VLCFA inhibitors. This would suggest that pyroxasulfone has a higher water solubility, which is not the case; it has lower water solubility and yet still has reduced soil binding (Westra 2012). Pyroxasulfone use rate is approximately 7 times less than that of other LCFA inhibitors, which may be due either to increased activity per gram or reduced soil adsorption. While pyroxasulfone does have reduced soil adsorption, the correlation with activity has not yet been established (Westra 2012).

Herbicide properties are not the only factor when considering why herbicides bind to soil, soil properties play a large role as well. Soil organic matter (SOM) can affect herbicide binding. High SOM may increase herbicide adsorption by increasing binding sites. Westra (2012) examined herbicide binding of pyroxasulfone and s-metolachlor across 25 different soil types, and adsorption was strongly correlated with amount of soil SOM. Generally, herbicide adsorption to soil is seen to increase in slightly acidic soil, but this is unlikely to be the case with VLCFA inhibitors because they do not have a dissociable ion in soil pH's range (5-8.5) (Carter

2000). Soil moisture also has an effect on adsorption because water competes with herbicide binding site on soil colloids: desorption will increase as soil moisture increases (Carter 2000). This could be one of the factors influencing the increased herbicide activity of VLCFA inhibitors with ample soil moisture (Szmigielski et al. 2014). Triallate control of wild oat increased from 5-28% with good moisture conditions, and reduced control was seen in dry cool conditions (Hamblin 1977).

Herbicide binding in the soil can be directly related to its movement in the soil profile. Herbicides will generally move in soil solution, which suggests less movement with increased binding. Inhibitory activity on a sandy loam soil was greatly decreased when triallate was more than 3 mm away from the germinating seed (Beestman and Deming 1976). Downward movement of triallate did not exceed 5 cm in loam and clay soils (Smith and Fitzpatrick 1970). In another study, 95% of triallate remained in the top 1 cm of the soil profile unless it was applied with an emulsifier, which still only allowed movement down to 3 cm (Beestman and Deming 1976). The downward movement of pyroxasulfone and s-metolachlor have been directly compared in recent year's, and across all sites, pyroxasulfone moved further which is in support of the sorption coefficients of these two compounds (Westra 2012). The movement of both these herbicides was most influenced by soil type compared to moisture or irrigation (Westra 2012).

Herbicide residual activity in the soil can be beneficial for weed control, but if a herbicide persists too long it can be detrimental to future crops as well as the environment. There are two major routes of herbicide dissipation, microbial being the main mechanism, and

chemical. Microbial decomposition takes place because microorganisms use herbicides as a carbon source and can vary with SOM, soil moisture, temperature, pH and cropping systems. Chemical decomposition is a nonenzymatic oxidation, reduction or hydrolysis of the chemical in the soil. Szmigielski et al. (2014) reported pyroxasulfone's half-life in the soil decreased with both increases in SOM and increased pH. The effect of pH can be explained by changes in the charge of the organic matter and clay colloids (Szmigielski et al. 2014). As pH decreases, there is a decrease in the amount of negative charges on SOM and colloids, which would lead to a greater sorption of herbicides and decreased availability in the soil solution (Szmigielski et al. 2014). Microbial degradation may be the main dissipation mechanism for pyroxasulfone because of SOM's important role (Westra 2012).

Microbial populations can increase in the soil in response to addition of a carbon source such as a herbicide. This can lead to a shorter half-life's in the soil with consecutive applications. The half-life of triallate decreased from 52 days to 32-38 days in soil that had been treated annually with triallate for 23 years compared to that in soil that had never received a treatment of triallate (Anderson 1981). Herbicides can also be degraded by sunlight through a process is called photodegradation. This degradation process appears to be insignificant in thiocarbamates such as triallate (Hambin 1977), and its affects have not been reported for pyroxasulfone.

Before degradation, herbicides can become unavailable by dissipating away from the target weed or being lost to the atmosphere. If a herbicide is applied to the soil surface, there will be a chance for volatilization. Volatilization is the loss of herbicides to the atmosphere as a

vapor, and is influenced by a herbicides vapor pressure (Carter 2000) and the environmental conditions. Losses are greatest if soil surface is bare and moist, but can be decreased with incorporation. Volatilization is not thought to be a factor in dissipation of either pyroxasulfone or triallate. Surface run-off and leaching are both ways in which herbicides can move away from an area where it will be taken up by desired plants. Surface run-off will occur when infiltration capacity of an application area is exceeded or there is a very heavy rainfall and excess water plus soluble residues/residues sorbed to fine particles move across the surface (Carter 2000). Surface runoff movement will typically only result in losses of less than 0.05% of applied active ingredients (Carter 2000). Leaching is when the soil water and herbicide dissolved in it move downward in the soil profile and eventually will end up in ground water. Rate of leaching will increase in coarse-textured soils or when movement is through cracks or macro-pores (Carter 2000). Losses can be up to 5%, but in a normal situation will be less than 1% of applied active ingredient.

2.2.2.7. Herbicide resistance. The mode of action of triallate is not fully understood but it is known that multiple steps of biosynthesis are affected and a target site mutation may be detrimental to the plant, thus decreasing the probability of selection (Babczinski et al. 2012). Triallate resistance in wild oats has been previously discussed and resistance in other weed species is primarily due to enhanced metabolism (Babczinski et al. 2012). Pyroxasulfone was introduced much later than triallate; it is believed to have several sites of action, which could potentially slow the development of target site resistance (Westra 2012). Even though pyroxasulfone is considered a “low risk” herbicide for development of resistance, a rapid (3 generations) shift towards pyroxasulfone resistance was reported in a multiple-resistant annual

ryegrass population that was resistant to other modes of action through enhanced metabolism (Busi et al. 2012). This resistance was later characterized as non-target site based and conferred by one semi-dominant allele (Busi et al. 2014). When a completely susceptible biotype of annual ryegrass was used, this shift towards resistance was not observed and high dose screening did not reveal any resistance, suggesting any major genes potentially responsible for resistance either have very low penetrance or are extremely rare (Busi et al. 2012). The annual ryegrass population selected for resistance by pyroxasulfone was later reported to have a corresponding shift towards prosulfocarb and triallate resistance (Busi and Powles 2013). This indicated cross-resistance from a singe mechanism is likely conferring resistance to all three herbicides (Busi and Powles 2013). If this cross-resistance is consistent and observed in other species, such as wild oat, which have previously been selected for resistance to triallate in western Canada, the utility of pyroxasulfone could be limited for wild oat control.

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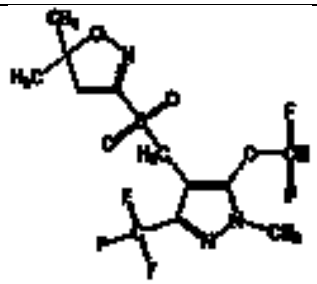
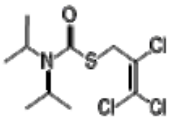
Table 2-1 Physical-chemical properties of pyroxasulfone and triallate &.

	Pyroxasulfone	**Triallate**
Group	15(K3)	8 (N)
Chemical family	Isoxazoline	Thiocarbamate
Example trade name	Zidua	Avadex BW
Formula	C12H14F5N3O4S	C10H16Cl3NOS
Structure		
pKa	*	*
log Kow	2.39	4.6
Water solubility (mg L^{-1} @ 20°C)	3.49	4
Koc (L kg-1)	113	2400
Kd (sorption coefficient; L kg^{-1})	1.725	5.3 L kg-1
Half-Life (d)	16-69	50
Vapour pressure (Pa @ 25°C)	2x10-6	14.7 X 10-2
Acute toxicity (LD50; mg kg^{-1})	>2000	2150

* no pKa in accessible pH range

& Adapted from Westra 2012

Table 2-2 Uses and applications of Pyroxasulfone and Triallate

	Pyroxasulfone	Triallate
Group	15 (K3)	8 (N)
Chemical Family	Isoxazoline	Thiocarbamate
Example Trade Name	Zidua	Avadex BW
Weeds controlled	Grasses and small seeded broadleaves	Wild oat
Crops	Corn, Soybeans, Wheat, Sunflower, spring wheat, winter wheat, and field pea	Barley, wheat, winter wheat, green pea, field pea, lentils, canola, sugar beats, flax
Application	Pre-emergence (preplant/postplant) spring or fall	Pre-emergence (preplant/postplant) spring or fall. Incorporation recommended
Symptoms	Prevent establishment, growth inhibited and distorted. Root growth can be inhibited but less sensitive than shoot growth.	

Chapter Three: Influence of tillage factors on control of wild oat (*Avena fatua* L.) by the soil-applied herbicide pyroxasulfone[1]

3.1. Introduction

Wild oat is the most economically detrimental weed in western Canada (Beckie et al. 2012) with over $500 million spent on herbicides each year for its control (Leeson et al. 2006). Due to the repeated use of herbicides, wild oat populations have evolved resistance to several herbicidal sites of action (Heap 2016). Cross-resistance, resistance to more than one herbicide by a single mechanism of resistance, and multiple resistance mutations to several target sites and enhanced metabolism, have also been reported (Beckie and Tardif 2012). Beckieet al. (2013) estimated that herbicide-resistant wild oat occurred on about 40% of all cropland in western Canada in late 2000s. As resistance incidence increases, there is a need for new chemical and integrated management solutions for wild oat. The soil-applied herbicide pyroxasulfone is being investigated as an alternative control option for wild oat in western Canada.

Pyroxasulfone, a very-long chain fatty acid biosynthesis inhibitor (Group 15/K3), offers pre-emergent control of several grasses and small-seeded broadleaves with acceptable tolerance in corn, soybean, spring wheat, sunflower winter wheat, and field pea (Tanetani et al. 2011). Pyroxasulfone has a low water solubility of 3.49 mg L^{-1} @ 20°C and there is a strong correlation between its soil binding and soil organic matter content (Westra et al 2014). Higher

[111] A version of this research "Influence of tillage factors on control of wild oat (*Avena fatua*) by the soil-applied herbicide pyroxasulfone. Amy R. Mangin, Linda M. Hall1, Jeff J. Schoenau, Hugh J Beckie" has been submitted to Weed Science.

rates of pyroxasulfone were required to achieve similar control of wild oats and cleavers (*Galium aparine*) at locations in Alberta and Saskatchewan as soil organic matter increased (Tidemann et al. 2014). Szmigielski et al. (2014) measured pyroxasulfone bioactivity across 25 different prairie soils and reported decreased activity in soils with low pH, although Westra et al (2014) observed no correlation between soil adsorption and soil acidity. Pyroxasulfone is non-ionizable, and therefore soil pH does not affect dissociation. However, soil pH may influence bioactivity due to fewer negative charges on organic and clay surfaces in soils with lower pH (Szmigielski et al. 2014).

Reduced tillage has been widely adopted across the Canadian Prairies and is typically characterized by increased soil moisture content, vertical water movement (Arshad et al. 1999; Blevins et al. 1983), soil organic matter content, microbial activity, stable surface soil aggregates (Azooz and Arshad 1996; Blevins et al. 1983; Locke and Bryson 1997; Reicosky et al. 1995; Wu et al. 1992), and soil acidity (Dick 1983; Levanon et al. 1994) relative to intensive-tilled soils. Generally, reduced tillage results in higher adsorption of herbicides to soils and, for water-soluble herbicides, increased leaching (Chauhan et al. 2006; Locke and Bryson 1997).

Tillage can also redistribute weed seeds vertically throughout the soil profile. In the absence of tillage, weed seeds are generally distributed within the top 5 cm of the soil profile (Chauhan et al. 2006; Clements et al. 1996). A survey of cropland in western Canada showed wild oat seedlings being recruited from 1.5 cm deeper in the soil profile in intensive vs. reduced or no-till management (du Croix Sissons et al. 2009). Vertical placement of weed seeds in the soil profile will determine the portion(s) of the seedling (leaves, coleoptile, crown node, or

rooting zone) that intercepts a concentrated layer of a soil-applied herbicide and thus may affect efficacy. Herbicides with low water solubility and strong soil binding, like pyroxasulfone, tend to remain near the soil surface (Locke and Bryson 1997). Westra et al (2014) reported >90% of pyroxasulfone and *s*-metolachlor remained in the top 7.5 cm of the soil profile at two field sites in Colorado over the growing season.

Due to a relatively large seed size and mesocotyl elongation, wild oats can germinate from depths up to 20 cm as well as on the soil surface (reviewed by Beckie et al. 2012). Medd (1990) compared tillage effects on wild oat population growth in wheat, and reported the greatest increase in intensive tillage compared with zero or minimal tillage. Increased persistence of the wild oat seed bank with burial depth could partially account for this population increase (Banting 1966; Mohler 1993; Wilson and Cussans 1975).

Triallate is a thiocarbamate, soil-applied, fatty acid biosynthesis inhibitor (Group 8/N) that has been used across western Canada for selective control of wild oat since the 1960s, and has similar physical-chemical properties as pyroxasulfone (Table 1). Decreased control of wild oat by triallate was reported as depth between incorporated herbicide layer and seed increased (Banting 1967). Studies looking at wild oat interception with soil-applied thiocarbamate herbicides, such as triallate, diallate and EPTC, showed greatest emerging seedling sensitivity occurring when the coleoptile came in contact with the herbicide layer (Banting 1967; Friesen et al. 1962; Hannah et al. 1960; Knake et al. 1967). Friesen et al (1962) noted that sensitivity to diallate was greatest during either the initial 1.2 cm of coleoptile growth or later during initiation of the crown node.

Comparisons of pyroxasulfone efficacy in tilled and no-tilled fields in Ontario showed increased control on velvetleaf (*Abutilon theophrasti*) (20%), redroot and smooth pigweed (*Amaranthus retroflexus*; *Amaranthus hybridus*) (33%), common ragweed (26%), and lambsquarters (58%) in tilled vs. no-tilled soils (Mahoney et al. 2014). Weed control differences were attributed to increased binding and/or degradation by increased soil organic matter and microbial activity in no-till fields, which reduced herbicide phytotoxicity.

Interaction of tillage effects on soil properties, wild oat seed position and herbicide interception may all influence efficacy. To isolate these effects, we conducted a series of greenhouse and field trials. The objectives of this study were to (1) determine the effect of wild oat seed depth on control by pyroxasulfone and triallate, (2) determine the effective site of interception of pyroxasulfone for control of wild oat, (3) quantify leaching of pyroxasulfone in the soil after a simulated rainfall event, and (4) determine pyroxasulfone and triallate control of deep- and shallow-seeded wild oats in tilled and untilled soil under field conditions.

3.2. Materials and Methods

3.2.1. Soil and Plant Material

Wild oat seeds, susceptible to fatty acid biosynthesis inhibitors, were collected from the University of Alberta Ellersllie Research Farm in 2011 (S2011) and Edmonton Research Station in 1988 (S1988). Germinations tests were conducted prior to the experiment, and germination was 85 and 87% for S1988 and S2011, respectively. Soil used in greenhouse trials was collected (0-15 cm depth) from the University of Alberta Kinsella Research Farm in fall, 2013, and was

moistened and homogenized in a soil mixture before use. Kinsella soil texture was sandy loam (58% sand, 30% silt, 12% clay) with an organic matter content of 6% and pH 7.

3.2.2. Effect of Seed Depth on Herbicidal Control

To isolate the effect of wild oat seed depth on control by pyroxasulfone and triallate, a greenhouse experiment was conducted to reduce microclimatic and soil variations that would normally be present across different tillage systems. Experimental design was a randomized split-plot, herbicide type as main plot effect and rate with seeding depth as the split-plot effect. The trial had four replicates and the entire trial was conducted twice with two susceptible wild oat populations (S2011 and S1988). Pots (8.25-cm diameter) were filled either to 6 cm or 0.5 cm from the top, and 15 wild oat seeds were placed on soil. The pots were then filled to the brim to simulate either a shallow or deep seeding. After planting, pots were sprayed with either pyroxasulfone (85% WG) (150 or 300 g ai ha^{-1}) or triallate (480 g L^{-1} EC) (1672 or 3344 g ai ha^{-1}). Untreated controls were included. Herbicides were applied using a moving-track cabinet chamber sprayer calibrated for 200 L ha^{-1} at 207 kPa using an Air Bubble Jet 110015 nozzle. One pot of each seeding depth was placed together in a tray and sprayed simultaneously for each herbicide treatment, and pots were placed in a greenhouse. Natural light was supplemented with 16 h of artificial light (275 mmol m^{-2} s^{-1}) at a temperature of 21 °C. Trays were top-watered and rotated daily to avoid positional effects. Seedling emergence per pot was recorded on a weekly basis for 4 weeks. Destructive sampling occurred 28 days after treatment (DAT) and shoot length, root length and shoot fresh weight were measured per plant. An analysis of variance (ANOVA) was conducted using the statistical software R (v. 0.98.1091) on

untransformed data, as transformations did not improve distribution or variance. Data was fit to a linear mixed model and subjected to ANOVA in the nlme package, and lsmeans analysis in the lsmeans package of R (v. 0.98.1091). Fixed factors included herbicide treatment, seeding depth and the interaction between herbicide treatment and seeding depth, with trial and replicate as random factors. Contrasts of interest were completed with a 0.95 confidence level using the *cld()* function in the lsmeans package, and P-values were adjusted when necessary using Tukey's method for multiple comparisons.

3.2.3. Effective Pyroxasulfone Interception Site

A randomized complete block experiment with four replicates was conducted to determine the effects of site of interception of pyroxasulfone on wild oat control. A thin layer (1-2 mm) of activated charcoal (Fisherbrand #05-690A, 50-200 mesh) was used as a barrier to isolate herbicide contact with specific parts of the wild oat seedling (Blair 1978). At the time of seeding, 12 wild oat seeds (S1988) were placed 2 cm from the soil surface and an activated charcoal layer was placed either 1 cm below seed level (trt 3), at seed level (trt 4), 1 cm above seed (trt 5) or 2-3 mm below the soil surface (trt 6) for surface-applied pyroxasulfone, and at seed level after herbicide application (trt 7) (Figure 1). Pyroxasulfone was applied at 150 g ai ha^{-1} as described previously. Two controls were present, untreated (trt 1) and treated with pyroxasulfone (trt 2) in the absence of activated charcoal. Plants were uprooted and washed 11 days after treatment, and shoot length, root length and shoot fresh weight recorded per plant. Each pot was considered an experimental unit and the trial was replicated three times. Data was combined for the three trials after ANOVA results indicated no significant effect of trial.

The statistical software R (v. 0.98.1091) was used to complete the ANOVAs and derive least squares means estimates for each treatment in the nlme and lsmeans packages. Tukey's HSD pairwise comparisons were used to determine treatment differences using shoot length and root length as response variables with a confidence level of 0.95.

3.2.4. Pyroxasulfone Leaching with Rainfall

To reveal how pyroxasulfone may be redistributed in the soil profile from precipitation, a soil bioassay was conducted to determine the downward movement of pyroxasulfone in potted soil with and without a simulated rainfall event. Trays (15 cm wide X 30 cm long x 12 cm deep) were filled to the brim with moistened, homogenized Kinsella soil in 2.5-cm deep intervals. A thin plastic mesh layer was used to separate depth intervals to allow for easy separation, but not obstruct water infiltration or herbicide movement. Trays were then sprayed with pyroxasulfone at 150 g ai ha^{-1} as described previously. After herbicide application, trays were placed in the greenhouse for 24 hours, after which one tray was subjected to a 2.54-cm rainfall simulation applied to the soil surface slowly over 10 minutes from a graduated cylinder and one tray did not receive any rainfall. Trays were retained for 1 week without any water and then were divided into individual 2.5-cm depth increments. Soil samples for each depth were placed in paper bags and oven-dried at 45 °C for 7 days. Samples were then transferred to plastic bags and transported to the University of Saskatchewan for completion of the soil bioassay. The soil bioassay was conducted using the bioassay technique for pyroxasulfone as described in Szmigielski et al (2014).

Soil samples from each layer were hand-mixed and divided into five subsamples for each replicate. Subsamples were then transferred to 59 mL (2 oz) WhirlPak®bag (VWR International, Mississauga, ON, Canada). Soil in the bag was gently packed to form a layer approximately 8 cm high, 6 cm long and 1 cm wide. Six canola (Liberty Link 154) seeds were planted at a 2-mm depth and the soil surface was covered with a 5-mm layer of plastic beads to reduce soil drying. Plants were grown in the laboratory under fluorescent lights that had photosynthetic photon flux density of 16µmol m^{-2} s^{-1} at plant level, and plants were watered daily to 100% field capacity by adding water to a predetermined weight. At harvest time, intact plants were recovered from soil after the WhirlPak® bag was opened, and soil was washed away with water. Canola shoot length was measured with a ruler. The trial had four replicates and subsamples within each replicate were averaged. Two-sample t-tests in R (v. 0.98.1091) were used to determine significance between the two treatments at each depth.

3.2.5. Effect of Tillage, Seed Depth and Herbicide in the Field

To explore the potential influencing factors associated with tilled and untilled soil on pyroxasulfone and triallate control of shallow and deep wild oat seeds, field trials were established at the University of Alberta's research stations at Edmonton and St. Albert, AB during the summer of 2014. Organic matter content, pH, soil texture and seasonal rainfall were similar at both sites (Table 2). Trials were designed as a split-split plot with four replicates, where main plot was tillage, first split was herbicide type and rate and the second split was wild oat seeding depth. In the spring of 2014, soil was either cultivated or was left undisturbed. Cultivation was performed to a depth of approximately 8 to 10 cm by a single pass of 10-cm

wide A-shaped shovels mounted on a custom-built 2-m wide plot seeder. Tillage was performed on May 7th and May 9th at Edmonton and St. Albert, respectively, and wild oat was seeded the next day at both sites. Wild oat was hand-seeded either shallow (0.5 cm) or deep (5 cm) in micro plots (0.25 m^2) using a 5 by 10 cm grid-seeding pattern (equivalent of 50 plants m^2). Two sub-microplots of each depth were established in each tillage (herbicide) treatment. After seeding, plots were treated with either pyroxasulfone (85 WG) applied at 150 g a.i. ha^{-1} or 300 g a.i. ha^{-1}, or triallate (480 gL^{-1} EC) at 1170 g ai ha^{-1} or left untreated. Herbicide application was performed using a 2-m wide CO_2 backpack sprayer with 100 L ha^{-1} water volume with Air Bubble Jet (110015) nozzles and a screen mesh size of 100. Herbicide treatments occurred after seeding on May 8th and May 13th at Edmonton and St. Albert, respectively. Bromoxynil/MCPA mixture was applied on May 23rd at 553 g ai ha^{-1} for control of broadleaf weeds across the entire trial at the St. Alberta research station, but no broadleaf herbicide was applied at Edmonton Research Station. Each tillage (herbicide(depth)) treatment was an individual experimental unit.

The number of wild oats that had emerged was quantified weekly. At 42 days after treatment (DAT), destructive sampling was used to measure individual plant above ground and below ground. Fresh weight data was recorded for each micro plot, and plants were placed in paper bags and put in a drier at 42°C for 7 days.

Data was fit to a linear mixed model in R with fixed factors of tillage, herbicide, seeding depth, tillage:herbicide interaction, tillage:seeding depth interaction, herbicide:seeding depth interaction and the three-way interaction of tillage:herbicide:seeding depth. Location and

replicate were considered to be random. Contrasts of interest were completed with a 0.95 confidence level using the *cld()* function in the lsmeans package in R (v.0.98.1091), and p-values were adjusted when necessary using tukey's method for multiple comparisons.

3.3. Results and Discussion

3.3.1. Effect of Seed Depth on Herbicidal Control

When wild oats were seeded shallow or deep and treated with pyroxasulfone or triallate, there was a significant effect of herbicide, seeding depth and herbicide:seeding depth interaction ($p < 0.05$). There was no interaction with wild oat population, therefore results from each trial were combined. Due to the interaction between seeding depth and herbicide, the effect of seeding depth was compared within herbicide treatments, and herbicide effects examined within a particular seeding depth.

There was no significant difference between the average shoot lengths of shallow or deep wild oats that were left untreated. This result was expected due to large seed size and ability for mesocotyl elongation that allows wild oat to be recruited from varying depths (Blair 1978; Boyd and Van Acker 2003; Sharma and Vander Born 1978). Within each depth, the recommended field rate of pyroxasulfone and triallate both significantly reduced wild oat shoot length compared with the untreated controls (Figure 2). In shallow-seeded wild oats, average shoot length was reduced by 66.5% and 77.5% by the recommended field rate of pyroxasulfone and triallate, respectively. Deep-seeded wild oats treated with pyroxasulfone (150 g ai ha^{-1}) had an average shoot length reduction of 10.0 cm, while triallate reduced shoot length by 7.6 cm.

Within each depth, there was no significant difference between triallate and pyroxasulfone at the recommended field rate or between rates of pyroxasulfone or triallate.

Herbicide efficacy of both pyroxasulfone and triallate was significantly reduced in deep-seeded compared to shallow-seeded wild oats (Figure 2). Shoot length was decreased an additional 3.6 and 9.4 cm in shallow-seeded compared with deep-seeded plants when treated with the recommended field rate of pyroxasulfone and triallate, respectively. The effect of depth on herbicidal activity indicates that when wild oats are deeper in the soil profile, they are less vulnerable to both triallate and pyroxasulfone. The decreased activity on deep-seeded wild oat is most likely due to the physical-chemical herbicide interaction with the soil, as well as wild oat site of uptake of these herbicides (Knake et al. 1967). With similar low water solubility values of pyroxasulfone (3.49 mg L-1 @ 20 °C) and triallate (4.0 mg L-1 @ 20 °C) (Table 1), we expect both herbicides to strongly bind to the soil and remain near the soil surface unless incorporated (Beestman and Deming 1976; Westra et al 2014). This would result in different areas of a wild oat seeding contacting the concentrated herbicide layer when recruited from various depths in the soil profile. Our results agree with a previous study by Banting (1967) that reported increased shoot length of wild oat with increased distance between wild oat seeds and triallate-treated soil.

3.3.2. Effective Pyroxasulfone Interception Site

Inhibition of wild oat varied, depending on what portion of the wild oat seedling came in contact with the pyroxasulfone-treated soil. When pyroxasulfone was applied in the absence of active charcoal, shoot length was reduced by 6.4 cm (Table 3). When pyroxasulfone was

allowed to reach wild oat seed level (Trt. 4) and 1 cm below seed level (Trt. 3), similar herbicidal symptoms to the pyroxasulfone application without an isolation zone were observed. When pyroxasulfone was isolated to either the soil surface or rooting zone below the seed, there was little effect of pyroxasulfone on wild oat shoot length (reductions of only 1.51 and 1.26 cm, respectively). Even though all treatments significantly decreased shoot length compared to the untreated check, these results indicate that the critical point of pyroxasulfone and seedling interception is either the seed itself or within the first 1 cm of shoot growth. Root length of wild oat was not significantly reduced by pyroxasulfone applied to the soil surface with or without active charcoal isolation (Table 3). When pyroxasulfone was applied before seeding and isolated to the rooting zone (Trt. 7), there was a significant decrease in root length (3.02 cm) compared with the untreated check.

Banting (1967) investigated the effect of depth of seed placement on control of wild oat when treated with triallate and diallate, and observed an increase in wild oat shoot length as the distance between triallate- or diallate-treated soil layer and wild oat seed increased. This was attributed to a longer period of shoot development before the stem apex reached the herbicide layer. Friesen et al (1962) reported that either the initial 1.5 cm of coleoptile growth or the initiation of the crown node to be the most sensitive growth stages of wild oat and diallate interception. Our results suggest that when wild oats are being recruited from deep in the soil profile, the critical site of interception may not come in contact with the concentrated herbicide layer, allowing for seedlings to avoid herbicidal effects.

3.3.3. Pyroxasulfone Leaching with Rainfall

When comparing a single 2.54-cm rainfall event after application to no rainfall in a canola shoot bioassay, we observed that in both treatments the majority of the pyroxasulfone activity was localized in the top 2.5 cm of soil with no significant difference of shoot inhibition at this depth ($p > 0.05$) (Figure 3). In the 2.5 to 5-cm and 5 to 7.5-cm depths, there were significant differences in canola shoot inhibition. Canola that was grown in the soil that had a rainfall event had greater shoot length at both depth intervals. This would suggest that there is more bioactive pyroxasulfone available in the soil that did not receive any rainfall after application. The effect of rainfall contradicts what would be expected, possibly due to movement of soil and pyroxasulfone along the sides of the tray with the rainfall event. Westra et al (2014) looked at vertical movement of pyroxasulfone in field soils and reported the majority of pyroxasulfone remained in the top 7.5 cm of the soil profile throughout the growing season. Our bioassay results suggest that with or without a single rainfall event, the majority of bioactive pyroxasulfone is present in the top 2.5 cm of the soil profile. This would support our previous work indicating that deep-seeded wild oats are escaping herbicidal effects because the critical point of interception is below the concentrated herbicide layer in the soil profile.

3.3.4. Effect of Tillage, Seed Depth and Herbicide in the Field

With the additional factor of tilled and untilled soil on control of wild oat seeded shallow and deep, deep-seeded wild oats emerged earlier than shallow-seeded wild oats in both Edmonton and St. Albert sites. Additionally, earlier emergence was observed in wild oats seeded deep in no-till soil compared with tilled soil (Figure 4). These differences in emergence may be attributed to increased soil moisture availability when seeds are placed deep compared

to shallow, and slower soil drying in no-till compared with tilled soil (Chauhan et al. 2006; Roberts 1984).

Above ground plant length was used to determine treatment differences. Tillage ($p = 0.0223$), herbicide ($p < 0.0001$), seed depth ($p < 0.0001$), tillage:herbicide interaction ($p = 0.0027$), tillage:seed depth interaction ($p < 0.0001$) and herbicide:seed depth interaction ($p < 0.0001$) were all significant, while the three-way interaction between tillage, seed depth and herbicide was not ($p = 0.4917$). Due to interactions, treatment effects were examined within an individual depth and tillage combination.

Wild oat shoot length was significantly reduced when seeded shallow vs. deep in all herbicide and tillage treatments, including the untreated control (Figure 5). In the absence of herbicides, mean shoot length was, 16.4 cm shorter in shallow-seeded wild oat compared to deep-seeded wild oat in no-till soil, and shallow-seed wild oat was 11.2 cm shorter than deep-seeded wild oat in tilled soil. This difference is most likely due to delayed emergence of shallow-seeded wild oats due to rapid soil drying in tilled soil compared to untilled soil, thereby decreasing available moisture for plant growth.

Herbicide treatments all significantly reduced wild oat shoot length in each tillage and depth treatment except for shallow-seeded wild oats in tilled soil (Figure 5). The lack of herbicidal activity on shallow-seeded wild oats in tilled soil may be attributed to the absence of moisture for herbicidal activation (Walker 1971) due to rapid soil drying. Pyroxasulfone and triallate applied at the recommended rate to shallow- and deep-seeded wild oats in no-till soil, as well as deep seeded in tilled soil all significantly decreased wild oat shoot length compared

to the untreated control in that seeding depth/tillage treatment. Similar to the effect of seed depth experiment, there were no significant differences in shoot inhibition between pyroxasulfone rates or between the recommended rates of pyroxasulfone and triallate.

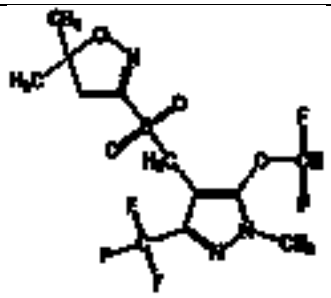
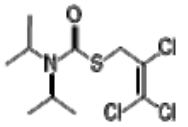
Across all depth and herbicide combinations, there was no significant effect of a single tillage pass on wild oat shoot length (Figure 5). By comparing soil directly adjacent with or without a single tillage event, we reduced differences in soil properties, such as organic matter content, and isolated tillage effects mainly to affects on soil moisture and temperature. An established no-till field will generally have slower soil drying and cooler soil temperatures, but also increased soil organic content and microbial activity (Blevins et al. 1983; Locke and Bryson 1997; Reicosky et al. 1995; Wu et al. 1992), which can additionally influence the efficacy of a soil-applied herbicide. Differences in control of velvetleaf, pigweed, common ragweed and lambsquarters by pyroxasulfone and flumioxazin between tilled and no-tilled systems have been reported previously (Mahoney et al. 2014).

3.4. Conclusion

The efficacy of soil-applied herbicides is influenced by edaphic and climatic factors along with the position of weed seeds relative to the herbicide-treated layer. Herbicides with low water solubility, like pyroxasulfone, have limited ability to penetrate the soil profile. For adequate control, wild oat seeds need to germinate either in the concentrated herbicide layer or within 1 cm below it to allow for effective herbicide interception by the seedling. Tillage influences many edaphic factors and weed seed position within the soil profile. Soil moisture is required to allow pyroxasulfone to partition into the seedlings and for seed germination and

emergence, but it varies with depth and tillage. Therefore, efficacy of pyroxasulfone on wild oat is likely to be variable between years, sites, and tillage regime used by growers.

Table 3-1 Physical-chemical properties of pyroxasulfone and triallate.

	Pyroxasulfone	**Triallate**
Group	15(K3)	8 (N)
Chemical family	Isoxazoline	Thiocarbamate
Example trade name	Zidua	Avadex BW
Formula	C12H14F5N3O4S	C10H16Cl3NOS
Structure		
pKa	*	*
log Kow	2.39	4.6
Water solubility (mg L^{-1} @ 20°C)	3.49	4
Koc (L kg^{-1})	113	2400
Kd (sorption coefficient; L kg^{-1})	1.725	5.3 L kg-1
Half-Life (d)	16-69	50
Vapour Pressure (Pa @ 25°C)	2x10-6	14.7 X 10-2
Acute toxicity (LD50; mg kg^{-1})	>2000	2150

* no pKa in accessible pH range

Table 3-2 Soil properties and precipitation data for trial locations

Location	Soil OM	Soil pH	Soil Classification	Soil Texture			Accumulated from May 1-Aug 1	Long term Average (LTA)	% of LTA
	-%-			---------- % ----------			----------mm----------		-%-
				Sand	Silt	Clay			
Edmonton	12.3	6.4	Black Chernozemic	20	39	41	230.5	215	107
St. Albert	11.2	6.2	Black Chernozemic	13	58	29	210	210	112

Table 3-3 Shoot length (cm) and root length (cm) of wild oat with isolated pyroxasulfone exposure.

Trt	Herbicide Isolation	Shoot Length (cm) (±SE)		Root Length (cm) (±SE)	
1	Untreated check	8.92 (0.28)	a	8.16 (0.64)	a
2	Pyroxasulfone check	2.50 (0.28)	d	6.88 (0.64)	ab
3	Surface – 1 cm below seed	2.13 (0.28)	d	7.81 (0.64)	a
4	Surface – seed level	2.53 (0.28)	d	8.31 (0.64)	a
5	Surface – 1 cm above seed	4.04 (0.28)	c	8.40 (0.64)	a
6	Top 0.25 cm of soil	7.41 (0.28)	b	8.73 (0.64)	a
7	Below seed	7.66 (0.28)	b	5.14 (0.64)	b

Values in a column followed by a different letter are significantly different ($p < 0.05$) according to tukey HSD pairwise comparisons.

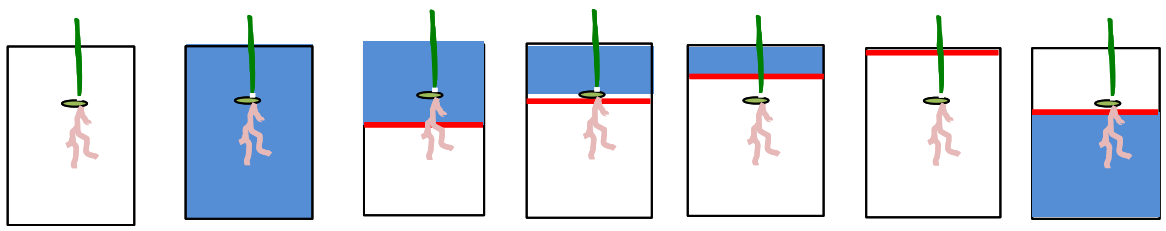


Figure 3-1 Illustration of treatments (Trt) in effective site of interception trial, where the line is the activated charcoal layer with the shaded area being the area in the soil that pyroxasulfone was allowed to move throughout. In trt's 3-6 pyroxasulfone was applied to the soil surface and was allowed to reach 1 cm below the seed, seed level, 1 cm above the seed and one the soil surface. Pyroxasulfone was applied before seeding in trt 7 and was isolated to below the soil surface.

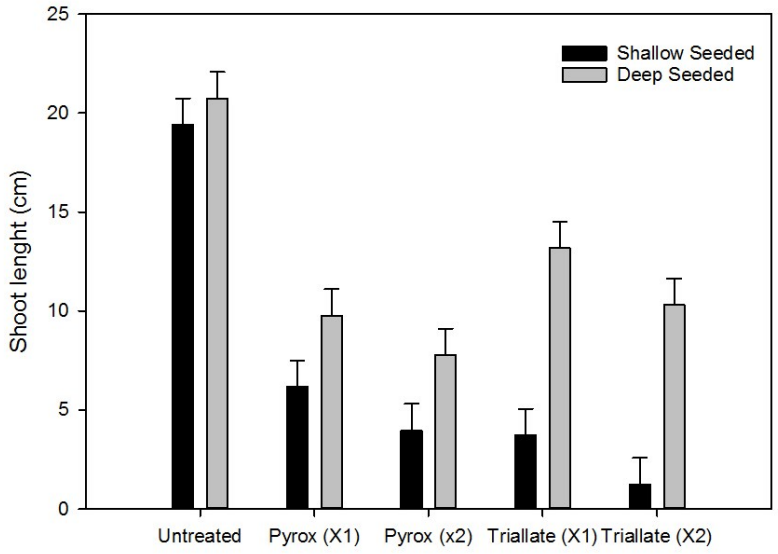


Figure 3-2 Average shoot length of wild oat seedlings seeded shallow and deep when treated with the soil-applied herbicides pyroxasulfone and triallate (bars denote SE).

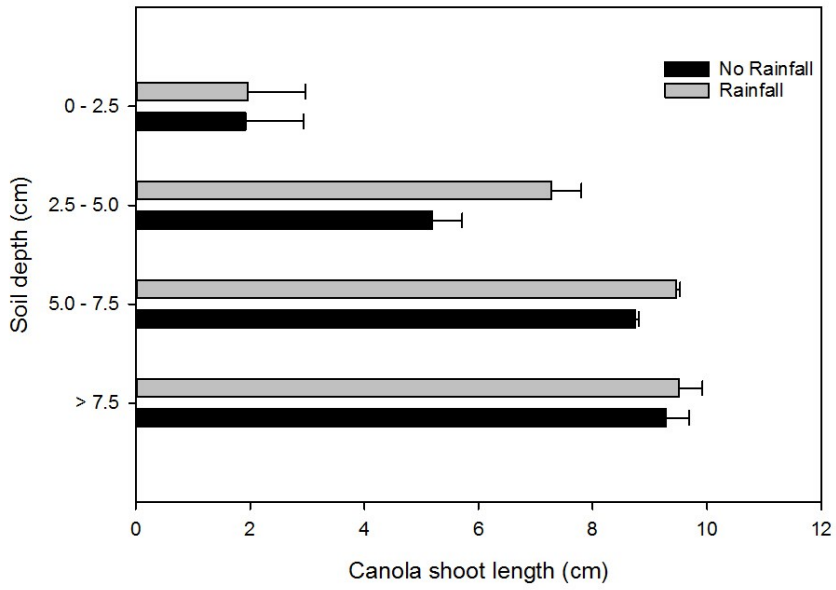


Figure 3-3 Soil bioassay results with canola shoot length (cm) indicating distribution of bioactive pyroxasulfone in the soil profile after application with and without a simulated rainfall event (bars denote SE).

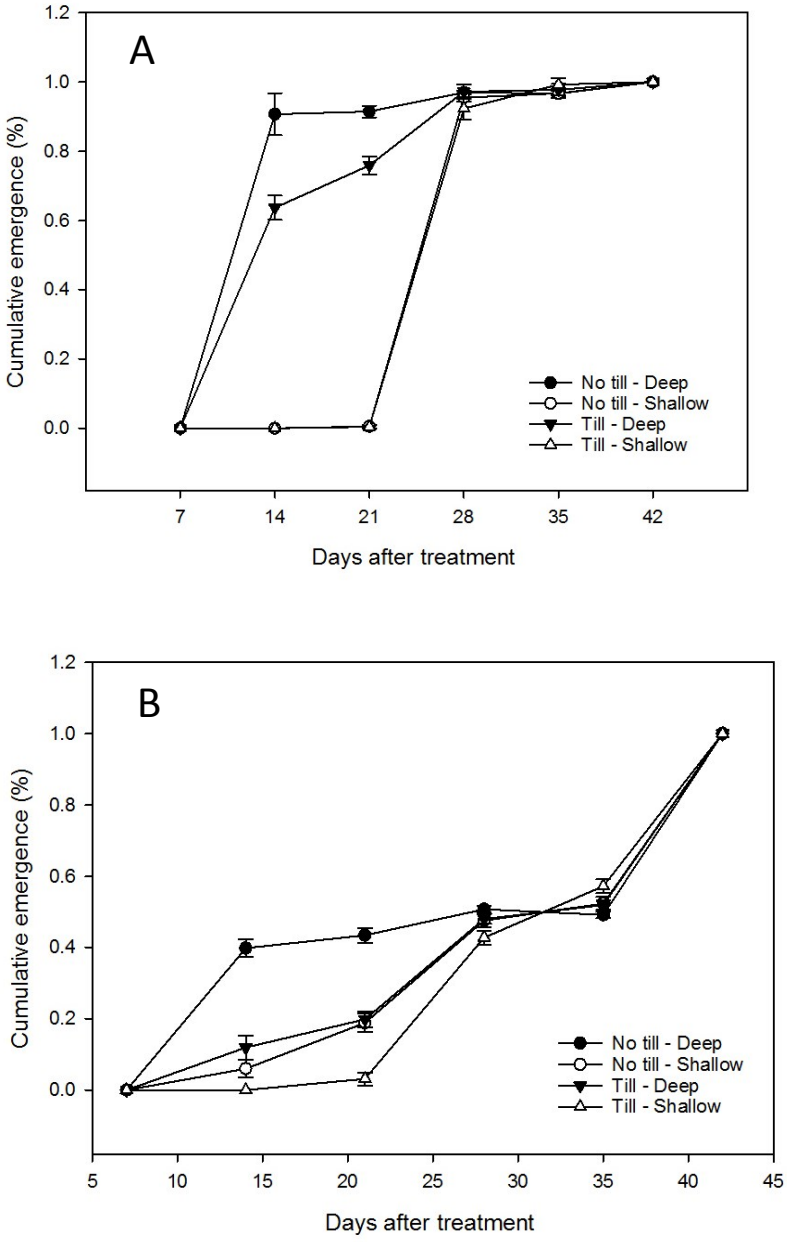


Figure 3-4 Proportion of wild oat emergence following pyroxasulfone treatment at Edmonton (A) and St. Albert (B) (bars denote SE).

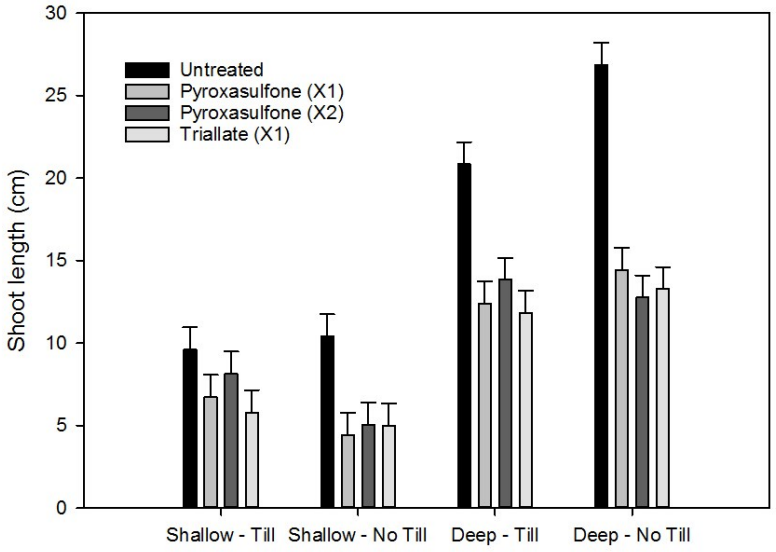


Figure 3-5 Wild oat shoot length (cm) measured from the soil surface when seeded shallow or deep in tilled or untilled soil and treated with pyroxasulfone or triallate (bars denote SE).

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Chapter Four: Influence of tillage on wild oat control by pyroxasulfone and vertical seed distribution

4.1. Introduction

In the past decades, there has been an increasing shift towards reduced or zero-tillage from conventional tillage in Western Canada (Larney et al. 1994). Adoption of reduced tillage can be contributed to the short-term benefits of decreased fuel cost and time savings as well as long-term benefits such as reductions in soil erosion (Cogo et al. 1984), increased soil moisture (Blevins et al. 1983; Griffith et al. 1986), increased soil organic matter (Arshad et al. 1999; Blevins et al. 1983; Franzluebbers and Arshad 1996; Janzen et al. 1998; Woods 1989), improved soil structure and increased microbial activity (House and Brust 1989; Karlen et al. 1994). Even with the proven benefits of reduced tillage, there remains a number of producers that use tillage for weed control of problematic species, residue management, and for seedbed preparation.

In addition to alterations to soil properties, changes in tillage practices affect the vertical placement of weed seeds in the soil (Cousens and Moss 1990; Starica et al. 1990). Tillage practices can also redistribute weed seeds throughout the soil profile to the depth of tillage. Seed depth influences seed survival, germination and emergence (Chauhan et al. 2006; Mohler 1993). Generally, increased numbers of weed seeds will remain on or near the soil surface when tillage is reduced. Yenish et al (1992) reported 60% of the total weed seed bank in the top 1 cm of the soil profile in zero-till fields and only 30% in conventional tillage fields. Additionally, Yenish et al (1996) reported 90% of the total weed seed bank in the top 2 cm of

the soil profile in zero-tillage fields. The depth of weed seeds in the soil profile may have different effects on weed species due to differences in seed size, germination requirements and recruitment success from depths in the soil profile.

Wild oat is the most economically important weed in Canada and the second most abundant grassy weed across the Prairie Provinces (Beckie et al 2012; Leeson et al. 2006). Populations of wild oat have been reported to be greater in tilled fields compared to no-till (Medd 1990), which may be attributed to its increased seed bank persistence as burial depth increases, reduced seed predation and reported stimulation of emergence with cultivation (Peltzer and Matson 2002; Wilson and Cussans 1975). A survey of cropland in Western Canada reported wild oat were, on average, being recruited from 1.5 cm deeper in the soil profile in conventional tillage fields compared to reduced or zero tillage fields (du Croix Sissons et al. 2009). Wild oats have the ability to emerge from depths up to 20 cm in the soil profile due to elongation of the first internode through mesocotyl elongation (Beckie et al. 2012).

With reduced tillage there is an increased reliance on herbicides for weed control (Chauhan et al. 2006), but repeated applications of herbicides with similar modes of action for control of wild oat have selected for herbicide-resistant populations across the Prairies (Beckie et al. 2013). With over 40% of the fields in Western Canada having wild oat populations resistant to ACCase, ALS or fatty acid synthesis inhibitors, the development of new herbicides for control is important (Beckie et al. 2013). Pyroxasulfone is a soil-applied, very long chain fatty acid inhibitor (Group 15) that has potential to be used for control of wild oat in Western Canada (Tanetani et al. 2009; Tidemann et al. 2014). Acceptable tolerance had been reported in corn,

soybean, sunflower and field pea, while general weed spectrum controlled includes small-seeded broadleaves and grasses (Tanetani et al. 2011). A low water solubility (3.49 mg L^{-1} @ 20°C) and strong correlation between adsorption and soil organic matter (r^2=0.94) indicates that pyroxasulfone is strongly bound to the soil and therefore, unless incorporated, will remain near the soil surface (Westra 2012). Performance of a soil-applied herbicide, such as pyroxasulfone, may be influenced by weed seed position in the soil profile, soil disturbance, degree of incorporation and residue present on the soil surface (Chauhan et al. 2006)

Tillage influence on soil properties, such as increase in soil organic matter with reduced tillage, has been reported to increase persistence and reduce efficacy of soil-applied herbicides with low water solubility (Harrison and Weber 1978; Lambert 1967; Leopold and Neal 1960). The increased presence of crop residue on the soil surface can also have a significant effect on the efficacy of soil applied herbicides by intercepting applied herbicide making them unavailable for uptake by weeds (Chauhan et al. 2006). Soil moisture is required for herbicidal activity of soil-applied herbicides (Walker 1971). Therefore, reduced activity may be observed in soils under conventional tillage fields because of a rapid rate of drying compared to no-till fields (Blevins et al. 1983; Griffith et al. 1986).

The persistence of soil-applied herbicides in the soil can also be influenced by tillage practices. By strongly binding herbicides, increased levels of soil organic matter can protect herbicides from degradation (Locke and Bryson 1997). Alternatively, the increased microbial population with increased soil organic matter (Doran 1980) causes a faster dissipation rate, as it

is the primary factor responsible degradation of many herbicides in the soil (Beestman and Deming 1974; Mueller et al. 1992).

The control of wild oat by pyroxasulfone must be evaluated across tillage systems for a better understanding of its potential utility in Western Canada. Prediction of control of a specific weed species in various tillage systems is difficult due to the interactions between the herbicide physical-chemical properties, soil properties, microclimatic differences and the vertical weed seed distribution in the soil. Therefore, the research objectives of this study include (1) to compare the efficacy of pyroxasulfone on wild oat control when applied in the fall and spring to tilled and untilled soil and (2) effects on vertical distribution of wild oat in the soil in tilled compared to untilled soil.

4.2. Materials and Methods

4.2.1. Locations and Design

In the fall of 2014, pyroxasulfone efficacy and wild oat seed distribution as affected by tillage trials were established at 3 locations in Alberta: Edmonton, Kinsella, and Lacombe. Soil organic matter content ranged from 6.2% in Kinsella, AB to 12.4% at the Edmonton Research Station (Table 1). Soil texture ranged from a loam at Kinsella (51% sand, 33% silt, 16% clay) to silty clay at Edmonton (18% sand, 41% silt, 42% clay). Soil pH was similar across all sites with the lowest pH being at Lacombe (5.9) and the highest pH in Edmonton (6.4) (Table 1). Edmonton and Lacombe trials were both established on barley silage stubble, Edmonton with light straw residue and Lacombe with heavy residue, while Kinsella was placed on canola stubble with light straw residue.

4.2.2. Pyroxasulfone Efficacy Trials

To better understand control of wild oat by pyroxasulfone when applied in the spring and fall to soil with fall tillage verses direct seeding, efficacy trials were established in a split-split plot with 4 replicates. The main plot was tillage type (fall tillage or no-till), the first split plot being application timing (fall or spring) and the second split plot being pyroxasulfone rate.

Wild oat seed, susceptible to group 1, 2 and 8 herbicide (refer to chapter 3), was broadcast in the fall before tillage to emulate natural seed dispersal at a rate of 200 seeds m^{-2} (87% germination) across the entire trial at all locations between October 3rd and 8th, 2014 (Table 2). Fall tillage treatment in Edmonton and Kinsella was done using a 3-point hitch 8-foot wide plot cultivator with A-shaped shovels (12 cm wide) to a depth of approximately 8-10cm at a speed of 5 km h^{-1}. Tillage at Lacombe was conducted using a 3.66-m wide 3-point hitch cultivator with spike style openers, 5 cm wide at a speed of 5 km/h due to soil that was difficult to cultivate with shovels.

Pyroxasulfone 85 WDG was applied at 0, 50, 100, 200 and 400 g a.i. ha^{-1} either in fall after tillage or in spring shortly after seeding. It was applied after fall tillage on either October 8th (Edmonton and Lacombe) or October 9th (Kinsella). Herbicide application in both spring and fall was performed using a 2-m wide CO2 backpack sprayer with 100L ha^{-1} water volume at all sites with Air Bubble Jet (110015) nozzles and a screen mesh size of 100.

Field peas, cultivar Thunderbird, were seeded at all sites to a depth of 2-2.5 inches using a custom-built 2-m wide plot seeder with a target plant population of 90 plants m^{-2}. Field peas were fertilized based on spring soil-test results, seed placed phosphorus was applied at seeding

at all locations and Kinsella had side-banded potash applied at seeding (Table 2). Seeding was performed perpendicular to tillage passes. Dates of seeding for all sites ranged from May 5th to May 21st. Spring pyroxasulfone treatments were completed as soon as possible after seeding between May 11th to May 23rd (Table 2). Maintenance herbicide application was completed as necessary either pre-seeding or in crop to control broadleaf weeds (Table 3).

4.2.2.1. Data Collection. Wild oat populations were counted once a week after initial emergence and continued for 4 weeks in 2 permanently marked 0.25 m^{-2} quadrats placed in the front and the back of each plot, excluding outside crop rows. Visual control ratings of entire plots were done weekly after initial wild oat emergence up to 4 weeks after emergence. Plots were given a rating on a scale of 0-100% control of wild oat compared to the untreated control of that replicate. Four weeks after initial wild oat emergence, all individual wild oat plants present within the permanently marked quadrats were dug and shoot length, root length and seed to soil surface length of each plant measured. Wild oat fresh weight biomass was taken per quadrat. Staging of wild oat plants at all sites ranged between 2 leaves to flowering at the time of final sampling, because of a wide range of emergence timings. Crop counts where conducted once in all plots two weeks after crop emergence by randomly placing a 0.25m^{-2} quadrat in 3 locations throughout the plot (data not shown).

4.2.2.2. Statistical analysis. Each location was analyzed separately in the statistical software R (v.0.98.1091) due to treatment interactions with location. Analysis of variance (ANOVA) was conducted on wild oat biomass data, where tillage, application timing, rate, tillage:application timing, tillage:rate, application timing:rate and tillage:application timing:rate were considered

fixed factors and replicate, replicate:tillage, and replicate:tillage:application timing were considered to be random. All wild oat fresh weight biomass data was square root transformed to improve the distribution and variance. The cld() function in the lsmeans package of R (v.0.98.1091) was used to determine least square means (lsmeans) and significant treatment effects of fixed factors by conducting contrasts of interest within the lsmeans package. All data was back-transformed for presentation of results.

4.2.3. Vertical Seed Distribution with Tillage

A second trial at each location was used to examine wild oat vertical distribution in the soil with and without fall cultivation. Experimental design was a 2 treatment (fall-till or direct seeding) randomized complete block experiment with 4 replicates. Before the fall tillage application, wild oat seed was broadcast (200 seed m^{-2}). Due to seed limitations, 1 – 1.5 cm long coloured pieces of wooden skewers were also broadcast across plots at a rate of 400 pieces m^{-2} to emulating wild oat seeds. Fall tillage and seeding of field pea was completed with the same methods and timing as the pyroxasulfone efficacy trial at each location (Table 2).

4.2.3.1. Data collection. After seeding a 10.16-cm in diameter cylinder was used to sample wild oat seed movement with tillage. The cylinder was marked at 2.5 cm intervals from 0 – 15 cm, and each sample was divided into depth layer relative to the soil surface. Within each core, individual depths were removed by repeated sampling in the same location and placed in separate paper bags. Five subsamples were taken per plot. All samples were allowed to air-dry and then sieved to remove any wild oat or skewer pieces from each the sample depth per subsample.

4.2.3.2. Statistical analysis. Number of wild oat seeds and skewers retrieved from each depth of subsamples within the same plot were combined and averaged across replicates. Data was subjected to 2-sample test for equality of proportions in R to determine significant differences in vertical distribution of total retrieved wild oat and skewers between fall tillage and direct-seeded plots as skewers acted similar to wild oats.

4.3. Results And Discussion

4.3.1. Pyroxasulfone Efficacy Trials

All trial locations had precipitation similar to the 30 year long-term average (LTA) from October 1, 2014 to March 31, 2015, but precipitation was limited from April to June, with monthly precipitation less than 50% of the long term averages (LTA) at all sites (Table 4). Due to lack of precipitation in May and June and later seeding dates, Kinsella and Lacombe had delayed emergence of wild oats. Kinsella had a low number of wild oats emerge 20 days after planting but after a heavy rainfall event of 22.8 mm on July 1, 2015 a second cohort of wild oats emerged. Effects of tillage, application timing, pyroxasulfone rate and their interactions on wild oat control were quantified using wild oat biomass.

The effect of tillage at all sites had a similar trend of increased wild oat biomass in untilled soil compared to tilled soil (Figure 1). At Edmonton, tillage ($p = 0.0126$) as well as the interaction between tillage and application timing ($p = 0.0087$) were significant while interaction of tillage with herbicide rate ($p = 0.5575$) and the three-way interaction with application timing and herbicide rate ($p = 0.7438$) were not significant (Table 5). When comparing tilled and untilled soil within application timing we observed increased wild oat

biomass in untilled soil compared to tilled soil (Figure 1). There was a significant difference ($p < 0.05$) when pyroxasulfone was applied in the spring with wild oat biomass being 87.96 g m^{-2} in untilled soil and 22.64 g m^{-2} in tilled soil.

At the Kinsella location there was a significant effect of tillage ($p = 0.0326$) but no significant interactions with tillage. Untilled soil had a significantly higher wild oat biomass compared to tilled soil ($p < 0.05$).

Lacombe had a low wild oat population compared to Edmonton and Kinsella due to heavy competition by other grassy weeds including volunteer barley and green foxtail. There was a significant effect of tillage ($p = 0.0325$) and the interaction between pyroxasulfone rate and tillage ($p = 0.0039$) but no significant effect of tillage interaction with application timing ($p = 0.7885$) or the 3-way interaction with application timing and pyroxasulfone rate ($p = 0.4132$) (Table 5). Within each pyroxasulfone rate, there was a trend of untilled soil having greater wild oat biomass, but this difference was only significant in the absence of pyroxasulfone ($p < 0.05$) (Figure 1).

By comparing control of pyroxasulfone on soil directly adjacent with or without a fall tillage application, we reduced the effects of long term direct seeding effects on soil properties and focused on the influence tillage has on soil moisture, temperature and wild oat distribution in the soil profile. The relationship between tillage and wild oat seedling recruitment depth was explored in this trial. No relationship was observed, but previous studies have reported wild oat recruitment from deeper in the soil profile in conventional-tillage fields compared to direct seeded fields (du Croix Sissons et al. 2009). The increase in wild oat biomass between untilled

and tilled soil is likely attributed to increased soil drying in tilled soil compared to untilled soil. During the growing season, spring precipitation was very limited across all sites compared to the long-term averages (Table 4) and this led to soil moisture conservation being critical for plant growth. Even though our study showed a decreased wild oat biomass with fall tillage, wild oat populations and seed bank survival tend to increase under conventional tillage (Banting 1966; Medd 1990; Wilson and Cussans 1975) and cultivation can stimulate emergence (Peltzer and Matson 2002)

The effect of application timing of pyroxasulfone on control of wild oats varied across locations. Application timing ($p = 0.0002$), the interaction between application timing and pyroxasulfone rate ($p = 0.0087$) as well as the interaction between application timing and tillage ($p= 0.0095$) had a significant effects on wild oat biomass at Edmonton (Table 5). Across both tillage types and all pyroxasulfone rates, there was a similar trend of decreased wild oat biomass when pyroxasulfone was applied in the fall compared to the spring (Figure 1). In untilled soil the effect of application was significant ($p < 0.05$) for all pyroxasulfone rates and in the tilled soil it was not ($p> 0.05$). In the absence of pyroxasulfone there was no difference between wild oat biomass in spring vs. fall application timing in tilled or untilled soil, which was expected.

In Kinsella, application timing ($p = 0.0261$) and application timings interaction with pyroxasulfone rate ($p = 0.0293$) had a significant effect on wild oat biomass, while application timing interaction with tillage ($p = 0.0734$) and the 3-way interaction with pyroxasulfone rate and tillage ($p = 0.9110$) were not significant (Table 5). Similar to Edmonton, wild oat biomass

was reduced when pyroxasulfone was applied in the fall compared to the spring (Figure 1). Higher rates of pyroxasulfone (200 and 400 g ai ha^{-1}) had a significant effect of application timing ($p < 0.05$) while lower rates did not ($p > 0.05$). There was no significant effect of application timing ($p = 0.3167$) or any interaction including application timing at the Lacombe site.

Previous studies on pyroxasulfone control of wild oats in Alberta and Saskatchewan showed an inconsistent effect of application timing at various locations (Tidemann et al. 2014). Lower ED_{50} values, indicating greater inhibition by pyroxasulfone, were observed in fall-applied pyroxasulfone for 2 out of 5 sites and in the spring-applied pyroxasulfone for 1 site. Soil-applied herbicides require adequate soil moisture for weed control (Walker 1971). Therefore, in a growing season like 2015 with limited spring precipitation, it is expected that fall application would provide better control due to the influence of spring snowmelt. This was observed in 2 out of the 3 locations in this study. The lack of application timing effect in Lacombe may be attributed to a very low wild oat population in the absence of herbicide due to competition by other grassy weeds. Increased control by pyroxasulfone applied in the fall could have additionally been influenced by incorporation at seeding as spring application was done after seeding operations. Pyroxasulfone has a low water solubility and remains near the soil surface after application due to adsorption to the soil. Additionally, wild oat seedlings can emerge from various depths in the soil profile, which can affect the portion of the seedling that contacts this concentrated herbicidal layer. It has been reported that for effective control of wild oat by pyroxasulfone, the wild oat must intercept herbicide either at seed level or 1 cm above or they may escape lethal herbicidal effects (Chapter 3). With seeding operations happening after

pyroxasulfone application, the herbicide may be incorporated to the depth of seeding, this could potentially result in the wild oats that are being recruited from deeper in the soil profile intercepting pyroxasulfone sooner and at the critical site of interception for effective control to take place.

Wild oat biomass was affected similarly at Edmonton and Kinsella with increasing rates of pyroxasulfone, but differed at Lacombe. In Edmonton and Kinsella, there was a significant effect of pyroxasulfone rate ($p = <0.0001$) as well as the interaction between pyroxasulfone rate and application timing ($p= 0.0087, 0.0293$). The interaction between pyroxasulfone rate and tillage ($p= 0.5575, 0.2061$) and the 3-way interaction between tillage, application timing and pyroxasulfone rate ($p= 0.7438, 0.9110$) were not significant (Table 5). When applied in the fall, there was a significant response of decreasing wild oat biomass with an increasing pyroxasulfone rate ($p < 0.05$) (Figure 1). Wild oat biomass was reduced from 10.04 and 17.05 g 0.25 m^{-2} in the untreated check to 0.18 and 0.77 g 0.25 m^{-2} with 400 g ai ha^{-1} of pyroxasulfone in Edmonton and Kinsella, respectively. Pyroxasulfone application did not decrease wild oat biomass compared to the untreated check when applied in the spring at either location (Figure 1). A larger influence of increasing rate of pyroxasulfone on wild oat biomass when applied in the fall compared to the spring was expected due to limited spring precipitation available for herbicide activation.

In Lacombe, pyroxasulfone rate ($p < 0.0001$) and the interaction between tillage and pyroxasulfone rate ($p = 0.0039$) had a significant effect while the interaction with application timing ($p = 0.3870$) and the 3-way interaction ($p = 0.4132$) was not significant (Table 5). When

applied to untilled soil, wild oat biomass decreased as pyroxasulfone rate increased (Figure 1). Tilled soil had a very low population of wild oats in the absence of herbicide (1.08 g $0.25m^{-2}$), therefore the effect of increasing rates of pyroxasulfone was small and non-significant ($p > 0.05$). The greater influence of increasing pyroxasulfone rate in direct-seed soil is likely due to increased wild oat germination due to soil moisture conservation compared to tilled soil.

4.3.2. Vertical Seed Distribution with Tillage

To further understand how tillage and application timing is affecting wild oat control we examined vertical seed distribution in direct-seeded soil and with fall tillage. The proportion of recovered wild oats and skewers in each vertical 2.5 cm depth increment from the soil surface was not significantly different in direct-seeded soil compared to soil with a fall tillage pass at Edmonton or Kinsella (Table 6). The proportion of wild oat and skewers in the top 2.5 cm of the soil profile was significantly higher in no-till soil compared to tilled soil at Lacombe ($p = 0.0431$) (Table 6). This could be attributed to Lacombe being the only site were tillage was done using spike openers which may have created channels for movement of wild oat seeds and skewers deeper in the soil profile. The majority (approximately 60%) of wild oat seeds remained in the top 5 cm of the soil profile in both direct seeded and fall tillage across all locations (Figure 2). In a survey of wild oat recruitment depth in direct-seeded fields compared to conventional-tillage fields (minimum three years of cultivation), the average recruitment depth was 1.5 cm deeper in conventional tillage than direct seeded fields (du Croix Sissons et al. 2009)

4.4. Conclusions

The efficacy of a soil-applied herbicide is influenced by the vertical position of weed seeds in the soil profile, soil disturbance and the degree of incorporation of the herbicide after application (Chauhan et al. 2006). Efficacy of spring-applied, soil-applied herbicides such as pyroxasulfone may be largely compromised in years with limited spring precipitation, therefore making fall applications more reliable for herbicidal activity. Soil disturbance by tillage can increase rate of soil drying in the spring, which can limit moisture availability for pyroxasulfone activity as well as decrease wild oat germination and emergence. Tillage may dilute pyroxasulfone by increasing soil mixing and if herbicide application occurred before seeding operation some degree of incorporation is achieved. Wild oats being recruited from deeper in the soil profile in conventional-tillage fields will influence the efficacy of pyroxasulfone on their control. Pyroxasulfone is strongly adsorbed to the soil and will remain near the soil surface after application. The efficacy of pyroxasulfone on wild oat control is influence by edaphic and environmental factors and their interactions, therefore determining the most beneficial use patterns is difficult to predict.

Table 4-1 Soil characteristics at trial locations

Location	Soil OM	Soil pH	Soil Classification	Soil Texture		
	-%-			---------- % ----------		
				Sand	Silt	Clay
Edmonton	12.4	6.4	Eluviated Black Chernozem	17.6	40.1	42.3
Kinsella	6.2	6.2	Orthic Black Chernozem	50.6	33.2	16.2
Lacombe	8.1	5.9	Eluviated Black Chernozem	42.0	30.8	27.2

Table 4-2 Field operation dates, site description and fertilizer rates at each trial location from fall 2014 to summer 2015.

	Edmonton	**Kinsella**	**Lacombe**
Wild Oat/Skewer Seed Dispersal	Oct 3	Oct 9	Oct 8
Fall Tillage	Oct 3	Oct 9	Oct 8
Fall Pyroxasulfone Application	Oct 8	Oct 9	Oct 8
Stubble	Barley	Canola	Barley
Residue Coverage	Light	Light	Heavy
Pea Seeding	May 5	May 14	May 21
Phosphorus Fertilization	28 kg ha^{-1}	64 kg ha^{-1}	31 kg ha^{-1}
Potash Fertilization	-	-	83 kg ha^{-1}
Spring Pyroxasulfone Application	May 11	May 19	May 23
Depth Sampling	May 11	May 19	May 26
Biomass and Final Counts	June 22	July 9	July 13

Table 4-3 Herbicides, rates used and target species for all maintenance herbicide applications.

Location	Date	Product	Formulation	Rate	Target Species
Edmonton	Oct 17/14	Glyphosate	540 g ae L^{-1}	360 g ae ha^{-1}	Volunteer barley, Canada thistle
Kinsella	May 8/15	Carfentrazone-ethyl		17.78 g ai ha^{-1}	Volunteer canola
Lacombe	Oct 8/14	Glyphosate	540 g ae L^{-1}	360 g ae ha^{-1}	Volunteer barley
Lacombe	May 13/15	Glyphosate	540 g ae L^{-1}	360 g ae ha^{-1}	Volunteer barley, green foxtail

Table 4-4 Precipitation at field locations throughout the trial duration in 2015.

Location	Precipitation					
	---------- mm (% of 30 Year Long Term Average) ----------					
	Oct-Mar	April	May	June	July	Total
Edmonton	111.2 (106)	6.9 (30)	19.4 (43)	24.9 (34)	67.4 (75)	229.8 (68)
Kinsella	102.9 (100)	14.4 (58)	18.3 (46)	33.4 (44)	112 (154)	281 (107)
Lacombe	110 (117)	11.8 (54)	23.3 (45)	71 (91)	108.5 (125)	324.6 (98)

Table 4-5 P-values from analysis of variance for fixed effect including interactions for pyroxasulfone efficacy trials at all locations

Fixed Effect	Location		
	Edmonton	Kinsella	Lacombe
Tillage	0.0126	0.0326	0.0325
Application Timing	0.0002	0.0261	0.3167
Pyroxasulfone Rate	0.0008	<0.0001	<0.0001
Tillage : Application Timing	0.0095	0.0734	0.7885
Tillage : Pyroxasulfone Rate	0.5575	0.2061	0.0039
Application Timing : Pyroxasulfone Rate	0.0087	0.0293	0.3870
Tillage : Application Timing : Pyroxasulfone Rate	0.7438	0.9110	0.4132

Table 4-6 P-values for 2-sample test for equality of proportions when comparing total amount of retrieved wild oats and skewers to proportion in each depth interval for direct seeded compared to fall tillage.

Depth	Edmonton	Kinsella	Lacombe
0 – 2.5 cm	0.9243	O.5953	0.0431*
2.5 – 5 cm	0.7283	0.4008	0.6617
5 – 7.5 cm	0.3380	0.5970	0.1138
7.5 – 10 cm	0.7576	0.6433	0.3058
10 – 12.5 cm	0.1992	0.9200	0.4768
12.5 – 15 cm	0.4195	0.5357	0.8078

*Significant with a 95% confidence interval

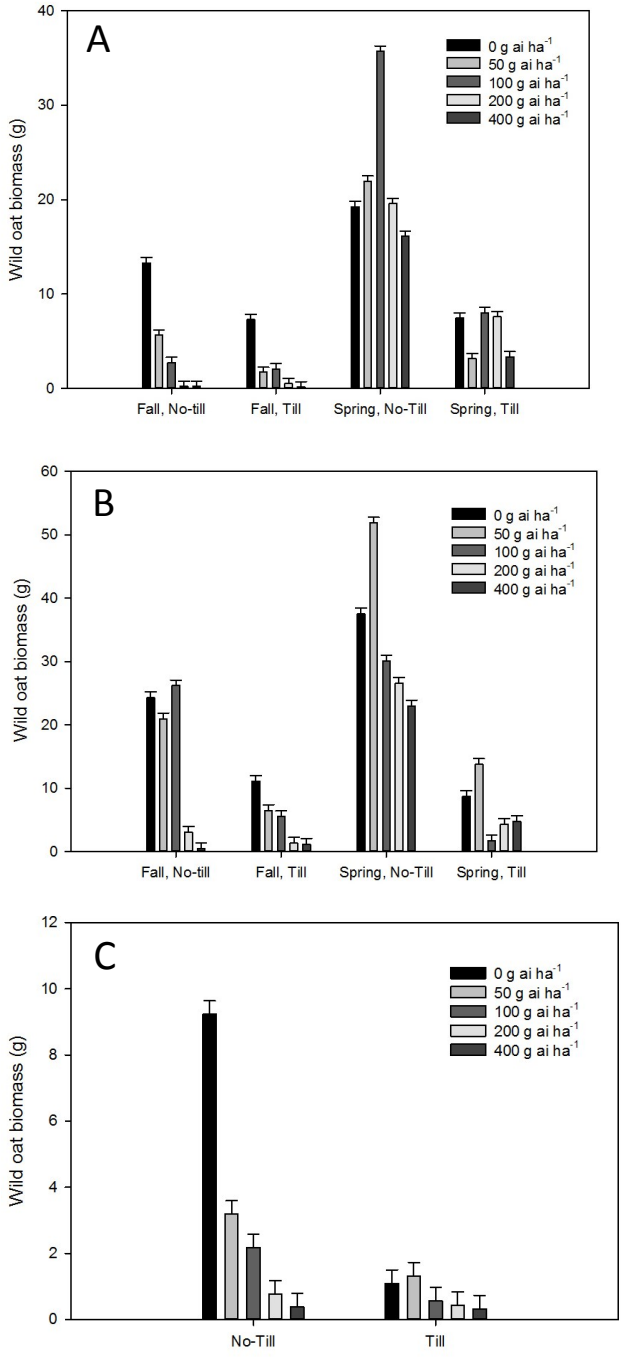

Figure 4-1 Wild oat biomass (g^{-1} 0.25 m^{-2}) at Edmonton (A), Kinsella (B), and Lacombe (C) as affected by tillage, application timing and pyroxasulfone rate.

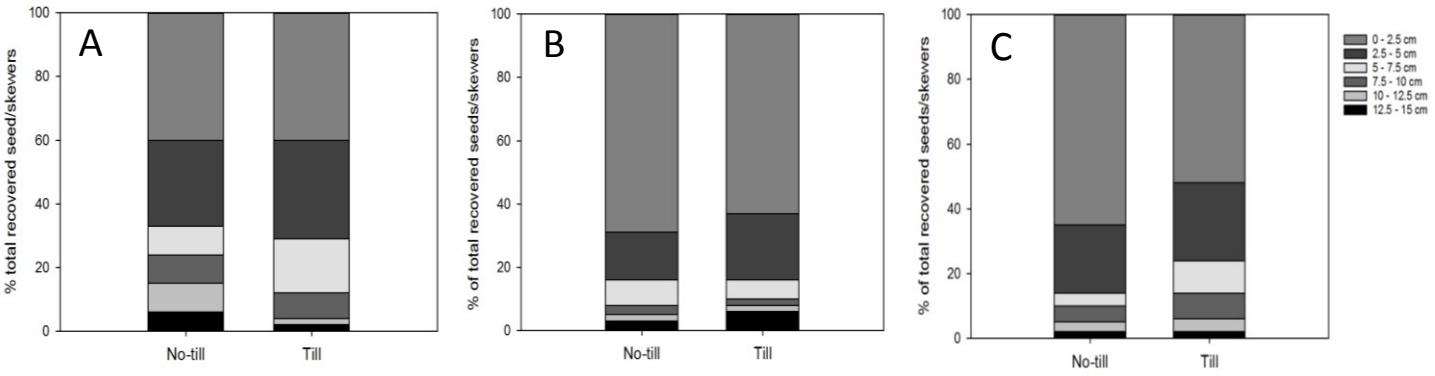

Figure 4-2 Percent of total recovered wild oat seeds and skewers in each 2.5 cm depth interval form the soil surface at Edmonton (A), Kinsella (B) and Lacombe (C).

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Chapter Five: Triallate-resistant wild oat (*Avena fatua L.*): Unexpected resistance to pyroxasulfone and sulfentrazone[2]

5.1. Introduction

Wild oat *(Avena fatua L.)* is one of the 15 most important herbicide-resistant (HR) weed species worldwide (Heap 2016), and the most abundant HR weed species in the Canadian Prairies (Beckie et al. 2013). Triallate (Group 8/N), a soil-applied, lipid synthesis inhibitor, was first introduced in 1961 for pre-emergent selective wild oat control and was used extensively until more efficacious ACCase inhibitors (Group 1/A) and ALS inhibitors (Group 2/B) were introduced beginning in the late 1970s (Beckie et al. 1999). Wild oat resistant to triallate and cross-resistant to difenzoquat was reported by O'Donovan et al. (1994), and reports of resistance to ACCase inhibitors and ALS inhibitors followed (Beckie et al. 1999; Joseph et al. 1990). Wild oat resistance is now common across the prairies; Beckie et al. (2013) reported 28, 12, and 8% of fields surveyed between 2007 and 2009 contained wild oat populations resistant to ACCase inhibitors, ALS inhibitors and triallate, respectively. Multiple-resistant HR populations reported were ACCase + ALS (8%), ACCase + triallate (5%), ALS + triallate (2%) and ACCase + ALS + triallate (3%).

Cross-resistance between herbicides with different sites of action may be conferred by non-target site resistance such as herbicide metabolism, or multiple-resistance can be conferred by several target sites and/or non-target site resistance mechanisms (Beckie and

[2] A version of this research "Triallate-resistant wild oat (*Avena fatua* L.): Unexpected resistance to pyroxasulfone and sulfentrazone. Amy R. Mangin, Linda M. Hall1, Hugh J. Beckie" has been accepted by the Canadian Journal of Plant Science.

Tardif 2012; Preston et al. 2001). The mechanisms of resistance in wild oat have not all been clarified. Rashid et al. (1998) proposed that increased levels of endogenous gibberellin in triallate-resistant wild oat increased germination and accelerated shoot growth, allowing for seedlings to avoid toxic herbicidal effects. Alternatively, Kern et al. (1996) reported a correlation between reduced triallate metabolic activation (sulfoxidation) and triallate resistance in wild oat. However, reduced metabolism does not explain resistance to other selective herbicides or to difenzoquat, as difenzoquat does not require sulfoxidation for activation (Kern et al. 1996; Sharma et al. 1976). Kern and Dyer (1998) proposed a separate resistance mechanism of cell wall sequestration was conferring resistance to difenzoquat in these populations. Upon further investigation into triallate mechanism of resistance, Kern et al. (2002) suggested that two independently segregating, recessive genes (*TRR1, TRR2*) controlled resistance. Target-site mutations and increased metabolism are the primary mechanisms responsible for HR ACCase and ALS populations (Beckie et al. 2012).

Due to the widespread resistance to current wild oat herbicides, Canadian producers are looking for options to control these HR populations. Pyroxasulfone (Group 15/K3), a very-long chain fatty acid (VLCFA) biosynthesis inhibitor, has recently been introduced in Canada. It is a pre-emergent herbicide registered for control of grasses and small-seeded broadleaves in corn, soybean, sunflower and field pea (Tanetani et al. 2009). Pyroxasulfone has shown success in managing other problematic HR weeds in the United States and Australia due to its ability to inhibit multiple steps in the elongation pathways of VLCFA compared to other lipid synthesis inhibitors (Busi et al. 2012, 2014; Tanetani et al. 2009). Pyroxasulfone control of wild oat was

reported by Tidemann et al. (2014) in combination with the soil-applied PPO inhibitor sulfentrazone (Group 14/E).

Resistance to sulfentrazone has been reported in populations of smooth pigweed (*Amaranthus hybridus*), common ragweed (*Ambrosia artmisiifolia*) and common waterhemp species (*Amaranthus tuberculatus*; *Amaranthus rudis*) that were also resistant to multiple other PPO inhibitors. Sulfentrazone resistance was attributed to target site mutations to protoporphyrinogen oxidase, a single codon deletion (ΔG210 PPX2L) in waterhemp and a point mutation in common ragweed (R98L PPXL) (Patzoldt et al. 2006; Rousonelos et al. 2012).

Worldwide, four grass species, including annual ryegrass (*Lolium rigidum* L. Gaud.), have been reported to be resistant to VLCFA inhibitors (other than pyroxasulfone)(Heap 2016). Screening of susceptible annual ryegrass with high doses of pyroxasulfone did not select for resistant individuals, indicating that any major genes potentially responsible for resistance either have very low penetrance or are extremely rare (Busi et al. 2012). However, low-dose selection of a multi-resistant (metabolism based) population of annual ryegrass by pyroxasulfone showed a shift towards pyroxasulfone resistance, with LD_{50} increasing from 46 g ai ha^{-1} in the parent to 208 g ai ha^{-1} by the third generation (Busi et al. 2012). This resistance was later characterized as non-target site-based and conferred by one semi-dominant allele (Busi et al. 2014). This shift towards pyroxasulfone resistance showed a corresponding increase in resistance to prosulfocarb and triallate (Busi and Powles 2013), suggesting cross-resistance from a single mechanism. The annual ryegrass population with the highest resistance to pyroxasulfone showed 81% and 39% survival for prosulfocarb and triallate, respectively, at label

rates (Busi and Powles 2013). Enhanced metabolic herbicide detoxification is thought to be the main mechanism of resistance in annual ryegrass and is also the main mechanism of selectivity in wheat (Busi and Powles 2013; Shimabukuro et al. 1979; Shimabukuro and Hoffer 1991; Tanetani et al. 2013).

The objective of this study was to determine if two Canadian wild oat populations that have been selected for resistance to triallate exhibited cross-resistance to pyroxasulfone similar to annual ryegrass in Australia. Additionally, cross-resistance to the pre-emergent PPO-inhibitor sulfentrazone was quantified because it may be an additional tool for managing HR weed populations.

5.2. Materials and Methods

5.2.1. Plant Material

Seed of the susceptible wild oat population was collected from the University of Alberta farm in the 1988 growing season (S1988). Two resistant wild oat populations were submitted to Saskatoon Research Centre, Agriculture and Agri-Food Canada by growers for testing with suspected resistance to triallate: HR08-210 population from Olds, AB and HR11-151 population from Rivers, MB. In 2014, resistant seeds were grown in the absence of herbicide in separate locations in the field at the University of Alberta and progeny were collected. Germination tests were conducted to ensure each population had a minimum 60% germination prior to initiation of dose-response experiments.

5.2.2. Resistance Screening

Wild oat biotypes were screened with selective and non-selective ACCase and ALS inhibitors (Table 1) to distinguish between the resistance mechanisms that could be present. Wild oats were seeded in 24.75-cm X 41.25 cm trays using Sunshine Professional Growing Mix® and populations were screened with the ACCase-inhibitor herbicides fenoxaprop and quizalofop, and ALS-inhibitor herbicides imazamethabenz and imazapyr (Table 1). Fenoxaprop and imazamethabenz are selective herbicides and metabolized in wheat, while quizalofop and imazapyr are not metabolized. If a biotype is resistant only to the herbicide that is metabolized, it is an indication that metabolism may be responsible for resistance rather than a target-site mutation. Herbicides were applied with a moving track cabinet chamber sprayer calibrated for 200 L ha^{-1} at 207 kPa using an Air Bubble Jet 110015 nozzle at the three-leaf stage of wild oat. After treatment, plants were returned to a greenhouse and natural light was supplemented with 16 h of artificial light and at a temperature of 21 °C.

Screening methods were adapted from Beckie et al. (2013); individual plants were visually assessed as HR (2, some injury but new growth, or 3, no injury) or herbicide susceptible (HS) (0=dead or 1= nearly dead) at 21 days after treatment (DAT). Approximately 50 plants were screened in each resistance test and treatments were replicated four times. We assumed wild oat populations submitted for testing were heterogeneous consisting of both HS and HR individuals having one or multiple herbicide resistance mechanisms.

5.2.3. Dose Response

Dose-response experiments were completed for pyroxasulfone, sulfentrazone and triallate. Because activity of these herbicides may be affected by soil organic matter, field soil collected from Kinsella Research Station in the fall of 2014 was homogenized in a soil mixer and used to plant 15 intact seeds of each population 1 cm deep in 8.25-cm diameter pots. The soil was a sandy loam with 58% sand, 30% silt and 12% clay with an organic matter content of 6%. Immediately after seeding the soil surface was sprayed with a proportion of the label rate of pyroxasulfone (150 g ai ha^{-1}), sulfentrazone (140 g ai ha^{-1}) or triallate (1180 g ai ha^{-1}). Application rates were either pyroxasulfone (85% WG) at 0, 37.5, 75, 150, 300, and 600 g ai ha^{-1}, sulfentrazone (470 g/L SC) at 0, 35, 70, 140, 280, and 560 g ai ha^{-1}, or triallate (480 g/L EC) at 0, 295, 590, 1180, 2360, and 4720 g ai ha^{-1}. Herbicides were applied using a moving track cabinet chamber sprayer calibrated for 200 L ha^{-1} at 207 kPa using an Air Bubble Jet 110015 nozzle. Immediately after treatment, pots were placed in a greenhouse. Natural light was supplemented with 16 h of artificial light and at a temperature of 21 °C. Pots were watered and rotated daily to reduce positional effects. Emergence counts were conducted 2 weeks after seeding. At 4 weeks, plants were uprooted and washed. Plants were classified as either dead if they ceased growth due to injuries, or healthy if growth continued. Survival was determined by dividing the number of wild oats healthy and alive at 4 weeks by the total number of germinated seeds per pot. Shoot length, root length (data not shown) and fresh weight were quantified for each plant. Each pot was a considered an experimental unit.

The experimental design was a randomized complete block with four replicates for each dose-response experiment, and replicates were seeded one per week for 4 weeks. Survival data was analyzed using a binomial two-parameter log-logistic model using the *drm()* function in the *drc* package in the software program R (v. 0.98.1091). Population response to herbicide treatments was measured as the R/S (resistant/susceptible) ratio of estimated LD_{50} values.

5.3. RESULTS AND DISCUSSION

5.3.1. Herbicide Screening

Results from the screen with ACCase and ALS inhibitors indicated that HR08-210 and HR11-151 were resistant to ACCase and ALS inhibitors (Table 2). HR08-210 and HR11-151 wild oat populations acted similar in response to all herbicides. All populations exhibited visual symptoms after fenoxaprop application, but resistant populations showed regrowth, suggesting metabolism-based resistance. A high level of resistance to the non-selective herbicide quizalofop indicates an ACCase target-site mutation is also conferring resistance to ACCase inhibitors. HR08-210 and HR11-151 were highly resistant to the ALS inhibitor imazamethabenz yet susceptible to imazapyr. This suggests that resistance to ALS inhibitors was due to enhanced metabolism and not an ALS target-site mutation. Resistance to ALS inhibitors in wild oat is more commonly due to enhanced metabolism than target-site mutation; in contrast, ACCase inhibitor resistance in the species is often due to target-site mutation, followed by enhanced metabolism by P450 monooxygenases (Beckie et al. 2012).

5.3.2. Dose Response

Both wild oat biotypes HR08-210 and HR11-151 were resistant to triallate, with R/S ratios of 3.39 (P = 0.0006) for HR11-151 and 2.53 (P = 0.0088) for HR08-210 (Table 3). Wild oat population HR11-151 showed cross-resistance to both pyroxasulfone and sulfentrazone with R/S ratio of 2.78 (P = 0.0063) and 2.0 (P = 0.0290), respectively. HR08-210 population was not classified as resistant, with R/S ratios of 1.13 (P = 0.7429) for pyroxasulfone and 1.28 (P = 0.1740) for sulfentrazone (Figure 1).

Pyroxasulfone and sulfentrazone are both soil-applied pre-emergent herbicides, but have different target sites. The wild oat populations tested have never been exposed to pyroxasulfone or sulfentrazone, so the possibility of target-site mutations conferring multiple-resistance mechanisms for these three herbicides is unlikely. Cross-resistance between triallate and pyroxasulfone in annual ryegrass is believed to be due to increased metabolism because cross-resistance was not observed for herbicides not metabolized by wheat (Busi and Powles 2013). The resistance mechanism for triallate-resistant wild oat populations is believed to be increased levels of endogenous gibberellin (Rashid et al. 1998). Increased endogenous gibberellin would allow for rapid germination and meristematic growth, allowing plants to avoid toxic levels of herbicide from reaching its target site (O'Donovan et al. 1999). It is probable that an increased level of endogenous gibberellin in triallate-resistant wild oat population are allowing seedlings to avoid toxic effects of both soil-active herbicides pyroxasulfone and sulfentrazone. Alternatively, resistance to pyroxasulfone and/or sulfentrazone could be conferred by enhanced metabolism by P450 monoxygenases selected for by previous use of ACCase and ALS inhibitors.

When the resistant populations were treated with higher rates of triallate, pyroxasulfone and sulfentrazone, a few individuals appeared unaffected (Figure 2). Although LD_{50} values suggest that the overall resistance level was low in HR11-151 (R/S = 2.78) and not significantly different in HR08-210 relative to the S1988 population (Table 3), presence of some individuals unaffected by herbicide within the two HR populations suggest that further selection for resistance may be rapid. These populations will be further selected by pyroxasulfone to determine if variance was due to random error or to genetic differences in the population. Rapid selection of resistance to pyroxasulfone in annual ryegrass has been reported previously by Busi et al (2012).

This is the first report of a grass species resistant to pyroxasulfone or sulfentrazone in North America. However, resistance to triallate, ACCase inhibitors, and ALS inhibitors is widespread in wild oat and some other grasses (Heap 2016). Research is required to determine if triallate-resistant wild oat commonly exhibits resistance to these herbicides having different sites of action. If it is a common occurrence, cross-resistance to pyroxasulfone and sulfentrazone will further limit wild oat control options in the Canadian Prairies. In response to widespread herbicide resistance in wild oat to ACCase and ALS inhibitors, producers are looking to newer herbicides of differing sites of action, such as pyroxasulfone and sulfentrazone. Alternatively, they may return to older soil-applied products such as triallate or trifluralin. Nevertheless, this research suggests that alternative herbicides will likely have a short utility in controlling this important grass weed due to the prevalence of non-target-site resistance mechanisms conferring resistance across multiple herbicide sites of action. Knowledge of cross-resistance patterns is important to facilitate management of herbicide-resistant wild oat

populations. Repeated selection of the experimental populations is underway to quantify the rate of selection for pyroxasulfone resistance in these wild oat populations.

Table 5-1 Herbicides used in wild oat resistance screening

Herbicide	Formulation	Mode of action	Selective in wheat	Rate
				(g ai ha^{-1})
Fenoxaprop	120 g/L EC	ACCase Inhibitor (Aryloxyphenoxy propionate)	Yes	150
Quizalofop	96 g/L EC	ACCase Inhibitor (Aryloxyphenoxypr pionate	No	35
Imazamethabenz	300 g/L SC	ALS inhibitor (Imidazolinone)	Yes	500
Imazapyr	240 g/L SN	ALS inhibitor (Imidazolinone)	No	717

Table 5-2 Resistant screening of wild oat populations S1988, HR08-210 and HR11-151. The number in brackets indicated the mean % of R (resistant) individuals present in each screen (N=4).

Herbicide	Site of Action	Population		
		S1988	HR08-210	HR11-151
		----------%----------		
Fenoxaprop	ACCase	S (0)	R (35)	R (36)
Quizalofop	ACCase	S (0)	R (85)	R (86)
Imazamethabenz	ALS	S (0)	R (86)	R (87)
Imazapyr	ALS	S (0)	S (0)	S (0)

R: resistant; S: susceptible

Table 5-3 Estimated LD_{50} values and R/S ratios for wild oat populations S1988, HR08-210 and HR11-151 in a dose response study with triallate, pyroxasulfone and sulfentrazone.

Biotype	LD_{50} (±SE)[z]	R/S[y]	P-value
	--------(g ai ha^{-1})---------		
Triallate			
S1988	270 (50)	-	-
HR08-210	681 (95)	2.53	0.0088
HR11-151	915 (81)	3.39	0.0006
Pyroxasulfone			
S1988	41 (8)	-	-
HR08-210	46 (13)	1.13	0.7429
HR11-151	114 (13)	2.78	0.0063
Sulfentrazone			
S1988	183 (13)	-	-
HR08-210	236 (34)	1.28	0.1740
HR11-151	375 (83)	2.0	0.0290

[z]LD_{50}, lethal dose required for 50% survivorship of wild oat biotypes. The values in parentheses are standard errors.

[y]R/S, ratio (resistant/susceptible) referred to the standard susceptible population S1988.

[x]P-value, indicated significance between LD_{50} values when treated with a particular herbicide.

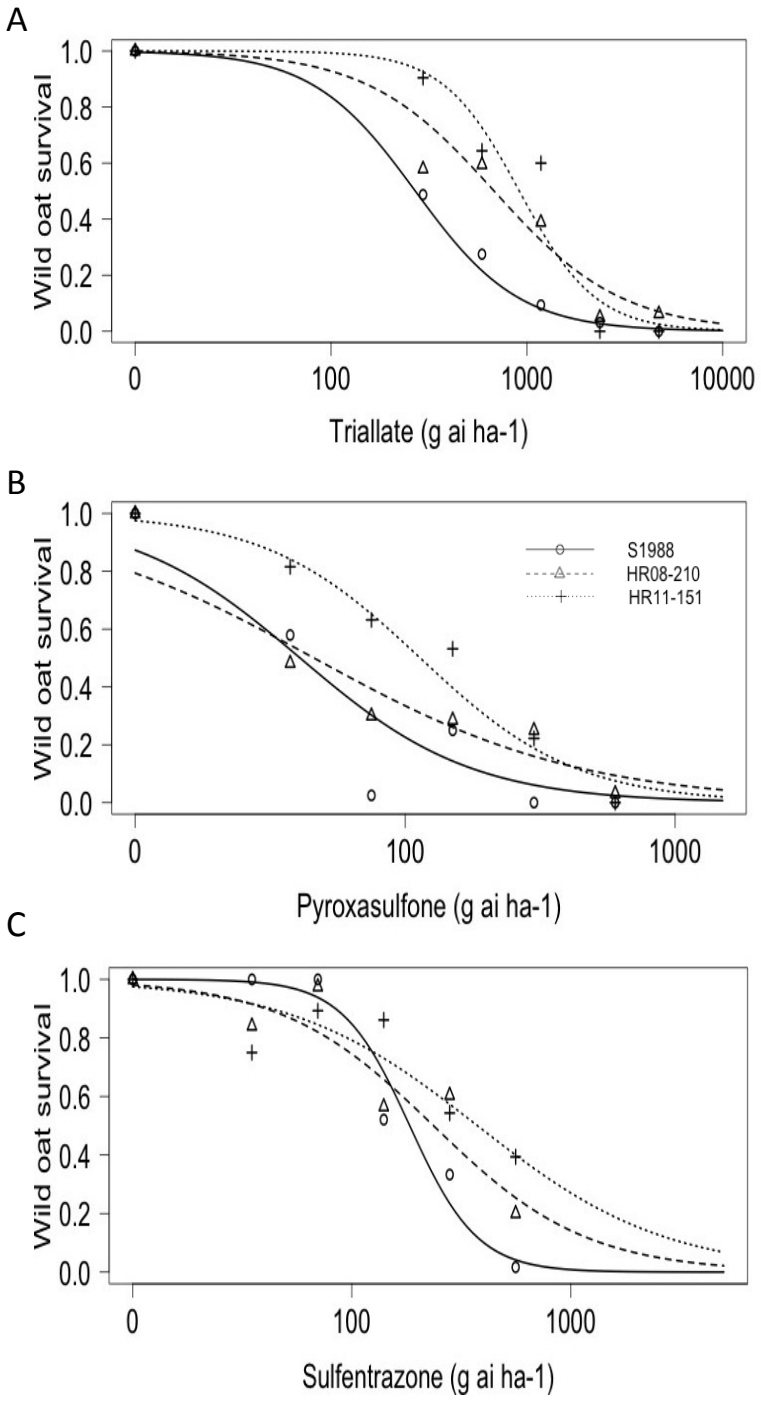

Figure 5-1 Wild oat biotypes S1988, HR11-151 and HR08-210 survival in a dose-response study to triallate (A), pyroxasulfone (B), and sulfentrazone (C). Survival was calculated by live plants divided by the total number of germinated seeds in each pot.

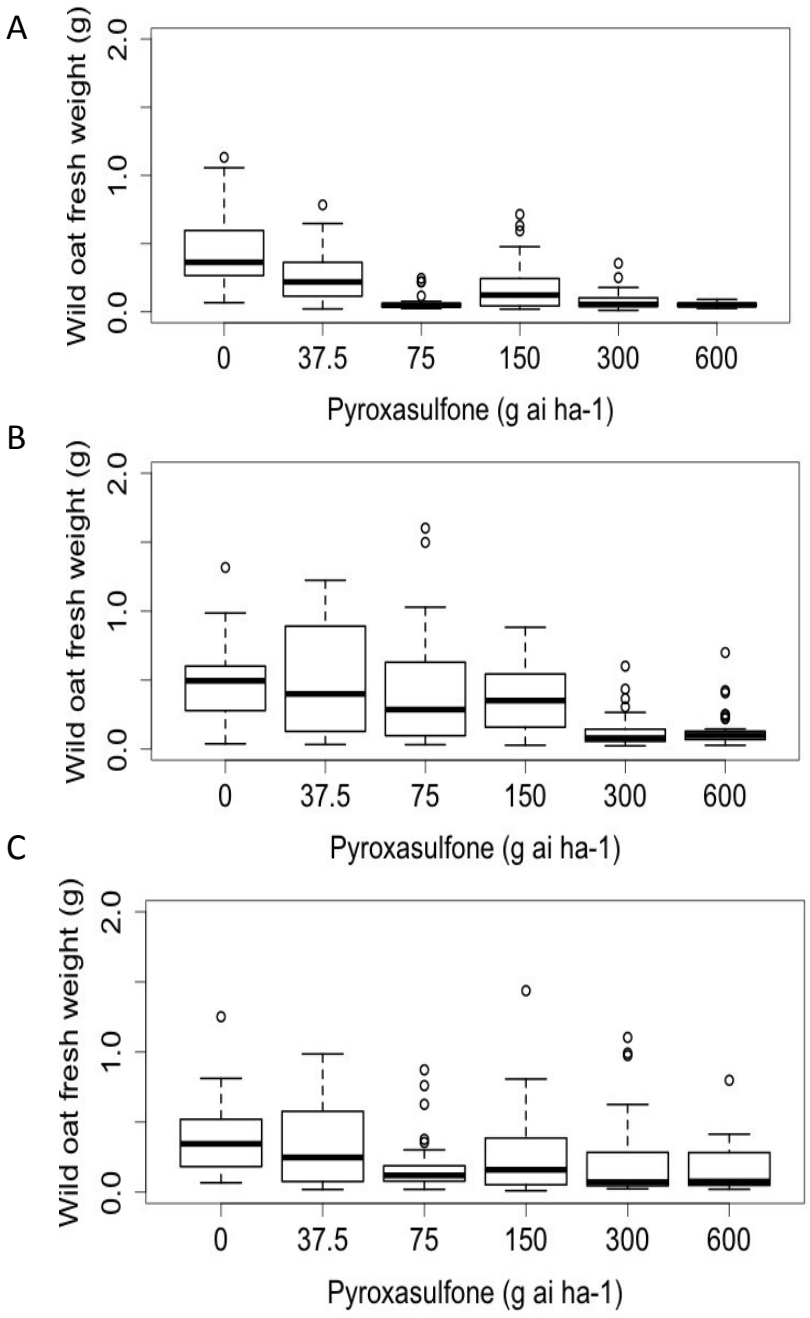

Figure 5-2 Fresh weight of wild oat populations S1988 (A), HR11-151 (B), and HR08-210 (C), 28 days after treatment with increasing rates of pyroxasulfone (N = 60). Centerline on each box indicates the median value and upper and lower box edges describe upper and lower quartiles, respectively. Maximum and minimum values indicated by whiskers and outliers indicated by hollow circles.

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Chapter Six: General Discussion and Conclusion

6.1. Summary of Results

This research suggests pyroxasulfone efficacy on wild oat will vary across western Canadian cropping systems. The effects of wild oat seed depth, seed/seedling and herbicide interception site, tillage, and application timing on pyroxasulfone efficacy on wild oat were determined. Pyroxasulfone vertical movement in the soil and wild oat seed movement with tillage were also studied.

When applied to the soil surface pyroxasulfone binds strongly to the soil and remains near the soil surface. This may allows wild oats recruited from deep in the soil profile to avoid lethal herbicidal effects because the seedling/herbicide site of interception required for control to take place is below the concentrated herbicidal layer. Soil disturbance through tillage redistributes wild oat seeds deeper in the soil profile and increases the rate of soil drying, reducing soil moisture. Moisture availability has a large impact on activity of pyroxasulfone as well as wild oat germination and emergence. Efficacy of pyroxasulfone was reduced in tilled soil compared to untilled soil due to reduced soil moisture in 1 of 3 sites in the 2015 growing season. In western Canada, when pyroxasulfone is applied in the fall spring snowmelt is available to provide herbicidal activation, while spring applications rely on spring precipitation for activation. Due to dry spring conditions in the 2015 growing season there was a clear advantage of fall over spring application of pyroxasulfone with limited herbicidal activity when pyroxasulfone was applied in the spring. This suggests that pyroxasulfone may be better suited for fall application in western Canada to ensure herbicidal activation for the following growing

season. Seed location in the soil profile and the soil conditions will interact to influence pyroxasulfone efficacy on wild oats, which is critical information to further our understanding of the variability in control of wild oat by pyroxasulfone in various cropping systems across western Canada.

Cross-resistance between wild oat populations previously selected for resistance to common wild oat herbicides (Groups 1, 2 & 8) and pyroxasulfone and sulfentrazone was identified in one wild oat population from Rivers, Manitoba. If this cross-resistance pattern is consistence across resistant wild oat populations in Canada the use of pyroxasulfone and sulfentrazone for management of resistant populations could be limited. Pyroxasulfone will not replace in-crop wild oat herbicides but is a potential tool for managing herbicide resistant populations. Pyroxasulfone has a unique mechanism of action compared to in crop wild oat herbicides, therefore by applying pyroxasulfone as a pre emergent herbicide in addition to other in crop controls we can avoid selection by a single mechanism of action in a single growing season, decreasing selection for further herbicide resistant populations.

6.2. Results Summarized by Research Objective

6.2.1. Determine effect of wild oat seed depth on control by pyroxasulfone and triallate

The effect of wild oat seed depth on control by pyroxasulfone and triallate was investigated in Chapter 3 under controlled conditions. Wild oat shoot length inhibition by pyroxasulfone and triallate was significantly lower when wild oat seeds were emerging from deep within the soil profile compared to a shallow depth. There was no difference in wild oat shoot length when seedlings emerged from either deep or shallow in the soil profile in the

absence of herbicides. The efficacy on deep-seeded wild oats was not improved by doubling the application rate of either herbicide. This suggests that wild oats emerging from deep in the soil profile are able to mitigate herbicidal effects of both pyroxasulfone and triallate.

6.2.2. Determine the effective site of interception of pyroxasulfone by wild oat seedlings for control to occur

In Chapter 3, active charcoal was used to isolate the interception site of wild oat seed/seedling and pyroxasulfone in the soil profile. It was found that the site of interception resulting in the greatest shoot inhibition to be either the wild oat seed itself or 1 cm above the seed.

6.2.3. Quantify vertical movement of pyroxasulfone in the soil with and without a simulated rainfall event

The majority of pyroxasulfone applied to the soil surface remained in the top 2.5 cm of the soil profile with and without a simulated rainfall event. We were not able to accurately quantify the difference between pyroxasulfone movement with and without a simulated rainfall.

6.2.4. Determine pyroxasulfone and triallate control of shallow- and deep-seeded wild oats on tilled and untilled soil

Chapter 3 describes a split-split plot field experiment used to determine the influence of seeding depth, tillage, herbicides and their interactions on control of wild oat. ANOVA results indicated significant effects of all factors and interactions, except a three-way interaction

between herbicide, tillage and seeding depth. Reduced herbicide efficacy was observed on shallow seeded wild oat, which was attributed to decreased soil moisture for herbicide activation due to increased soil drying. Deep seeded wild oat had longer shoot lengths and exhibited earlier emergence than shallow-seeded wild oat in both treated and untreated plots.

6.2.5. Determined wild oat vertical distribution in the soil with and without tillage

Vertical movement of wild oat seeds in the soil profile with a single tillage pass was determined at three locations in Alberta in Chapter 4. There was no difference in wild oat seed distribution after seeding in tilled or untilled soil at Edmonton or Kinsella, but in Lacombe a significantly higher proportion was found in the top 2.5-cm layer of untilled soil compared to tilled soil.

6.2.6. Determine the efficacy of pyroxasulfone applied in the fall and spring on tilled and untilled soil for wild oat control

Chapter 4 describes a split-split plot field experiment conducted to determine efficacy of pyroxasulfone when applied in the fall and spring to tilled and untilled soil. Untilled soil had significantly greater wild oat biomass than tilled soil across all locations; this was attributed to increased soil drying in tilled soil paired with little spring precipitation. The effect of application timing across sites varied, but increased control was observed when pyroxasulfone was fall-applied in 2 out of 3 sites and there was no influence of application timing at 1 of the 3 sites. LD_{50} values were not determined due to the large influence of tillage and application timing on control.

6.2.7. Determine if triallate-resistant Canadian wild oat populations exhibit cross-resistance to pyroxasulfone and the PPO-inhibitor sulfentrazone.

Chapter 5 reports the first case of wild oat resistance to pyroxasulfone and sulfentrazone reported worldwide, and the first reported resistance of a grass species to pyroxasulfone or sulfentrazone in North America. This resistance was found in a wild oat population that had previously been selected for resistance to ACCase-, ALS- and fatty acid biosynthesis inhibitors. These results indicate that previously selected resistant Canadian wild oat populations have potential to influence wild oat control by new herbicides such as pyroxasulfone and sulfentrazone.

6.3. Future Research

- Our study looked at a single tillage pass to isolate the effects of seeding depth, moisture, temperature and residue on pyroxasulfone efficacy. Long-term conventional/reduced tillage fields would have additional difference, even in adjacent fields, including soil organic matter content, soil water infiltration and soil structure differences that could also affect the efficacy of pyroxasulfone. Therefore, pyroxasulfone efficacy on wild oat populations in long term conventional and reduced tillage fields should be evaluated to examine the effects of long-term soil factors.

- To better understand the effects tillage placement of the wild oat seed bank in the soil profile has on pyroxasulfone efficacy research should be conducted on long-term conventional/reduced tillage fields with natural wild oat populations.
- Further selection by pyroxasulfone and sulfentrazone of the wild oat populations tested for resistance is required to determine the rate of selection in these populations. While selected populations were retained, the time frame of the project did not permit completion.
- Cross-resistance patterns of wild oat need to be examined in wild oat populations that have been previously selected for resistance by a single herbicidal group (1, 2, or 8) to further guide producer choices and aid in determining the mechanism conferring cross-resistance to these populations.
- Research is required to determine if this cross-resistance to pyroxasulfone and sulfentrazone is commonly exhibited in resistant wild oat populations in western Canada. If this cross-resistance pattern is consistent the potential use of pyroxasulfone and sulfentrazone for managing herbicide resistant populations will be limited. This may be considered as a component of the 2016/7 resistance surveys in Western Canada.

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Appendix

First report: spotted knapweed (*Centaurea stoebe*) resistance to auxinic herbicides[3]

Spotted knapweed (*Centaurea stoebe subsp micranthos*) is a prohibited noxious invasive species establishing and replacing native rangeland habitat critical for sustaining wildlife and domestic animals across the northwestern United States and southern British Columbia and Alberta (Sheley et al. 1998; Watson and Renney 1974). Spotted knapweed is a biennial or short-lived perennial that can form a monoculture in poor range conditions in as little as one season (USDA 2015). Spotted knapweed is a prolific seed producer and survives winter as a rosette and bolts to produces seed in the spring. It tends not to be grazed by animals. It thrives under a wide range of conditions and can easily withstand disturbances, all of which contribute to its rapid establishment and invasiveness (Sheley et al. 1998; Watson and Renney 1974).

Provincial laws regulate invasive species to protect endangered habitats. Spotted knapweed is a noxious species in British Columbia under the Weed Control Act (Government of British Columbia 2011) and a prohibited noxious species under the Alberta Weed Act (Government of Alberta 2010) which requires all plants to be destroyed and rendered non-viable. Herbicides are currently the most common method of control for spotted knapweed; currently registered herbicide active ingredients include 2,4-D, dicamba, picloram, clopyralid,

[3] A version of this research "First report: spotted knapweed (Centaurea stoebe) resistant to auxinic herbicides. Amy R. Mangin and Linda M. Hall" has been accepted by the Canadian Journal of Plant Science.

aminopyralid, and aminocyclopyrachlor all of which are synthetic auxin herbicides (Senseman 2007). Synthetic auxins mimic naturally occurring plant hormones (indole acetic acid (IAA)) and may use the same auxin-binding protein (ABP) sites to enter the plasma membrane.

Auxins regulate gene expression of auxin responsive factors (ARFs) through promotion of degradation of Aux/IAA (auxin/indole acetic acid) transcription factors via binding to the complex of the TIR (Transport Inhibitor Response)/AFB (auxin-signaling F-box), which are protein components of the SCF (Skip, Cullin, F-Box) (Christoffoleti et al. 2015). Different types of auxinic herbicides exhibit different binding affinity to the TIR complex. Uncontrolled gene expression of ARFs leads to increased synthesis of plant hormones (abscissic acid (ABA) and ethylene), resulting in excessive plant growth, epinasty and cell death in sensitive plants (Christoffoleti et al. 2015).

Heavy reliance and repeated use of herbicides with similar modes of action for weed control has greatly increased the selection of herbicide resistant weed populations (Heap 2015). Resistance to synthetic auxins has been reported in 32 weed species worldwide, seven include resistance to herbicides in the picolinic acid chemical family, most of which are also resistant to multiple synthetic auxin chemical families (Heap 2015). Two *Centaurea* species have been reported to be resistant to synthetic auxins. *Centaurea solstitialis* in Washington State was selected for resistance to picloram along roadsides and later classified as cross-resistant to clopyralid, triclopyr, dicamba and 2,4-D (Fuerst et al. 1996; Miller et al. 2001). *Centaurea cyanus* was observed in Poland to be resistant to dicamba in winter wheat fields (Heap 2015).

In the fall of 2012, a putative clopyralid resistant spotted knapweed biotype was identified from an extensively managed rangeland in the East Kootenay's of British Columbia at Mount Broadwood. A detailed herbicide history was not available, but this area has received repeated applications of clopyralid and plants has survived the recommended rate is 252 g a.i. ha^{-1}. During the winter of 2013, a small replicated greenhouse experiment with limited seed from this site was conducted and >95% of spotted knapweed seedlings survived a clopyralid rate of 600 g a.i. ha^{-1}..Survivors were vernalized and allowed to flower for a seed increase and seed was harvested in winter 2013 for the resistant (R) population. Susceptible seed was obtained from mature plants the summer of 2013 near Vernon, BC from an area with no known herbicide history.

Separate dose-response experiments for clopyralid, 2,4-D, picloram, and aminopyralid were conducted to further quantify the level of herbicide resistance in the Mount Broadwood spotted knapweed population. Resistant (6 seeds) and susceptible (4 seeds) spotted knapweed were seeded in 4-inch square pots and allowed to grow for approximately 2 weeks until plants had three true leaves. The number of plants per pot varied from 3-5 depending on emergence of each biotype. Biotype-specific herbicide doses were applied as fractions of the following recommended label rates: clopyralid (120 g a.i. ha^{-1}), 2,4-D LV ester (560 g a.i. ha^{-1}), picloram (250 g a.i. ha^{-1}), aminopyralid (120 g a.i. ha^{-1}). Resistant biotypes were treated with 0, 1, 2, 4, 8, 16, or 32 times the recommended label rates and susceptible biotypes received 0, 0.25, 0.5, 1, 2, or 4 times recommended label rates. Herbicides were applied using a moving track cabinet chamber sprayer calibrated to deliver 200 L ha^{-1} at 207 kPa using an Air Bubble Jet 110015 nozzle. Immediately after treatment trays were placed in a greenhouse. Natural light was

supplemented with 16h of artificial light and temperature was maintained at 21 °C. Pots were watered and shifted daily to limit positional effects.

Experimental design was a nested randomized complete block design with 4 replicates, where individual plants were nested within an individual pot, with 3-5 plants per pot and each pot was a separate experimental unit. Above ground fresh weight was quantified 15 days after treatment (DAT) for each plant. Data was first fit to a linear mixed mixed model in the lsmeans package of R (v.0.98.1091) to account for nested effects. Least-square means for individual herbicide treatments were used to create dose-response curves in the dose-response curve (drc) package of R (v.0.98.1091) for each experiment by fitting data to a four-parameter log-logistic curve. Dose-response curves were used to determine the effective dose required to reduce above ground biomass by 50% compared to untreated controls (GR_{50}). The resistance ratio (resistant/susceptible, R/S) of GR_{50} values for resistant and susceptible populations was calculated.

Clopyralid resistance was confirmed in the resistant spotted knapweed population with the estimated GR_{50} value 32 times higher than the recommended label rate, while the susceptible population were controlled at the label rate (252 g a.i. ha^{-1}) and had a GR_{50} value of 0.148 of the recommended label rate (Table 1). Resistance ratios show strong cross-resistance to picloram (27.85), less to aminopyralid (2.25) and no cross-resistance to 2,4-D (1.76). In the absence of herbicide, there were no consistent morphological differences between susceptible and resistant biotypes. Considerable variation in individual plant biomass was observed in spotted knapweed treated with clopyralid and picloram. Some plants were injured while others

appear unaffected. This suggests a heterogeneous biotype, which could be further selected for herbicide resistance.

For aminopyralid and 2,4-D, chosen rates were higher than those required for a 50% reduction in above ground biomass, which resulted in high standard error values for GR_{50} estimates (Table 1). Clopyralid rates of 32 times the recommended rate did not significantly decrease above ground biomass of the resistant biotype and higher rates would be required to accurately estimate GR_{50} for this biotype. Limited amounts of susceptible seed prevented the use of additional doses and replication of dose-response experiments.

Resistance to clopyralid has only been reported twice worldwide and both cases were detected in populations selected for resistance by picloram (Heap 2015). Yellow starthistle (*Centaurea solstitialis*) from Washington State was reported to be resistant to picloram (5.6), clopyralid (3.7), triclopyr (2.4), dicamba (5.1), and 2,4-D (3.2) (Miller et al. 2001). Resistance inheritance in this population of yellow starthistle was attributed to a single recessive gene (Sabba et al. 2003). They suggested resistance was due to an altered auxin receptor (AFB) that could confer selective resistance to picolinic acids, similar to that identified in picloram-resistant *Arabidopsis* mutants (Walsh et al. 2006). Unlike auxinic resistance in wild mustard (Hall and Romano 1995), resistant knapweed plants showed normal growth; however, following herbicide treatment, plants showed some epinasty without tissue necrosis. This may indicate a resistance mechanism that interferes with the complex between TIR1, AFBs or Aux/IAA (Christoffoleti et al. 2015). This is the first resistant spotted knapweed population reported and the first case of a weed reporting a high level of clopyralid resistance. More research is needed to investigate

possible resistance mechanisms. Auxinic resistance will limit the potential herbicidal control options for spotted knapweed, which if left uncontrolled may potentially affect up to 10 million ha of western Canada, with projected forage losses of $13 million annually (USDA 2015).

Table 1. Estimate of GR_{50} and resistance ratio (R/S) values of resistant (R) and susceptible (S) spotted knapweed populations to clopyralid, picloram, aminopyralid and 2,4-D in dose response experiments.

Herbicide	Population		Resistance ratio	Recommended label use rate
	R	S		
	--- GR_{50} [z] (g a.i. ha^{-1}) ---		*R/S*[y]	---- g a.i. ha^{-1} ----
Clopyralid	> 3,840	0.15 (0.08)	> 25,600	120
Picloram	1,293 (5855)	46.6 (14.7)	27.85	250
Aminopyralid	9.26 (6)	4.16 (4.1)	2.25	120
2,4-D	122.96 (62)	69.5 (16)	1.76	560

[z] GR_{50} refers to the herbicides rate (g a.i. ha^{-1}) required for 50% above ground fresh weight reduction compared with nontreated control. SE in parentheses.

[y] Resistance ratio *(R/S)* was calculated by dividing GR_{50} value of resistant population by that of the susceptible population.

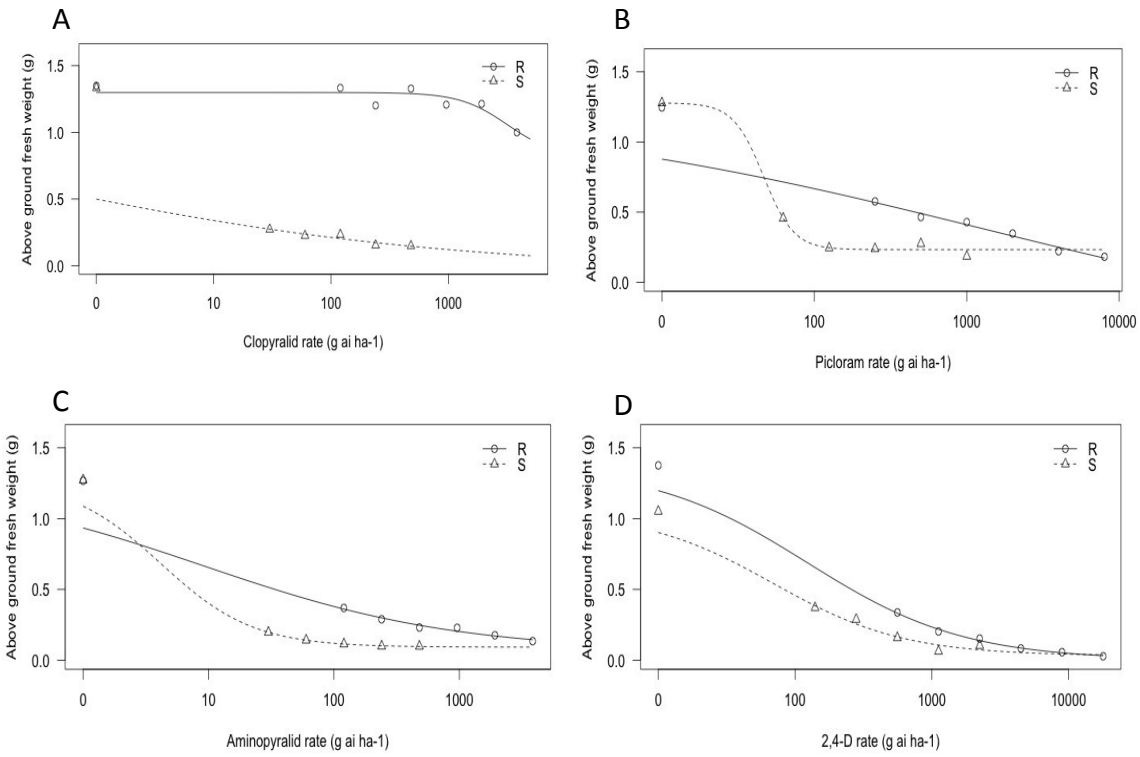

Figure 1: Dose response curves (on logarithmic dose scale) of above ground fresh weight per plant (g) of resistant (R) and susceptible (S) spotted knapweed biotypes when treated with (A) clopyralid, (B) picloram, (C) aminopyralid, and (D) 2,4-D.

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