University of Alberta

Recovery of Legumes in Northern Temperate Pastures Following the Application of Broadleaf Herbicides

by

Amanda Jo-Ann Miller

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

Master of Science

in

Rangeland and Wildlife Resources

Department of Agricultural, Food and Nutritional Science

Fall 2013
Edmonton, Alberta

‘Let me tell you the secret that has led me to my goals: my strength lies solely in my tenacity.’

- Louis Pasteur

‘The Earth does not belong to us. We belong to the Earth.’

- Chief Seattle

Abstract

Field and greenhouse trials were conducted to assess the breakdown of soil residues of two broadleaf herbicide bioactives, aminopyralid and aminocyclopyrachlor, as well as associated legume reestablishment/recovery and pasture sward production dynamics. Greenhouse trials indicated legume seedling germination and emergence was unaffected 15 months-after-treatment (MAT), while field trials showed recovery 24 MAT. Short-term variable dose trials suggest that herbicide rates below recommended rates will not allow legume reestablishment during the growing season of application. Herbicide bioactives were functionally indistinguishable, and legume species of interest, alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.), had similar responses to herbicide application. The effects of mowing on legume recovery were dependent on legume identity, with increased density of clover, and neutral effects on alfalfa. Total forage production was unaffected by herbicide application, with increases in biomass noted over the length of the study. Recovery of weedy species (dandelion) was similar to that of legumes, at 22 MAT.

Acknowledgements

This thesis would not have been possible without the combined efforts of many individuals and organizations. I would like to thank DuPont, DowAgrosciences, and NSERC for funding this research project. Thank you to my committee members, Dr. Linda Hall and Dr. Hernandez-Ramirez. I would like to say a special thanks to my supervisor Dr. Edward Bork for his infectious optimism and enthusiasm, for always having an open door and a smile, and for all the patience and support on this project. He always had my back and I couldn't have asked for a better supervisor.

I would like to acknowledge all the assistance provided by the research team at F-62. They were instrumental in setting up research trials, and for all the assistance relating to herbicide application, thank you so much Lisa Raatz, Jamie Crowe and Judy Irving, you guys were great! My field assistants were wonderful, and I would like to acknowledge the efforts of Scott Dunn, Meaghan Dunn, and Katelyn Céh, and thank them for their dedication. I would like to thank Jill Kaufmann for helping me ease into my masters, and my lab mates Tanner Broadbent, Lysandra Pyle, and Mark Lyseng for their support and friendship.

My family and friends have been an amazing source of love and support throughout this process; I couldn't have done it without them. A special thanks to my parents, Jackie and Gary Miller for instilling the importance of hard work and education at a young age. Mom, thanks for always being a phone call away and helping me through the tough times, and celebrating the good times. A thank you to my aunt Cathy Hirst for her love and support throughout this process. Thanks to my two sisters/best friends Michelle and Katie Miller, for keeping me laughing (sometimes inadvertently) throughout my degree, I couldn't have done it without you girls. A special thanks to my amazing grandmothers for their twisted humour and complete disregard for propriety, for the phone calls that had me in tears from laughter, and the amazing amount of pride they had in my achievements that always kept me striving for my best. A special thanks to Jeff O'Brien for all the support, for his unwavering faith in me, and for bringing me chocolate when I needed it. To everyone who was a part of my life during this process, thank you.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

a.i.	Active Ingredient
AMCP	Aminocyclopyrachlor
AMP	Aminopyralid
ANOVA	Analysis of variance
^{o}C	Celsius
EC	Electrical conductivity
MAT	Months after treatment
OM	Organic matter
PPB	Parts per billion
SE	Standard error
WAT	Weeks after treatment

1. WHY IS LEGUME RECOVERY IMPORTANT?

1.1 Background/Introduction

Many ranches and farms of western North America rely on hayfields, pastures, and rangelands for livestock production. These plant communities are typically composed of a mixture of cool season grasses and legumes such as alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.), important pasture components with significant ecological and economic functions (Vogel et al. 1983; Merou and Papanastasis 2009). Productive pastures and rangelands are essential for profitable ruminant production, and the integration of a legume component can result in economic gains for producers (Groya and Sheaffer 1981; Brummer and Moore 2000; Seguin et al. 2001).

Legumes are well known for their ability to increase the overall quantity and quality of forage as a result of the high crude protein content of legumes, as well as the transfer of biologically fixed nitrogen to associated grasses (McCloud and Mott 1953). Legumes are such a valuable asset in pasture and rangeland settings due primarily to their nitrogen fixing properties. They are able to convert atmospheric nitrogen into a readily available form (ammonia NH_3) through a symbiotic relationship with *Rhizobium* bacterium, found in the root nodules of various leguminous plants (Kunelius et al. 1982; Seguin et al. 2001; Frame 2005). Nitrogen accumulated through fixation can be transferred to the associated plant community through root and nodule death and decomposition (Ta and Faris 1987; Burity et al. 1989; Heichel and Henjum 1991; Dubach and Russelle 1994). Annual nitrogen fixation estimates for alfalfa range from 85 to 360 kg N ha^{-1}, while white clover has been found to fix between 100 to 400 kg ha^{-1}, with a wide range of variance depending on climatic and soil characteristics (Witty et al. 1983; Kunelius and Campbell 1984; Heichel and Henjum 1991; Frame 2005). Nitrogen inputs of legumes can minimize or replace the need for nitrogen fertilizers, helping to reduce labour efforts and ultimately costs for producers (Vogel et al. 1983; Popp et al. 2000).

Although nitrogen fixation is often the main reason for incorporating legumes into pasture communities there are several additional benefits. Over-yielding, where legume-grass mixtures yield more biomass than monocultures of either community, has been well documented (McCloud and Mott 1953; Gökkuş et al. 1999; Frame 2005). This effect is likely a product of the increased diversity associated with grass-legume complexes, and the effective use of environmental resources; light, moisture, nutrients, etc., possible when a mixture of plants utilize different niches (Hay and Walker 1989). Inclusion of legumes also stabilizes inter- and intra-annual forage production, a result of the differences in timing of yield contributions between grass and legume species (Groya and Sheaffer 1981; Seguin 1998; Gökkuş et al. 1999; Katepa-Mupondwa et al. 2002). The increased diversity of grass-legume mixtures also results in increased resistance and resilience to weed invasion, a function of more thorough niche exploitation (Sleugh et al. 2000; Sanderson et al. 2005). Grass-legume mixtures are less susceptible to erosion and may have greater stand longevity due to the complimentary nature of grass-legume communities (Droslom and Smith 1976). Legume plants also have greater crude protein content and increased palatability relative to their graminoid counterparts, characteristics that can increase intake and improve animal performance (Groya and Sheaffer 1981; Kunelius et al. 1982; Kunelius and Campbell 1984; Sleugh et al. 2000).

Undesirable broadleaf weeds are often a problem in range and pasture systems, reducing forage productivity and introducing potentially unpalatable or dangerous weed species (Masters and Sheley 2001). Pasture systems in Alberta have been shown to experience yield losses of 2kg ha^{-1} for each kilogram of Canada thistle (*Cirsium arvense* L.) biomass present, and 4.3 ka ha^{-1} for each additional thistle stem per m^{-1}, illustrating the magnitude of forage loss associated with the presence of noxious weeds (Grekul and Bork 2004). The use of selective herbicides for control of nuisance or noxious weeds in range and pasture settings has been associated with the loss of beneficial legumes from the plant community (Enloe et al. 2007; Grekul et al. 2005). Although grasses have been shown to exhibit limited compensatory biomass responses to legume removal, research in the Edmonton, Alberta, area has found an overall negative forage response in correlation with legume removal (McLeod 2011). This phenomenon represents a considerable opportunity cost of weed control in grass-legume systems, where producers risk reducing pasture productivity and quality for an indeterminate time following weed control.

Aminopyralid (AMP) and aminocyclopyrachlor (AMCP) are two relatively new broadleaf selective herbicides designed for use in range and pasture settings (DowAgroSciences 2005; DuPont 2012). Both herbicides are part of the pyrimidine carboxylic acid family, and are post emergence systemic synthetic auxin herbicides. They have low use rates relative to other herbicides, and low levels of leaching due to tight adsorption to soil colloidal surfaces (Bunkun et al. 2010). Both herbicides also have soil residual properties which inhibit the establishment and survival of broadleaf species following application (DowAgroSciences 2005; DuPont 2012).

Withdrawal periods, defined as the interval between herbicide application and successful legume reestablishment (where residual bioactives have broken down to the point that negative effects are not seen on emerging legume seedlings), are highly variable depending on localized climatic and soil characteristics, as well as soil residual properties of herbicides themselves (Blackshaw et al. 2006). As producers may be hesitant to apply herbicides for control of broadleaf weed species due to the loss of beneficial legumes, it is important to understand the withdrawal periods of herbicides, and identify safe reseeding intervals to help mitigate the forage quality and quantity losses associated with legume removal.

1.2 Study Purpose and Objectives

The questions of interest for this thesis were “What is the length of time required for herbicide residues to degrade sufficiently to permit legume seedling establishment?” and “What factors in addition to soil residues affect legume recovery?” in regards to disturbance of neighboring grasses, environmental factors such as light and moisture, as well as seed availability from the seedbank and established plant communities. Three complementary experiments addressed these questions through the use of two new herbicides, aminopyralid and aminocyclopyrachlor, and two common legume species, alfalfa and white clover, at several research sites. Specific objectives were to:

1) Quantify the response of legume emergence from seed following application of herbicide at various doses (Chapter 3).
2) Quantify the natural long-term sward dynamics in pastures following the application of residual broad-leaf herbicides, including the role of secondary disturbance and environmental factors (Chapter 4).
3) Use a soil bioassay to quantify the potential for seedling establishment in response to herbicides degrading under field conditions (Chapter 5).
4) Develop reseeding and management recommendations for the reestablishment of legumes (Chapter 6).

1.3 Literature Cited

Blackshaw, R. E., O'Donovan, J. T., Harker, K. N., Clayton, G. W., Stougaard, R. N. 2006. Reduced herbicide doses in field crops: A review. Weed Biol. Manag. **4**: 10-17.

Brummer, E. C. and Moore, K. J. 2000. Persistence of perennial cool-season grass and legume cultivars under continuous grazing by beef cattle. Agron. J. **92**: 466-471.

Bukun, B., Shaner, D. L., Nissen, S. J., Westra, P., Brunk, G. 2010. Comparison of the interactions of aminopyralid vs. cloyralid with soil. Weed Sci. **58**: 473-477.

Burity, H. A., Ta, T. C., Faris, M. A., Coulman, B. E. 1989. Estimate of nitrogen fixation and transfer from alfalfa to associated grasses in mixed swards under field conditions. Plant & Soil **114**: 249-255.

Droslom, P. N. and Smith, D. 1976. Adapting species for mixtures. multiple cropping. Papendick, R. I., (ed.) 1st ed. ASA, CSSA, SSSA, Madison, WI. 223-234 pp.

Dow AgroSciences. 2009. Milestone fact sheet. R01-879-028 (5/09)DAS 010-56950.

Dubach, M., and Russelle, M. 1994. Forage legume roots and nodules and their role in nitrogen transfer. Agron. J. **86**: 259-266.

DuPont. 2012. DuPont Rejuvra XL herbicide. Research Update: 1-2.

Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., Moechnig, M., Peterson, V., Masters, R. A., Halstvedt, M. 2007. Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop areas. Weed Technol. **21**: 890-894.

Frame, J. 2005. Forage legumes for temperate grasslands. Science Publishers Inc., Enfield, NH, United States of America. 309 p pp.

Gökkuş, A., Koç, A., Serin, Y., Çomakli, B., Tan, M., Kantar, F. 1999. Hay yield and nitrogen harvest in smooth bromegrass mixtures with alfalfa and red clover in relation to nitrogen application. Eur. J. Agron. **10**: 145-151.

Grekul, C. W. and Bork, E. W. 2004. Herbage yield losses in perennial pasture due to Canada thistle (*Cirsium arvense*). Weed Technol. **18**(3): 784-794.

Grekul, C. W., Cole, D. E., Bork, E. W. 2005. Canada thistle (*Cirsium arvense*) and pasture forage responses to wiping with various herbicides. Weed Technol. **19**: 298-306.

Groya, F. L. and Sheaffer, C. C. 1981. Establishment of sod-seeded alfalfa at various levels of soil moisture and grass competition. Agron. J. **73**: 560-565.

Hay, R. K. M. and Walker, A. J. 1989. An introduction to the physiology of crop yield. Harlow, England: Longman Scientific and Technical, 292 pp.

Heichel, G. H. and Henjum, K. I. 1991. Dinitrogen fixation, nitrogen transfer and productivity of forage legume-grass communities. Crop Sci. **31**: 202-208.

Katepa-Mupondwa, F., Singh, A., Smith Jr., S. R., McCaughey, W. P. 2002. Grazing tolerance of alfalfa (*Medicago* spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (*Bromus riparious* Rehm. syn. *B. biebersteinii* Roem & Schult). Can. J. Plant Sci. **82**: 337-347.

Kunelius, H. T. and Campbell, A. J. 1984. Performance of sod-seeded temperate legumes in grass dominant swards. Can. J. Plant Sci. **64**: 643-650.

Kunelius, H. T., Campbell, A. J., McRae, K. B., Ivany, J. A. 1982. Effects of vegetation suppression and drilling techniques on the establishment and growth of sod-seeded alfalfa and bird's-foot trefoil in grass dominant swards. Can. J. Plant Sci. **62**: 667-675.

Masters, R. A. and Sheley, R. L. 2001. Principles and practices for managing rangeland invasive plants. J. Range Manag. **54**(5): 502-517.

McCloud, D. E. and Mott, G. O. 1953. Influence of association upon the forage yield of legume-grass mixtures. Agron. J. **45**(2): 61-65.

McLeod, E. M. 2011. Forage responses to herbicide weed control in grass-legume swards. M.Sc. Thesis, University of Alberta, Edmonton, AB. 60 pp.

Merou, T. P. and Papanastasis, V. P. 2009. Factors affecting the establishment and growth of annual legumes in semi-arid mediterranean grasslands. Plant Ecol. **201**: 491-500.

Popp, J. D., McCaughey, W. P., Cohen, R. D. H., McAllister, T. A., Majak, W. 2000. Enhancing pasture productivity with alfalfa: A review. Can. J. Plant Sci. **80**(3): 513-519.

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., Goslee, S. C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J. **97**: 1465-1471.

Seguin, P. 1998. Review of factors determining legumes in sod-seeding outcome during pasture renovation in North America. Biotechnol. Agron. Soc. Environ. **2**(2): 120-127.

Seguin, P., Peterson, P. R., Sheaffer, C. C., Smith, D. L. 2001. Physical sod suppression as an alternative to herbicide use in pasture renovation with clovers. Can. J. Plant Sci. **81**: 255-263.

Sleugh, B., Moore, K. J., George, R., Brummer, E. C. 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agron. J. **92**: 24-29.

Ta, T. C. and Faris, M. A. 1987. Species variation in the fixation and transfer of nitrogen from legumes to associated grasses. Plant & Soil **98**: 265-274.

Vogel, K. P., Kehr, W. R., Anderson, B. E. 1983. Sod-seeding alfalfa into cool-season grasses and grass-alfalfa mixtures using glyphosate or paraquat. J. Range Manage. **36**(6): 700-702.

Witty, J. F., Minchin, F. R., Sheehy, J. E. 1983. Carbon costs of nitrogenase activity in legume root nodules determined using acetylene reaction. J. Exp. Bot. **34**: 951-963.

2. LITERATURE REVIEW

2.1 Importance of Legumes

2.1.1 Benefits

The inclusion of legumes in cool season pastures can yield several benefits, ranging from increases in forage quality and quantity, to a reduced need for nitrogen fertilizers (Kunelius et al. 1982; Seguin et al. 2001). The ability of legumes to biologically fix atmospheric nitrogen into a readily available form (ammonia, NH_3) is a well-known characteristic, and one of the most positive attributes legumes can impart to plant communities (Kunelius et al. 1982; Seguin 2001). The transfer of biologically fixed nitrogen to associated grasses increases overall pasture production and helps replace nitrogen fertilizers, reducing costs and labour efforts for producers (Olsen et al. 1981; Popp et al. 2000; Vogel et al. 1983). Seeded red clover has been shown to replace the equivalent of 100-150 kg N ha^{-1} for each post seeding year of production (Kunelius and Campbell 1984). As the costs of nitrogen fertilizers continue to increase, so does the need to maintain a legume component in pasture systems.

Legumes also increase forage quality, providing greater crude protein and increasing palatability, which can result in improved forage intake and associated animal gains (Groya and Sheaffer 1981; Kunelius et al. 1982; Kunelius and Campbell 1984; Sleugh et al. 2000; Merou and Papanastasis 2009). Legume presence also tends to stabilize forage production both inter- and intra-annually, a result of the differences in timing of yield contributions between grass and legume species (Groya and Sheaffer 1981; Seguin 1998; Seguin 1998; Gökkuş et al. 1999; Katepa-Mupondwa et al. 2002). This allows for greater sustained forage yields throughout the growing season when compared to grass-only pastures (Sleugh et al. 2000). Grass-legume mixtures have reduced invasion potential by weedy species and greater resiliency when compared to grass or legume monocultures: a function of greater niche exploitation under increased plant diversity (Sleugh et al. 2000; Sanderson et al. 2005). These communities are also less susceptible to erosion and can have greater stand longevity due to the complimentary nature of a legume-grass community (Droslom and Smith 1976).

The indirect and direct benefits of legumes in pasture systems can only be realized if the legumes are present in an adequate amount in the plant community (McCloud and Mott 1953; Evans et al. 1992). Clover content of 30-50% ground cover is optimal to impart benefits to the pasture community (Evans et al. 1992). Legumes have been shown to provide secondary beneficial effects on many associated species in pasture trials, and the inclusion of legumes has resulted in yields 100% or greater than controls in approximately half of the different plants species studied, indicating an interspecific benefit between legumes and their associates (McCloud and Mott 1953). This is a well-documented phenomenon known as 'over-yielding' (Gökkuş et al. 1999). The effect of over-yielding is likely a product of the increased diversity associated with grass-legume complexes and the more effective use of environmental resources, such as light, moisture, nutrients, etc., which is possible with a mixture of plants that utilize different niches (Hay and Walker 1989). Legumes clearly have positive attributes, which when integrated into cool season pastures; confer benefits to producers, making them a valuable part of any productive forage-based operation. The two major legume species utilized in western North America are alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.).

2.1.2 Alfalfa

Alfalfa is the oldest and highest yielding forage legume, with many cultivars and a large distribution (Frame 2005; USDA 2002a). It is also known as 'lucerne' and originates from

Europe and Asia. It is a productive plant, mineral rich with high crude protein content, and is palatable with elevated voluntary intake (Conrad and Klopfenstein 1988; Frame 2005).

Alfalfa has been found to increase yields, help achieve a longer growing season, and result in a more uniform nutrient supply when incorporated into grass pasture systems (Katepa-Mupondwa et al. 2002). It is the legume of choice for long-lived hay fields, helps to maximize forage productivity and quality, and replenish soil nitrogen (Olsen et al. 1981).

2.1.2.1 HABITAT. Alfalfa is one of the most widely distributed forage legumes, with an estimate of 10-11 million hectares of annual growth in the United States alone (Barnes and Sheaffer 1995), and is found across most temperate zones. It is adapted for survival in these areas and tolerates a wide range of environmental conditions, but deep well-drained fertile soils are required to reach maximum potential, and a relatively high soil pH of 6.0-6.5 is optimal (USDA 2002a; Frame 2005). Areas with high water tables or flooding are less than ideal and may result in mortality (USDA 2002a; Frame 2005). It is also able to tolerate saline soils and drought conditions better than many other forage species (Frame 2005).

2.1.2.2 BIOLOGY. Alfalfa is a perennial legume, 30-100 cm tall on average, with flowers that vary in colour from purple to yellow, arranged in loose clusters (USDA 2002a; Frame 2005). Stems have an erect form originating from a woody crown, which contains buds for new growth (USDA 2002a). Leaves are trifoliate with an alternate arrangement, while seed pods vary from sickle to spiral shaped, with 2-5 kidney shaped seeds per pod (USDA 2002a; Frame 2005). Alfalfa plants have a 2-4 m long tap root that can utilize resources deeper in the soil, although the majority of total root biomass, 60-70%, is found in the top 15 cm of the soil profile (Heichel 1982); USDA 2002a; Frame 2005). This taproot gives plants the ability to withstand temporary drought conditions (Groya and Sheaffer 1981). The nitrogen fixing root nodules associated with alfalfa plants are found on the fibrous roots in the upper soil level (Frame 2005). Alfalfa has been shown to exhibit autotoxic/autoallelopathic effects, where mature plants produce water-soluble substances which act to inhibit alfalfa germination and growth (Hall et al. 1989; Chung et al. 1995).

2.1.2.3 CULTIVAR – ALGONQUIN ALFALFA. Algonquin alfalfa is one of the main alfalfa cultivars utilized in the Central Parkland region of Alberta, mainly due to its winter hardiness (ability to withstand frosts and cooler temperatures) (Baenziger 1975; NPARA 2006). It was developed by Agriculture and Agri-Food Canada at their research station in Ottawa, Ontario, and is a standard type alfalfa. It is a 16 clone synthetic with resistance to bacterial wilt (Baenziger 1975). These plants have light purple flowers, with some that are almost white. The plants have wide crowns, which branch out to many fine stems (Baenziger 1975). This cultivar also boasts a deep taproot system, along with medium sized roots, has a wide range of adaptability and is prized for its longevity (NPARA 2006). When compared to the Vernal cultivar, Algonquin alfalfa was found to have 5-10% less winterkill during a trial in Ottawa (Baenziger 1975). Algonquin alfalfa was found to have the highest yield out of 10 different alfalfa cultivars over a two year study by the North Peace Applied Research Association, indicating that this cultivar is one of the highest producing, in addition to having favorable winter hardiness and longevity (NPARA 2006).

2.1.3 White Clover

White clover is a highly nutritious nitrogen fixing perennial forage crop that can be utilized by all types of livestock. It is easily digestible, with high crude protein content, and is very palatable (Clark 2007). Originating from Europe, it is well suited for grazing and adapted to a wide range of soil and environmental conditions (USDA 2002b; Frame 2005).

The benefits of introducing clover into pastures are evident in many different studies (Evans et al. 1992; Frame 2005; Clark 2007). Grass production has shown increases from 0.3 to 1.2 tons ha^{-1} over 2 years when comparing a pasture system with a white clover component to a grass monoculture system under similar management regimes (Evans et al. 1992). The increase in grass production was attributed to nitrogen transfer from nitrogen fixing clover to the grass species. These trials indicated that white clover has the ability to increase total production by up to 50% in comparison to grass monocultures (Evans et al. 1992).

2.1.3.1 HABITAT. White clover is ubiquitous across moist temperate regions of the world and is adapted to a wide range of conditions, but is best suited to cool, moist environments (USDA 2002b; Clark 2007). It grows well in clay and silt soils with humid to subhumid regimes, with a soil pH of 5.5-6.0 (USDA 2002b; Frame 2005). It is not drought tolerant and does not perform well on acidic soils (Frame 2005). White clover is naturalized across the moist regions of North America, including the Parkland of western Canada, and is a common volunteer plant in these areas (Zeven 1991; USDA 2002b; Frame 2005).

2.1.3.2 BIOLOGY. White clover is prostrate in form, with a stoloniferous habit, with both leaves and roots originating from nodes along the stolon (USDA 2002b). Roots are shallow, seldom rooting deeper than 1 m, but dense and extensively branched (USDA 2002b). These root systems can protect soil from erosion, as well as suppress weed invasions (Clark 2007). Leaves are trifoliate, often with a white crescent shaped watermark on the upper leaf surface (USDA 2002b). Flower heads are borne on long pedicels originating from leaf axils and often contain 40-100 florets, which are white in colour with an occasional pinkish hue (USDA 2002b; Frame 2005). Plants are relatively small, and grow to a maximum height of 15-30 cm (Clark 2007).

White clover has relatively slow growth in the spring, and can be outcompeted by aggressive grass species at this time (Frame 2005).

2.1.3.3 CULTIVAR – WHITE DUTCH CLOVER. White Dutch Clover is a widespread cultivar in North America, and is an intermediate clover type. It is also known as 'English Giant' and 'English Dutch', and originated from the Netherlands in the 16th century (Hawkins 1960; Caradus et al. 1989; Zeven 1991). It has a relatively large leaf size, and upright growth habit (Caradus et al. 1989). Dutch white clover is quite winter-hardy, but is considered to be a poor performer in terms of production when compared to other cultivars (Zeven 1991).

2.1.4 Nitrogen Fixation and Transfer

Legumes have the ability to biologically fix atmospheric nitrogen (N_2) into a readily available form (ammonia, NH_3) by the enzyme nitrogenase through their symbiotic association with *Rhizobium* bacterium (Kunelius et al. 1982; Seguin 2001). This is one of the most valuable functions of legumes in range and pasture systems.

Biological fixation of atmospheric nitrogen occurs in nodules formed on the fibrous roots of alfalfa in the upper soil layer, which form as a symbiotic relationship with various strains of *Rhizobium meliloti* (Frame 2005). Transfer of fixed nitrogen from alfalfa to associated grasses is through root death and turnover, and the subsequent mineralization of nitrogen from organic matter (Dubach and Russelle 1994). Annual nitrogen fixation estimates for alfalfa range from 85 to 360 kg N ha^{-1}, although there is a wide range of variance depending on climate and soil characteristics (Witty et al. 1983; Kunelius and Campbell 1984; Heichel and Henjum 1991).

White clover fixes atmospheric nitrogen in root nodules with the help of different strains of *Rhizobium leguminosarum*, which performs best in areas with high soil fertility and a history of white clover growth (Frame 2005). For white clover to effectively fix nitrogen a soil pH of at least 5.0 is needed to avoid toxic levels of exchangeable aluminum and manganese (Cooper et al. 1983). Annual nitrogen fixation for clover is generally between 100 to 400 kg ha^{-1}, where the amount of nitrogen fixation is related to the amount of clover present, as well as soil and climatic characteristics (Frame 2005).

2.2 Legume Reestablishment

Successful legume reestablishment is contingent on an array of factors, and low levels of emergence may occur even in cases where most conditions are favourable. Poor recruitment as a result of seedling death will result if resident vegetation is not suppressed adequately, light or nutrient competition is high, moisture regimes or temperatures are unfavourable, or the interval between herbicide application and legume seeding is too short for suitable herbicide degradation (Rioux 1994).

2.2.1 Vegetative Competition

Legume reestablishment, through reseeding or volunteer reestablishment from the seed bank, is dependent primarily on the effects of the resident plant community and its impacts on legume reestablishment (Seguin 1998). The suppression of competing vegetation through grazing/defoliation, or herbicide application is often the best way to facilitate the reestablishment of a productive legume component in pastures (Sheaffer and Swanson 1982; Vogel et al. 1983). Competing vegetation decreases the availability of soil moisture, light, and nutrients for the growth of legumes, resulting in seedling death, which can hamper successful legume reestablishment (Gist and Mott 1956; Leroux and Harvey 1985).

Legumes such as alfalfa and white clover tend to decline in grass-legume pastures relatively quickly, and significant declines have been noted within 2-4 years (Kunelius et al. 1982; Kunelius and Campbell 1984), and to maintain a desired legume component and prevent the encroachment of invasive plants, legumes must either be reseeded at frequent intervals or a seed bank must be present with the capacity for adequate volunteer reseeding (Kunelius et al. 1982; Kunelius and Campbell 1984). If legumes are not managed and are allowed to disappear from the pasture system, producers will lose the benefits associated with legumes and leave their pastures vulnerable to invasive plants through the creation of gaps within the plant community.

Grass species in particular are highly competitive with both alfalfa and white clover, and have the capacity to reduce legume survival and the forage value of pastures (Katepa-Mupondwa et al. 2002). There is, in fact, a negative relationship between grass and alfalfa yields, and when grass and competing vegetation is not adequately suppressed, or suppression is reduced, there is a corresponding reduction in alfalfa seedling survival and corresponding yields (Groya and Sheaffer 1981).

The height of competing grass species at the time of legume seeding and during reestablishment has a direct effect on the success or failure of legume introduction into a pasture system. Even in cases when grasses have been suppressed by herbicide application, they have the potential to negatively impact legume reestablishment (Seguin 1998). Alfalfa establishes best in pastures with high levels of grass sod suppression, via herbicides or defoliation treatments, and pastures with lower suppression have decreased alfalfa yields (Rioux 1994).

As seedlings germinate and reestablish, their relationship with other seedlings, spatially and temporally, governs their success (Skinner 2005). In clusters of seeds, micro environmental conditions can limit emergence and established seedlings can suppress the emergence of close neighbouring seedlings (Skinner 2005). The competitive relationships between plants are complex and may vary with seed availability, microsite differences, levels of disturbance and across ecosystems.

2.2.2 Environmental Factors

The reestablishment of legumes is highly dependent on the availability of four main factors: soil moisture, light, nutrients, and space – as addressed in the previous section (Gist and Mott 1956). Legume seedlings compete with each other for these resources, or conversely, may facilitate the establishment of other seedlings by mitigating undesirable conditions, depending on the environment and the species used (Skinner 2005). The interactions between light intensity and soil moisture in particular are significant to legume seedling development, where increased light and moisture availability leads to increased seedling success (Gist and Mott 1956; Vough and Marten 1971). Temperature effects have also been shown to weigh significantly on the success or failure of legume reestablishment, where temperatures either too high or too low will reduce the successful reestablishment (Pearson and Hunt 1972).

2.2.2.1 LIGHT. Seedling death is most often associated with a lack of light availability, usually a function of competition from the surrounding vegetative community (Pritchett and Nelson 1951; Wing-To and MacKenzie 1971; Groya and Sheaffer 1981). As light availability decreases, a general decrease in overall plant vigour occurs (Pritchett and Nelson 1951; Byers and Templeton Jr. 1988). Alfalfa plants that receive abundant light are more vigorous and produce more leaves than shaded plants, while decreases in light intensity result in decreases in the overall dry weight of alfalfa plants, most noticeably in the roots (Pritchett and Nelson 1951). Decreases in root growth can have negative impacts on alfalfa survival via reductions in the ability of plants to utilize moisture and nutrient resources found in the deeper soil profile (Pritchett and Nelson 1951). Alfalfa biomass yields also decline significantly when shaded at different levels of soil moisture, indicating that light restrictions will prevent a yield response to soil moisture, and that light is the dominant factor regulating alfalfa growth (Wing-To and MacKenzie 1971; Groya and

Sheaffer 1981). White clover can survive as an understory plant if necessary, but growth and productivity are markedly reduced under low light intensity (Frame 2005; Clark 2007).

2.2.2.2 SOIL MOISTURE. Available moisture is another factor necessary for successful legume establishment. Precipitation, both distribution and amount, was found to have the greatest effect on legume establishment in Lexington, Kentucky when compared to the other environmental factors (Taylor et al. 1969). Low soil moisture levels significantly reduce alfalfa germination rates, while higher moisture levels aid establishment (Groya and Sheaffer 1981). Following germination, growth of legume seedlings is reduced with increases in moisture stress (Gist and Mott 1956). Successful alfalfa reestablishment is more likely when seeded during high moisture periods and at lower temperatures (Vough and Marten 1971; Groya and Sheaffer 1981; Taylor et al. 1969).

Increases in moisture can reduce light availability to alfalfa seedlings by causing increases in the growth of competing vegetation (Groya and Sheaffer 1981). Similarly, when light is a limiting factor, increases in soil moisture availability have the potential to decrease legume seedling vigour due to increases in the growth of competing vegetation (Gist and Mott 1956). When grass is suppressed, or shading is eliminated and light availability increases, soil moisture then becomes the limiting factor for alfalfa growth and yield (Groya and Sheaffer 1981). The growth responses associated with increases in light are realized only when seedlings have adequate soil moisture available (Gist and Mott 1956).

Once alfalfa plants are established, moisture becomes less of a limiting factor for plant growth and survival. In grass-alfalfa mixtures, favourable moisture will result in grass dominance due to fibrous root systems which exploit surface moisture and may outcompete legumes, but in areas where moisture is limited, deep alfalfa taproots allow the latter to utilize subsurface moisture, giving the advantage to alfalfa plants and allowing them to maintain or even increase their population size (Lardner et al. 2001). However, low available moisture will decrease shoot production and lead to reduced yield of alfalfa plants, as well as impair photosynthesis, respiration, and nitrogen fixation (Durand et al. 1989).

Due to its shallow root system and relatively less effective control over transpiration, white clover does not tolerate drought and is subject to early senescence when faced with dry conditions (Skinner et al. 2004). Effects of moisture stress on white clover increase over time; drought stressed clover has reduced growth rates over the growing season, with no effect in comparison to controls in May, a 33% decrease in July, and a 43% decrease in September (Skinner et al. 2004). In mixed grass/legume swards, clover intolerance to drought may be aided by grass foliage acting to protect and shade clover from solar radiation, as well as helping to reduce temperatures at ground level (Frame 2005). Although clover is best suited to humid and subhumid climates, it cannot survive long periods of flooding (Frame 2005).

2.2.2.3 NUTRIENTS. Both clover and alfalfa tend to decrease in pasture systems with abundant levels of available nitrogen (Wing-To and MacKenzie 1971; Evans et al. 1992; Collins et al. 1996; Schwinning and Parsons 1996; Lardner et al. 2001; Frame 2005). Both alfalfa and clover maintain greater relative growth rates compared to grass in systems that have low soil nitrogen levels due to their ability to supplement mineral uptake with nitrogen fixation (Schwinning and Parsons 1996). In areas with higher levels of available soil nitrogen, this effect disappears as available nitrogen uptake is more efficient than the combination of nitrogen uptake and nitrogen fixation (Schwinning and Parsons 1996). Plentiful nitrogen is the desired state for pasture systems in regards to overall production, but grass plants tend to exploit plentiful available nitrogen and suppress legume growth through light and moisture competition, resulting in decreases in the legume population (Schwinning and Parsons 1996). Although nitrogen is the main nutrient of concern, it is important to note that both clover and alfalfa need adequate levels of available phosphorus for seedling development (Frame 2005).

2.2.2.4 TEMPERATURE. The optimum temperature for plant growth is specific to certain growth stages and varies as the plant matures throughout the growing season (Pearson and Hunt 1972). Alfalfa establishment and early growth is optimal between 10-15^{o}C in the North American cool humid continental zone, the temperature at which dry matter accumulation and leaf area expansion were most rapid (Pearson and Hunt 1972). In additional studies similar results have been found; alfalfa forage quality and quantity increase with decreases in temperature (16^{o}C day / 10^{o}C night) Vough and Marten (1971). Different alfalfa cultivars have varying degrees of cold tolerance (Frame 2005).

White clover grows best at temperatures between 20-25^{o}C, with growth rates increasing along with temperature increases up to these levels (Boller and Nösberger 1985; Frame 2005). Similarly, White Dutch clover experiences increases in growth rate in correlation with temperature increases, but being a winter hardy cultivar, it is able to withstand low winter temperatures without extreme die offs (Zeven 1991).

2.2.3 Reestablishment – When?

With all the factors that must be taken into account when aiming for successful legume reestablishment, when is the best time to seed? In the Central Parkland, during years where moisture stress is not a concern, the best time to seed/reseed alfalfa is during June, which coincides with a period of rapid plant growth (Bowes and Zentner 1992). Early spring sowings are better for legume reestablishment, due in part to increased moisture availability and the decreased presence of slugs and other insects, which predate on seeds in later spring (Byers and

Templeton Jr. 1988). Fall is another advantageous reseeding time as legumes can take advantage of winter snow melt and germinate in early spring before competition becomes a major limitation (Malik and Waddington 1990).

Both of these scenarios take advantage of high levels of available soil moisture. Regardless of the timing, ensuring that the problems associated with moisture stress and vegetative competition are reduced will allow for successful legume reintroduction (Bowes and Zentner 1992).

2.3 Herbicide Use in Pastures

Under the Weed Control Act of Alberta (Province of Alberta 2008) producers are legally required to take action to control noxious weeds, destroy prohibited noxious weeds, and take measures to prevent their spread. The weeds of concern are often broadleaf species (*Cirsium arvense* L., *Ranunculus acris L., Sonchus arvensis* L., etc.) which can be managed and reduced effectively through the application of broadleaf herbicides (Enloe et al. 2007; Grekul et al. 2005), often resulting in increased forage production (Bork et al. 2007). Herbicides are the principal method of weed control in rangeland and pasture systems due to the lack of efficient, cost-effective alternatives (Masters and Sheley 2001; Almquist and Lym 2010). Although they are a straightforward solution to weed control, the use of broadleaf herbicides often results in the reduction or removal of broadleaf legumes, such as alfalfa and white clover, in addition to the intended target species. This places producers in a difficult position: they are legally obligated to manage weed species, but in doing so may risk reducing pasture productivity and quality for an indeterminate amount of time due to legume removal.

The degradation of residual herbicide bioactive stored in soil is a necessary precursor to legume reestablishment (Renz 2010). Residual herbicides are desired by some producers as an extra measure of control against future weed emergence, but this is tempered by the desire for residual soil-active herbicides that degrade suitably for sensitive crops, such as alfalfa and white clover, to be reintroduced (Strachan et al. 2011). The period of time that must elapse before successful reestablishment can occur will vary widely with site characteristics, herbicide degradation and the residual properties of herbicides.

Herbicide decay rates tend to increase with increases in organic matter content and associated microbial activity (Ou 1984; Veeh et al. 1996; Picton and Farenhorst 2004), high soil temperatures that assist in biochemical breakdown (Walker and Zimdahl 1981; Ou 1984; Goetz et al. 1990; Veeh et al. 1996;), greater soil moisture levels (Walker and Zimdahl 1981; Parker and Doxtader 1983; Ou 1984; Goetz et al. 1990), and higher soil pH (Loux and Reese 1992; Aichele and Penner 2005). Auxinic herbicides are broken down primarily by soil microbes, and the rate of degradation varies depending on changes in the aforementioned conditions, as well as the adsorption of herbicides to soil surfaces.

Increases in soil organic matter are associated with increased microbial populations which are the mechanism of breakdown for auxinic herbicides (Veeh et al. 1996). This relationship clarifies why increased organic matter leads to increased herbicide decay rates (Veeh et al. 1996; Voos and Groffman 1997). Increased microbial populations have been shown to result in faster degradation of diallate and triallate (thiocarbamate herbicides) and 2,4-D herbicide residues (Anderson 1984; Ou 1984). Faster dissipation of 2,4-D and dicamba have also been noted in soils with higher microbial biomass (Voos and Groffman 1997). Although microbial biomass and microbial activity are not always directly related, increased levels of microbial activity are often associated with increases in overall microbial biomass.

Both 2,4-D and imazethapyr have demonstrated decreased degradation rates in conjunction with decreases in soil temperature (Goetz et al. 1990; Veeh et al. 1996) Increases in temperature result in increased microbial activity, the primary mechanism of herbicide breakdown, which explains why herbicide degradation increases with temperature. AMP has shown faster (2-8 times) dissipation rates at soil temperatures of 24^{o}C vs. 8^{o}C (Mikkelson 2010).

Abundant moisture is associated with faster degradation of imazethapyr herbicide residues (Goetz et al. 1990), while cooler dryer soils have shown reduced degradation rates for atrazine, linuron and metolachlor (Walker and Zimdahl 1981). Moisture availability plays a major role in herbicide degradation.

Soil pH also plays an important role in herbicide degradation. Herbicides degraded by microbial activity show increased persistence with decreases in pH (Loux and Reese 1992; Aichele and Penner 2005). This phenomenon is a function of herbicide adsorption properties, where adsorption of herbicides to soil colloidal surfaces increases at lower pH levels, resulting in reduced accessibility for microbial degradation (Loux and Reese 1992; Aichele and Penner 2005). The adsorption and leaching properties of weak acid herbicides, such as auxinic herbicides, vary slightly with variations in pH.

Considering the impact these factors have on herbicide degradation, longer withdrawal periods are to be expected after fall applications relative to spring applications as very little degradation occurs when soil temperatures are cooler or below freezing during the fall and winter months (Mikkelson and Lym 2011).

Recommendations for seeding legumes following herbicide application either specify a bioassay prior to seeding or are unavailable (USEPA 2010; DowAgrosciences 2012). Soil dissipation studies for AMP prior to registration were limited to two study sites, each located in the southern USA (USEPA 2005). The estimated half-life of 34.5 days was at variance with laboratory reports of 31.5 to 533.2 days. AMCP half-life was indicated between 22 and 126 days (USEPA 2010). Field trials in Colorado reported AMP and AMCP half-lives of 28.9 and 32.5 days, respectively (Lindenmayer 2012). Herbicide degradation is expected to slow in areas with shorter growing seasons, colder average temperatures, as well as different soil conditions and precipitation regimes; extending half-lives and increasing the withdrawal period of these herbicides in Alberta. Specific knowledge on AMP and AMCP degradation rates in northern temperate pastures is pivotal to help producers understand how to effectively manage weeds in pasture and range systems, while maintaining economic viability.

2.3.1 Herbicide Bioactives

2.3.1.1 AMINOPYRALID. Marketed as Milestone™, aminopyralid (AMP) is a product developed by Dow AgroSciences, which provides immediate and prolonged control of broadleaf weed species through initial weed removal partnered with soil residual activity (Dow AgroSciences 2009). It is part of the pyrimidine carboxylic acid family, and is a postemergence, systemic synthetic auxinic herbicide. It works by translocation from leaves and roots to meristematic tissues, where it mimics auxin hormones. Symptoms include leaf-cupping, loss of apical dominance, epinastic growth form, unregulated plant growth, and eventual plant death (Bussan and Dyer 1999; USEPA 2005). Milestone™ provides a means of control of weedy broadleaf species in range and pasture settings. It is a liquid formula that is mixed with water (0.4 to 5.5 mL per litre) and used for ground, aerial and spot applications (Dow AgroSciences 2005; Dow AgroSciences 2012).

AMP has relatively low application rates to reduce herbicide loading, and a lighter environmental footprint due to adsorption to soil particles and reduced leaching when compared to other herbicides (Dow AgroSciences 2005; Bunkun et al. 2010). These qualities have been found to allow for use in riparian areas, up to the water's edge, representing a viable control option in areas where other herbicides are unsuitable (Enloe et al. 2007). In regular field conditions AMP remains in the upper portion of the soil profile due to its tight adsorption to soil particles, and when compared to clopyralid, it has greater adsorption to soils, resulting in a lower potential for leaching due to a more lipophilic nature, and minimal concerns regarding herbicide leaching (Bukun et al. 2010). AMP is part of the same family of herbicides as clopyralid and picloram, two herbicides that have been known to exhibit carry over effects of soil residues (Bukun et al. 2009). The increased adsorption rates of AMP to soil colloidal surfaces is likely due to the chemical structure of the pyridine ring, and the amino substitution found there in. This structure appears to interact strongly with soil surfaces and/or divalent cations found in the soil, allowing for strong bonds to form between the herbicide and the soil surface (Bukun et al. 2010). In comparison to picloram, AMP has greater sorption to soil and clay minerals, with an average K_{oc} value of 10.8 vs. 0.026 to 100 (picloram), resulting in less potential for movement to off-target areas (Dow AgroSciences 2005; Fast et al. 2010; TCI 2010). Picloram has been found to persist in northern soils for over a year, and alfalfa can be safely replanted in the fifth year following picloram application in Alberta soils, indicating a long withdrawal period (Keys and Friesen 1968; Vanden Born 1969).

AMP has been used to control Canada thistle in restored tallgrass prairie, with favourable results in regards to preservation of the natural system's integrity (Almquist and Lym 2010). Application of AMP led to a decrease in the richness and diversity of the system overall, but the majority of the species removed were invasive weeds or undesirable, which had positive impacts on the function of the system. Native grass species were able to increase once unwanted species were removed by AMP application, which was believed to increase the plant community's ability to resist future encroachment by invasive and/or undesirable plant species (Almquist and Lym 2010).

2.3.1.2 AMINOCYCLOPYRACHLOR. Aminocyclopyrachlor (AMCP) is a synthetic auxin herbicide for use in pastures, rangelands, and industrial right-of-ways for the control of broadleaf weeds and select types of unwanted brush and tree species in forestry applications (DuPont 2009; Strachan et al. 2011). This herbicide is part of the pyrimidine carboxylic acid family and is absorbed by leaves and roots, then translocated to meristematic tissues, where it mimics auxin hormones (DuPont 2009). DuPont is in the process of developing their AMCP product, known as DPX-MAT28™, for use in Canada as the primary ingredient in three different herbicides: Rejuvra™ XL, Truvist, and Navius (DuPont 2012; Forsythe 2012).

For pasture and rangeland settings Rejuvra™XL is currently under development. It is a mixture of the synthetic auxin herbicide, AMCP (known industrially as DPXMAT28™), and a sulfonylurea herbicide (DuPont 2012). This herbicide is a granular formula which is mixed with water and then applied post emergence to foliage for general broadleaf weed control in non-crop (pasture and rangeland) applications. It is absorbed by the roots and foliage of plants, and inhibits the growth of vulnerable broadleaf weeds, as well as some types of woody brush species. It provides broad spectrum post-emergence control of broadleaf weeds, including perennials. It remains active in the soil profile after application, and can be taken up by roots for post application control. Although this herbicide does not prevent germination, it is readily taken up by germinating weeds via both the roots and shoots (DuPont 2012). AMCP has low use rates and a low potential for vapor drift and subsequent non-target responses (DuPont 2009; Strachan et al.

2010). AMCP has also shown less potential for leaching than clopyralid or AMP, indicating that it is likely safe for use near riparian areas (Lindenmayer 2012).

2.3.2 Impacts of Herbicides on Legume Reestablishment

Active residues of both AMP and AMCP can impede the successful reestablishment of broadleaf plants, including legumes such as alfalfa and white clover. Alfalfa has been safely replanted 20 and 23 months following AMP application in trials in Fargo, North Dakota (Mikkelson and Lym 2011). Alfalfa did display injury when seeded 8 and 11 months after AMP treatment (Mikkelson and Lym 2011). It is not prudent to plant alfalfa within a year following the application of AMP in North Dakota, AMP seems to have a longer soil residual time in northern environments than originally predicted.

The reestablishment of forage legumes (alfalfa, white and red clover) has demonstrated significant reductions relative to controls, with reductions of 74, 45, and 81%, respectively, relative to untreated controls when seeded in the spring following fall AMP application (Renz 2010). Even small concentrations of AMCP in soil can cause damage to alfalfa plants, with 25% phytotoxicity (measured as plant death) of alfalfa plants recorded were AMCP soil concentrations were only 5.4 ppb (Strachan et al. 2011).

2.4 Defoliation

The persistence of legumes under grazing regimes is often limited, and few of the perennial legume varieties have the capacity for reliable long-term production under grazing pressure (Peterson et al. 1994). Stoloniferous or rhizomatous forage legumes, such as white clover, can withstand grazing better than species with an erect form, such as alfalfa (Frame 2005). Erect legumes like alfalfa are better suited for haying practices, where defoliation is infrequent. Despite species type and composition, continuous grazing over time can have severe negative impacts on legume stands (Evans et al. 1992; Brummer and Moore 2000).

The impacts of defoliation may vary with regards to defoliation type, mowing vs. various types of livestock grazing. Cattle grazing has been shown to impact plants differently than mowing, by increasing the amount of bare ground and changing grass species composition (De La Hoz and Wilman 1981). This is due to the fundamental differences between these types of defoliation: grazing has the added impacts of trampling and mineral deposition (urine and feces), while mowing does not. Grazing is a non-uniform type of defoliation, with repeated selection of palatable plants, such as legumes, which may lead to their decline over time, while mowing is a uniform type of defoliation which has significantly different impacts on plant community composition than selective grazing (Schwinning and Parsons 1996; Rutter 2006; Holechek et al. 2010)

Suppression of weeds by herbicide use can result in a loss of potential forage sources, a decrease in pasture productivity, and an undesirable legume to grass ratio (Taylor et al. 1969; Seguin et al. 2001). Physical sod suppression is an alternative to herbicide use, via mowing or grazing, prior to and during establishment, and can mitigate many of the problems associated with competing vegetation (Seguin et al. 2001). In comparison to herbicide application, defoliation sod suppression methods were found not to reduce forage yields during pasture renovation with clover, and did not result in invasion by weedy species (Seguin et al. 2001). Contrary to these results, some studies have found that the form of vegetation suppression, herbicide vs. mowing, did not ultimately have a significant effect on legume yield (Kunelius et al. 1982).

2.4.1 Alfalfa

Although a good choice for hay fields, alfalfa does not persist well when exposed to consistent grazing pressure, and when included in a grass pasture mixture, its persistence is further reduced, regardless of the grazing system (rotational vs. continuous) (Katepa-Munpondwar et al. 2002). This does not necessarily indicate that alfalfa is a poor choice, as it has multiple benefits and can be easily reseeded into pastures as the population begins to decline. Rather, it is best suited to infrequent defoliation, and although persistence generally decreases under any grazing regime, productivity and persistence is maintained longer in rotational grazing systems than in continuous systems (Walton et al. 1981; Frame 2005). Rotational grazing systems may prolong alfalfa persistence, or even result in increases in alfalfa biomass, as was noted in a brome-alfalfa-creeping red fescue pasture system at the University of Alberta Kinsella research ranch (Walton et al. 1981). Loss of alfalfa from pasture stands occurs mainly during the first year of grazing, with smaller losses seen in following years (Brummer and Moore 2000).

2.4.2 White Clover

White clover tends to become the main legume in heavily grazed pastures, despite the preferential selection for clover in grazed systems (Schwinning and Parsons 1996; Brummer and Moore 2000). Once clover has been established in pasture systems it has the ability to thrive under defoliated conditions, and can stand up to high levels of animal traffic and trampling (Clark 2007).

Both cattle and sheep grazing tends to favour the growth of clover in pastures, although cattle grazing does so to a greater extent (Evans et al. 1992). Grazing by cattle is less damaging to white clover when compared to sheep grazing due to the differences in grazing selection and associated foraging behaviour between cattle and sheep, where sheep are more selective and put heavier pressure on clover (Evans et al. 1992). Rotational grazing has been found to favour white clover growth, regardless of the type of livestock present (Evans et al. 1992).

Mowing appears to result in greater clover populations than grazing, likely due to the uniform defoliation provided by mowing compared to uneven defoliation as characterized by grazing, or possibly due to the damage to clover seedlings by livestock trampling (Seguin et al. 2001). Although clover content is impacted less by cattle grazing than sheep grazing, grazing still results in less clover than mowing (Evans et al. 1992).

Although clover does have the ability to persist in grazed situations, frequent and severe defoliation can reduce plant growth, resulting in shorter petioles, stolon internodes, and reduced stolon branching, as total available carbohydrate content is decreased (Jones and Davies 1988). Clover production declines each year under grazing pressure, with yields highest in the spring then gradually declining as the growing season progresses (Peterson et al. 1994). Consequently, maintenance of rest periods between grazing may help increase the clover content of pastures (Evans et al. 1992). However, incorporation of rest periods poses its own problems in that infrequent defoliation can reduce clover cover, as grass plants can easily outcompete clover for light (Frame 2005).

2.5 Literature Cited

Aichele, T. M. and Penner, D. 2005. Adsorption, desorption, and degradation of imidazolinones in soil. Weed Technol. **19**(1): 154-159.

Almquist, T. L. and Lym, R. G. 2010. Effect of aminopyralid on Canada thistle (*Cirsium arvense*) and the native plant community in a restored tallgrass prairie. Invas. Pl. Sci. and Manage. **3**: 155-168.

Anderson, J. P. E. 1984. Herbicide degradation in soil: Influence of microbial biomass. Soil Biol. Biochem. **16**(5): 483-489.

Baenziger, H. 1975. Algonquin alfalfa. Can. J. Plant Sci. **55**(4): 1093-1094.

Barnes, D.K., and Sheaffer, C.C. 1995. An Introduction to Grassland Agriculture. Barnes, R.F., Miller, D.A. and Nelson, C.J. (eds) Forages, 5th edn., Iowa State University Press, Ames, Iowa, 1: 205-216 pp.

Boller, B.C. and Nösberger, J. 1985. Photosynthesis of white clover leaves as influenced by canopy position, leaf age and temperature. Ann. Bot. **56:** 19-27.

Bork, E. W., Grekul, C. W., DeBrujin, S. L. 2007. Extended pasture forage sward responses to Canada thistle (*Cirsium arvense*) control using herbicides and fertilization. Crop Prot. **26**(10): 1546-1555.

Bowes, G. G. and Zentner, R. P. 1992. Effect of vegetation suppression on the establishment of sod-seeded alfalfa in the aspen parkland. Can. J. Plant Sci. **72**(4): 1349-1358.

Brummer, E. C. and Moore, K. J. 2000. Persistence of perennial cool-season grass and legume cultivars under continuous grazing by beef cattle. Agron. J. **92**: 466-471.

Bukun, B., Shaner, D. L., Nissen, S. J., Westra, P., Brunk, G. 2010. Comparison of the interactions of aminopyralid vs. cloyralid with soil. Weed Sci. **58**: 473-477.

Bukun, B., Gaines, T. A., Nissen, S. J., Westra, P., Brunk, G., Shaner, D. L., Sleugh, B. B., Peterson, V. F. 2009. Aminopyralid and clopyralid absorption and translocation in Canada thistle (*Cirsium arvense*). Weed Sci. **57**: 10-15.

Bussan, A. J. and Dyer, W. E. 1999. Herbicides and rangeland. Pages 116-132. Sheley, R. L. and Petroff, J. K., (eds.) Biology and management of noxious rangeland weeds. Oregon State University Press., Corvallis, OR:.

Byers, R. A. and Templeton Jr., W. C. 1988. Effects of sowing date, placement of seed, vegetation suppression, slugs, and insects upon establishment of no-till alfalfa in orchargrass sod. Grass Forage Sci. **43**: 279-289.

Caradus, J. R. 1986. World checklist of white clover varieties, N. Z. J. Ex. Ag. **14:**2, 119-164.

Caradus, J. R., MacKay, A. C., Woodfield, D. R., VandenBosch, J., Wewala, S. 1989. Classification of a world collection of white clover cultivars. Euphytica **42**: 183-196.

Chung, I., Miller, D. A. 1995. Effect of alfalfa plant and soil extracts on germination and growth of alfalfa. Agron. J. **87**: 762–767.

Clark, A. 2007. White clover (*Trifolium repens*). Pages 179-184. Clark, A., (ed.) Managing cover crops profitably. 3rd ed. Sustainable Agriculture Network, Beltsville, MD.

Collins, R. P., Fothergill, M., Rhodes, I. 1996. Interactions between seedlings of perennial ryegrass and white clover cultivars in establishing swards. Grass Forage Sci. **51**(2): 163-169.

Conrad, H.R. and Klopfenstein, T.J. 1988. Role in livestock feeding – greencrop, silage, hay, and dehy. Hanson, Barnes and Hill Jr. (eds) Alfalfa and Alfalfa Improvement. Agronomy Monograph No. 29, ASA/CSSA/SSSA, Madison, Wisconsin. 539-566 pp.

Cooper, J.E., Wood, M. and Holding, A.J. 1983. The influence of soil acidity factors on rhizobia. D.F. Jones and D.R. Davies (eds). Temperate Legumes. London, Pitman, UK.

De La Hoz, V. M. B. and Wilman, D. 1981. Effects of cattle grazing, sheep grazing, cutting, and sward height on a grass-white clover sward. J. Agric. Sci. (Camb.) **97**: 699-706.

Droslom, P. N. and Smith, D. 1976. Adapting species for mixtures. multiple cropping. Papendick, R. I., (ed.) 1st ed. ASA, CSSA, SSSA, Madison, WI. 223-234 pp.

Dow AgroSciences. 2012. Milestone specialty herbicide. Specimen Label: 1-9.

Dow AgroSciences. 2009. Milestone fact sheet.

Dow AgroSciences. 2005. Aminopyralid technical bulletin. An introduction to aminopyralid. V45-313-001 (01/05) BOD. 010-50433. 19 pp.

Dubach, M., and Russelle, M. 1994. Forage legume roots and nodules and their role in nitrogen transfer. Agron. J. **86**: 259-266.

DuPont. 2012. DuPont Rejuvra XL herbicide. Research Update: 1-2.

DuPont. 2009. DPX-MAT28 technical bulletin: 1-7.

Durand, J.L., Lemaire, G., Gosse, G., and Charter, M. 1989. Analysis of the conversion of solar energy to dry matter by a population of Lucerne (*Medicago sativa* L.) subject to a water shortage. Agronomie, **9:** 599-607.

Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., Moechnig, M., Peterson, V., Masters, R. A., Halstvedt, M. 2007. Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop areas. Weed Technol. **21**: 890-894.

Evans, D. R., Williams, T. A., Evans, S. A. 1992. Evaluation of white clover varieties under grazing and their role in farm systems. Grass Forage Sci. **47**: 342-352.

Fast, B. J., Ferrell, J. A., MacDonald, G. E., Krutz, L. J., Kline, W. N. 2010. Picloram and aminopyralid sorption to soil and clay minerals. Weed Sci. **58**: 484-489.

Fick, G.W., Holt, D.A. and Lugg, D.G. 1988. Environmental physiology and crop growth. Hanson, A.A., Barnes, D.K. and Hill, R.R. Jr (eds) Alfalfa and Alfalfa Improvement. Agronomy Monograph No. 29, ASA/CSSA/SSSA, Madison, Wisconsin, 163-191 pp.

Forsythe, P. 2012. Personal communication.

Frame, J. 2005. Forage legumes for temperate grasslands. Science Publishers Inc., Enfield, NH, United States of America. 309 p pp.

Gist, G. R. and Mott, G. O. 1956. Some effects of light intensity, temperature, and soil moisture on the growth of alfalfa, red clover and birdsfoot trefoil seedlings. Agron. J. **49**(1): 33-36.

Goetz, A. J., Lavy, T. L., Gbur, J.,E.E. 1990. Degradation and field persistence of imazethapyr. Weed Sci. **38**(4/5): 421-428.

Gökkuş, A., Koç, A., Serin, Y., Çomakli, B., Tan, M., Kantar, F. 1999. Hay yield and nitrogen harvest in smooth bromegrass mixtures with alfalfa and red clover in relation to nitrogen application. Eur. J. Agron. **10**: 145-151.

Grekul, C. W., Cole, D. E., Bork, E. W. 2005. Canda thistle (*Cirsium arvense*) and pasture forage responses to wiping with various herbicides. Weed Technol. **19**: 298-306.

Groya, F. L. and Sheaffer, C. C. 1981. Establishment of sod-seeded alfalfa at various levels of soil moisture and grass competition. Agron. J. **73**: 560-565.

Hall, M. H., Henderlong, P. R. 1989. Alfalfa autotoxic fraction characterization and initial separation. Crop Sci. **29**: 425–428.

Hawkins, R.P. 1960. Investigation of local strains (varieties) of herbage plants. IV. White clover. J. British Grassland Soc. **15:** 28-33.

Hay, R. K. M. and Walker, A. J. 1989. An introduction to the physiology of crop yield. Harlow, England: Longman Scientific and Technical, 292 pp.

Heichel, G. H. 1982. Alfalfa. crop-water relations. Teare, I. D. and Peet, M. M., (eds.) Wiley, New York, NY, United States of America. 127-155 pp pp.

Heichel, G. H. and Henjum, K. I. 1991. Dinitrogen fixation, nitrogen transfer and productivity of forage legume-grass communities. Crop Sci. **31**: 202-208.

Holechek, J. L., Pieper, R. D., Herbel, C. H. 2010. Range management: Principles and practices. 6th Edition. Prentice Hall, Englewood Cliffs, NJ. 456 pp.

Jones, D. R. and Davies, A. 1988. The effects of simulated continuous grazing on development and senescence of white clover. Grass Forage Sci. **43**(4): 421-425.

Katepa-Mupondwa, F., Singh, A., Smith Jr., S. R., McCaughey, W. P. 2002. Grazing tolerance of alfalfa (*Medicago* spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (*Bromus riparious* Rehm. syn. *B. biebersteinii* Roem & Schult). Can. J. Plant Sci. **82**: 337-347.

Keys, C. H. and Friesen, H. A. 1968. Persistence of picloram activity in soil. Weed Sci. **16**(3): 341-343.

Kunelius, H. T. and Campbell, A. J. 1984. Performance of sod-seeded temperate legumes in grass dominant swards. Can. J. Plant Sci. **64**: 643-650.

Kunelius, H. T., Campbell, A. J., McRae, K. B., Ivany, J. A. 1982. Effects of vegetation suppression and drilling techniques on the establishment and growth of sod-seeded alfalfa and bird's-foot trefoil in grass dominant swards. Can. J. Plant Sci. **62**: 667-675.

Lardner, H. A., Wright, S. B. M., Cohen, R. D. H., Curry, P., MacFarlane, L. 2001. The effect of rejuvenation of aspen parkland ecoregion grass-legume pastures on botanical composition. Can. J. Plant Sci. **81**: 673-683.

Leroux, G. D. and Harvey, R. G. 1985. Herbicides for sod-seeding establishment of alfalfa (*Medicago sativa*) in quack grass (*Agropyron repens*) infested alfalfa swards. Weed Sci. **33**(2): 222-228.

Lindenmayer, R. B. 2012. Understanding aminocyclopyrachlor behavior in soil and plants. Ph.D. Thesis, Colorado State University, Fort Collins, CO. 82 pp.

Loux, M. M. and Reese, K. D. 1992. Effect of soil pH on adsorption and persistence of imazaquin. Weed Sci. **40**(3): 490-496.

Malik, N. and Waddington, J. 1990. No-till pasture renovation after sward suppression by herbicides. Can. J. Plant Sci. **70**: 261-267.

Masters, R. A. and Sheley, R. L. 2001. Principles and practices for managing rangeland invasive plants. J. Range Manag. **54**(5): 502-517.

McCloud, D. E. and Mott, G. O. 1953. Influence of association upon the forage yield of legume-grass mixtures. Agron. J. **45**(2): 61-65.

Merou, T. P. and Papanastasis, V. P. 2009. Factors affecting the establishment and growth of annual legumes in semi-arid mediterranean grasslands. Plant Ecol. **201**: 491-500.

Mikkelson, J. R. 2010. Effect of aminopyralid on crop rotations and native forbs. M.Sc. Thesis, North Dakota State University, Fargo, ND.

Mikkelson, J. R. and Lym, R. G. 2011. Aminopyralid soil residues affect crop rotation in North Dakota soils. Weed Technol. **25**(3): 422-429.

NPARA. 2006. Forage yield of alfalfa varieties in north peace region. North Peace Applied Research Association : 1-2.

Olsen, F. J., Jones, J. H., Patterson, J. J. 1981. Sod-seeding forage legumes in a tall fescue sward. Agron. J. **73**: 1032-1036.

Ou, L. 1984. 2,4-D degradation and 2,4-D degrading microorganisms in soils. Soil Sci. **137**(2): 100-107.

Parker, L. W. and Doxtader, K. G. 1983. Kinetics of the microbial degradation of 2,4-D in soil: Effects of temperature and moisture. J. Environ. Qual. **12**(4): 533-558.

Pearson, C. J. and Hunt, L. A. 1972. Effects of temperature on primary growth of alfalfa. Can. J. Plant Sci. **52**: 1007-1015.

Peterson, R. P., Sheaffer, C. C., Jordan, R. M., Christians, C. J. 1994. Responses of kura clover to sheep grazing and clipping: I. yield and forage quality. Agron. J. **86**: 655-660.

Picton, P. and Farenhorst, A. 2004. Factors influencing 2,4-D sorption and mineralization in soil. J. Environ. Sci. & Health **39**(3): 367-379.

Popp, J. D., McCaughey, W. P., Cohen, R. D. H., McAllister, T. A., Majak, W. 2000. Enhancing pasture productivity with alfalfa: A review. Can. J. Plant Sci. **80**(3): 513-519.

Pritchett, W. L. and Nelson, L. B. 1951. The effect of light intensity on the growth characteristics of alfalfa and bromegrass. Agron. J. **43**: 172-177.

Province of Alberta. 2008. Weed control act. Alberta Queen's Printer: W-5.1.

Renz, M. J. 2010. Establishment of forage grasses and legumes after fall herbicide applications. Forage and Grazinglands Available: doi:10.1094/FG-2010-0806-01-RS [March 10, 2013].

Rioux, R. 1994. Direct seeding of alfalfa in grain stubble and bromegrass sod. Can. J. Plant Sci. **74**(4): 773-778.

Rutter, S. M. 2006. Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. Appl. Anim. Behav. Sci. **97**(1): 17-35.

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., Goslee, S. C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J. **97**: 1465-1471.

Schwinning, S. and Parsons, A. J. 1996. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. J. Ecol. **84**(6): 799-813.

Seguin, P. 1998. Review of factors determining legumes in sod-seeding outcome during pasture renovation in North America. Biotechnol. Agron. Soc. Environ. **2**(2): 120-127.

Seguin, P., Peterson, P. R., Sheaffer, C. C., Smith, D. L. 2001. Physical sod suppression as an alternative to herbicide use in pasture renovation with clovers. Can. J. Plant Sci. **81**: 255-263.

Sheaffer, C. C. and Swanson, D. R. 1982. Seeding rates and grass suppression for sod-seeded red clover and alfalfa. Agron. J. **74**: 355-358.

Skinner, R. H. 2005. Emergence and survival of pasture species sown in monocultures or mixtures. Agron. J. **97**(3): 799-805.

Skinner, R. H., Gustine, D. L., Sanderson, M. A. 2004. Growth, water relations, and nutritive value of pasture species mixtures under moisture stress. Crop Sci. **44**: 1361-1369.

Sleugh, B., Moore, K. J., George, R., Brummer, E. C. 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agron. J. **92**: 24-29.

Strachan, S. D., Nanita, S. C., Ruggiero, M., Casini, M. S., Heldreth, K. M., Hageman, L. H., Flanigan, H. A., Ferry, N. M., Pentz, A. M. 2011. Correlation of chemical analysis of residual levels of aminocyclopyrachlor in soil to biological responses of alfalfa, cotton, soybean and sunflower. Weed Technol. **25(2)**: 239-244.

Strachan, S. D., Casini, M. S., Heldreth, K. M., Scocas, J. A., Nissen, S. J., Bukun, B., Lindenmayer, R. B., Shaner, D. L., Westra, P., Brunk, G. 2010. Vapour movement of synthetic auxin herbicides: Aminocyclopyrachlor, aminocyclopyrachlor-methyl ester, dicamba and aminopyralid. Weed Sci. **58**: 103-108.

Taylor, T. H., Smith, E. M., Templeton Jr., W. C. 1969. Use of minimum tillage and herbicide for establishing legumes in kentucky bluegrass (*Poa pratensis* L.) swards. Agron. J. **61**: 761-766.

TCI America. 2010. Picloram material safety data sheet.

USDA. 2002a. Alfalfa (*Medicago sativa* L.) plant fact sheet. United States Department of Agriculture Natural Resources Conservation Service.

USDA. 2002b. White clover (*Trifolium repens* L.) plant fact sheet. United States Department of Agriculture Natural Resources Conservation Service.

USEPA. 2005. Environmental fate and ecological risk assessment for the registration of aminopyralid. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances.

USEPA. 2010. Registration of the new active ingredient aminocyclopyrachlor for use on non-crop areas, sod farms, turf, and residential lawns. U.S. Environmental Protection Agency, Office of Pesticide Programs, Registration Division.

Vanden Born, W. H. 1969. Picloram residues and crop production. Can. J. Plant Sci. **49**: 628-629.

Veeh, R. H., Inskeep, W. P., Camper, A. K. 1996. Soil depth and temperature effects on microbial degradation of 2,4-D. J. Environ. Qual. **25**: 5-12.

Vogel, K. P., Kehr, W. R., Anderson, B. E. 1983. Sod-seeding alfalfa into cool-season grasses and grass-alfalfa mixtures using glyphosate or paraquat. J. Range Manage. **36**(6): 700-702.

Voos, G. and Groffman, P. M. 1997. Relationship between microbial biomass and dissipation of 2,4-D and dicamba in soil. Biol. Fertil. Soils **24**: 106-110.

Vough, L. R. and Marten, G. C. 1971. Influence of soil moisture and ambient temperature on yield and quality of alfalfa forage. Agron. J. **63**: 40-42.

Walker, A. and Zimdahl, R. L. 1981. Simulation of the persistence of atrazine, linuron and metolachlor in soil at different sites in the USA. Weed Res. **21**: 255-265.

Walton, P. D., Martinez, R., Bailey, A. W. 1981. A comparison of continuous and rotational grazing. J. Range Manag. **34**(1): 19-21.

Wing-To, C. and MacKenzie, A. F. 1971. Effects of shading and nitrogen on growth of grass-alfalfa pastures. Agron. J. **63**: 667-669.

Witty, J. F., Minchin, F. R., Sheehy, J. E. 1983. Carbon costs of nitrogenase activity in legume root nodules determined using acetylene reaction. Exp. Bot. **34**: 951-963.

Zeven, A. C. 1991. Four hundred years of cultivation of dutch white clover landraces. Euphytica **54**: 93-99.

3. SHORT-TERM DOSE TRIALS OF LEGUME SENSITIVITY TO HERBICIDE

3.1 Introduction

Alfalfa *(Medicago sativa* L.) and white clover (*Trifolium repens* L.) are two important legume species of northern temperate pastures and hayfields in the Central Parkland natural sub-region of western Canada. The presence of these legumes increases the quantity of available forage through greater overall pasture productivity (Groya and Sheaffer 1981; Kunelius et al. 1982; Seguin et al. 2001). Productivity is increased directly through either the complementary use of soil resources when grown in diverse swards (Olsen et al. 1981; Vogel et al. 1983; Popp et al. 2000), or the nitrogen fixing properties of legumes (Kunelius et al. 1982; Seguin 2001). Additionally, legumes can indirectly increase production through the transfer of fixed nitrogen to associated grasses (Kunelius et al. 1982; Seguin 2001). Legume presence may also result in stabilized forage production between years (Groya and Sheaffer 1981; Seguin 1998), as well as more consistent intra-annual seasonal forage production. The latter results from differences in the timing of yield contribution between grass and legume species throughout the growing season (Sleugh et al. 2000; Katepa-Mupondwa et al. 2002).

Maintenance of a legume component also increases forage quality, as legume plants have greater crude protein concentration and are more palatable than their graminoid counterparts (Groya and Sheaffer 1981; Kunelius et al. 1982; Kunelius and Campbell 1984; Merou and Papanastasis 2009). Other benefits of grass-legume communities include reduced susceptibility to erosion, greater stand longevity through the addition of community diversity as well as greater resiliency and reduced invasion potential when compared to grass or legume monocultures (Sleugh et al. 2000; Sanderson et al. 2005). A significant challenge in the maintenance of beneficial legume communities is that they are often removed from pastures when herbicides are applied to control broadleaf weeds (Grekul and Bork 2005; Enloe et al. 2007).

Producers in the province of Alberta are legally required to control noxious weeds, destroy prohibited noxious weeds, and take measures to prevent their spread (Province of Alberta 2008). These weeds are often broadleaf species (*Cirsium arvense* L., *Ranunculus acris* L., *Sonchus arvensis* L., etc.) and can be effectively reduced in pastures through the application of broadleaf herbicides (Grekul et al. 2005), leading to increased forage production (Bork et al. 2007). However, these herbicides also remove broadleaf legumes such as alfalfa and white clover, placing producers in the difficult position of balancing weed control with maintaining legume communities. While legally required to manage weed species, doing so may risk reducing pasture productivity and quality for an indeterminate time.

The Central Parkland natural sub-region is a productive area of Alberta which has been subject to high levels of cultivation and agricultural development due to several factors which result in a highly favourable agricultural environment. Fertile Black Chernozemic soils, with high organic matter content, in conjunction with favourable precipitation (350-450 mm year^{-1}, falling primarily during the growing season), create an ecosystem well suited for agricultural development (Soil Classification Working Group 1998; Natural Regions Committee 2006). The combination of fertile soils and high summer precipitation coincides with a short but favorable growing season. To maximize forage availability legumes should be reseeded as quickly as possible into forage swards following herbicide application. Seeding success may be affected by herbicide residues, and an understanding of the specific herbicide degradation rates, as well as the minimum interval before legumes can be safely reintroduced for this agro-climatic region is required. Reestablishment of legumes depends on the presence of favourable conditions for germination, emergence, and growth, including the degradation of residual herbicide bioactives to levels lower than those that are toxic to seedlings. Degradation rates and associated ‘legume withdrawal periods’ defined as the interval between herbicide application and successful legume reestablishment, vary depending on the residual properties (half-life) of herbicides, along with fundamental decay rates. Most herbicide decay rates increase with increases in organic matter content and associated microbial activity (Ou 1984; Veeh et al. 1996; Picton and Farenhorst 2004), warmer soil temperatures which assist in biochemical breakdown through increased microbial activity (Walker and Zimdahl 1981; Ou 1984; Goetz et al. 1990; Veeh et al. 1996;), moderate soil moisture levels (Walker and Zimdahl 1981; Parker and Doxtader 1983; Ou 1984; Goetz et al. 1990), as well as higher soil pH levels in the case of weak acid herbicides ($K_{ow} < 4.5$) (Loux and Reese 1992; Aichele and Penner 2005). Together with herbicide degradation, environmental factors and vegetative competition play important roles in legume reestablishment, and success can vary across different climatic regimes (Gist and Mott 1956; Vough and Marten 1971; Mikkelson and Lym 2011).

Recommendations for seeding legumes following application of aminopyralid (AMP) or aminocyclopyrachlor (AMCP) either specify a bioassay be done prior to seeding or are unavailable (USEPA 2010; DowAgrosciences 2012). Soil dissipation studies for AMP prior to registration were limited to two studies, each located in the southern USA (USEPA 2005). The estimated half-life of 34.5 days was at variance with laboratory reports of 31.5 to 533.2 days. AMCP half-life was indicated to be between 22 and 126 days (USEPA 2010). Lindenmayer (2012) reported AMP and AMCP half-lives of 28.9 and 32.5 days, respectively, for field trials in Colorado. Although herbicide degradation will slow in areas with shorter growing seasons, colder average temperatures, as well as different soil conditions and precipitation regimes, possibly extending half-lives and increasing the withdrawal period. Currently available data are insufficient to estimate legume withdrawal periods, defined as the interval between herbicide application and successful legume reestablishment, for Western Canada.

The effects of defoliation on legume establishment and persistence are varied depending on legume species, as well as the frequency and intensity of defoliation (Evans et al. 1992; Peterson et al. 1994; Brummer and Moore 2000). Alfalfa does not persist well under consistent or frequent grazing pressure, and tends to drop out of grazed pasture systems over time (Katepa-Mupondwa et al. 2002), while white clover does well under grazing, and can become the main legume in heavily grazed pastures (Schwinning and Parsons 1996; Brummer and Moore 2000). The effects of defoliation, herbicide use, and associated soil residues may have additive (positive or negative) effects on legume establishment and persistence following herbicide application. For example, changes in resource availability (light, moisture) could increase legume competitive ability, while the damage done by defoliation may reduce legume viability (Jones and Davies 1988; Seguin 1998; Katepa-Munpondwar et al. 2002, Frame 2005).

Recommended herbicide application rates are chosen to provide effective treatment under a wide range of conditions, but herbicide efficacy is often maintained when application rates are reduced to below recommended rates (Zhang et al. 2000; Blackshaw et al. 2006). Producers may take advantage of this in an attempt to reduce weed control costs. There are significant concerns associated with reduced rate herbicide use, mainly centered around herbicide resistance and increases in the weed seed bank, problems that may take years to manifest, but have the potential to become issues (Doyle and Stypa 2004; Blackshaw et al. 2006). Little information exists on the reestablishment of legumes at below-label herbicide rates, and practical information on legume reestablishment in instances of below-label herbicide use would be a useful tool for producers to mitigate the costs associated with use of herbicides at full rates, as well as facilitate reseeding of legumes removed by herbicide application.

The two main objectives of this study were to 1) determine the herbicide rate at which seedlings of two key forage legumes (alfalfa and white clover) are not affected, and 2) evaluate the additive effect of environmental factors (specifically light and moisture, as regulated by defoliation), on legume emergence, growth and survival within treated areas.

3.2 Materials and Methods

3.2.1 Study Sites

Experiments were initiated May 2010 and 2011 at each of two locations within the Central Parkland natural sub-region. Sites were located at University of Alberta research stations, and treatments were applied to older hayfields that had not experienced any grazing pressure, were uniform in slope, and homogenous in plant composition with a legume component of 10-30% ground cover. One study site was located at the Ellerslie Research Station (53° 25' 6.02" N, 113° 32' 29.79" W), and the other at the St. Albert Research Station (53° 41' 34.31" N, 113° 38' 5.40" W).

The 2011 trials were located in the same fields as the 2010 trials, approximately 100 m apart. Sites differed in plant community composition. The Ellerslie site was dominated by reed canary grass (*Phalaris arundinacea* L.) while the St. Albert site was composed primarily of orchard grass (*Dactylis glomerata* L.). Both sites also contained smooth brome (*Bromus inermis* Leyss), Kentucky bluegrass (*Poa pratensis* L), and quackgrass (*Elytrigia repens* (L.) Beauv.). Precipitation and temperature data for the years of study are provided in Appendix B.1 and B.5. St. Albert experienced higher precipitation that usual in the summer of 2011, and the Ellerslie site experienced more precipitation than usual compared to the 30 year average during both the 2010 and 2011 study years.

3.2.2 Experimental Design and Treatments

Experiments were designed as a strip-split plot randomized complete block design (Appendix A.1). Within each site-year, four replicate blocks were established in May, each containing a mowed main plot (5 x 36 m in size) randomly assigned to half of the block, split by a randomly assigned herbicide sub-plot (3 x 10m). Either AMP or AMCP were applied at six different rates: 0x, 0.0625x, 0.125x, 0.25x, 0.5x, and 1x, randomized to one side of each plot. AMP 1x field rates were 120 g active ingredient per hectare (a.i. ha^{-1}), while AMCP 1x field rates were 60 g a.i. ha^{-1}.

Sites were laid out with a five meter buffer and initially mowed using a gas powered ride-on tractor to approximately 10 cm height to facilitate seeding and spraying, and ensure all vegetation was at the same phenological stage at the start of the trial. Sites were then mechanically raked to remove excess litter, and broadcast seeded using a Valmar seeder with a 50:50 mix of white clover[1] (Common #1) and alfalfa (cv Algonquin) seed[1], at a rate of 16 kg ha^{-1}. A high seeding rate was used to ensure an abundant legume seed bank was present throughout the study areas. Germination tests performed prior to seeding indicated alfalfa and clover germination rates of 87.2% and 91.7%, respectively. Following seeding, sites were raked twice with a mechanical hay rake in perpendicular directions to ensure good seed to soil contact.

Seven to 10 days following seeding, herbicide subplots were sprayed at the appropriate rates using a high clearance self-propelled Spider Trac sprayer[2] on June 8th 2010 and July 4th 2011. Herbicide was applied to plots in a two meter wide strip using Tee Jet® XR110015 nozzles[3] (spaced 50 cm apart on the 2 m boom), delivering 100 L ha^{-1} with CO_2 for each herbicide. A one meter buffer was maintained between herbicide treatments to reduce the influence of neighbouring treatments. Shortly following seeding and spraying, permanent m^{-2} quadrats were established within each plot to monitor legume density. Mowed treatments were mowed every four weeks with a gas powered ride-on tractor to a height of 10cm every 4 weeks to mimic repeated light grazing.

3.2.3 Field Measurements

Legume seedlings were counted within the permanent quadrats twice during the growing season, once in late July and again at the end of August or beginning of September. The number of alfalfa and white clover seedlings within each quadrat were counted up to a maximum of 100 seedlings.

Light measurements µm m^{-2} sec^{-1} were recorded for each quadrat using a 1 m long AccuPAR model LP-80 PAR/LAI Ceptometer light wand[4] just prior to each mowing treatment (i.e. monthly). Soil moisture was also measured monthly for each quadrat using an ML2X-ThetaProbe[5] soil moisture meter. Moisture readings were taken at least 7 days after rain to assess differences between treatments in the absence of recent precipitation.

3.2.4 Soils

Soil samples were collected in May of each year for each study site (2010 and 2011) to characterise growing conditions (Table 3.1). Each site was sampled using a W-shaped pattern as outlined by Thomas (1985), with a minimum of 10 cores taken to 30 cm depth, and then bulked to provide a composite sample. Soil samples were analyzed for soil texture (% sand, silt and clay), soil organic matter (%), soil pH, electrical conductivity (salinity) (µS cm^{-1}), total carbon (%), total nitrogen (%) and available nitrogen (NH_4 + NO_3 mg kg^{-1}), using the methods outlined by the Canadian Society of Soil Science (Carter and Gregorich 2008). Finally, soil pits were dug at each site to describe the soil profile and identify the soil type (Table 3.2; Appendix C).

3.3 Analysis

Legume count data were initially tested for normality prior to analysis using a Kolmogorov-Smirnov test in Proc UNIVARIATE (SAS Institute Inc. 2008) and found to be non-normal (*P*<0.10), and efforts to transform the data were unsuccessful. However, examination of the data indicated the non-normality occurred from an obvious tendency for all rates of herbicide above 0x to all but eliminate legume emergence, resulting in large numbers of zeros for legume emergence. General linear mixed models (i.e. Proc GLIMMIX) in SAS 9.2 were subsequently used to conduct an analysis of variance to assess legume recovery in response to the fixed effects of herbicide type, rate and mowing, with blocks and trial year random. Separate analyses were conducted on Ellerslie and St. Albert data. Subsequent measures in each plot within the same growing season were included as a repeated measure using a simple covariance structure. Although clover and alfalfa counts could not be fit to a distribution recognized by GLIMMIX according to chi-squared goodness-of-fit statistics (*P*<0.05), the closest fitting distribution (normal/Gaussian) was used to run the data. Non-parametric procedures were considered, but the loss of explanatory power was deemed undesirable, and thus, data were run without satisfying all assumptions. For significant main effects and their interactions (*P*<0.05), a Tukey's honest significance difference (HSD) test was performed among treatments to adjust for multiple comparisons among lsmeans (*P*<0.05).

Finally, to assess the relationship between legume establishment and environmental factors (light and moisture), correlations were performed between legume density counts and each environmental variable (*P*<0.05). Prior to analysis all light and moisture data were tested for normality, and found to be non-normal (*P*<0.10). As a normal distribution could not be achieved using transformations (square root, arcsine), a Kendall's Tau rank correlation test was used. Only control (0x) plots were used in the environmental correlation to remove the obvious confounding effects of herbicide, which markedly reduced legume establishment.

3.4 Results

Herbicide type (AMP vs. AMCP) had a significant (*P*<0.05) impact on clover densities at both sites, and a near-significant (*P*=0.057) effect on alfalfa at the Ellerslie site (Tables 3.2, 3.3). Clover densities were lower (*P*<0.005) in plots treated with AMP at both sites (Fig. 3.1). Overall reductions in clover density from AMP relative to AMCP were 37% and 49% at Ellerslie and St. Albert, respectively. Alfalfa density at the Ellerslie site was 15.7 plants m^{-2} (±2.6 SE) in AMCP treatments and 12.3 plants m^{-2} (±2.6 SE) in AMP treatments. A single interaction was observed between herbicide type and application rates (*P*=0.03) at the Ellerslie study site for clover density only. Closer examination revealed that the lone reduction (*P*<0.002) in clover density occurred at the 0x rate, or non-sprayed control, which could not be attributed to the impact of herbicide itself but rather to natural variation in clover establishment. Clover densities at AMCP 0x (control) plots were 49.4 plants m^{-2} (±36.3 SD) while clover densities in AMP control plots were 32.0 plants m^{-2} (±31.5 SD). Notably, this trend of no mean segregation between AMCP and AMP treatments at any rate of herbicide application (excluding the 0x control plots) was observed in clover and alfalfa densities at the St. Albert study site, as well as alfalfa at the Ellerslie study site. This indicates that any differences in herbicide type were attributable to differences between non-sprayed controls as a function of natural variation, and not herbicide type.

At both sites, the density of clover and alfalfa responded to rate of herbicide (Tables 3.2, 3.3). Both legumes declined significantly (P<0.05) in response to increasing herbicide application rates (Table 3.5). Application rates as low as 0.0625x decreased legume density at Ellerslie and St. Albert by 64-68% and 39-54%, respectively. Rates of 0.125x further decreased clover and alfalfa density at both sites relative to the non-sprayed control (Table 3.4). Legume densities for both species in the short-term generally reached a minimum at rates as conservative as 0.25x of recommended levels or greater at both locations (Table 3.4).

At both sites, alfalfa density varied by season of sampling (P≤0.06). At Ellerslie, mean alfalfa density was 11.7 plants m^{-2} (±5.6 SE) in July, increasing to 16.3 (±2.6 SE) by the end of August (P=0.009). At the St. Albert site, a different pattern was evident, with mean alfalfa density decreasing from 10.4 plants m^{-2} (±4.0) in July, to 8.0 (±4.04) by the end of August (P=0.059). Alfalfa density also responded to the rate x season interaction at Ellerslie (Table 3.3). This effect resulted from limited emergence of additional alfalfa plants between summer and fall sampling periods at Ellerslie, but only within plots that received herbicide application. Alfalfa densities at Ellerslie increased by 20.5 plants m^{-2} (from 43.2±3.8 to 63.7±3.8) within non-sprayed plots during this time. In contrast, increases in alfalfa within plots receiving as little as 0.0625x herbicide application rate were limited to 2.5 plants m^{-2}. Thus, the presence of herbicide at Ellerslie greatly impeded alfalfa recruitment during the growing season. Similarly, herbicide rate also interacted with season of sampling (P<0.05) to impact clover density, but only at the St. Albert site. Closer examination of this effect indicated there were no differences between rates of herbicides within each season of sampling, and clover densities generally decreased from 43 plants m^{-2} in spring to 30.7 plants m^{-2} in fall (±7.0), but only in the absence of herbicide, as plots with herbicide contained little to no clover throughout the year (data not shown).

Finally, legume density demonstrated limited responses to mowing, with the only effect seen in alfalfa at St. Albert (P<0.0001) (Table 3.2). Mowed plots contained an average density of 6.7 (±4.0) alfalfa plants m^{-2}, while non-mowed plots had an average density of 12.1 (±4.0) alfalfa plants m^{-2}.

Clover density at both study sites remained positively correlated with soil moisture (P<0.05), particularly at the Ellerslie site (Table 3.5). In contrast, alfalfa density was negatively associated with soil moisture (P<0.05) at both locations. The negative correlation of alfalfa with moisture at Ellerslie was particularly apparent in late summer (August). Light increased by 48% with mowing, from an average of 358.6 µm m^{-2} sec^{-1} (±29.8 SE) in non-mowed plots to 692.3 µm m^{-2} sec^{-1} (±19.9 SE). Legume density however, exhibited no correlation with light availability (µm m^{-2} sec^{-1}) at either site (Table 3.5).

3.5 Discussion

Legumes showed a consistent negative correlation with herbicide rate, where legume density decreased with increasing rates of herbicide application, and rates as low as 6.25% of the recommended label rate markedly reduced legume establishment. While rates of 6.25 and 12.5% generally improved legume recovery compared to higher rates, the weed control efficacy of such low rates is questionable; moreover, producers are unlikely to cut rates by more than 25 or 50%, which still led to poor levels of legume recovery, similar to that of the full 1x rate. These results may negate the option of using these herbicides at reduced rates in an attempt to increase the likelihood of legume reestablishment, at least in the short-term.

Although below-label herbicide use has been shown to provide effective control of weeds (Zhang et al. 2000; Blackshaw et al. 2006), in the case of legume reestablishment during the first growing season this study indicates that reduced herbicide rates will not allow for faster or increased levels of reestablishment. White clover and alfalfa reestablishment has been shown to differ under varied application rates of AMP, with slightly greater reestablishment noted under lower rates of application (74% recovery of alfalfa relative to untreated control with AMP application at 122 g ha^{-1}, vs. 97% at 54 g ha^{-1}) (Renz 2010). The findings of the current study indicate that any weed control by broadleaf herbicides is not conducive to legume reestablishment in the short-term. Herbicide application at any useful rate will result in legume removal, as well as residual control throughout the growing season of application as a side effect of soil residues.

Rate structure was chosen to reflect half-life amounts, and can be used as a proxy for half-lives, eg. 50% application rate is 1 half-life, 25% is 2 half-lives, etc. These results indicate that legume plants start to respond with significant increases in legume density (plants m^{-2}) after 4 half-lives, or at the 6.25% application rate (Table 3.4). Estimated half-lives vary widely for these two bioactives (USEPA 2005; USEPA 2010; Lindenmayer 2012) but a conservative estimate of 50 growing season days is not unreasonable. This would indicate a period of roughly 200 growing season days following application before increases in legume density were noted. Plants responded to herbicide application and residues mainly by way of seedling death, with some symptoms of herbicide use, such as leaf cupping and epinastic twisting, noted on established seedlings.

Although herbicide type had an apparent effect on clover recovery, with greater recovery seen in AMCP treatments, when examined closely these differences were found to be a product of natural variation in clover establishment in control (0x) plots, rather than actual differences in plots receiving herbicide. This indicates that the two herbicides function in a similar fashion, complementing previous research that has found no difference between the half-lives of AMP and AMCP in field trials in Colorado (Lindenmayer 2012). Although no differences were seen between herbicides in the current study, AMCP has been shown to provide more effective residual control of spiny amaranth (*Amaranthus spinosus* L.) than AMP (Edwards 2010) with similar results noted for kudzu (*Pueraria montana var. lobata* (Willd.)) control (Minogue et al. 2011). These studies suggest that AMCP may have longer-lasting residual effects than AMP. This was not the case in the present study however, which might be due to differences in the susceptibility of bioindicator plants. Alternatively, other explanations exist, including that key differences in residual effects may have occurred between bioactives at periods not captured by our sampling times (1 and 2 MAT). Similarly, longer term monitoring would be needed to determine if low herbicide rates (i.e. ≤50%) lead to more rapid legume return in the following growing seasons.

Both herbicides are pyrimidine carboxylic acids, synthetic auxinic herbicides with similar chemistries (Appendix D), are selective for broadleaf plants, and degrade primarily by soil microbial action. They are systemic herbicides, translocated from leaves and roots to meristematic tissues, where they mimic auxin hormones, resulting in leaf cupping, loss of apical dominance, epinastic growth, and unregulated plant growth culminating in plant death (Bussan and Dyer 1999; USEPA 2005). The similarities between bioactives suggest that they would have similar functional responses, with similar withdrawal periods in northern temperate pastures. This hypothesis is further supported by the greenhouse bioassay results in Chapter 5, where herbicide type did not have a significant impact on the recovery of alfalfa or white clover, as determined by density (a measure of legume germination, emergence, and survival), or biomass (a measure of plant vigour and forage productivity). Results of the long-term trials in Chapter 4 also support this finding, as bioactive identity did not have an impact on legume recovery, biomass responses, or weed community dynamics.

Reestablishment over time was also variable between sites, with increases in legume density between summer and fall sampling periods at the Ellerslie study site, and decreases noted at the St. Albert study site. Recruitment at Ellerslie coincided with lower levels of vegetative competition, while decreases in legume at St. Albert between summer and fall were attributed to a highly competitive plant community that shaded out and smothered legume seedlings as the growing season progressed. This trend was noted primarily in control (0x) plots, while sprayed plots had limited emergence of legumes due to residual bioactive presence in the soil. Legume reestablishment in this case seems to be an outcome of vegetative competition, and the less competitive plant community at Ellerslie allowed for greater establishment, a trend noted in other studies (Bowes and Zentner 1992; Seguin 1998; Muto and Martin 2000; Cuomo et al. 2001).

In many studies mowing/defoliation has been found to have a significant effect on the reestablishment dynamics of clover and alfalfa (Evans et al. 1992; Peterson et al. 1994; Brummer and More 2000; Seguin et al. 2001; Frame 2005). The effects of mowing here were limited to one legume species at one site (alfalfa density decreased as a response to mowing at St. Albert), and this lack of response is likely due to the short nature of the study, where monitoring was limited to one growing season. Mowing effects therefore may not have had the time to manifest through complex effects of changes in competition and environment. For example, by reducing moisture use via transpiration, mowing may increase moisture and favor clover increases in the long-term.

The lone response of alfalfa to the mowing treatment at St. Albert is consistent with previous responses of alfalfa under mowing/defoliation regimes. Alfalfa is relatively intolerant to defoliation and has reduced persistence under any type of defoliation (Brummer and Moore 2000; Katepa-Mupondwa et al. 2002; Frame 2005), which explains why alfalfa density decreased as survival was reduced under mowing treatments at the St. Albert study site. The absence of this effect at Ellerslie may be due to the reduced vegetative competition at this site, which may have offset the detrimental impact of mowing itself. Alternatively, as mowing occurred to 10 cm height, slower establishment of alfalfa at Ellerslie would also reduce the physical impact of mowing on slower growing alfalfa seedlings.

The effects of soil moisture on legume recovery dynamics were as anticipated. Clover had a positive association with increased soil moisture, reflecting the moisture-loving nature of this plant (Frame 2005; USDA 2002b). Alfalfa was negatively associated with increases in soil moisture; a reflection of the different moisture management strategy alfalfa has adapted through the development of a deep taproot (Heichel 1982; USDA 2002a; Frame 2005). Grass plants take advantage of abundant surface soil moisture, while alfalfa plants have adapted a taproot system that allows them to access subsurface moisture (Groya and Sheaffer 1981; Heichel 1982; USDA 2002a). In areas with higher surface moisture, grass plants may outcompete alfalfa plants that remain at a competitive disadvantage (Gist and Mott 1956). Surprisingly, light was not a significant factor for both legumes at either site, suggesting light availability was not a constraint for legume reestablishment in this study.

Complex legume recovery dynamics arising between sampling periods may not have been effectively captured with our sampling interval, and results should therefore be considered within the context of both temporal and spatial variability. Although results are likely representative of other northern temperate regions, they are truly only representative of the dynamics noted at study sites, during the years of study. Caution is warranted in extrapolating these results beyond the scope of the study sites. As herbicide degradation and legume establishment are dependent on a myriad of factors, ranging from microsite to climatic variation (temperature and precipitation), factors which change across time and space, the effects of these sources of variance may never be truly ascertained. For example, our study sites were unusual in that they were relatively high in soil organic matter with moderately alkaline pH values in comparison to other soils in the region.

3.6 Conclusions and Management Recommendations

Knowledge on legume reestablishment dynamics specific to the northern temperate pastures of Alberta could help producers to effectively manage both weed and legume communities in their pasture systems. Incorporating the effects of below-label herbicide usage increases the applicability of the study for producers who may wish to take advantage of reduced herbicide application rates. Seedlings of both species, alfalfa and white clover, were sensitive to even the lowest herbicide rates. Consequently, it appears that below label herbicide rates are likely to lead to small increases in legume reestablishment, though not to levels approaching the untreated check plots, and only under major reductions in herbicide rates (6.25 and 12.5% of label recommendations). In contrast, herbicides applied at levels likely to be considered in agricultural systems (25, 50, 100%) led to no increase in legume reestablishment, at least in the first year. For successful legume reestablishment, herbicide residue levels would need to degrade after spraying to levels beyond those noted in this study.

From these results, it also was apparent that there was no functional difference between herbicide type, AMP vs. AMCP, on legume reestablishment, legume dynamics, and withdrawal periods during the year of application.

This research gives valuable insight into the dynamics of legume communities in response to variable rate broad leaf herbicide application rates. In any case, legume reestablishment is sharply reduced over one growing season in northern temperate pastures, even when herbicide is applied at rates as low as 6.25% of recommendations. Use of herbicides at below-label rates may be economically beneficial for the producer, but marked differences in withdrawal periods were not noted over in the growing season immediately after spraying.

3.7 Sources of Materials

[1]Seed from Viterra, Fort Saskatchewan, Alberta, Canada.

www.viterra.ca

[2]Spider Trac Sprayer, West Texas Lee, Co., Idalou, TX USA,

www.westtexaslee.com

[3]Tee Jet® XR 110015 flat fan nozzles, Spraying Systems Co., Wheaton, IL, USA.

www.teejet.com/english/home.aspx

[4] AccuPAR model LP-80 PAR/LAI Ceptometer light wand. Decagon Devices Inc., Pullman, Washington, USA.

http://www.decagon.com/

[5] ML2X-ThetaProbe soil moisture sensor. DeltaT Devices, Cambridge, United Kingdom.

http://www.delta-t.co.uk/

3.8 Literature Cited

Aichele, T. M. and Penner, D. 2005. Adsorption, desorption, and degradation of imidazolinones in soil. Weed Technol. **19**(1): 154-159.

Blackshaw, R. E., O'Donovan, J. T., Harker, K. N., Clayton, G. W., Stougaard, R. N. 2006. Reduced herbicide doses in field crops: A review. Weed Biol. and Manage. **4**: 10-17.

Bork, E. W., Grekul, C. W., DeBrujin, S. L. 2007. Extended pasture forage sward responses to canada thistle (C*irsium arvense*) control using herbicides and fertilization. Crop Prot. **26**(10): 1546-1555.

Bowes, G. G. and Zentner, R. P. 1992. Effect of vegetation suppression on the establishment of sod-seeded alfalfa in the aspen parkland. Can. J. Plant Sci. **72**(4): 1349-1358.

Brummer, E. C. and Moore, K. J. 2000. Persistence of perennial cool-season grass and legume cultivars under continuous grazing by beef cattle. Agron. J. **92**: 466-471.

Bussan, A. J. and Dyer, W. E. 1999.
Herbicides and rangeland. Pages 116-132 *in:* Sheley, R. L. and Petroff, J. K., eds. Biology and management of noxious rangeland weeds. Oregon State University Press., Corvallis, OR.

Carter, M.R., Gregorich, E.G. 2008. Soil sampling and methods of analysis. Second edition Canadian Society of Soil Science. CRC Press. Taylor & Francis Group, Boca Raton, FL.

Cuomo, G. J., Johnson, D. G., Head Jr., W. A. 2001. Interseeding kura clover and birdsfoot trefoil into existing cool-season grass pastures. Agron. J. **93**: 458-462.

Dow AgroSciences. 2012. Milestone specialty herbicide. Specimen Label : 1-9.

Dow AgroSciences. 2005. Aminopyralid technical bulletin. An introduction to aminopyralid. V45-313-001 (01/05) BOD. 010-50433. 19 pp.

Doyle, P. and Stypa, M. 2004. Reduced herbicide rates - a Canadian perspective. Weed Technol. **18**(4): 1157-1165.

Edwards, M. E. 2010. Spiny amaranth control and aminopyralid persistence in Kentucky pastures. M.Sc. Thesis, University of Kentucky, Lexington, Kentucky, United States of America. 85 pp.

Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., Moechnig, M., Peterson, V., Masters, R. A., Halstvedt, M. 2007. Canada thistle (C*irsium arvense*) control with aminopyralid in range, pasture, and noncrop areas. Weed Technol. **21**: 890-894.

Evans, D. R., Williams, T. A., Evans, S. A. 1992. Evaluation of white clover varieties under grazing and their role in farm systems. Grass Forage Sci. **47**: 342-352.

Frame, J. 2005. Forage legumes for temperate grasslands. Science Publishers Inc., Enfield, NH, United States of America. 309 pp.

Gist, G. R. and Mott, G. O. 1956. Some effects of light intensity, temperature, and soil moisture on the growth of alfalfa, red clover and birdsfoot trefoil seedlings. Agron. J. **49(1)**: 33-36.

Goetz, A. J., Lavy, T. L., Gbur, J.,E.E. 1990. Degradation and field persistence of imazethapyr. Weed Sci. **38**(4/5): 421-428.

Grekul, C. W. and Bork, E. W. 2004. Herbage yield losses in perennial pasture due to canada thistle (*cirsium arvense*). Weed Technol. **18**(3): 784-794.

Grekul, C. W., Cole, D. E., Bork, E. W. 2005. Canda thistle (C*irsium arvense*) and pasture forage responses to wiping with various herbicides. Weed Technol. **19**: 298-306.

Groya, F. L. and Sheaffer, C. C. 1981. Establishment of sod-seeded alfalfa at various levels of soil moisture and grass competition. Agron. J. **73**: 560-565.

Heichel, G. H. 1982. Alfalfa. crop-water relations. Teare, I. D. and Peet, M. M., eds. Wiley, New York, NY, United States of America. 127-155 pp.

Jones, D.R., and Davies, A. 1988. The effects of simulated continuous grazing on development and senescence of white clover. Grass Forage Sci. **43**: 421-425.

Katepa-Mupondwa, F., Singh, A., Smith Jr., S. R., McCaughey, W. P. 2002. Grazing tolerance of alfalfa (M*edicago* spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (B*romus riparious* Rehm. syn. *B. biebersteinii* Roem & Schult). Can. J. Plant Sci. **82**: 337-347.

Kunelius, H. T. and Campbell, A. J. 1984. Performance of sod-seeded temperate legumes in grass dominant swards. Can. J. Plant Sci. **64**: 643-650.

Kunelius, H. T., Campbell, A. J., McRae, K. B., Ivany, J. A. 1982. Effects of vegetation suppression and drilling techniques on the establishment and growth of sod-seeded alfalfa and bird's-foot trefoil in grass dominant swards. Can. J. Plant Sci. **62**: 667-675.

Lindenmayer, R. B. 2012. Understanding aminocyclopyrachlor behavior in soil and plants. Ph.D. Thesis, Colorado State University, Fort Collins, CO. 82 pp.

Loux, M. M. and Reese, K. D. 1992. Effect of soil pH on adsorption and persistence of imazaquin. Weed Sci. **40**(3): 490-496.

Merou, T. P. and Papanastasis, V. P. 2009. Factors affecting the establishment and growth of annual legumes in semi-arid mediterranean grasslands. Plant Ecol. **201**: 491-500.

Mikkelson, J. R. and Lym, R. G. 2011. Aminopyralid soil residues affect crop rotation in north dakota soils. Weed Technol. **25**(3): 422-429.

Minogue, P. J., Enloe, S. F., Osiecka, A., Lauer, D. K. 2011. Comparison of aminocyclopyrachlor to common herbicides for kudzu (P*ueraria montana*) management. Invas. Pl. Sci. and Manage. **4**: 419-426.

Muto, P. J. and Martin, R. C. 2000. Effects of pre-treatment, renovation procedure and cultivar on the growth of white clover sown into a permanent pasture under both grazing and mowing regimes. Grass Forage Sci. **55**: 59-68.

Natural Regions Committee. 2006. Natural regions and sub-regions of Alberta. Government of Alberta, 264 pp.

Olsen, F. J., Jones, J. H., Patterson, J. J. 1981. Sod-seeding forage legumes in a tall fescue sward. Agron. J. **73**: 1032-1036.

Ou, L. 1984. 2,4-D degradation and 2,4-D degrading microorganisms in soils. Soil Sci. **137**(2): 100-107.

Parker, L. W. and Doxtader, K. G. 1983. Kinetics of the microbial degradation of 2,4-D in soil: Effects of temperature and moisture. J. Environ. Qual. **12**(4): 533-558.

Peterson, R. P., Sheaffer, C. C., Jordan, R. M., Christians, C. J. 1994. Responses of kura clover to sheep grazing and clipping: I. yield and forage quality. Agron. J. **86**: 655-660.

Picton, P. and Farenhorst, A. 2004. Factors influencing 2,4-D sorption and mineralization in soil. J. Environ. Sci. & Health **39**(3): 367-379.

Popp, J. D., McCaughey, W. P., Cohen, R. D. H., McAllister, T. A., Majak, W. 2000. Enhancing pasture productivity with alfalfa: A review. Can. J. Plant Sci. **80**(3): 513-519.

Province of Alberta. 2008. Weed control act. Alberta Queen's Printer: W-5.1.

Renz, M. J. 2010. Establishment of forage grasses and legumes after fall herbicide applications. Forage and Grazinglands Available: doi:10.1094/FG-2010-0806-01-RS [March 10, 2013].

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., Goslee, S. C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J. **97**: 1465-1471.

SAS Institute Inc. 2008. SAS/STAT® 9.2 user’s guide. SAS Institute Inc., Cary, NC.

Schwinning, S. and Parsons, A. J. 1996. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. J. Ecol. **84**(6): 799-813.

Seguin, P. 1998. Review of factors determining legumes in sod-seeding outcome during pasture renovation in north america. Biotechnol. Agron. Soc. Environ. **2**(2): 120-127.

Seguin, P., Peterson, P. R., Sheaffer, C. C., Smith, D. L. 2001. Physical sod suppression as an alternative to herbicide use in pasture renovation with clovers. Can. J. Plant Sci. **81**: 255-263.

Sleugh, B., Moore, K. J., George, R., Brummer, E. C. 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agron. J. **92**: 24-29.

Soil Classification Working Group. 1998. The Canadian system of soil classification. Haynes, R. H., ed. 3rd ed. Agriculture and Agri-Food Canada, NRC Research Press, Ottawa, ONT, Canada. 187 pp.

Thomas, A. G. 1985. Weed survey system used in Saskatchewan for cereal and oilseed crops. Weed Sci. **33**(1): 34-43.

USDA. 2002a. Alfalfa (M*edicago sativa* L.) plant fact sheet. United States Department of Agriculture Natural Resources Conservation Service.

USDA. 2002b. White clover (T*rifolium repens* L.) plant fact sheet. United States Department of Agriculture Natural Resources Conservation Service.

USEPA. 2005. Environmental fate and ecological risk assessment for the registration of aminopyralid. Office of Prevention, Pesticides, and Toxic Substances.

USEPA. 2010. Registration of the new active ingredient aminocyclopyrachlor for use on non-crop areas, sod farms, turf, and residential lawns. U. S. Environmental Protection Agency Office of Pesticide Programs, Registration Division.

Veeh, R. H., Inskeep, W. P., Camper, A. K. 1996. Soil depth and temperature effects on microbial degradation of 2,4-D. J. Environ. Qual. **25**: 5-12.

Vogel, K. P., Kehr, W. R., Anderson, B. E. 1983. Sod-seeding alfalfa into cool-season grasses and grass-alfalfa mixtures using glyphosate or paraquat. J. Range Manage. **36**(6): 700-702.

Vough, L. R. and Marten, G. C. 1971. Influence of soil moisture and ambient temperature on yield and quality of alfalfa forage. Agron. J. **63**: 40-42.

Walker, A. and Zimdahl, R. L. 1981. Simulation of the persistence of atrazine, linuron and metolachlor in soil at different sites in the USA. Weed Res. **21**: 255-265.

Zhang, J., Weaver, S. E., Hamill, A. S. 2000. Risks and reliability of using herbicides at below-labeled rates. Weed Technol. **14**(1): 106-115.

Table 3.1 Physical site (i.e., soil) characteristics of short-term dose trials, as sampled in May 2010 and 2011.[z] All sites are level, with negligible slope.

Site	Lat.-Long.[y]	Texture/Soil Type	N (%)	Available N ($N0_3$+NH_4) (mg kg^{-1})	C (%)	OM (%)	pH	EC (µS cm^{-1})
St. Albert (2010)	53° 41' 34.31" N 113° 38' 5.40" W	Silty Clay, Eluviated Black Chernozem	0.5	7.8	5.4	16.1	8.4	532.5
St. Albert (2011)	53° 41' 34.31" N 113° 38' 5.40" W	Silty Clay, Eluviated Black Chernozem	0.4	7.0	4.5	16.4	8.4	467.0
Ellerslie (2010)	53° 25' 6.02" N 113° 32' 29.79" W	Loam, Eluviated Black Chernozem	1.1	18.6	13.4	39.9	8.0	1757.5
Ellerslie (2011)	53° 25' 6.02" N 113° 32' 29.79" W	Loam, Eluviated Black Chernozem	0.8	24.7	11.0	29.9	8.1	3185.0

[z]Values represent the average of 10 soil cores collected at 0-30cm depth.
[y]Lat.-Long. Represent exact coordinates of site.

Table 3.2 Summary of F-statistic and significance values associated with clover and alfalfa density at the St. Albert study site. Data represent two duplicate trials in consecutive years.

	Clover Density		Alfalfa Density	
Treatment Effect	F-stat	P-value	F-stat	P-value
Mow	0.09	0.76	26.2	**<0.0001**
Herb	15.8	**<0.0001**	3.1	0.08
Rate	24.4	**<0.0001**	42.6	**<0.0001**
Season	0.03	0.87	3.6	0.059
Herb*Rate	1.9	0.096	0.2	0.97
Herb*Mow	0.0	0.99	2.5	0.12
Herb*Season	0.8	0.38	0.04	0.85
Rate*Mow	0.4	0.84	1.9	0.10
Rate*Season	2.4	**0.04**	0.2	0.98
Mow*Season	2.3	0.13	2.7	0.10
Herb*Rate*Mow	0.2	0.96	0.3	0.93
Herb*Rate*Season	0.3	0.91	1.4	0.23
Herb*Mow*Season	0.6	0.44	1.4	0.24
Rate*Mow*Season	0.7	0.71	0.7	0.63
Herb*Mow*Rate*Season	0.4	0.83	0.7	0.66

Table 3.3 Summary of F-statistic and significance values associated with clover and alfalfa density responses at the Ellerslie study site. Data represent two duplicate trials in consecutive years.

	Clover Density		Alfalfa Density	
Treatment Effect	F-stat	P-value	F-stat	P-value
Mow	0.02	0.87	0.0	0.96
Herb	7.8	**0.0056**	3.6	0.057
Rate	59.7	**<0.0001**	91.8	**<0.0001**
Season	2.6	0.11	6.9	**0.009**
Herb*Rate	2.4	**0.034**	0.3	0.92
Herb*Mow	0.02	0.89	2.1	0.14
Herb*Season	0.05	0.82	1.6	0.21
Rate*Mow	0.3	0.92	0.06	0.10
Rate*Season	1.1	0.36	3.4	**0.005**
Mow*Season	0.6	0.42	0.04	0.84
Herb*Rate*Mow	0.5	0.79	1.3	0.29
Herb*Rate*Season	0.08	1.0	0.5	0.78
Herb*Mow*Season	0.03	0.87	1.2	0.28
Rate*Mow*Season	0.3	0.94	0.1	0.99
Herb*Mow*Rate*Season	0.4	0.84	1.1	0.39

Table 3.4 Mean densities of legume seedlings (plants m^{-2}) in response to varied rate applications at the Ellerslie and St. Albert study sites. Results are combined across the AMCP and AMP treatments and over years (2010 and 2011).

		Herbicide Rate						SE
Site	Species	0x	0.0625x	0.125x	0.25x	0.5x	1x	
Ellerslie	Clover	40.7*a*[z]	14.8*b*	5.0*c*	1.2*c*	0.1*c*	0.1*c*	5.8
	Alfalfa	53.4*a*	18.0*b*	9.6b*c*	2.8*cd*	0.0*d*	0.0*d*	3.1
St. Albert	Clover	36.8*a*	22.4*b*	17.3*b*	6.4*c*	2.1*c*	0.8*c*	6.4
	Alfalfa	24.5*a*	11.2*b*	10.2*bc*	5.3*cd*	3.5*d*	1.6*d*	4.2

[z]Within a row, means with different letters differ based on a Tukey HSD test ($P \leq 0.05$).

Table 3.5 Correlation between environmental factors (% soil moisture, µm m^{-2} sec^{-1}) in each sampling period on clover and alfalfa density within control (0x) plots. Correlation coefficient (Kendall’s Tau-b statistic) indicated in table.

	Ellerslie Site		St. Albert Site	
Environmental Factor	Clover	Alfalfa	Clover	Alfalfa
June Moisture	+0.19*	-0.12	+0.12	-0.16
August Moisture	+0.18*	-0.22**	+0.15	-0.18*
Average Moisture	+0.19*	-0.17	+0.18*	-0.16
Light	+0.15	-0.08	-0.05	-0.05

*P<0.05, **P<0.01

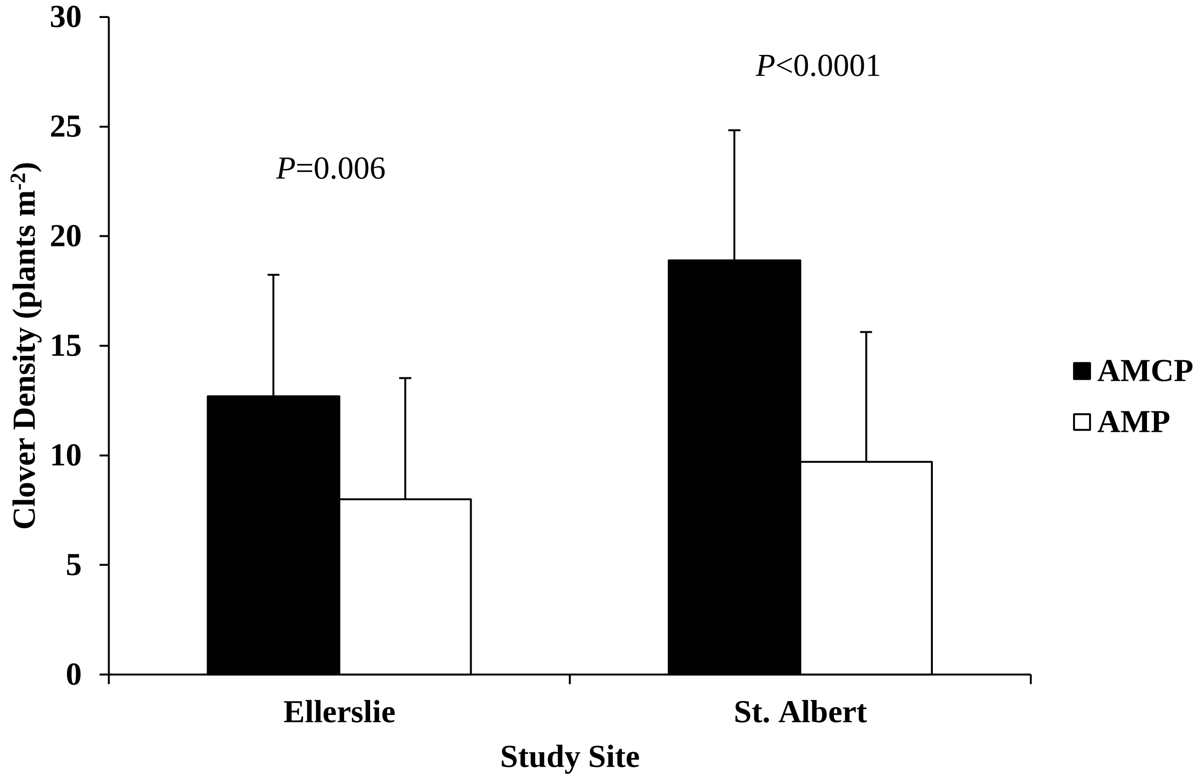

Figure 3.1 Establishment of clover at each site under different bioactive identities, including associated p-values. Means within a site differ based on Tukey's HSD test ($P \leq 0.05$).

4. LONG-TERM LEGUME RECOVERY DYNAMICS IN PASTURES SPRAYED WITH RESIDUAL BROADLEAF HERBICIDE

4.1 Introduction

The incorporation and maintenance of a legume component in pastures is a goal for many producers who strive to maximize forage production. Legumes such as alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.) may increase the productivity of pastures as well as the quality and palatability of forage (Groya and Sheaffer 1981; Kunelius et al. 1982; Seguin et al. 2001). Many western North American pastures are composed of a mixture of cool season grasses and legumes, a combination that imparts several ecological and economic benefits (Vogel et al. 1983).

Legumes are well known for their nitrogen fixing properties, and the ability to convert atmospheric nitrogen into a biologically available form (ammonia, NH_3) is the most positive attribute they impart to plant communities (Kunelius et al. 1982; Seguin 2001). The transfer of biologically fixed nitrogen to associated grasses increases the overall production of pasture and range systems, negating or alleviating the need for nitrogen fertilizers, which are otherwise necessary in cool season swards to maximize productivity (Olsen et al. 1981; Vogel et al. 1983; Popp et al. 2000). Legumes therefore have the ability to reduce the cost and labour associated with forage production. Legume plants themselves also have greater crude protein content than grasses, are palatable to livestock, and can increase forage intake and improve animal performance (Groya and Sheaffer 1981; Kunelius et al. 1982; Kunelius and Campbell 1984; Merou and Papanastasis 2009).

Pastures containing legumes have stabilized forage production between years, as well as more consistent seasonal forage production, resulting from differences in the timing of yield contributions between grass and legume species over the growing season (Groya and Sheaffer 1981; Katepa-Mupondwa et al. 2002; Seguin 1998; Sleugh et al. 2000). Additional benefits of grass-legume communities include reduced susceptibility to erosion, greater community resiliency after disturbance, a reduced potential for invasion from weeds, and greater stand longevity when compared to either grass or legume monocultures (Sleugh et al. 2000; Sanderson et al. 2005). Despite these benefits, the maintenance of legumes in pasture and range systems can be problematic in that they are susceptible to decline, including removal during herbicide application (Grekul and Bork 2004; Enloe et al. 2007).

In the province of Alberta, weed control is a legal obligation: producers must control noxious weeds, destroy prohibited noxious weeds, and take measures to prevent their spread (Province of Alberta 2008). These weeds are often broadleaf species (*Cirsium arvense* L., *Ranunculus acris* L., *Sonchus arvensis* L., etc.) that can be effectively reduced in pastures through the application of broadleaf herbicides (Grekul et al. 2005); in turn leading to increased forage production (Bork et al. 2007). The downside of herbicide use is that broadleaf legumes such as alfalfa and white clover are typically reduced or removed from systems with this type of herbicide application (Grekul and Bork 2004; Enloe et al. 2007). This places producers in a difficult position, as they are legally mandated to control weed species, but in doing so risk reducing pasture productivity and quality for an indeterminate time through legume loss.

The reestablishment of legumes following herbicide application is an important goal of many producers. Successful legume establishment depends on favourable conditions for germination, emergence, and growth, including the absence of deleterious substances for legume seedling growth. This includes the degradation of residual herbicide bioactives within the soil (Renz 2010). The ‘legume withdrawal period’, defined as the time period between herbicide application and successful legume reestablishment, is likely to be associated with herbicide degradation rates, which vary widely with the residual properties of herbicides and environmental conditions. Herbicide decay rates tend to increase with increases in organic matter content and associated microbial activity (Ou 1984; Veeh et al. 1996; Picton and Farenhorst 2004), high soil temperatures which assist in biochemical breakdown (Walker and Zimdahl 1981; Ou 1984; Goetz et al. 1990; Veeh et al. 1996;), higher soil moisture levels (Walker and Zimdahl 1981; Parker and Doxtader 1983; Ou 1984; Goetz et al. 1990), as well as higher soil pH levels (Loux and Reese 1992; Aichele and Penner 2005). In addition to herbicide degradation, environmental factors and vegetative competition both play key roles in legume reestablishment, with varied success over different climatic regimes (Gist and Mott 1956; Mikkelson and Lym 2011; Vough and Marten 1971).

Legume reseeding recommendations are limited for aminopyralid (AMP) and aminocyclopyrachlor (AMCP). Either a bioassay prior to seeding is recommended (AMP) or data are unavailable (AMCP) (USEPA 2010; Dow AgrSciences 2012). Soil dissipation studies for AMP prior to product registration were limited to two studies, each located in the southern United States (USEPA 2005). The estimated terrestrial half-life of 34.5 days was at odds with the highly variable laboratory reports of half-lives varying between 31.5 to 533.2 days. AMCP half-life was indicated between 22 and 126 days in terrestrial field dissipation studies (USEPA 2010). AMP and AMCP half-lives of 28.9 and 32.5 days, respectively, have been reported for field trials in Colorado (Lindenmayer 2012). It is reasonable to assume that herbicide degradation will slow in areas with colder average temperatures and shorter growing seasons, extending half-lives and increasing the legume withdrawal period, but data are not available for western Canada.

The Central Parkland natural subregion of Alberta is highly productive, and has been subject to relatively high levels of agricultural development compared to other regions of Alberta (Natural Regions Committee 2006). The Central Parkland has several characteristics that make it favourable for agriculture. The area is comprised primarily of fertile Black Chernozemic soils with high organic matter content, with a favourable precipitation regime (350-450 mm/year, falling primarily during the growing season), factors which work in combination to create an agro-ecosystem well suited for forage production (Soil Classification Working Group 1998; Natural Regions Committee 2006). However, these conditions also correspond with a short growing season and relatively low temperatures (Natural Regions Committee 2006). To help maximize forage availability during this condensed growing season, the incorporation of cool-season legumes is desirable, and rapid reintroduction of legumes as soon as possible following herbicide application is a major goal. To maximize economic benefits to producers, an understanding of the specific herbicide degradation rates for this agro-climatic region is necessary, including identification of the minimum safe interval between herbicide application and legume reintroduction (legume 'withdrawal period'). Knowledge of the long-term sward dynamics of pastures following herbicide application and legume seeding will aid producers in their decisions regarding weed control and legume maintenance, and help gain a better understanding of the legume losses associated with the use of broadleaf herbicides in pastures.

Our goal was to evaluate the individual and combined effects of various mechanisms regulating long-term legume recovery in northern temperate pastures following broadleaf herbicide application. The three main objectives of this study are to, 1) indirectly quantify the degradation of two herbicide bioactives (aminopyralid (AMP) and aminocyclopyrachlor (AMCP)) applied at recommended field rates by assessing their effect on the ongoing emergence and survival of two key forage legumes (alfalfa and white clover) at varied intervals following herbicide application, 2) evaluate the additive effect of environmental factors (specifically light and moisture, as regulated by defoliation), on legume emergence, growth and survival, within herbicide treated areas, and 3) document long-term pasture community dynamics, including weed composition, following spraying, and link those responses to herbicide application, secondary disturbance (mowing) and environmental factors.

4.2 Materials and Methods

4.2.1 Study Sites

Field experiments were conducted from 2010 to 2012 in five separate fields located in the Central Parkland natural sub-region. All study sites were hayfields of various ages which did not experience any grazing pressure, were uniform in slope, homogenous in plant composition, and had an initial legume component of 10-30% cover. Hayfields rather than pastures were used to avoid the confounding effects of ongoing livestock grazing.

Three study sites were established in May 2010, with an additional two sites established in June of 2011. The original three study sites were located near Stony Plain (53° 27' 17.18" N, 114° 8' 12.42" W), Fort Saskatchewan (53° 47' 18.94" N, 113° 20' 38.73" W) and at the University of Alberta St. Albert research station (53° 41' 34.31" N, 113° 38' 5.40" W), while the two additional study sites were located near the towns of Lamont (53° 44' 29.84" N, 112° 31' 43.28" W) and Millet (Glenpark site) (53° 10' 38.58" N, 113° 24' 42.64" W). All sites differed slightly in plant composition, but were dominated by some combination of orchard grass (*Dactylis glomerata* L.), smooth brome (*Bromus inermis* Leyss), Kentucky bluegrass (*Poa pratensis* L), quackgrass (*Elytrigia repens* (L.) Beauv.), timothy (*Phleum pratense L.*), and intermediate wheatgrass (*Thinopyrum intermedium* Host) in varying quantities.

Precipitation and temperature data were compiled for all study sites for the years of study (Appendix B). Above average precipitation occurred for all sites in the growing season of 2011, with some variation in precipitation in other years. Temperatures closely followed the long term norms for all sites.

4.2.2 Experimental Design and Treatments

All five sites consisted of a strip-split-split plot randomized complete block design, with four replicate blocks per site (Appendix A.2). Mowed main plots (6x12 m) were randomly assigned to one half of each block, and then split by herbicide subplots (3x12 m). Herbicide bioactives (AMP and AMCP) were randomly assigned to herbicide subplots, and applied at full field rates (1x treatment: 120g a.i. ha^{-1} of AMP, and 60g a.i. ha^{-1} of AMCP) or maintained as an untreated control (0x treatment). Within each mowing/herbicide subplot three seeding sub-subplots were established (2 x 3 m). Seeding treatments included alfalfa and clover overseeding, as well as natural recovery where no seed was applied.

4.2.3 Site Preparation

Sites were laid out with a two meter buffer and initially mowed to a height of approximately 10 cm during spring to facilitate seeding and spraying, as well as reduce all vegetation to an equal initial height. Sites were then raked either by hand or mechanically to remove litter. Seeded sub-subplots were then seeded by hand at a rate of 16 kg ha^{-1} with either white dutch clover[1] (Common #1) or alfalfa[1] (cv. Algonquin, Certified #1). Plots were promptly hand raked to ensure good seed to soil contact. Germination tests indicated overall germination rates of 87.2% for alfalfa, and 91.7% for clover.

Seven to ten days after seeding, herbicide treatments were applied using a two meter handboom, equipped with Air Bubble Jet 110010 nozzles[2], mounted on a CO_2 backpack sprayer delivering 100 L of herbicide solution ha^{-1}, applied at a height of 50 cm above the plant canopy. Spraying occurred June 21st 2010 for St. Albert, Stony Plain, and Fort Saskatchewan sites, June 22nd 2011 for Glenpark, and July 2nd 2011 for Lamont. Spraying treatments were randomized across repetitions, and a 50 cm buffer maintained between sprayed and control plots. After spraying plots were monitored for the remainder of the field season. Permanently marked m^{-2} quadrats were established in each plot following seeding and spraying, to monitor over time. Mowing main plots were mowed every 4 weeks using a string trimmer[3] to mimic repeated light grazing. Litter was raked and removed from mowed plots to more closely mimic grazing and avoid the potential for seedlings to be smothered by litter.

4.2.4 Field Measurements

Seedling counts were done in permanent plots twice each growing season, once in late June and again at the end of August or beginning of September. Alfalfa and white clover seedlings within permanent plots were tallied separately up to a maximum of 100 seedlings per species per quadrat.

Biomass was harvested from a randomly located 50x50 cm area of the plot (located outside of the permanent quadrat) during peak growth, typically between mid-July to mid-August of each year. Vegetation was clipped to a height of 2 cm, sorted to grass, alfalfa, white clover, and forbs, then dried to a constant mass and weighed.

To assess changes in weed composition within each plot, the percent canopy cover of dandelion (*Taraxacum officinale* Web.), the most prevalent weed at each site, was recorded prior to herbicide application, and again each following growing season, recorded to the nearest 1%.

Light measurements ($\mu m\ m^{-2}\ sec^{-1}$) were recorded for each plot using a one meter AccuPAR model LP-80 PAR/LAI Ceptometer light wand[4] prior to mowing, and again immediately following mowing. This was done on a monthly basis just prior to maintenance mowing. Soil moisture was also recorded on a monthly basis for each plot in each study area using an ML2X-DeltaT moisture probe[5]. Moisture readings were taken a minimum of 7 days after rain to get a representative reading of average soil moisture.

Soil samples were collected for each site in the year of establishment to provide baseline information on physical site characteristics. Soil samples were taken at depths of 0-15, and 15-30 cm from 10 points evenly spaced along a W pattern as outlined by Thomas (1985) across each site. Prior to analysis, soil samples were pooled and combined for analysis. Soil samples were analyzed for soil texture (% sand, silt and clay), organic matter (%), pH, electrical conductivity (salinity) ($\mu S\ cm^{-1}$), total carbon (%), total nitrogen (%), and available nitrogen ($NH_4 + NO_3$ mg kg^{-1}), using the methods outlined by the Canadian Society of Soil Science (Carter and Gregorich 2008). Finally, soil pits were dug at each site to describe the soil profile and identify soil type (Table 4.1; Appendix C).

4.3 Analysis

The relationship between herbicide application and legume recovery, as represented by legume density and biomass, was explored using a strip-split-split plot analysis of variance. A repeated measures design was incorporated to account for variance and covariance associated with multiple measurements taken on the same experimental unit (i.e. plot) over time. Density and biomass were both assessed using the general linear mixed models (GLIMMIX) procedure of SAS 9.2 to evaluate significance of main effects and interactions ($P<0.05$). For all significant effects, a Tukey's HSD (honest significance difference) test was used to compare means and minimize the risk of a type one error.

Prior to analysis legume densities (clover and alfalfa) were tested for normality using the Kolmogorov-Smirnov statistic (SAS Institute Inc. 2008) and found to be non-normal ($P<0.10$). Although efforts to transform the data were unsuccessful, the GLIMMIX procedure is relatively robust to non-normality (SAS Institute Inc. 2008). Attempts to fit density data to one of the specified distributions in GLIMMIX were also unsuccessful (Chi squared tests $P<0.05$), and consequently, the closest fitting distribution, (normal/Gaussian), was used for the density analysis. While non-parametric procedures were considered, the loss of explanatory power associated with these techniques was deemed undesirable (Goonewardene 2012, personal communication). Clover densities were assessed only in clover seeded treatments, where responses were likely to be observed when compared to other seeding treatments. The same process was followed for biomass in all plots, with similar results. Data were analyzed with year as a random factor, and site maintained as a random factor due to limited differences between sites, and a desire for broadly applicable results.

To evaluate the relationship between legume recovery and environmental factors (light and moisture), correlations were performed to identify significant associations, their magnitude, and directionality. Correlations were performed for clover and alfalfa density, as well as forb, grass, legume, and total forage biomass, against environmental variables. Prior to correlation, both vegetation and environmental data were tested for normality, and found to be non-normal ($P<0.10$), with transformations unable to attain normality. Thus, a Kendall's Tau rank correlation was used to measure the association between variables. Environmental correlations were limited to the control (0x) non-seeded treatments to remove the obvious confounding effects of herbicide and seeding treatments.

To investigate changes in sward composition and herbicide efficacy over time, percent cover of the most ubiquitous weed, dandelion, was assessed. Significant main effects and interactions were analyzed using a mixed model analysis of variance with a repeated measures design. Only non-seeded treatments were used to prevent confounding effects of seeding. Cover was initially tested for normality using the Kolmogorov-Smirnov statistic, and found to be non-normal (P<0.10). Data were transformed using the arcsine transformation, and although transformed data did not satisfy normality (P<0.10), it did satisfy the assumption of homogeneity of variance based on a Levene's test (P>0.05). Transformed data were subsequently run using the MIXED procedure in SAS 9.2, and a Tukey's HSD test used to minimize the risk of a type one error in post-hoc mean comparisons.

4.4 Results

4.4.1 Legume Density

4.4.1.1 ALFALFA. Alfalfa density was influenced by all of the main experimental treatments tested, excluding mowing, with numerous 2-way interactions primarily involving rate and seeding, as well as a 3-way interaction of rate, seeding and time (Table 4.2). Averaged across all other factors (time, mowing, herbicide type, and seeding) herbicide application (1x) reduced alfalfa density (P<0.0001) from 17.3 (±2.2) plants m^{-2} to 1.0 (±2.2) plants m^{-2}. The impact of herbicides was most apparent 1 MAT (0x=25.5 (±2.4) plants m^{-2}; 1x=1.7 (±2.4) plants m^{-2}) and 2 MAT (0x=21.7 (±2.4) plants m^{-2}; 1x=1.5 (±2.4) plants m^{-2}).

When averaged over other factors (time, mowing, herbicide type and rate) seeding had a significant effect on average alfalfa density (P<0.0001) with 13.5 (±2.2) plants m^{-2} in alfalfa seeded treatments, compared to 6.6 (±2.2) plants m^{-2} in clover seeded plots and 7.4 (±2.2) plants m^{-2} in non-seeded plots. Seeding of legumes interacted with herbicide rate and time, along with a significant interaction with all three effects (*P*=0.0001). In the absence of herbicide application, seeding of legumes had a significant impact on alfalfa density. Alfalfa seeded 0x treatments had more alfalfa plants m^{-2} than clover and non-seeded treatments 1 and 2 MAT, but alfalfa density in alfalfa seeded plots had decreased and converged with clover seeded and non-seeded treatments by 12 MAT (Table 4.4). In contrast, 1x treatments showed no separation between seeding treatments or across sampling times, largely due to a very low number of alfalfa plants (≤3.2 plants m^{-2}). Not surprisingly, marked reductions in alfalfa density (P<0.05) were found in most 1x herbicide plots (excluding clover seeded and non-seeded areas 24 and 26 MAT) relative to the 0x treatments in all seeding treatments, with decreases ranging from 67 to 99%.

Averaged over other treatments, AMCP treatment had an average of 9.9 (±2.2) plants m^{-2}, which was greater (P=0.03) than plots treated with AMP (8.4±2.2 plants m^{-2}) Interactions of herbicide type with rate (P<0.01) revealed that alfalfa density was similar between AMCP and AMP plots at the 1x rate (P>0.05), with the only difference found between 0x treatments, a response that was likely independent of herbicide application. This indicates that herbicide type had no true effect on alfalfa density.

Finally, the interaction between mowing and seeding illustrated that mowing did not have a significant impact on alfalfa density (Table 4.5). Non-significant numerical increases and decreases were noted between mowing treatments, within seeding regimes.

4.4.1.2 CLOVER. Preliminary assessment of clover densities indicated there were overwhelming effects of seeding on the density of this species (F=334.8; P<0.0001). As clover emerged nearly exclusively in plots seeded to clover (20.1 ± 1.6 plants m^{-2}) compared to non-seeded plots (0.2 ± 1.6 plants m^{-2}) and plots seeded to alfalfa (0.3 ± 1.6 plants m^{-2}), the clover data were re-analyzed using only seeded plots. Subsequent analyses indicated clover density responded to mowing and rate of herbicide application, as well as their interaction, with marked density changes over time (Table 4.2). In addition, rate and mowing effects varied with time since herbicide application.

Clover densities in clover seeded plots were markedly lower (P<0.0001) in plots treated with 1x herbicide (5.7 ± 4.3 plants m^{-2}) than non-sprayed plots (33.5 ± 4.3 plants m^{-2}). This overall trend was significant over time, with differences between 1x and 0x treatments through to 14 MAT (Table 4.6). Similar values between sprayed and non-sprayed plots after that time were due to decreases in the density of clover plants in non-sprayed plots. The interaction of herbicide rate and mowing in clover seeded treatments indicated that clover densities peaked in mowed 0x treatments, while non-mowed 0x treatments had significantly less clover (Figure 4.2). With herbicide application, mowing maintained greater clover density.

Averaged over all other factors (herbicide type, rate, time, and mowing) clover density was greater (P <0.0001) in mowed plots (28.2 ± 4.3 plants m^{-2}) than non-mowed plots (11.0 ±4.3 plants m^{-2}). When taking time into consideration, average clover density declined from 31.3 ± 4.6 plants m^{-2} at the first sampling time (1 MAT), to 16.5 ± 5.1 plants m^{-2} at the final sampling time (26 MAT) (Figure 4.1). The interaction of mowing with sampling time revealed that clover density did not vary in the mowed portions of clover seeded treatments (Table 4.6), with differences in clover density found only at 12 MAT. Non-mowed treatments however, showed a distinct decline over sampling periods; with clover densities declining from an average of 30.4 plants m^{-2} (±5.1) 1 MAT, to 0 plants m^{-2} (±5.9) 24 and 26 MAT.

4.4.2 Biomass Responses

Legume biomass varied in response to herbicide rate and seeding, both of which also interacted with time of sampling, along with a 3-way interaction between these factors (P<0.05; Table 4.3). Herbicide rate effects on legume biomass were evident in all three years, with plots treated with herbicide (1x) yielding lower legume biomass (*P*<0.001) than those not sprayed (0x) (Figure 4.3). However, legume biomass also consistently declined throughout the study period in both 0x and 1x herbicide treatments (Figure 4.3). Inclusion of seeding effects with rate and sampling time indicated that rate by seeding effects were limited to the first sampling period (Figure 4.4): increases in legume biomass occurred only in unsprayed (0x) plots that were seeded to either alfalfa or clover (Figure 4.4).

Grass biomass did not respond to any of the experimental treatments (Table 4.3), but did differ across sampling times (*P*<0.0001). Grass biomass increased over the study period from an average of 312.5 (±46.1) g m^{-2} in year one, to an average of 437.4 (±46.6) g m^{-2} in year three.

Unlike grasses, forb biomass responded to rate of herbicide, sampling time and the interaction between these two effects (*P*<0.01; Table 4.3) Rate effects were only apparent during the first sampling period (2 MAT) where average forb biomass in the 1x treatment (7.0± 5.9 g m^{-2}) the first year remained below (*P*<0.0001) that of the 0x treatment (29.58 ±5.87 g m^{-2}). Forb biomass in 0x treatments decreased significantly (*P*=0.0006) between 2 MAT and 14 MAT, with no difference between 14 MAT and 26 MAT sampling periods (P=0.64). Finally, total forage biomass varied in response to herbicide rate and sampling time (*P*<0.05). Control (0x) treatments had an average total forage yield of 410.3 (±46.1) g m^{-2}, while 1x treatments were lower (*P*<0.05) with an average yield of 382.27 (±46.04) g m^{-2}. Total forage biomass increased (*P*<0.05) from an average of 356.7 (±46.7) g m^{-2} in the first year, to 449.9 (±46.7) g m^{-2} in year 3.

4.4.3 Dandelion Responses

The primary weed species found at all study sites was dandelion, which permitted assessment of the long-term impact of herbicide and mowing on this species. Dandelion cover did not differ between the 0x (15.7% ± 5.4) and 1x (16.3% ± 5.4) treatments (P=0.82) prior to herbicide application. After treatment, dandelion cover was influenced by herbicide rate (F=5.12; P=0.24), sampling time (F=4.79; P=0.009) and their interaction (F=10.87; $P \leq 0.0001$). Mean dandelion cover one year after application was lower (P<0.05) in the 1x treatment (8.3% ± 5.4) than the 0x treatment (18.1% ± 5.4) pooled over herbicide type, mowing, and seeding treatments, but promptly recovered in the former by the second year (14.1% ± 5.6) to levels similar (P=0.78) to those without herbicide (14.2% ± 5.6).

Dandelion cover also responded to mowing (F=34.71; $P \leq 0.0001$) and its interaction with sampling time (F=24.74; $P \leq 0.0001$) when pooled over bioactive type and seeding treatment. While dandelion was similar between mowed (15.1% ± 5.4) and non-mowed (17.0% ± 5.4) plots prior to the initiation of treatments (P=0.99), non-mowed plots declined markedly (P<0.05) in dandelion cover during years 2 and 3 to levels below (P<0.05) that of mowed plots. One year after mowing began, mowed and non-mowed plots had 17.1% (±5.4) and 9.3% (±5.4) dandelion cover, respectively, a pattern that became increasingly evident in the second year (mowed and non-mowed plots had 24.7% (±5.6) and 3.7% (±5.6), respectively).

4.4.4. Environmental Effects

Environmental effects were assessed only in non-sprayed plots to avoid the confounding effects of herbicide application. Both alfalfa and clover densities (clover densities assessed from clover seeded treatments only), as well as all biomass responses, were generally positively correlated with early season (i.e. June) soil moisture (P<0.05) (Table 4.7). In contrast, clover and alfalfa densities, and most biomass variables, were negatively associated with light availability (µm m^{-2} sec^{-1}) averaged over the growing season. Light increased by 49%, from an average of 243.1 (±6.6) µm m^{-2} sec^{-1} in non-mowed plots to 478.3 (±6.6) µm m^{-2} sec^{-1} in mowed plots.

4.5 Discussion

Although various factors played a role in legume recovery, herbicide application was the main overriding effect. Herbicide application caused a large reduction in legume density and biomass, regardless of bioactive identity. Effects of additional factors (mowing and seeding) on legume establishment were more subtle when interpreted within the context of herbicide use. In the absence of herbicide application, secondary factors (mowing and seeding) became the primary determinants of legume abundance.

4.5.1 Legume Recovery/Resiliency to Bioactives

Legume responses were not found to vary with respect to bioactive identity, indicating that the two herbicide types had functionally similar impacts on legume reestablishment. AMP and AMCP have similar chemistries (Appendix D), are both pyrimidine carboxylic acids, have an auxinic mode of action, select for broadleaf plants, and are broken down by microbial activity (Dow AgroSciences 2005; DuPont 2009). They are systemic herbicides that translocate from leaves and roots to meristematic tissues, where they mimic auxin hormones and cause leaf cupping, loss of apical dominance, epinastic growth form, unregulated plant growth, and death (Bussan and Dyer 1999; USEPA 2005). These characteristics suggest that the bioactives should have similar responses in ecosystems, with comparable withdrawal periods in treated soils, where legume re-emergence and survival would be indistinguishable. This hypothesis is supported by the greenhouse bioassay results in Chapter 5, where herbicide type did not have any significant impact on recovery of alfalfa or white clover. Similarly, the short-term field trial results of Chapter 3 also indicate no difference in legume response due to bioactive identity.

The range of half-lives for the bioactives tested here is large, 31.5-533.2 days for AMP and 22-126 days for AMCP based on pre-registration studies conducted by the Environmental

Protection Agency (USEPA 2005; USEPA 2010). Studies directly comparing these bioactives are limited; half-lives were found to be 28.9 and 32.5 days for AMP and AMCP respectively in field trials performed in Colorado, with equivalent control of Canada thistle provided, suggesting functional similarity between bioactives (Lindenmayer 2012), as was noted in our study. Other studies have indicated that AMCP provides more effective residual control, as was the case for spiny amaranth (*Amaranthus spinosus* L.) and kudzu (*Pueraria montana* (Lour.) Merr.) control (Edwards 2010; Minogue et al. 2011). The comparatively similar impact of bioactives in our study might be due to differences in bioindicator plants (i.e. their sensitivity to herbicide), or to differences in climatic regimes: the aforementioned studies were conducted in Kentucky, Alabama and Florida. Alternatively, functional differences in residual effects between bioactives may have existed in the interim between sampling times, and would not have been detected in this study.

Clover showed full recovery, with no difference between 1x and 0x herbicide treatments, by 24 MAT, suggesting that bioactives had degraded to a point allowing successful establishment and survival of white clover over the period of two growing seasons. This indicates that white clover can be successfully reintroduced to sprayed pastures by the second spring following herbicide application. Similar rates of recovery have been noted in other studies; alfalfa and sunflower were found to show successful reestablishment when replanted into chisel-plowed fields replanted 20 or 23 months following AMP application (120 g a.i. ha^{-1}, the same rate as this study) with little to no negative effects (Mikkelson and Lym 2011). Notably, the soils tested in that study were relatively high in organic matter (>5%), not unlike those tested in the current study (Table 4.1). AMCP has shown more rapid rates of degradation in studies investigating the control of forbs when applied at similar rates (70 g a.i. ha^{-1}, vs. 60 g a.i. ha^{-1} in the current study) with 60-78% control of various forbs (*Ambrosia psilostachya* DC, *Ratibida columnifera* (Nutt.) Woot. & Standl., *Connyza canadensis* (L.) Cronq.) seen one year after application in Kansas shortgrass prairie (Harmoney et al. 2012). This reflects more rapid degradation than seen in the current study; where clover still demonstrated 91.9% suppression at 12 MAT. The rapid rate of AMCP degradation may be due to the difference in climate and/or bioindicator plants (native forb species vs. agronomic legume species).

Differences among studies highlight variation in herbicide degradation across climatic regimes. Both bioactives are broken down by microbial activity, a process regulated by soil organic matter content, moisture, temperature, and pH levels. Herbicide decay rates tend to increase with increases in organic matter content and associated microbial activity (Ou 1984; Veeh et al. 1996; Picton and Farenhorst 2004), high soil temperatures which assist in biochemical breakdown (Walker and Zimdahl 1981; Ou 1984; Goetz et al. 1990; Veeh et al. 1996;), higher soil moisture levels (Walker and Zimdahl 1981; Parker and Doxtader 1983; Ou 1984; Goetz et al. 1990), as well as higher soil pH levels (Loux and Reese 1992; Aichele and Penner 2005). Different combinations of these factors will result in different decay rates.

The minimum legume withdrawal period found here (24 MAT for white clover only) is longer than that noted in Chapter 5 for the greenhouse bioassays (15 MAT). This may be due to two major factors: inherent differences between greenhouse and field conditions (temperature, light, moisture, nutrient availability, and vegetative competition), as well as seed input. For example, the presence of vegetative competition for soil and light resources may have reduced legume performance and/or survival by imposing additional stress on recovering seedlings, and thereby extended the impact of bioactives. While light levels in mowed plots were sufficient for legume growth and development, light levels in non-mowed treatments were, on average, 243.1

(±6.6) µm m^{-2} sec^{-1}, low enough to cause light stress for legume seedlings. This, in turn, may account for lower legume establishment. Light availability was negatively correlated with clover and alfalfa density in this study (Table 4.7), an odd finding as increased light availability is generally associated with increases in legume productivity (Gist and Mott 1956). This finding could be attributed to moisture stress associated with high light levels; a possible side effect of increased evapo-transpiration in mowed treatments, or may be due to the confounded effects of mowing and light. Both clover and alfalfa density showed a positive correlation with early season and average moisture levels, suggesting that abundant moisture may result in increased legume establishment and survival (Table 4.7).

Degradation of bioactives has also been found to increase with temperature. Half-lives of AMCP averaged 20 days in warmer sites of the Northern Great Plains (Conklin and Lym 2013), while AMP has shown an increase in degradation rates of 2-8 fold at soil temperatures of $24^{o}C$ in comparison to $8^{o}C$ (Mikkelson 2010). Reductions in herbicide degradation under cooler temperatures have been found for 2,4-D (Veeh et al. 1996) and imazethapyr (Goetz et al. 1990). Few studies have assessed the impact of soil moisture on degradation of AMP or AMCP, though AMCP degradation is known to increase with soil moisture (Conklin and Lym 2013), and other studies have found that elevated moisture levels result in more rapid degradation rates of other auxinic herbicides (Walker and Zimdahl 1981; Goetz et al. 1990). Greenhouse conditions may have accelerated bioactive degradation, as soil temperatures were held constant at relatively high levels between $17\text{-}25^{o}C$, and moisture was never a limiting factor due to daily watering. The six week growing period in the greenhouse trials, where temperature and moisture levels were consistently favourable, allowed for ongoing breakdown of herbicide residues during a period of legume growth, and may account for the more favourable recovery. It is also possible that the six week greenhouse trials may be analogous to a much longer period of time in the field.

Faster legume recovery in greenhouse trials may also be due to seed inputs. While field plots were seeded at high rates at the beginning of trials, greenhouse trials had seed added at the beginning of each experiment; with less opportunity for seed breakdown or predation, while seed used in field trials was exposed to many factors that may have resulted in loss of seed, or seed viability. Seed predation, disease, breakdown, and losses through germination followed by unsuccessful establishment (germination is not inhibited by herbicide residues, resulting in a flush of seedlings which is then partially removed) are all factors which contribute to seed losses.

Unexpectedly, alfalfa did not show recovery after herbicide application throughout the study, under any conditions. While alfalfa may be more susceptible to herbicide application than clover, this is unlikely based on results from Chapters 3 and 5, where no difference was evident between legumes based on short-term dose trials and greenhouse bioassays, respectively. The most probable explanation for the lack of alfalfa recovery is that this species may be constrained by a combination of exposure to herbicide in conjunction with competition from grasses, as noted in other studies (Groya and Sheaffer 1981; Katepa-Mupondwa et al. 2002). Moreover, although mowing was intended to reduce competition from neighbors, this benefit may have been offset by the destructive physical impact of defoliation to seedlings, as alfalfa is known to be intolerant of grazing, with reduced persistence under frequent defoliation (Katepa-Munpondwa et al. 2002). Additionally, alfalfa has been shown to exhibit autotoxic/autoallelopathic effects, where mature plants produce water-soluble substances which act to inhibit alfalfa germination and growth (Hall et al. 1989; Chung et al. 1995). Although the reasons behind the lack of alfalfa establishment and survival are not clear, it may be due to the cumulative negative impacts of herbicide application, vegetative competition, autotoxic effects, and mowing.

Reestablishment of alfalfa in the absence of herbicide application indicates that this species has the ability to successfully reestablish from the soil seed bank of the area. Moreover, the lack of segregation between alfalfa densities in non-sprayed plots, irrespective of seeding regime, reinforces the fact that alfalfa can reestablish successfully even in the absence of alfalfa seeding. Also surprising was that augmentation of the seed bank did not have long-lasting effects on alfalfa density. Within 0x plots, seeding led to an initial increase of alfalfa seedlings during the first year of study, but subsequent mortality resulted in no difference in alfalfa density between all non-sprayed plots, regardless of seeding regime. Alfalfa has not previously demonstrated natural recovery from the soil seedbank, as was evident in trials from western North Dakota although 'recovery' was considered to be 200 seedlings m^{-2}, a level not reached during our study (densities < 50 m^{-2}) (Carr et al. 2005). Alfalfa establishment from the natural seed bank is attributed to the relatively large seeds and hard seed coat of this species, allowing it to maintain viability over long periods. Alfalfa seeds are viable even after extended periods of time; seeds of up to 70 years old have had successful, albeit low levels of germination (Lewis 1973; Wilton et al. 1978). The longevity of alfalfa seeds indicates that this species has the ability to wait for favorable conditions (i.e. high moisture, low competition, etc.) before germination. Seed abundance in the natural seed bank is a function of ongoing seed rain inputs coupled with seed predation, viability, and dormancy (Maron and Simms 1997), and more information is needed on the combined effects of these factors on the demography of alfalfa in seed banks of the Central Parkland.

Unlike alfalfa, mowing was a major determinant of clover survival, with clover reestablishment heavily dependent on the suppression of competing vegetation by mowing, as well as the increased availability of light resources associated with mowing. In the absence of mowing, clover initially re-established, but then promptly declined 24 months after seeding. Neighboring vegetation, primarily tall statured grasses, appeared to ‘smother’ clover plants via shading and competition for other resources (USDA 2002b; Clark 2007). As noted earlier, in the absence of mowing, average light levels fell to 243.1 (±6.6) μm m^2 sec, a level that can markedly reduce clover growth and productivity (Frame 2005). The favorable relationship between clover recruitment and defoliation or other disturbances has been well documented (Barrett and Silander Jr. 1992; Evans et al. 1992; Schwinning and Parsons 1996). White clover content and herbage production have been shown to be greater in mowed areas when compared to non-mowed areas (Muto and Martin 2000). In the present study, clover densities remained stable over time in mowed plots (Table 4.6), indicating that mowing (and presumably defoliation by livestock) may be one of the most important factors regulating clover establishment.

The lack of clover in plots not seeded to the species indicates that clover has limited ability to recover naturally from the soil seed bank. Thus, some form of seed input, either from seeding or volunteer seed input from neighboring areas, is likely necessary for periodic clover reestablishment and persistence in the Central Parkland. Even at sites where clover was naturally present in the sward, and conditions were considered favourable for clover germination and growth (i.e. cool and moist silt soils) there was little to no natural clover recruitment. Although not the case in the current study, white clover has demonstrated the ability to perpetuate in grazed pastures through recruitment from the seed bank in the northeastern US (Tracy and Sanderson 2000). Seed longevity may be an issue for this species, as white clover has relatively small seeds, which would limit recruitment in the absence of seeding, but long-term seed viability has been shown to be greater for clover than alfalfa (Lewis 1973). In this case, seed availability rather than viability may be the constraint for natural clover establishment in our study.

Legumes may be reintroduced to pastures through a variety of methods, including broadcast seeding (i.e. oversowing), (sod) interseeding, recovery from the natural seed bank, and complete tillage with reseeding. Legume reestablishment from broadcast seeding is possible, but requires a favourable environment for germination, seedling emergence and survival, but has demonstrated the same reestablishment and survival rates as mechanical drilling (Bryan and Prigge 1990; Schleuter 2011). Interseeding of legumes has also been found to reestablish white clover and alfalfa in permanent pastures without conventional tillage (Decker et al. 1969; Mortenson et al. 2005; Muto and Martin 2000; Olsen et al. 1981; Schellenberg et al. 1994). When combined with vegetation suppression, such as herbicide application or defoliation (via mowing or grazing), legume establishment and productivity increases (Bowes and Zentner 1992; Seguin 1998; Muto and Martin 2000). Additionally, alfalfa has been successfully interseeded into grass dominated rangeland and led to increases in total forage biomass of 42-143% (Mortenson et al. 2005). Interseeded alfalfa does not establish well in bromegrass sod when compared to conventional planting (Rioux 1994), suggesting that successful legume reestablishment remains dependent on the suppression of competing vegetation (Cuomo et al. 2001).

Natural legume recovery following herbicide application is dependent on seed bank characteristics, including species presence, abundance of seeds, quality of microsites, and competing vegetation (Barbera et al. 2006; Chambers and MacMahon 1994; Groya and Sheaffer

1981). The potential for natural recovery can be quite high, as has been the case for birdsfoot trefoil, suggesting that self-seeding is possible for legumes in pasture systems where there is an appropriate seed bank (Carr et al. 2005). Conventional tillage and reseeding often result in successful legume reestablishment due to the removal of competition, creation of favourable substrate and microsites. Greater legume establishment is typically evident under conventional tillage regimes than interseeding due to reduced competition for resources such as light, moisture, and space, factors important for small seeded legume species (Rioux 1994).

4.5.2 Forage Production Responses

Legume biomass within the sward, assessed as the sum of both clover and alfalfa biomass, decreased over time in both sprayed and control treatments in this study. This was likely a function of grass plants outcompeting legumes for light and moisture resources (Wing-To and MacKenzie 1971; Groya and Sheaffer 1981; Kunelius et al. 1982; Rioux 1994; Sanderson et al. 2005). Legume biomass was positively correlated with soil moisture, indicating the latter was important for legume establishment and growth (Table 4.7). However, legume biomass decreased over time, necessitating reseeding even in non-sprayed areas (Peterson et al. 1994). These decreases are neither unexpected nor out of the ordinary for pasture and rangeland systems, especially as the study sites were all older hayfields with well-established, highly competitive graminoid communities.

Forb biomass also experienced a temporary decline following herbicide application, particularly immediately after spraying (2 MAT). The quick rebound in forb biomass the following year is likely due to the nature of the forb seed bank in these pastures. Agricultural lands are prone to species invasion as increases in resource availability (moisture, light, nutrients and space) are often associated with anthropogenic disturbance and can increase the susceptibility of a community to invasion (Daehler 2003). Increases in grazing intensity have been associated with increases in weed populations in central Alberta, and the abundance of weed seeds in the seed bank of the Central Parkland has been noted to range between 207 to 580 plants m^{-2}, although weed abundance is a localized characteristic (Harker et al. 2000; Cathcart et al. 2006). Although there is variation in weed density, even the lowest density (207 plants m^{-2}) suggests that there is a robust weed seed bank in the Central Parkland area, which helps explain why the forb community was able to rebound so quickly from herbicide application.

Grass and total forage biomass both increased over the study period, regardless of herbicide application. Increases in total forage biomass, even under spraying, suggest that the grass component of these swards compensated for legume removal, and potentially benefited from 1) the removal of competition from legumes, forbs, or weeds, and 2) nitrogen mineralization from the decay of affected plant roots, particularly that of legumes. In the case of compensatory responses to legume removal, it is likely that grasses moved in and utilized vacant space resulting from broadleaf removal (Wing-To and Mackenzie 1971; Groya and Sheaffer 1981; Sanderson et al. 2005). A 'flush' of available nitrogen is associated with legume root decay, and could also explain the increases in total forage biomass. The incorporation of legume residues (*Medicago sativa* and *Trifolium subterraneum* L.) into cropping systems has been associated with increased nitrogen mineralization and availability, allowing for greater crop yields one to two years after legume incorporation (Bolger et al. 2003). This supports our finding that the decay of legume roots may increase total forage biomass. In doing so, it also suggests that the penalty of legume loss due to herbicide application is not as large as expected. However, the slow return of legumes in the long run may still decrease production once nitrogen becomes more limiting. Although grass biomass may exhibit limited compensatory responses to legume removal, total forage productivity has been shown to decrease in mixed swards after legume removal, with decreases in crude protein yield as well as biomass (McLeod 2011). Thus, although forage production did not decrease over the length of our study, it most likely will in future years in the absence of legume presence.

4.5.3 Weed Community Dynamics

Although herbicide application initially decreased dandelion cover, this species recovered by the second growing season to control and pre-spraying levels. This suggests that these bioactives do not have lasting residual effects on dandelion, and control can be expected for less than two growing seasons. The application of AMP at the same rate as in this study (120 g a.i. ha^{-1}) has been shown to reduce dandelion cover by 50 and 70% in Canada thistle infested and native tallgrass prairie communities in Manitoba, during the year of application. However, dandelion recovered to pre-treatment levels by 22 MAT, corroborating our findings on dandelion responses to AMP. This suggests that for effective control of dandelion in pastures, herbicides would have to be reapplied every second growing season, a relatively costly management strategy, which would also result in a system devoid of legumes.

Although herbicide application was anticipated to be the main determinant of dandelion abundance, it became apparent that mowing was far more important in regulating this species. Increases in dandelion under mowing treatments were not unexpected as this weed has the ability to tolerate and even thrive under high defoliation (Mølgaard 1977). However, the pronounced reductions in dandelion cover within non-mowed plots, both sprayed and non-sprayed, was unexpected. Although reduced dandelion in non-mowed treatments was expected, and is attributed to increased competition from grasses (Mølgaard 1977), the extent of reductions and duration of declines seen here were not.

In contrast, increases in dandelion in mowed treatments were likely a result of abundant light availability, which allowed dandelion to successfully emerge and grow, subsequently forming wide-spreading rosettes capable of suppressing neighbouring plants (Godoy et al. 2011). Successful dandelion establishment in grasslands is only possible when grass cover is low, with open spaces between plants and light infiltration through competing vegetation (Mølgaard 1977). Dandelion plants tend to be smaller and less vigorous in areas with strong competition from grasses, which explains why dandelion cover decreased over time in non-mowed treatments (Mølgaard 1977).

Light levels in the absence of mowing were generally below the light compensation point of dandelion (243.1 (±6.6) µm m^{-2} sec^{-1} in non-mowed plots, vs. ~300 µm m^{-2} sec^{-1} light compensation point), accounting for why dandelion decreased in non-mowed treatments (Godoy et al. 2011). Competitive vegetation as well as crop residues can shade out dandelion plants, reducing their germination and emergence, as noted in this study (Derksen et al. 2002).

Overall, increases in light availability associated with defoliation allowed dandelion to recover and proliferate, while decreased light availability in non-mowed treatments decreased dandelion cover. These results suggest that successful dandelion control cannot be expected in areas with frequent grazing or defoliation. Consequently, herbicide application in conjunction with low intensity defoliation may allow for the greatest decreases in dandelion abundance.

4.6 Management Implications

Documentation of the effects of broadleaf herbicide application on legume communities as well as associated post-spraying sward dynamics, including withdrawal periods of residual bioactives, is an important step to take in efforts to mitigate the negative effects of chemical weed control in pastures. Moreover, lack of information exists regarding the withdrawal periods of some broadleaf herbicides, AMP and AMCP in particular, in northern temperate grasslands. This study attempted to address this lack of information by documenting the effects of broadleaf herbicide application on two common pasture legumes, alfalfa and white clover, as well as the overall sward dynamics over time as herbicide residues degrade.

Herbicide withdrawal in this study, as represented by legume establishment, was noted at 24 MAT for clover, indicating this species can be reintroduced the second full growing season after spraying. Unfortunately, dandelion was able to recover to pre-treatment levels 24 MAT as well. There was also no functional difference between the two herbicide bioactives tested in this study (AMP and AMCP), with similar impacts on both legume recovery and apparent weed control.

Forage biomass was not impacted by herbicide application and the associated removal of legumes, and instead increased over the three year study. Increased forage was attributed to a nitrogen flush from decomposing forbs, including legume dry matter, though increased productivity after herbicide application should not be counted on in the long term, particularly if legumes do not naturally reestablish within the sward.

The results of this study indicate that although legume removal by broadleaf herbicides is an unfavourable consequence of weed control, it seems that pasture productivity in the Central Parkland region is not compromised during the withdrawal period between herbicide application and a return to the ‘safe’ reseeding time (24 MAT). Although forage quality may decrease due to lack of legumes, overall forage quantity will not, and was actually found to increase over time in both sprayed and non-sprayed treatments in this study. Consequently, use of herbicides such as AMP and AMCP can be an economical and effective way to manage weeds while maintaining pasture productivity in the interim. Successful legume reintroduction should be possible after two growing seasons (24 MAT) under normal field conditions, with no reduction in forage quantity in the interval between herbicide application and reseeding. These findings should lead to improved legume management practices in pastures of the Central Parkland, and increased economic benefits for producers of the region.

Finally, recovery dynamics occurring between sampling times may not have been captured in this study, and thus, the final recommendation on the legume re-cropping interval after herbicide application should be tempered by the limited temporal and spatial sampling available in the present study. Variation in soil and climatic conditions, coupled with the resident vegetation characteristics, may all lead to substantial variation in minimum withdrawal periods for clover and alfalfa, which should be further modified depending on the impacts of secondary disturbances, including mowing/grazing. Differences in temperature and precipitation, variation in microsites, as well as limitations of the seed bank itself, will inherent determine the extent of legume recovery following spraying and long-term forage dynamics.

4.7 Materials Used

[1]Seed from Viterra, Fort Saskatchewan, Alberta, Canada.

www.viterra.ca

[2]Air Bubble Jet 110010 nozzles, ABJ Agri Products, Brandon, Manitoba, Canada.

www.abjagri.com

[3]Ardisam Earthquake Rolling String Trimmer, Briggs & Stratton 190cc Engine, Model# 600050B. Earthquake, Cumberland, Wisconsin, USA.

http://www.getearthquake.com

[4] AccuPAR model LP-80 PAR/LAI Ceptometer light wand. Decagon Devices Inc., Pullman, Washington, USA.

http://www.decagon.com/

[5] ML2X-ThetaProbe soil moisture sensor. DeltaT Devices, Cambridge, United Kingdom.

http://www.delta-t.co.uk/

4.8 Literature Cited

Aichele, T. M. and Penner, D. 2005. Adsorption, desorption, and degradation of imidazolinones in soil. Weed Technol. **19**(1): 154-159.

Almquist, T. L. and Lym, R. G. 2010. Effect of aminopyralid on Canada thistle (*Cirsium arvense*) and the native plant community in a restored tallgrass prairie. Invas. Pl. Sci. and Manage. **3:** 155-168.

Barberá, G. G., Navarro-Cano, J. A., Castillo, V. M. 2006. Seedling recruitment in a semi-arid steppe: The role of microsite and post-dispersal seed predation. J. Arid Environ. **67:** 701- 714.

Barrett, J. P. and Silander Jr., J. A. 1992. Seedling recruitment limitation in white clover (*Trifolium repens:* Leguminosae). Am. J. Bot. **79(6):** 643-649.

Bolger, T. P., Angus, J. F., Peoples, M. B. 2003. Comparison of nitrogen mineralisation patterns from root residues of *Trifolium subterraneum* and *Medicago sativa.* Biol. Fertil. Soils **38:** 296-300.

Bork, E. W., Grekul, C. W., DeBrujin, S. L. 2007. Extended pasture forage sward responses to Canada thistle (*Cirsium arvense*) control using herbicides and fertilization. Crop Prot. **26(10):** 1546-1555.

Bowes, G. G. and Zentner, R. P. 1992. Effect of vegetation suppression on the establishment of sod-seeded alfalfa in the aspen parkland. Can. J. Plant Sci. **72(4):** 1349-1358.

Bryan, W. B. and Prigge, E. C. 1990. Effects of stocking rate and overseeding with red clover on productivity of native pasture continuously grazed by yearling steers. J. Agron. & Crop Sci. **165(4):** 273-280.

Bussan, A. J. and Dyer, W. E. 1999. Herbicides and rangeland. Pages 116-132 in: Sheley, R. L. and Petroff, J. K., eds. Biology and management of noxious rangeland weeds. Oregon State University Press, Corvallis, OR.

Carr, P. M., Poland, W. W., Tisor, L. J. 2005. Dryland cropping systems: forage legume regeneration from the soil seed bank in western North Dakota. Agron. J. **97:** 505-513.

Carter, M. R. and Gregorich, E. G., eds. 2008. Soil sampling and methods of analysis. second edition. Canadian Society of Soil Science.CRC Press.Taylor & Francis Group, Boca Raton, Florida.

Cathcart, J., Topinka, A. K., Kharbanda, P., Lange, R., Yang, R., Hall, L. M. 2006. Rotation length, canola variety and herbicide resistance affect weed populations and yield. Weed Sci. **54(4):** 726-734.

Chambers, J. C. and MacMahon, J. A. 1994. A day in the life of a seed: Movements and fates of seeds and their implications for natural and managed systems. Annu. Rev. Ecol. Syst. **25:** 263-292.

Chung, I., Miller, D. A. 1995. Effect of alfalfa plant and soil extracts on germination and growth of alfalfa. Agron. J. **87**: 762–767.

Clark, A. 2007. White clover (T*rifolium repens*). Pages 179-184 *in:* Clark, A., ed. Managing cover crops profitably. 3rd ed. ed. Sustainable Agriculture Network, Beltsville, MD.

Conklin, K. L. and Lym, R. 2013. Effect of temperature and moisture on aminocyclopyrachlor soil half-life. Weed Technol. (In-Press).

Cuomo, G. J., Johnson, D. G., Head Jr., W. A. 2001. Interseeding kura clover and birdsfoot trefoil into existing cool-season grass pastures. Agron. J. **93:** 458-462.

Daehler, C. C. 2003. Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. Ann. Rev. of Ecol., Evolu. and Syst. **34:** 183-211.

Decker, A. M., Retzer, H. J., Sarna, M. L., Kerr, H. D. 1969. Permanent pastures improved with sod-seeding and fertilization. Agron. J. **61:** 243-247.

Derksen, D. A., Anderson, R. A., Blackshaw, R. E., Maxwell, B. 2002. Weed dynamics and management strategies for cropping systems in the northern great plains. Agron. J. **94:** 174-183.

Dow AgroSciences. 2005. Aminopyralid technical bulletin. An introduction to aminopyralid. V45-313-001 (01/05) BOD. 010-50433. 19 pp.

Dow AgroSciences. 2012. Milestone specialty herbicide. Specimen Label: 1-9.

DuPont. 2009. DPX-MAT28 technical bulletin: 1-7.

Edwards, M. E. 2010. Spiny amaranth control and aminopyralid persistence in Kentucky pastures. M.Sc. Thesis, University of Kentucky, Lexington, Kentucky, United States of America. 85 pp.

Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., Moechnig, M., Peterson, V., Masters, R. A., Halstvedt, M. 2007. Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop areas. Weed Technol. **21:** 890-894.

Evans, D. R., Williams, T. A., Evans, S. A. 1992. Evaluation of white clover varieties under grazing and their role in farm systems. Grass Forage Sci. **47:** 342-352.

Frame, J. 2005. Forage legumes for temperate grasslands. Science Publishers Inc., Enfield, NH, United States of America. 309 pp.

Gist, G. R. and Mott, G. O. 1956. Some effects of light intensity, temperature, and soil moisture on the growth of alfalfa, red clover and birdsfoot trefoil seedlings. Agron. J. **49(1):** 33-36.

Godoy, O., Valladares, F., Castro-Diez, P. 2011. Multispecies comparison reveals that invasive and native plants differ in their traits but not in their plasticity. Funct. Ecol. **25:** 1248-1259.

Goetz, A. J., Lavy, T. L., Gbur, J.,E.E. 1990. Degradation and field persistence of imazethapyr. Weed Sci. **38**(4/5): 421-428.

Goonewardene, L. 2012. Personal communication. Biostatistician, AFNS Department, Faculty of ALES, University of Alberta, Edmonton, Alberta.

Grekul, C. W. and Bork, E. W. 2004. Herbage yield losses in perennial pasture due to Canada thistle (*Cirsium arvense*). Weed Technol. **18(3):** 784-794.

Grekul, C. W., Cole, D. E., Bork, E. W. 2005. Canada thistle (*Cirsium arvense*) and pasture forage responses to wiping with various herbicides. Weed Technol. **19:** 298-306.

Groya, F. L. and Sheaffer, C. C. 1981. Establishment of sod-seeded alfalfa at various levels of soil moisture and grass competition. Agron. J. **73:** 560-565.

Hall, M. H., Henderlong, P. R. 1989. Alfalfa autotoxic fraction characterization and initial separation. Crop Sci. **29**: 425–428.

Harker, K. N., Baron, V. S., Chanasyk, D. S., Naeth, M. A., Stevenson, F. C. 2000. Grazing intensity effects on weed populations in annual and perennial pasture systems. Weed Sci. **48**(2): 231-238.

Harmoney, K. R., Stahlman, P. W., Geier, P. W., Rupp, R. 2012. Effects of a new herbicide (aminocyclopyrachlor) on buffalograss and forbs in shortgrass prairie. Weed Technol. **26(3):** 455-459.

Haystead, A. and Marriott, C. 1979. Transfer of legume nitrogen to associated grass. Soil Biol. Biochem. **11:** 99-104.

Katepa-Mupondwa, F., Singh, A., Smith Jr., S. R., McCaughey, W. P. 2002. Grazing tolerance of alfalfa (*Medicago* spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (*Bromus riparious* Rehm. syn. *B. biebersteinii* Roem & Schult). Can. J. Plant Sci. **82:** 337-347.

Kunelius, H. T. and Campbell, A. J. 1984. Performance of sod-seeded temperate legumes in grass dominant swards. Can. J. Plant Sci. **64:** 643-650.

Kunelius, H. T., Campbell, A. J., McRae, K. B., Ivany, J. A. 1982. Effects of vegetation suppression and drilling techniques on the establishment and growth of sod-seeded alfalfa and bird's-foot trefoil in grass dominant swards. Can. J. Plant Sci. **62:** 667-675.

Lewis, J. 1973. Longevity of crop and weed seeds: Survival after 20 years in soil. Weed Res. **13:** 179-191.

Lindenmayer, R. B. 2012. Understanding aminocyclopyrachlor behavior in soil and plants. Ph.D. Thesis, Colorado State University, Fort Collins, CO. 82 pp.

Loux, M. M. and Reese, K. D. 1992. Effect of soil pH on adsorption and persistence of imazaquin. Weed Sci. **40**(3): 490-496.

Maron, J. L. and Simms, E. L. 1997. Effect of seed predation on seed bank size and seedling recruitment of bush lupine (*Lupinus arboreus*). Oecologia **111(1):** 76-83.

McLeod, E. M. 2011. Forage responses to herbicide weed control in grass-legume swards. M.Sc. Thesis, University of Alberta, Edmonton, AB. 60 pp.

Merou, T. P. and Papanastasis, V. P. 2009. Factors affecting the establishment and growth of annual legumes in semi-arid mediterranean grasslands. Plant Ecol. **201:** 491-500.

Mikkelson, J. R. 2010. Effect of aminopyralid on crop rotations and native forbs. M.Sc. Thesis, North Dakota State University, Fargo, ND**.**

Mikkelson, J. R. and Lym, R. G. 2011. Aminopyralid soil residues affect crop rotation in North Dakota soils. Weed Technol. **25(3):** 422-429.

Minogue, P. J., Enloe, S. F., Osiecka, A., Lauer, D. K. 2011. Comparison of aminocyclopyrachlor to common herbicides for kudzu (*Pueraria montana*) management. Invas. Pl. Sci. and Manage. **4:** 419-426.

Mølgaard, P. 1977. Competitive effect of grass on establishment and performance of *Taraxacum officinale*. Oikos **29(2):** 376-382.

Mortenson, M. C., Schuman, G. E., Ingram, L. J., Nayigihugu, V., Hess, B. W. 2005. Forage production and quality of a mixed-grass rangeland interseeded with *Medicago sativa* ssp. *falcata*. Rangeland Ecol. Manage. **58:** 505-513.

Muto, P. J. and Martin, R. C. 2000. Effects of pre-treatment, renovation procedure and cultivar on the growth of white clover sown into a permanent pasture under both grazing and mowing regimes. Grass Forage Sci. **55:** 59-68.

Natural Regions Committee. 2006. Natural regions and sub-regions of Alberta. Government of Alberta, 264 pp.

Olsen, F. J., Jones, J. H., Patterson, J. J. 1981. Sod-seeding forage legumes in a tall fescue sward. Agron. J. **73:** 1032-1036.

Ou, L. 1984. 2,4-D degradation and 2,4-D degrading microorganisms in soils. Soil Sci. **137(2):** 100-107.

Parker, L. W. and Doxtader, K. G. 1983. Kinetics of the microbial degradation of 2,4-D in soil: Effects of temperature and moisture. J. Environ. Qual. **12(4):** 533-558.

Peterson, R. P., Sheaffer, C. C., Jordan, R. M., Christians, C. J. 1994. Responses of kura clover to sheep grazing and clipping: I. yield and forage quality. Agron. J. **86:** 655-660.

Picton, P. and Farenhorst, A. 2004. Factors influencing 2,4-D sorption and mineralization in soil. J. Environ. Sci. & Health **39(3):** 367-379.

Popp, J. D., McCaughey, W. P., Cohen, R. D. H., McAllister, T. A., Majak, W. 2000. Enhancing pasture productivity with alfalfa: A review. Can. J. Plant Sci. **80(3):** 513-519.

Province of Alberta. 2008. Weed control act. Alberta Queen's Printer: W-5.1.

Renz, M. J. 2010. Establishment of forage grasses and legumes after fall herbicide applications. Forage and Grazinglands Available: doi:10.1094/FG-2010-0806-01-RS [March 10, 2013].

Rioux, R. 1994. Direct seeding of alfalfa in grain stubble and bromegrass sod. Can. J. Plant Sci. **74(4):** 773-778.

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., Goslee, S. C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J**. 97:** 1465-1471.

SAS Institute Inc. 2008. SAS/STAT® 9.2 user’s guide. SAS Institute Inc., Cary, NC.

Schellenberg, M. P., Waddington, J., King, J. R. 1994. Direct seeding of alfalfa into established russian wildrye pasture in southwest Saskatchewan. Can. J. Plant Sci. **74(3):** 539-542.

Schleuter, D. H. 2011. Overseeding clovers into permanent pastures. M. Sc. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States of America. 99 pp.

Schwinning, S. and Parsons, A. J. 1996. Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. J. Ecol. **84(6):** 799-813.

Seguin, P. 1998. Review of factors determining legumes in sod-seeding outcome during pasture renovation in North America. Biotechnol. Agron. Soc. Environ. **2(2):** 120-127.

Seguin, P., Peterson, P. R., Sheaffer, C. C., Smith, D. L. 2001. Physical sod suppression as an alternative to herbicide use in pasture renovation with clovers. Can. J. Plant Sci. **81:** 255-263.

Sleugh, B., Moore, K. J., George, R., Brummer, E. C. 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agron. J. **92:** 24-29.

Soil Classification Working Group. 1998. The Canadian system of soil classification. Haynes, R. H., ed. 3rd ed. Agriculture and Agri-Food Canada, NRC Research Press, Ottawa, ONT, Canada. 187 pp.

Thomas, A. G. 1985. Weed survey system used in Saskatchewan for cereal and oilseed crops. Weed Sci. **33(1):** 34-43.

Tracy, B. F. and Sanderson, M. A. 2000. Seedbank diversity in grazing lands of the northeast United States. J. Range Manage. **53(1):** 114-118.

USDA. 2002b. White clover (*Trifolium repens* L.) plant fact sheet.

USEPA. 2005. Environmental fate and ecological risk assessment for the registration of aminopyralid. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances.

USEPA. 2010. Registration of the new active ingredient aminocyclopyrachlor for use on non-crop areas, sod farms, turf, and residential lawns. U.S. Environmental Protection Agency, Office of Pesticide Programs, Registration Division.

Veeh, R. H., Inskeep, W. P., Camper, A. K. 1996. Soil depth and temperature effects on microbial degradation of 2,4-D. J. Environ. Qual. **25:** 5-12.

Vogel, K. P., Kehr, W. R., Anderson, B. E. 1983. Sod-seeding alfalfa into cool-season grasses and grass-alfalfa mixtures using glyphosate or paraquat. J. Range Manage. **36(6):** 700-702.

Vough, L. R. and Marten, G. C. 1971. Influence of soil moisture and ambient temperature on yield and quality of alfalfa forage. Agron. J. **63:** 40-42.

Walker, A. and Zimdahl, R. L. 1981. Simulation of the persistence of atrazine, linuron and metolachlor in soil at different sites in the USA. Weed Res. **21:** 255-265.

Wilton, A. C., Townsend, C. E., Lorenz, R. J., Rogler, G. A. 1978. Longevity of alfalfa seed. Crop Sci. **18:** 1091-1093.

Wing-To, C. and MacKenzie, A. F. 1971. Effects of shading and nitrogen on growth of grass-alfalfa pastures. Agron. J. **63:** 667-669.

Table 4.1 Physical site (i.e., soil) characteristics of long-term trials, as sampled in May 2010 or 2011.[z] All sites are level with negligible slope.

Site	Location/ Lat.-Lon.[y]	Texture/Soil Type	N (%)	Available N ($N0_3$+NH_4) (mg kg^{-1})	C (%)	OM (%)	pH	EC (µs cm^{-1})
Fort Saskatchewan	53° 47' 18.94" N, 113° 20' 38.73" W	Silt Loam, Solodized Solonetz	0.3	5.5	3.6	9	7.1	482
Glenpark	53° 10' 38.58" N, 113° 24' 42.64" W	Silty Clay Loam, Gleyed Eluviated Black Chernozem	1.5	39.2	18.3	60.5	7.9	2580
Lamont	53° 44' 29.84" N, 112° 31' 43.28" W	Sandy Loam, Eluviated Black Chernozem	0.3	14.3	3.5	9	7.1	319.5
St. Albert	53° 41' 34.31" N, 113° 38' 5.40" W	Silty Clay, Eluviated Black Chernozem	0.4	7.8	4.5	16.4	8.4	467
Stony Plain	53° 27' 17.18" N, 114° 8' 12.42" W	Sandy Loam, Eluviated Black Chernozem	0.2	4.7	3.3	8.6	7.3	206.5

[z]Values represent the average of 10 soil cores collected at 0-30cm depth.

[y]Lat.-Long. Represent exact coordinates of site.

Table 4.2 Summary of ANOVA F-statistic and significance values associated with clover and alfalfa density (plants m^{-2}) responses to mowing, herbicide type and rate, and season of measurement, as well as all associated interactions, pooled over five long term study sites. Clover density data is taken from clover seeded treatments only. Data represent three years of assessment.

	Clover Density		Alfalfa Density	
Treatment Effect	F-stat	P-value	F-stat	P-value
Time	14.3	**<0.0001**	12.3	**<0.0001**
Rate	187.3	**<0.0001**	578.4	**<0.0001**
Mow	71.4	**<0.0001**	0.6	0.43
Seed	--	--	40.4	**<0.0001**
Herb	0.6	0.43	4.7	**0.031**
Time*Rate	11.9	**<0.0001**	8.6	**<0.0001**
Time*Mow	5.7	**<0.0001**	1.3	0.28
Time*Herb	0.3	0.93	1.0	0.43
Time*Seed	--	--	5.3	**<0.0001**
Rate*Mow	15.7	**<0.0001**	0.7	0.41
Rate*Herb	2.8	0.093	7.3	**0.0072**
Rate*Seed	--	--	31.8	**<0.0001**
Mow*Herb	1.1	0.30	3.8	**0.05**
Mow*Seed	--	--	4.5	**0.012**
Seed*Herb	--	--	1.1	0.35
Rate*Seed*Time	--	**--**	3.6	**0.0001**
Mow*Seed*Time	--	**--**	0.8	0.59
Rate*Mow*Time	1.5	0.18	0.6	0.67
Rate*Mow*Seed	--	**--**	2.8	0.061
Herb*Seed*Time	--	--	0.4	0.96
Herb*Rate*Time	0.5	0.80	0.4	0.85
Herb*Mow*Time	0.3	0.93	0.3	0.89
Herb*Mow*Seed	--	--	1.0	0.36
Herb*Rate*Seed	--	**--**	0.7	0.49
Herb*Rate*Mow	0.5	0.50	1.6	0.21
Rate*Mow*Seed*Time	--	**--**	1.3	0.21

Herb*Rate*Seed*Time	--	--	0.4	0.95
Herb*Rate*Mow*Time	0.5	0.81	0.3	0.89
Herb*Mow*Seed*Time	--	--	0.6	0.83
Herb*Rate*Mow*Seed	--	--	1.1	0.34
Herb*Rate*Mow*Seed*Time	--	--	0.6	0.82

Table 4.3 Summary of F-statistic and significance values associated with biomass (g m^{-2}) responses to mowing, herbicide type and rate, and time of measurement, as well as all associated interactions, pooled over all five long term study sites. Data represent three years of assessment.

	Grass Biomass		Legume Biomass		Total Forage Biomass		Forb Biomass	
Treatment Effect	F-stat	P-value	F-stat	P-value	F-stat	P-value	F-stat	P-value
Time	27.4	**<0.0001**	27.8	**<0.0001**	15.6	**<0.0001**	6.11	**0.002**
Rate	2.2	0.14	75.9	**<0.0001**	6.0	**0.015**	21.3	**<0.0001**
Seed	0.7	0.48	3.4	**0.03**	0.5	0.64	2.0	0.14
Herb	0.3	0.58	2.7	0.10	0.9	0.35	0.4	0.54
Rate*Time	2.3	0.10	6.1	**0.003**	2.2	0.12	8.3	**0.0003**
Seed*Time	0.5	0.71	2.5	**0.04**	0.1	0.98	1.0	0.40
Herb*Time	0.6	0.54	0.03	0.97	0.7	0.51	1.1	0.35
Herb*Rate	0.08	0.78	0.03	0.86	0.3	0.61	0.3	0.59
Rate*Seed	0.3	0.76	2.1	0.12	0.07	0.94	1.2	0.29
Herb*Seed	2.3	0.10	1.6	0.20	1.2	0.31	0.4	0.67
Rate*Seed*Time	1.2	0.29	2.5	**0.05**	1.0	0.40	0.6	0.64
Herb*Seed*Time	1.1	0.34	1.0	0.40	1.7	0.14	0.3	0.90
Herb*Rate*Time	1.2	0.31	0.9	0.40	1.3	0.29	0.2	0.79
Herb*Rate*Seed	0.6	0.55	1.1	0.33	0.1	0.90	0.3	0.73
Herb*Rate*Seed*Time	0.4	0.80	0.4	0.82	0.4	0.82	0.6	0.70

Table 4.4 Alfalfa density responses (plants m^{-2}) to the three-way interaction between herbicide rate, seeding regime, and sampling time in MAT (months after treatment).[z]

		Sampling Time					
Herbicide Rate	Seeding Regime	1 MAT	2 MAT	12 MAT	14 MAT	24 MAT	26 MAT
0x	Alfalfa	42.1*a*[x] A[y]	37.6*a A*	19.6*b A*	18.3*b A*	16.1*b A*	18.8*b A*
	Clover	17.7*a B*	12.8*a B*	13.2*a A*	10.8*a A*	9.5*a A*	10.4*a A*
	Non-Seeded	16.6*a B*	14.6*a B*	14.3*a A*	13.3*a A*	11.2*a A*	15.3*a A*
1x	Alfalfa	2.7*a A*	3.2*a A*	0.4*a A*	0.4*a A*	1.6*a A*	0.8*a A*
	Clover	1.9*a A*	0.5*a A*	0.3*a A*	0.2*a A*	1.4*a A*	0.8*a A*
	Non-Seeded	0.6*a A*	0.9*a A*	0.2*a A*	0.04*a A*	1.2*a A*	0.7*a A*
	Standard Error	2.8	2.8	2.8	2.8	3.3	3.3

[z]Means and pooled SE's. Tukey's Test ($P \leq 0.05$) used for treatment comparisons.

[y]Uppercase letters indicate significance between means within a column, within a rate treatment.

[x]Lowercase letters indicate significance between row means (i.e. among sampling times).

Table 4.5 Alfalfa density (plants m^{-2}) responses to mow by seed interaction.[z]

Seeding Treatment	Alfalfa	Clover	Non-Seeded
Mowed	15*a*[y]	5.6*b*	7.7*b*
P-value (mow)	(0.11)[x]	(0.56)	(1.0)
Non-Mowed	12.0*a*	7.6*b*	7.1*b*
Standard Error	2.3	2.3	2.3

[z]Means and pooled SE's. Tukey's test ($P<0.05$) was used for treatment comparisons.
[y]Within rows, means with different letters differ significantly based on a Tukey's HSD test ($P\leq0.05$).
[x]P-values indicate significance between mowing treatments within a seeding treatment.

Table 4.6 Responses in clover density (plants m^{-2}) in clover seeded plots only, to the interactions of mowing by sampling time, and herbicide rate by sampling time.[z]

		Sampling Time					
Treatment	Level	1 MAT	2 MAT	12 MAT	14 MAT	24 MAT	26 MAT
Mowing	Mowed	32.13*a*[y]	34.49*a*	14.97*b*	30.69*a*	22.76*ab*	34.18*a*
	Non-Mowed	30.44*a*	23.71*a*	6.03*b*	9.24*ab*	0*b*	0*b*
		(1.00)[x]	(0.37)	(0.67)	(<0.0001)	(0.002)	(<0.0001)
Herbicide							
Rate	0x	56.46*a*	52.43*a*	19.42*b*	31.56*b*	17.59*b*	23.66*b*
	1x	6.11*a*	5.78*a*	1.58*a*	8.36*a*	3.08*a*	9.4*a*
		(<0.0001)	(<0.0001)	(0.003)	(<0.0001)	(0.38)	(0.40)
	Standard Error	5.1	5.1	5.1	5.2	5.9	5.9

[z]Means and pooled SE's. Tukey's HSD Test ($P \leq 0.05$) used for treatment comparisons.
[y]Letters indicate significance within rows (i.e. across sampling times).
[x]P-values indicate significance between treatments within sampling times.

Table 4.7 Correlation of environmental factors, including soil moisture and light levels (μm m^{-2} sec^{-1}), on alfalfa density and clover density (plants m^{-2}), as well as legume, grass, total forage and forb biomass (g m^{-2}) in non-seeded, control (0x) plots only. Clover density data were taken from clover seeded treatments. Correlation coefficient (Kendall's Tau-b statistic) indicated in table.

Component	Response	June Soil Moisture	August Soil Moisture	Average Soil Moisture	Light Availability
Plant Density	Clover	+0.2***	+0.1***	+0.2***	-0.08*
	Alfalfa	+0.08***	-0.03	+0.05**	-0.1***
Biomass	Legume	+0.09*	+0.2**	+0.1**	-0.08
	Grass	+0.2***	+0.9*	+0.2**	-0.2***
	Total Forage[z]	+0.2***	+0.2***	+0.3***	-0.3***
	Forb	+0.2***	+0.2***	+0.2***	-0.4

*P<0.05, **P<0.01, P<0.0001***

[z]Total forage includes grass and legume biomass.

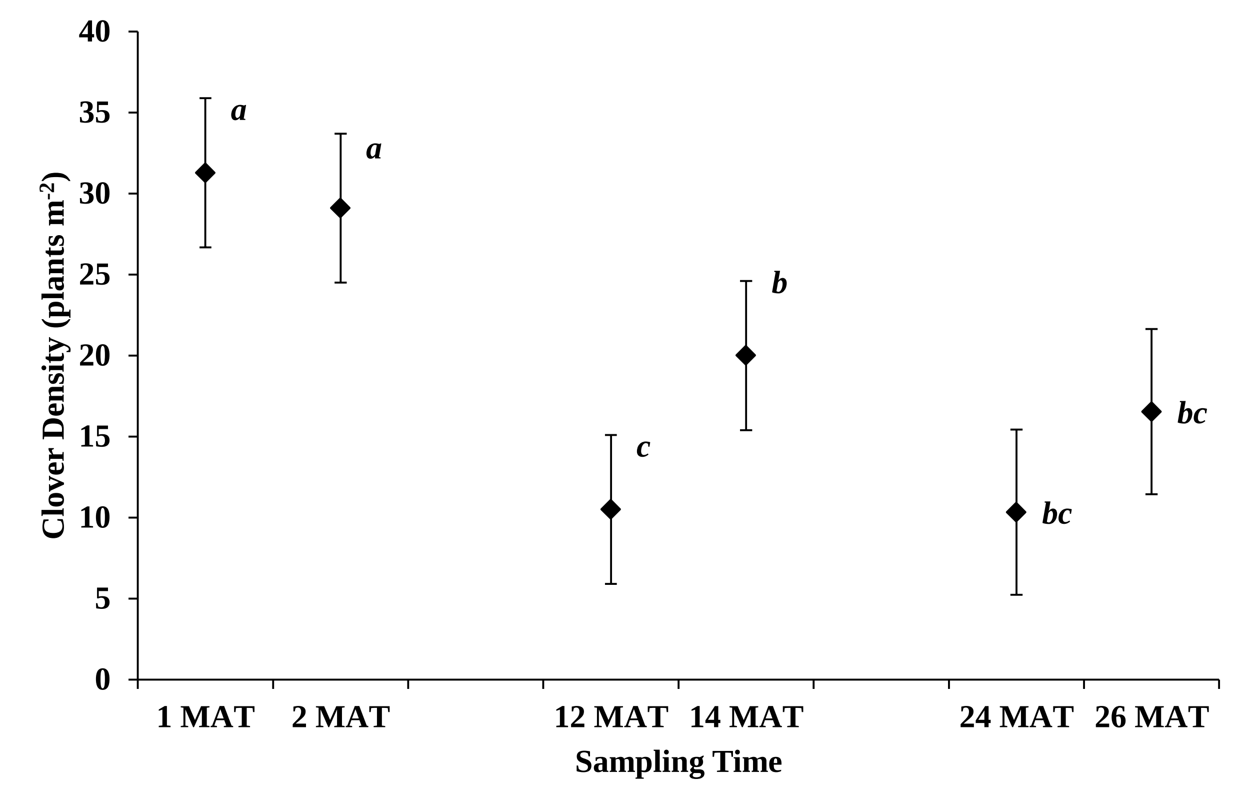

Figure 4.1 Changes in mean (±SE) clover density (plants m^{-2}) within clover seeded plots (0x and 1x) over time. Means with different letters differ based on Tukey's HSD test ($P \leq 0.05$).

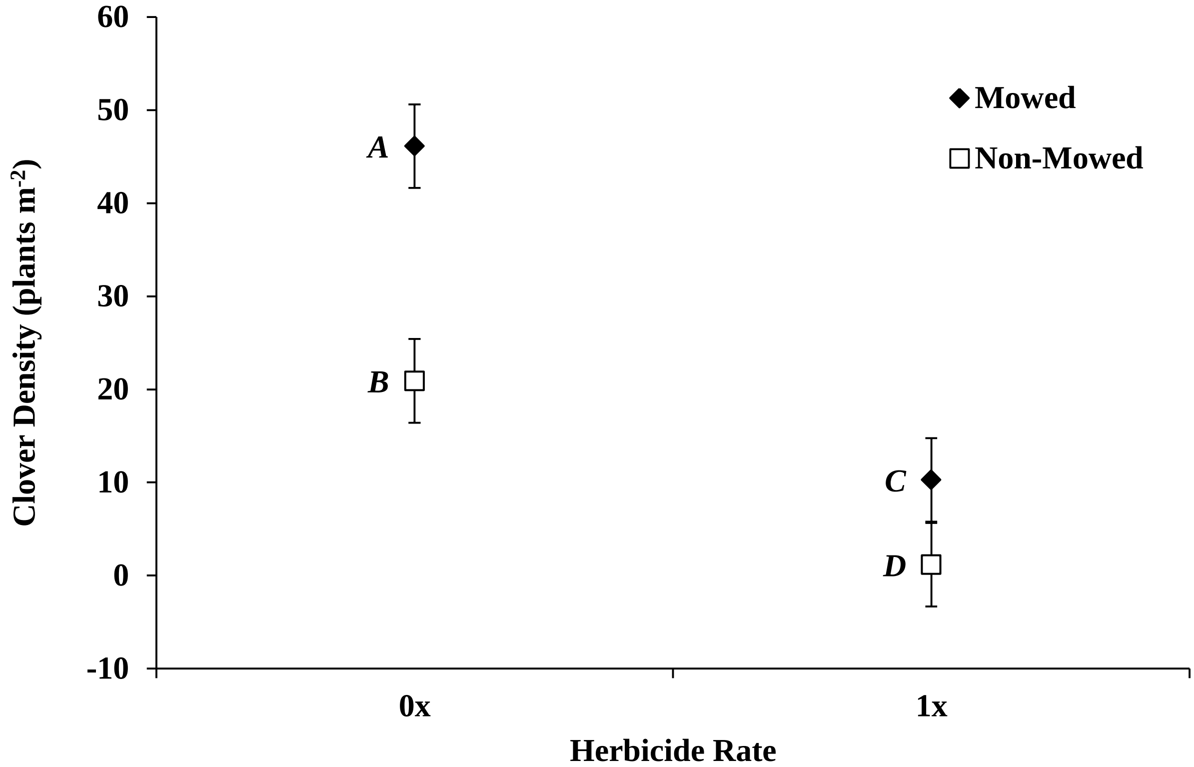

Figure 4.2 Responses in mean (±SE) clover density (plants m^{-2} in clover seeded plots only) to the interaction of herbicide rate and mowing treatment. Means with different letters differ based on a Tukey HSD test (P≤0.05).

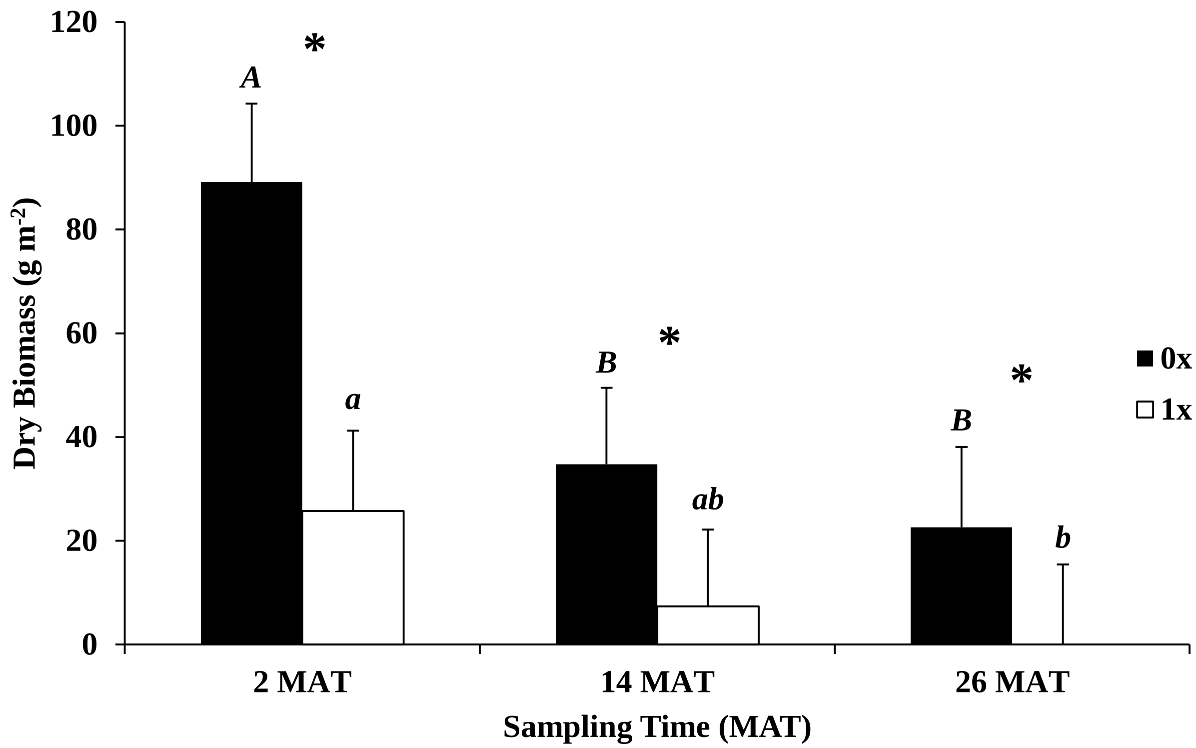

Figure 4.3 Mean (±SE) legume biomass (g m^{-2}) throughout the study period in 0x and 1x herbicide plots. Within a herbicide rate, means with different letters differ based on a Tukey HSD test ($P \leq 0.05$). Within sampling times, pairs of herbicide rates noted with an asterisk differ based on a Tukey HSD test ($P \leq 0.05$).

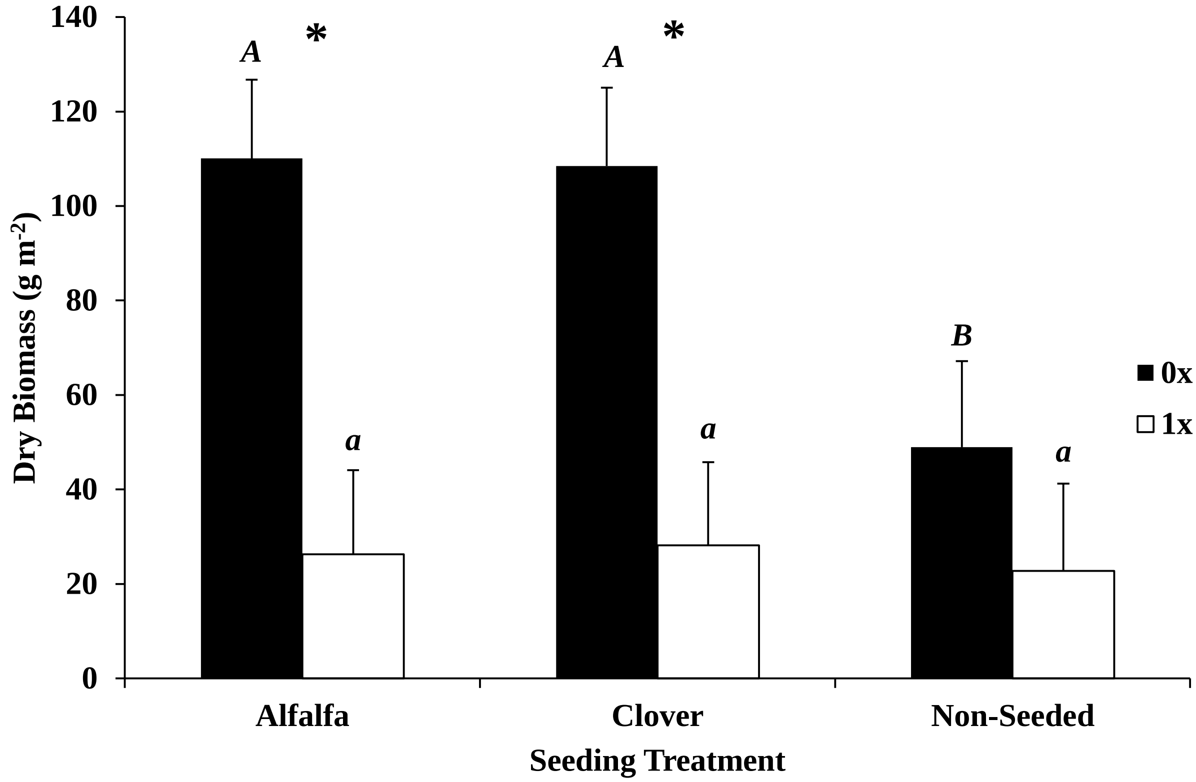

Figure 4.4 Seeding by rate effects on mean (±SE) legume biomass (g m^{-2}) in year one of the study (2 MAT). Within a rate, means with different letters differ based on a Tukey HSD test ($P \leq 0.05$). Within a seeding treatment, paired rates with an asterisk differ based on a Tukey HSD test ($P \leq 0.05$).

5. GREENHOUSE BIOASSAYS DEMONSTRATE HERBICIDE DEGRADATION IN NORTHERN TEMPERATE PASTURES

5.1 Introduction

Maintaining a legume component in pastures is an important objective for producers striving to maximize forage production. Alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.) are two legume species commonly utilized in hayfields and pastures of North America (Vogel et al. 1983). When combined with cool season grasses, legumes can increase the quality and palatability of forage, as well as the overall productivity of pastures (Groya and Sheaffer 1981; Kunelius et al. 1982; Seguin et al. 2001).

The ability to biologically fix atmospheric nitrogen into a readily available form (ammonia, NH_3) is one of the most positive attributes legumes impart to plant communities (Kunelius et al. 1982; Seguin 2001). Transfer of biologically fixed nitrogen to neighboring grasses increases overall pasture production and reduces or eliminates the need for nitrogen fertilizer (Kunelius and Campbell 1984; Frame 2005). As fertilizers are needed to maintain maximum productivity of cool season grasses, the inclusion of legumes reduces forage production costs (Olsen et al. 1981; Vogel et al. 1983; Popp et al. 2000). Legumes also tend to be greater in crude protein content than grasses, and are more palatable to livestock, characteristics that increase forage intake and improve animal gains (Groya and Sheaffer 1981; Kunelius et al. 1982; Kunelius and Campbell 1984; Merou and Papanastasis 2009).

Retention of legumes in pastures has the added benefit of stabilizing forage production both inter- and intra-annually, resulting from differences in the relative amount and timing of yield contributions between grass and legume species (Groya and Sheaffer 1981; Seguin 1998; Sleugh et al. 2000; Katepa-Mupondwa et al. 2002). By increasing plant community diversity, inclusion of legumes increases community resistance to weed invasion, improves recovery to disturbance (including drought), and prolongs stand longevity when compared to grass or legume monocultures (Sleugh et al. 2000; Sanderson et al. 2005). Despite the many benefits of legume incorporation, retention can be a major challenge, particularly in forage swards containing problematic weeds. Within the latter, legumes are often removed, in whole or in part, when herbicides are used for broadleaf weed control (Grekul and Bork 2004; Enloe et al. 2007).

Control of weeds, specifically noxious weeds, is a legal requirement in most jurisdictions of North America (including Alberta), and mandates that producers control noxious weed populations and take measures to prevent their spread (Province of Alberta 2008). Many problematic pasture weeds are broadleaf species (*Cirsium arvense* L.*, Ranunculus acris L..*, *Sonchus arvensis* L., etc.) that can be effectively reduced through the application of broadleaf herbicides (Grekul et al. 2005), which in turn, increases forage production (Bork et al. 2007). Although herbicides are a readily available and effective solution to weed control, an unintended and undesirable consequence to broadleaf herbicide use is that legumes such as alfalfa and white clover are also reduced or removed. This consequence places producers in the difficult position of deciding between obligations to control problematic weed species, and risking losses in productivity and quality within pastures containing legumes for an indeterminate time.

Recommendations for legume reseeding following herbicide application are either unavailable or specify a bioassay (USEPA 2010; DowAgrosciences 2012). Soil dissipation studies for AMP prior to registration were limited to two locations, each in the southern USA (USEPA 2005). The estimated half-life of 34.5 days reported from these trials was at variance with laboratory reports of 31.5 to 533.2 days. AMCP half-life was indicated between 22 and 126 days in pre-registration dissipation studies while AMP and AMCP half-lives of 28.9 and 32.5

days, respectively, were reported for field trials in Colorado (USEPA 2010; Lindenmayer 2012). Half-life data give insight into rates of herbicide breakdown, but these data are not adequate to estimate a withdrawal period for pasture and range systems in Alberta.

Areas with shorter growing seasons, colder average temperatures, lower soil fertility, and reduced precipitation, are likely to have slower degradation rates and longer soil half-lives (Vicari et al. 1994; Hall et al. 2009). Rates of bioactive degradation in northern temperate regions, including those of the Parkland in central Alberta, are unknown, but are likely longer than in warmer regions. The Parkland is a relatively productive agricultural area, and has been subject to high levels of development in comparison to other regions of Alberta (Natural Regions Committee 2006). Several characteristics of the Parkland create a favourable environment for arable agriculture, most notably the high soil organic matter and favorable growing season precipitation. Regional soils are primarily fertile Black Chernozems (Soil Classification Working Group 1998). These soil characteristics favorable for bioactive degradation (i.e. favorable soil chemistry and microbial activity) are accompanied by a short growing season and relatively low temperatures (2.5^{o}C annual average) (Natural Regions Committee 2006), which are likely to lead to longer associated withdrawal periods for bioactives such as AMP and AMCP in the Parkland, at least relative to warmer climates due with differing environmental conditions (Mikkelson and Lym 2011).

To maximize forage productivity and economic benefits for livestock producers during the relative short, rapid growing season of the Central Parkland, legumes should be reintroduced as quickly as possible following herbicide application. However, for this to take place, an understanding of the specific herbicide degradation rates of common bioactives, and the amount of herbicide residues that plants can tolerate, is necessary. Identification of the minimum withdrawal period needed prior to successful legume reintroduction is pivotal in both understanding the long-term impact of herbicides on sward composition dynamics, as well as identifying beneficial management practices (i.e. interseeding) that may facilitate legume recovery.

Bioassays performed using soils gathered from field sites treated with herbicide are an excellent way to isolate biological responses of specific plants (bioindicators) to stress factors in the environment (Strachan et al. 2011). The effects of herbicide residues on relevant bioindicators, typically plants with known sensitivity to herbicide, including legumes, provide relevant information for practitioners (Horowitz 1976; Sekutowski 2011). Using soils collected at varied time intervals following herbicide application allows for the identification of optimal withdrawal periods to facilitate recropping. Withdrawal periods represent the point where herbicide residues have reached a level where they no longer induce phytotoxic responses in bioindicator plants, signifying that reseeding (of legumes in this case) can be undertaken successfully (Sekutowski 2011).

We used soil samples taken at progressively longer intervals from field plots following one-time herbicide application to perform greenhouse bioassays for two common legume species (alfalfa and white clover). This was to evaluate the long-term effect of broadleaf herbicide residues on legume emergence and inferred seedling survival within northern temperate pastures. The main objective of this study was to indirectly quantify the degradation rates of two different herbicide bioactives (AMP and AMCP), by monitoring the emergence and survival of clover and alfalfa plants grown in field soils. In doing so, this information was expected to result in withdrawal period guidelines for soils treated with these bioactives.

5.2 Materials and Methods

5.2.1 Soil Sampling

Greenhouse bioassay trials were conducted using soil collected at various intervals from field plots sprayed with herbicide. Plots were sprayed June 21st 2010 at the St. Albert, Stony Plain, and Fort Saskatchewan sites, June 22nd 2011 at Glenpark, and July 2nd 2011 at Lamont using a 2 m handboom and CO_2 backpack sprayer delivering 100L ha^{-1} of herbicide solution, at a rate of 120g a.i. ha^{-1} AMP or 60g a.i. ha^{-1} AMCP. Duplicate soil cores (10 cm wide x 10 cm deep) were extracted from non-seeded subplots in mowed portions of each replicate block (Figure 5.1) within each site (total n=16 plots per site). See Chapter 4 for a detailed review of the methods associated with the field plot experimental design. Subplots were sampled at one and six weeks following herbicide application, and again in May and September of the two subsequent years, for a total of 6 sampling times per plot. Following extraction, cores were promptly frozen to arrest herbicide degradation.

Soil samples were collected for each site in the year of establishment to provide baseline information on physical site characteristics. Soil samples were taken at depths of 0-15, and 15-30 cm from 10 points evenly spaced along a W-shaped grid as outlined by Thomas (1985) across each site. Prior to analysis these samples were pooled and assessed for soil texture (% sand, silt and clay), organic matter (%), pH, electrical conductivity (salinity) (µS cm^{-1}), total carbon (%), total nitrogen (%), and available nitrogen (NH_4 + NO_3 mg kg^{-1}), using the methods outlined by the Canadian Society of Soil Science (Carter and Gregorich 2008). Finally, soil pits were dug at each site to describe the soil profile and identify the soil type (Table 5.1; Appendix C).

5.2.2 Experimental Design and Treatments

Bioassays were subsequently run using a fully randomized block design in the greenhouse. Separate bioassay trials were performed for soil taken at each interval following herbicide application (i.e. 1 week, 6 weeks, 11 months, 15 months, 23 months, and 26 months after spraying) from each site.

Eight different soil treatments were incorporated into the bioassay trials: four field treatments and four greenhouse 'controls'. The four field treatments were comprised of both AMP and AMCP at both 0x and 1x rates. Four additional greenhouse treatments were incorporated for comparison to biomass responses from the field data, also comprised of AMP and AMCP at 1x rates, with two additional 0x controls included, one for each bioactive, to maintain full randomization. The latter four greenhouse 'controls' were conducted using soil sourced from the University of Alberta Ellerslie research station, a highly fertile (16.6% organic matter – Table 5.1) loamy Black Chernozemic soil with high agricultural potential.

Each bioassay trial contained four repetitions of the field treatments and greenhouse control treatments, repeated within the four blocks of the bioassay trials for each site/field by sampling time combination. The complete list of all factors tested in each bioassay at each sampling time, is provided in Table 5.2. Additionally, each set of four treatment repetitions were conducted independently for each of two legume species: alfalfa and white clover. Two duplicates (i.e. cells) per herbicide treatment were seeded to alfalfa, and two were seeded to white clover, resulting in two sub-samples per seeding treatment. These sub-samples were used to obtain a mean value of response variables for analysis.

5.2.3 Soil Preparation & Planting Methodology

To prepare soil cores for use, frozen cores were thawed, large roots and debris removed by sifting, and cores mixed to produce a homogenous planting medium. Greenhouse control soils (1x rate) were sprayed with their respective bioactives using an indoor spray cabinet[1] at the University of Alberta, Agriculture-Forestry greenhouse. As with field cores, greenhouse control soils were sieved to remove large debris, and thoroughly mixed prior to use. Soil was placed in plastic bins to a depth of 10 cm and sprayed at 1x field rates with an application rate of 200 L ha^{-1}, using Air Bubble Jet 110015 nozzles[2], with the soil surface 50 cm from spray nozzles to mimic field spraying. Immediately following spraying, trays were sealed with plastic garbage bags to prevent herbicide volatilization. Prior to use in bioassays, soils were mixed in the same manner as field cores for uniform herbicide distribution.

After soil was placed in cells, three seeds of either white dutch clover[3] (Common #1) or alfalfa[3] (cv. Algonquin, Certified #1) were planted per cell (tests indicated overall germination rates were 87.2% for alfalfa and 91.7% for clover). Following planting, cells were covered with a thin layer of vermiculite to reduce evaporative losses. Cells were lightly misted on a regular basis for the six week duration of the study.

Styroblocks[4] (10 x 18 cells) were used to partition bioassay treatments into 60 ml volume cells. Greenhouse conditions were regulated in regards to temperature and photoperiod. The greenhouse maintained a 16 hour photoperiod, set from 6:00am to 10:00pm. Temperatures were maintained between 17^{o}C (night) and 25^{o}C (day), even in the hottest periods of summer.

5.2.4 Data Collection

Seedling emergence and density were measured for each cell once a week for the duration of the six week trials. Volunteer weeds were counted and removed on a weekly basis at the time of data collection. At the end of the trial (6 weeks after planting), seedlings were clipped at soil level within each cell to assess biomass. Harvested biomass from each cell was placed in labelled envelopes, dried at 60^{o}C for 48 hrs, and weighed. Dry weights were recorded for the total number of seedlings per cell to the nearest 0.0001 gram, and data relativized to mg per cell.

5.3 Analysis

To evaluate the relationship between herbicide application and legume recovery over time, a generalized linear mixed model (GLIMMIX) analysis of variance (ANOVA) with a repeated measures design was performed. Repeated measures were used to account for the lack of independence associated with sampling the same experimental unit (i.e. field plot) over time. The GLIMMIX procedure in SAS 9.2 (Statistical Analysis Software, 2008) was used to evaluate significant effects and interactions. Prior to analysis, data on the field plots were expressed as a percent of the greenhouse controls to remove disparities in density and biomass due to environmental influences arising from greenhouse trials run at different times. Controls were calculated as the average of the 0x greenhouse control for a particular herbicide (AMCP or AMP) for that particular bioassay trial. Both study site and replicate block were maintained as random factors in the analysis, while herbicide type, rate, and time interval following herbicide application were fixed factors. Each legume species was analyzed separately.

Density and biomass data (clover and alfalfa) were also tested for normality using the Kolmogorov-Smirnov statistic in Proc UNIVARIATE (SAS Institute Inc. 2008) prior to analysis, and found to be non-normal (P<0.10) and heteroscedastic. Efforts were made to transform the data, and although the assumption of normality could not be met, homogeneity of variance was satisfied (P>0.05) according to a Levene's test after undergoing a square root transformation. The GLIMMIX procedure was chosen to deal with the non-normal nature of the data. Several distributions are available in the GLIMMIX procedure to help analyze non-normal data, and attempts were made to fit density and biomass data (both transformed and non-transformed) to one of the distributions. Data did not fit any of the recognized distributions according to Chi-squared goodness-of-fit statistics (P<0.05). Consequently, the closest fitting distribution (normal/Gaussian) was used for the analysis of square root transformed data. Non-parametric procedures were considered, but the loss of descriptive power was deemed detrimental to the study, and data were therefore run without satisfying all statistical assumptions. This is justifiable as all other options were exhausted and a loss of descriptive power was deemed undesirable. Professional statisticians were consulted in coming to this conclusion (Goonewardene 2012, personal communication). There were differences between site, and although they were significant between some sites, a desire for broadly applicable results led to the grouping of sites. Finally, a Tukey honest significance difference (HSD) test was used to adjust for multiple comparisons among lsmeans for all significant treatment effects.

5.4 Results

5.4.1 Alfalfa

Final alfalfa density (density at the end of six week trials), expressed as a percent of greenhouse controls, was affected (P<0.05) by herbicide rate, time since spraying, and their interaction (Table 5.3). While no difference existed in alfalfa density over time within plots sprayed at the 0x rate, marked changes were apparent in alfalfa density within plots sprayed at the 1x rate (Fig. 5.2). Alfalfa density shortly after spraying remained less than 20% (P<0.0001) of that in the 0x treatment. While the density of alfalfa increased from that time onwards it remained below that of the 0x treatment (P<0.05) both 6 weeks after treatment (WAT) and 11 months after treatment (MAT) (Fig. 5.2). Alfalfa densities in the 1x sprayed plots were similar to those of the 0x treatment by 15 months after treatment.

A similar response was observed in alfalfa total dry matter biomass, with influences of herbicide rate, sampling time, and their interaction (Table 5.3). Like densities, alfalfa biomass in cells sprayed at the 1x rate remained below those of the 0x treatment during the first 3 sampling times, including 1 WAT, 6 WAT, and 11 MAT (Fig. 5.4). However, progressive increases in alfalfa biomass in the 1x treatment during the first 15 months led to similar biomass values to those in the 0x treatment from 15 MAT onward. Alfalfa biomass (as a % of greenhouse controls) also spiked in the final sampling time (27 MAT) in all plots, regardless of herbicide rate (Fig. 5.4). Responses to herbicide resides were noted following germination, where plants became chlorotic, exhibited leaf cupping and epinastic twisting, and in cases of high herbicide residue levels, death.

Finally, herbicide type and the interaction of herbicide type by sampling time also had an effect on alfalfa biomass (Table 5.3). Alfalfa total dry biomass (expressed as a percent of greenhouse control) was lower in AMCP treatments (55.5%±12.3) than in AMP treatments (77.8%±12.3) (P=0.001). Closer examination of the interaction of herbicide type by sampling time however, revealed the only difference ($P \leq 0.05$) in alfalfa biomass between herbicide types was found in bioassays performed using soils collected 26 months after herbicide application, and only at the 0x rate. The absence of differences between herbicides types actually sprayed at the 1x rates (AMP: 120g a.i ha^{-1} AMCP: 60g a.i. ha^{-1}) suggest these differences reflect natural variation in the environment rather than herbicide induced responses.

5.4.2 Clover

Clover responses closely paralleled those of alfalfa. Final clover density, expressed as a percent of greenhouse controls, was affected by herbicide rate and sampling time, as well as their interaction (Table 5.4). Clover density increased over time (P<0.0001) from an average of 47.9% of greenhouse controls (±8.0 SE) 1 WAT, to 103.8% of greenhouse controls (±8.0 SE) in trials using soils collected 26 MAT. Marked reductions in clover density were apparent in 1x herbicide treatments, particularly 1 WAT (Fig. 5.3). Despite some recovery in clover density within the 1x treatment at 6 WAT, clover densities remained significantly lower in the 1x treatment at 11 MAT. Clover densities were similar at 15 MAT and beyond with and without herbicide application (Fig. 5.3).

Although the main effect of herbicide type did not affect clover density, the interactions of herbicide type and sampling time, herbicide type and rate, as well as the three-way interaction between herbicide type, rate and time, all had effects on clover density (Table 5.4). The interaction between herbicide type and rate revealed that differences due to spraying were found largely between the 0x and 1x AMP treatments (91.9%±7.8 vs. 68.9%±7.7, respectively), while no difference existed between 0x and 1x AMCP treatments (82.3%±7.7 vs. 70.9±7.8, respectively). In addition, differentiation of clover densities between herbicide types occurred only at the 1 WAT sampling time (P=0.02), and only in the AMCP treatment (P<0.0001), thus accounting for the 3-way interaction (Table 5.5).

Clover biomass, expressed as a percent of control, responded to herbicide rate and type, and interactions of both rate and herbicide type with sampling time (Table 5.4). Clover biomass increased over time (P<0.0001) from an average of 54.7% of greenhouse controls (±22.3 SE) in soils collected 1 WAT, to an average of 283.6% of greenhouse controls (±24.1) in soils collected

26 MAT. Clover biomass showed responses to herbicide rate (*P*<0.0001), however, significant differences (*P*≤0.05) were limited to samples collected 1 and 6 WAT, as well as 11 MAT, but not thereafter (Figure 5.5). Significant differences in clover biomass were also noted between consecutive sampling periods in the 1x treatments, with progressive increases in clover biomass from trials run using 1 WAT soils to those done on soils removed 26 MAT. Responses to herbicide resides were noted following germination, where plants became chlorotic, exhibited leaf cupping and epinastic twisting, and in cases of high herbicide residue levels, death.

Herbicide type (AMCP vs. AMP) showed significant effects on clover biomass (*P*=0.001), where AMCP treatments had an average of 96.1% of greenhouse controls (±21.1 SE), and AMP an average of 129.1% of greenhouse controls (±21.1 SE), pooled over rates and times, which includes 0x and 1x treatments over all sampling periods. Differences in clover biomass between herbicide types were found specifically in bioassays performed with soils collected 26 MAT, where AMCP treatments had an average of 218.6% of the greenhouse controls (±27.2 SE) and AMP an average of 348.6% of greenhouse controls (±27.2). To further investigate this effect differences between herbicide type at the 1x rate over time were examined: there was no separation between AMCP and AMP treatments at the 1x rate at any of the sampling times run, indicating that differences in herbicide type were attributable to rate differences rather than true differences between the herbicide bioactives.

5.5 Discussion

Consistent recovery was observed in both alfalfa and white clover, as determined by plant density and biomass, by 15 MAT. Favorable density and biomass responses are encouraging, as they are indicative of the collective impacts of plant germination, emergence and survival, as well as individual plant vigor and associated forage productivity. In contrast, given the incomplete recovery during fall and the first spring following herbicide application, producers should not expect legumes to return in appreciable amounts during these times. However, it should also be noted that reestablishment as high as 16.4% were noted the following spring (11 MAT), and could therefore result in substantial legume presence.

Full recovery of 1x treatments to 0x levels by 15 MAT indicate that legume reestablishment, either through seeding or volunteers from the existing soil seed bank, should not be expected prior to the second fall following herbicide application. As such, seeding to reintroduce legumes to pastures should not be attempted until this time at the earliest. Legumes can be reintroduced using a variety of methods, including but not limited to: broadcast seeding (i.e. oversowing), (sod) interseeding, recovery from the natural seedbank, and complete tillage with reseeding.

There are limited bioassay studies regarding the withdrawal periods for AMCP and AMP. Full degradation of AMP applied at 120g a.i. ha^{-1} (same rate as the current study) was noted between 4 and 16 weeks after application in bioassays using soybeans as the bioindicator (Edwards 2010). These rapid degradation rates may be a function of the climatic conditions found in the soybean study. Situated in Kentucky, that area was subject to higher temperatures and precipitation levels than those of the Parkland in central Alberta. A more comparable field study conducted in Fargo, North Dakota, found alfalfa showed damage when seeded 8-11 months after AMP treatment (120 g a.i. ha^{-1}), but not when seeded 20-23 MAT (Mikkelson and Lym 2011). It is difficult to know if the slower degradation in North Dakota compared to Kentucky is due to the change in growing conditions, specifically drier, cooler conditions in the former location, or greater susceptibility of alfalfa than soybean to AMP. In any case, the withdrawal period of 20-23 months is significantly longer than the recovery period of 15 MAT found in the present bioassay experiment. While this may be explained by lower soil moisture and organic matter, coupled with reduced microbial activity, differences between studies may also arise due to lack of testing between the specified testing periods of 8, 11, 20, and 23 MAT. The lack of testing near the 15 MAT interval, the critical period of withdrawal in our study, may have resulted in the discrepancy between these studies. AMCP and AMP have both been shown to provide control of Canada thistle for up to 14 MAT at sites in Colorado, suggesting these bioactives have residual effects on susceptible plants for more than a year (Lindenmayer et al. 2009). Alfalfa and white clover establishment are reduced when planted in the spring following AMP application, and phytotoxic responses have been noted one year after treatment with AMCP (Renz 2010; Strachan et al. 2011).

Poor recovery of legumes during the first year of the present study (i.e. 1 and 6 WAT) was expected due to high levels of residual herbicide bioactive and the short period of the growing season available for bioactive degradation. However, little to no additional recovery in either legume species was noted from the first fall after treatment to the following spring, and suggests that limited herbicide degradation occurred over the winter months. This is not overly surprising as herbicide decay rates tend to decrease with cooler temperatures. Degradation of AMCP has been found to increase with temperature, with half-lives averaging less than 20 days in warmer sites on the Northern Great Plains (Conklin and Lym 2013). Aminopyralid has 2-8 times faster degradation rates at soil temperatures of 24^{o}C than 8^{o}C: soil temperatures are likely to be well below these levels in central Alberta from October through May, and are also frozen for 4 months or more during the winter months (Mikkelson 2010). Similar reductions in herbicide degradation under cooler temperatures have been found for 2,4-D and imazethapyr (Goetz et al. 1990; Veeh et al. 1996). Consequently, a significant portion of the increase in legume recovery during the ensuring growing season is likely to be associated with warmer soil temperatures from May through September.

In addition to temperature, herbicide degradation rates also increase directly with soil organic matter content and associated microbial activity (Ou 1984; Veeh et al. 1996; Picton and Farenhorst 2004), high soil temperatures which assist in biochemical breakdown (Walker and Zimdahl 1981; Ou 1984; Goetz et al. 1990; Veeh et al. 1996;), higher soil moisture levels (Walker and Zimdahl 1981; Parker and Doxtader 1983; Ou 1984; Goetz et al. 1990), as well as higher soil pH levels (Loux and Reese 1992; Aichele and Penner 2005). Auxinic herbicides, such as AMP and AMCP, are degraded mainly by soil microbes, and consequently, degradation of these bioactives is likely to increase with warmer soil temperatures during mid-summer, further accounting for the large improvement in legume recovery by the end of the second growing season. Increased soil organic matter content is associated with increased microbial populations, the mechanism of breakdown for auxinic herbicides (Veeh et al. 1996; Voos and Groffman 1997). This relationship explains why increased organic matter leads to increased herbicide decay rates (Veeh et al. 1996; Voos and Groffman 1997). Increases in both organic matter and associated microbial biomass has been shown to increase herbicide degradation, with specific examples apparent for 2,4-D (Ou 1984), diallate and triallate (thiocarbamate herbicides; Anderson 1984), and mixtures of 2,4-D and dicamba (Voos and Groffman 1997).

Moisture content is another factor known to regulate bioactive degradation. There are few studies assessing the impact of soil moisture on degradation of AMP or AMCP. AMCP degradation has been found to increase with soil moisture (Conklin and Lym 2013), and many other studies have found elevated moisture results in faster degradation of imazethapyr (Goetz et al. 1990), as well as atrazine, linuron and metolachlor (Walker and Zimdahl 1981). In the current study, soil moisture likely remained at favorable levels during most of the year, and given that precipitation peaks in June through August in central Alberta (Natural Regions Committee 2006), this would have favoured degradation of both bioactives during the growing season months.

Soil pH also plays an important role in herbicide degradation. Herbicides degraded by microbial activity show increased persistence with decreases in pH (Loux and Reese 1992; Aichele and Penner 2005). This phenomenon is a function of herbicide adsorption properties, where adsorption of herbicides to soil colloidal surfaces increases at lower pH levels, resulting in reduced accessibility for microbial degradation (Loux and Reese 1992; Aichele and Penner 2005).

Herbicide type did not have any significant impact on legume recovery, indicating that the two bioactives tested have similar impacts on legumes such as clover and alfalfa. Consequently, the safe recropping period for legumes is similar, if not the same, for AMP and AMCP, relative to both these legumes. This is not surprising, as both herbicides have related chemistries as auxinic herbicides in the pyrimidine carboxylic acid family, and both are degraded primarily by soil microbial action with similar half-lives, ranging from 31.5-533.2 days for AMP (USEPA 2005), and 22-126 days for AMCP (USEPA 2010). Additional studies have indicated half-lives of 28.9 and 32.5 days for AMP and AMCP respectively in Colorado field trials (Lindenmayer 2012).

Studies directly comparing AMP and AMCP residual effects are limited. AMCP has been shown to provide more effective residual control on spiny amaranth (*Amaranthus spinosus* L.) compared to AMP, with similar results for kudzu (*Pueraria montana* var. *lobata* (Willd.)) (Edwards 2010; Minogue et al. 2011). Combined, these studies indicate that AMCP should have longer residual effects than AMP. This was not the case in the present study, which might be due to differences in the susceptibility of bioindicator plants. Other explanations also exist, including that key differences in residual effects may have occurred between 11 and 15 MAT, and which were not captured by our sampling frequency in the second growing seasons.

Clopyralid and AMP have similar modes of action and half-lives (Bukun et al. 2010), although AMP has been shown to be more effective and have longer residual control on weeds like Canada thistle (Enloe et al. 2007). In bioassays performed using potatoes, sunflowers, soybeans, and lentils as bioindicators for clopyralid (280 g a.i. ha^{-1}), there were no negative effects seen in bioindicators planted 11 months or more after herbicide application in North Dakota soils (Thorsness and Messersmith 1991). These bioindicators are relatively different than legumes, but as auxinic herbicides target all broadleaf plants extrapolation is not out of the question. As clopyralid has relatively rapid degradation rates, it appears AMP would have withdrawal times shorter than 11 months for legumes such as clover and alfalfa.

No differences between alfalfa and clover recovery were noted in this study, with both legumes exhibiting the same recovery pattern relative to both herbicides. This indicates that recropping guidelines will be similar for the two legumes regarding AMP and AMCP application. Similarly, no differences were found in the establishment of white clover and alfalfa when planted the spring after AMP application (122 g a.i. ha^{-1}) in Wisconsin trials (Renz 2010). Alfalfa and clover have been shown to exhibit the same responses to picloram treatments as well, again suggesting that these legumes have similar susceptibility and recovery patterns to auxinic herbicides (Flater et al. 1974).

In terms of final legume densities and total dry matter biomass, Ellerslie control soils outperformed soils from the other field study (i.e. test) sites. This is likely due to the natural variation in soils evident among study sites, which on average had less favorable soils than the Ellerslie control soil. Ellerslie soils had higher levels of organic matter and higher nitrogen content, both factors which may increase bioactive degradation (Table 5.1). There was also one anomaly to this pattern seen in the 26 MAT bioassays for clover and alfalfa biomass, where field soils outperformed the greenhouse control soil. As the 26 MAT bioassays were run at the same time as the 15 MAT bioassays, greenhouse conditions were likely not to blame, leaving the mechanism for this deviation unknown.

Finally, it is important to note that this study may not have captured the full recovery dynamics arising between sampling periods. Thus, results should be interpreted within the context of the study sites assessed (across central Alberta) and the temporal resolution examined. Although these results are likely to be similar to, and thus representative of, other northern temperate regions, caution should be exercised in extrapolating beyond the area covered by the 5 study sites. Similarly, although precipitation during the years of study were representative of moisture conditions for the region (Appendix C), changes in precipitation and/or temperature may further alter herbicide degradation. Ultimately, herbicide degradation and legume recovery are dependent on many factors, from microsite variation to the effects of temperature and precipitation, all of which can change across the landscape and from year to year, representing a significant source of variance that may alter legume withdrawal intervals after herbicide application on a field by field basis.

5.6 Management Implications

Knowledge of withdrawal periods for residual herbicides can help producers mitigate the negative side effects of weed control on legume populations, and potentially develop management strategies to integrate weed control with the maintenance of beneficial legumes.

There is little information available regarding the withdrawal periods of AMP and AMCP in pasture and rangeland systems, and none for northern temperate grasslands of North America. This study attempted to address the lack of information regarding herbicide withdrawal periods necessary for legume recovery by examining the effects of soil residues on relevant bioindicator species, namely alfalfa and white clover.

Results indicate that legumes can be safely reintroduced into sprayed areas 15 months or more after treatment, a time period coinciding with the second fall after typical mid-summer herbicide application. This speedy rate of residue withdrawals may be due to an underestimation of herbicide effects by bioassays, likely due to the differences in greenhouse conditions relative to real field conditions (lack of competition, temperature, moisture, and light availability). Little functional difference was apparent in the withdrawal interval necessary for the two herbicide bioactives examined here (AMP and AMCP), nor between the different target legume species (alfalfa and white clover). However, caution should be exercised in extending these results to other herbicide chemistries, which may have longer or shorter withdrawal requirements. White clover and alfalfa recovery, both in terms of density and total dry biomass, followed the same pattern, with progressive signs of recovery from 1 WAT through 15 MAT, with no residual effects at the latter sampling time and beyond.

These findings suggest that while herbicides may reduce or eliminate legumes from existing forage swards, reintroduction is possible after a relatively short period of time. Based on our findings, successful legume reintroduction following the application of broadleaf herbicides is possible within two growing seasons under normal field conditions. This information should lead to improved guidelines for legume reestablishment, either following plough down and reseeding, as well as through overseeding or volunteer establishment following weed control, with corresponding economic benefits for producers.

5.7 Sources of Materials

[1]Indoor cabinet sprayer, Research Instrument MFG. CO. Ltd. Guelph, Ontario, Canada.

[2]Air Bubble Jet 110015 nozzles, ABJ Agri Products, Brandon, Manitoba, Canada.

www.abjagri.com

[3]Seed from Viterra, Fort Saskatchewan, Alberta, Canada.

www.viterra.ca

[4]Beaver Styroblocks, 4A-309A Superblocks. Stuewe & Sons, Tangent, Oregon, United States of America.

http://www.stuewe.com

5.8 Literature Cited

Bork, E. W., Grekul, C. W., DeBrujin, S. L. 2007. Extended pasture forage sward responses to canada thistle (*cirsium arvense*) control using herbicides and fertilization. Crop Prot. **26**(10): 1546-1555.

Bukun, B., Shaner, D. L., Nissen, S. J., Westra, P., Brunk, G. 2010. Comparison of the interactions of aminopyralid vs. cloyralid with soil. Weed Sci. **58**: 473-477.

Carter, M. R. and Gregorich, E. G., eds. 2008. Soil sampling and methods of analysis. second edition. Canadian Society of Soil Science.CRC Press.Taylor & Francis Group, Boca Raton, Florida.

Conklin, K. L. and Lym, R. 2013. Effect of temperature and moisture on aminocyclopyrachlor soil half-life. Weed Technol. (In-Press).

Dow AgroSciences. 2005. Aminopyralid technical bulletin. An introduction to aminopyralid. V45-313-001 (01/05) BOD. 010-50433. 19 pp.

Edwards, M. E. 2010. Spiny amaranth control and aminopyralid persistence in Kentucky pastures. M.Sc. Thesis, University of Kentucky, Lexington, Kentucky, United States of America. 85 pp.

Enloe, S. F., Lym, R. G., Wilson, R., Westra, P., Nissen, S., Beck, G., Moechnig, M., Peterson, V., Masters, R. A., Halstvedt, M. 2007. Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop areas. Weed Technol. **21**: 890-894.

Flater, R. L., Yarish, W., Vaartnou, H. 1974. Effects of picloram on germination and development of six crop species. Can. J. Plant Sci. **54**: 219-221.

Frame, J. 2005. Forage legumes for temperate grasslands. Science Publishers Inc., Enfield, NH, United States of America. 309 p pp.

Gist, G. R. and Mott, G. O. 1956. Some effects of light intensity, temperature, and soil moisture on the growth of alfalfa, red clover and birdsfoot trefoil seedlings. Agron. J. **49(1)**: 33-36.

Goonewardene, L. 2012. Personal communication. Biostatistician, AFNS Department, Faculty of ALES, University of Alberta, Edmonton, Alberta.

Grekul, C. W. and Bork, E. W. 2004. Herbage yield losses in perennial pasture due to Canada thistle (*Cirsium arvense*). Weed Technol. **18**(3): 784-794.

Grekul, C. W., Cole, D. E., Bork, E. W. 2005. Canada thistle (C*irsium arvense*) and pasture forage responses to wiping with various herbicides. Weed Technol. **19**: 298-306.

Groya, F. L. and Sheaffer, C. C. 1981. Establishment of sod-seeded alfalfa at various levels of soil moisture and grass competition. Agron. J. **73**: 560-565.

Hall, L., Beckie, H., Wolf, T. M. 2009. How herbicides work. biology to application. Kaulbars, C. and Vaillancourt, G., eds. 2nd ed. Alberta Agricuture and Rural Devlopment, Edmonton, AB. 134 pp.

Horowitz, M. 1976. Application of bioassay techniques to herbicide investigations. Weed Res. **16**: 209-215.

Katepa-Mupondwa, F., Singh, A., Smith Jr., S. R., McCaughey, W. P. 2002. Grazing tolerance of alfalfa (*Medicago* spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (*Bromus riparious* Rehm. syn. *B. biebersteinii* Roem & Schult). Can. J. Plant Sci. **82**: 337-347.

Kunelius, H. T. and Campbell, A. J. 1984. Performance of sod-seeded temperate legumes in grass dominant swards. Can. J. Plant Sci. **64**: 643-650.

Kunelius, H. T., Campbell, A. J., McRae, K. B., Ivany, J. A. 1982. Effects of vegetation suppression and drilling techniques on the establishment and growth of sod-seeded alfalfa and bird's-foot trefoil in grass dominant swards. Can. J. Plant Sci. **62**: 667-675.

Lindenmayer, R. B. 2012. Understanding aminocyclopyrachlor behavior in soil and plants. Ph.D. Thesis, Colorado State University, Fort Collins, CO. 82 pp.

Lindenmayer, B., Westra, P., Brunk, G. 2009. Selected invasive species control using aminocyclopyrachlor. Proc. West. Soc. Weed Sci. Abstr. **62**: 42.

Merou, T. P. and Papanastasis, V. P. 2009. Factors affecting the establishment and growth of annual legumes in semi-arid mediterranean grasslands. Plant Ecol. **201**: 491-500.

Mikkelson, J. R. 2010. Effect of aminopyralid on crop rotations and native forbs. M.Sc. Thesis, North Dakota State University, Fargo, ND. .

Mikkelson, J. R. and Lym, R. G. 2011. Aminopyralid soil residues affect crop rotation in north dakota soils. Weed Tech. **25(3)**: 422-429.

Minogue, P. J., Enloe, S. F., Osiecka, A., Lauer, D. K. 2011. Comparison of aminocyclopyrachlor to common herbicides for kudzu (P*ueraria montana*) management. Invas. Pl. Sci. and Manage. **4**: 419-426.

Natural Regions Committee. 2006. Natural regions and sub-regions of Alberta. Government of Alberta, 264 pp.

Olsen, F. J., Jones, J. H., Patterson, J. J. 1981. Sod-seeding forage legumes in a tall fescue sward. Agron. J. **73**: 1032-1036.

Ou, L. 1984. 2,4-D degradation and 2,4-D degrading microorganisms in soils. Soil Sci. **137**(2): 100-107.

Parker, L. W. and Doxtader, K. G. 1983. Kinetics of the microbial degradation of 2,4-D in soil: effects of temperature and moisture. J. Environ. Qual. **12**(4): 533-558.

Picton, P. and Farenhorst, A. 2004. Factors influencing 2,4-D sorption and mineralization in soil. J. Environ. Sci. & Health **39**(3): 367-379.

Popp, J. D., McCaughey, W. P., Cohen, R. D. H., McAllister, T. A., Majak, W. 2000. Enhancing pasture productivity with alfalfa: A review. Can. J. Plant Sci. **80**(3): 513-519.

Province of Alberta. 2008. Weed control act. Alberta Queen's Printer : W-5.1.

Renz, M. J. 2010. Establishment of forage grasses and legumes after fall herbicide applications. Forage and Grazinglands Available: doi:10.1094/FG-2010-0806-01-RS [March 10, 2013].

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., Goslee, S. C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J. **97**: 1465-1471.

SAS Institute Inc. 2008. SAS/STAT® 9.2 user's guide. SAS Institute Inc., Cary, NC.

Seguin, P. 1998. Review of factors determining legumes in sod-seeding outcome during pasture renovation in north america. Biotechnol. Agron. Soc. Environ. **2**(2): 120-127.

Seguin, P., Peterson, P. R., Sheaffer, C. C., Smith, D. L. 2001. Physical sod suppression as an alternative to herbicide use in pasture renovation with clovers. Can. J. Plant Sci. **81**: 255-263.

Sekutowski, T. 2011. Application of bioassays in studies on phytotoxic herbicide residues in the soil environment. Pages 253-272 *in:* Kortekamp, A., ed. Herbicides and environment. 1st ed. InTech, Rijeka, Croatia.

Sleugh, B., Moore, K. J., George, R., Brummer, E. C. 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agron. J. **92**: 24-29.

Soil Classification Working Group. 1998. The Canadian system of soil classification. Haynes, R. H., ed. 3rd ed. Agriculture and Agri-Food Canada, NRC Research Press, Ottawa, ONT, Canada. 187 pp.

Strachan, S. D., Nanita, S. C., Ruggiero, M., Casini, M. S., Heldreth, K. M., Hageman, L. H., Flanigan, H. A., Ferry, N. M., Pentz, A. M. 2011. Correlation of chemical analysis of residual levels of aminocyclopyrachlor in soil to biological responses of alfalfa, cotton, soybean and sunflower. Weed Technol. **25(2)**: 239-244.

Thomas, A. G. 1985. Weed survey system used in Saskatchewan for cereal and oilseed crops. Weed Sci. **33**(1): 34-43.

Thorsness, K. B. and Messersmith, C. G. 1991. Clopyralid influences rotational crops. Weed Technol. **5**(1): 159-164.

USEPA. 2010. Registration of the new active ingredient aminocyclopyrachlor for use on non-crop areas, sod farms, turf, and residential lawns. U. S. Environmental Protection Agency **Office of Pesticide Programs, Registration Division**.

USEPA. 2005. Environmental fate and ecological risk assessment for the registration of aminopyralid. Office of Prevention, Pesticides, and Toxic Substances.

Veeh, R. H., Inskeep, W. P., Camper, A. K. 1996. Soil depth and temperature effects on microbial degradation of 2,4-D. J. Environ. Qual. **25**: 5-12.

Vicari, A., Catizone, P., Zimdahl, R. L. 1994. Persistence and mobility of chlorsulfuron and metsulfuron under different soil and climatic conditions. Weed Res. **34**(3): 147-155.

Vogel, K. P., Kehr, W. R., Anderson, B. E. 1983. Sod-seeding alfalfa into cool-season grasses and grass-alfalfa mixtures using glyphosate or paraquat. J. Range Manage. **36**(6): 700-702.

Voos, G. and Groffman, P. M. 1997. Relationship between microbial biomass and dissipation of 2,4-D and dicamba in soil. Biol. Fertil. Soils **24**: 106-110.

Vough, L. R. and Marten, G. C. 1971. Influence of soil moisture and ambient temperature on yield and quality of alfalfa forage. Agron. J. **63**: 40-42.

Walker, A. and Zimdahl, R. L. 1981. Simulation of the persistence of atrazine, linuron and metolachlor in soil at different sites in the USA. Weed Res. **21**: 255-265.

Table 5.1 Physical site (i.e., soil) characteristics of long-term trials and bioassay source, as sampled in May of the year of establishment (2010 or 2011). All sites were on level ground with slopes <1%.

Site	Lat.-Lon.	Texture/Soil Type	N (%)	Available N (NH_4 + NO_3 mg kg^{-1})	C (%)	OM (%)	pH	EC (μs cm^{-1})
Fort Saskatchewan	53° 47' 18.94" N, 113° 20' 38.73" W	Silt Loam, Solodized Solonetz	0.3	5.5	3.6	9	7.1	482
Glenpark	53° 10' 38.58" N, 113° 24' 42.64" W	Silty Clay Loam, Gleyed Eluviated Black Chernozem	1.5	39.2	18.3	60.5	7.9	2580
Lamont	53° 44' 29.84" N, 112° 31' 43.28" W	Sandy Loam, Eluviated Black Chernozem	0.3	14.3	3.5	9	7.1	319.5
St. Albert	53° 41' 34.31" N, 113° 38' 5.40" W	Silty Clay, Eluviated Black Chernozem	0.4	7.8	4.5	16.4	8.4	467
Stony Plain	53° 27' 17.18" N, 114° 8' 12.42" W	Sandy Loam, Eluviated Black Chernozem	0.2	4.7	3.3	8.6	7.3	206.5
Ellerslie Bioassay	53° 25' 6.02" N, 113° 32' 29.79" W	Silt Loam, Eluviated Black Chernozem	0.5	32.4	6.5	16.6	7.4	352.5

[z]Values represent the average of 10 soil cores collected at 0-30cm depth.

[y]Latitude and longitude represent exact coordinates of each site.

Table 5.2 Summary of greenhouse soil bioassay tests performed in a full factorial design within each sampling time.

Site	Source	Herbicide	Rate	Block	Legume Spp.[z]	Sub sample
St. Albert	Field	AMP	0x	1	White clover	1
Stony Plain				2	Alfalfa	2
Glenpark	Greenhouse	AMCP	1x	3		
Fort Saskatchewan				4		
Lamont						

[z]Alfalfa and white clover responses include legume emergence/density and biomass.

Table 5.3 Summary of F-statistic and significance values associated with alfalfa density (plants/cell) and total dry matter biomass (mg/cell). Data represent a total of 26 greenhouse trials run for six different soil collection intervals.

	Alfalfa Density		Alfalfa Total Dry Biomass	
Treatment Effect	F-stat	P-value	F-stat	P-value
Sampling Time	8.2	**<0.0001**	28.5	**<0.0001**
Rate	35.9	**<0.0001**	70.70	**<0.0001**
Herb	0.02	0.90	10.90	**0.0011**
Time*Rate	10.6	**<0.0001**	9.3	**<0.0001**
Time*Herb	1.7	0.14	2.30	**0.048**
Herb*Rate	0.0	0.99	0.2	0.68
Time*Herb*Rate	1.40	0.21	1.3	0.27

Table 5.4 Summary of F-statistic and significance values associated with clover density (plants/cell) and total dry matter biomass (mg/cell). Data represent a total of 26 greenhouse trials run for six different soil collection intervals.

	Clover Density		Clover Total Dry Biomass	
Treatment Effect	F-stat	P-value	F-stat	P-value
Sampling Time	19.5	**<0.0001**	45.7	**<0.0001**
Rate	24.8	**<0.0001**	60.10	**<0.0001**
Herb	0.0	0.99	10.60	**0.0013**
Time*Rate	6.4	**<0.0001**	6.4	**<0.0001**
Time*Herb	4.8	**0.0003**	3.10	**0.0088**
Herb*Rate	5.4	**0.021**	0.0	0.97
Time*Herb*Rate	2.8	**0.017**	0.8	0.57

Table 5.5 Clover density responses to the three-way interaction between sampling time, herbicide type, and herbicide rate.

		Time Elapsed Following Herbicide Application					
Rate	Herbicide	1 WAT	6 WAT	12 MAT	16 MAT	24 MAT	28 MAT
0x	AMCP	67.5*a*[z]	69.6*a*	86.6*a*	94.1*a*	96.8*a*	79.3*a*
	AMP	76.4*a*	87.9*a*	95.2*a*	79.7*a*	88.2*a*	124.0*a*
	P-value	(1.0)[y]	(0.98)	(1.0)	(1.0)	(1.0)	(0.94)
1x	AMCP	43.7*b*	67.7*ab*	55.6*ab*	77.1*ab*	98.1*a*	83.1*ab*
	AMP	4.0*c*	60.6*b*	60.4*b*	75.9*ab*	83.2*ab*	129.0*a*
	P-value	(<0.0001)	(1.0)	(1.0)	(1.0)	(1.0)	(0.94)

[z]Letters indicate significance within treatments across sampling times, means with different letters differ ($P \leq 0.05$) based on a Tukey HSD test.

[y]P-values indicate significance between herbicide treatments within sampling time and rate.

	AMP - 0x	AMCP - 1x	AMP - 1x	AMCP - 0x
	ALF	NS	CLR	NS
Non-mowed	NS	ALF	NS	ALF
	CLR	CLR	ALF	CLR
	CLR	NS ★	ALF	ALF
Mowed	NS ★	CLR	CLR	NS ★
	ALF	ALF	NS ★	CLR

Figure 5.1 Example of a single replicate block at one of the study sites. Only starred plots were sampled for greenhouse bioassays (i.e. non-seeded and mowed subplots).

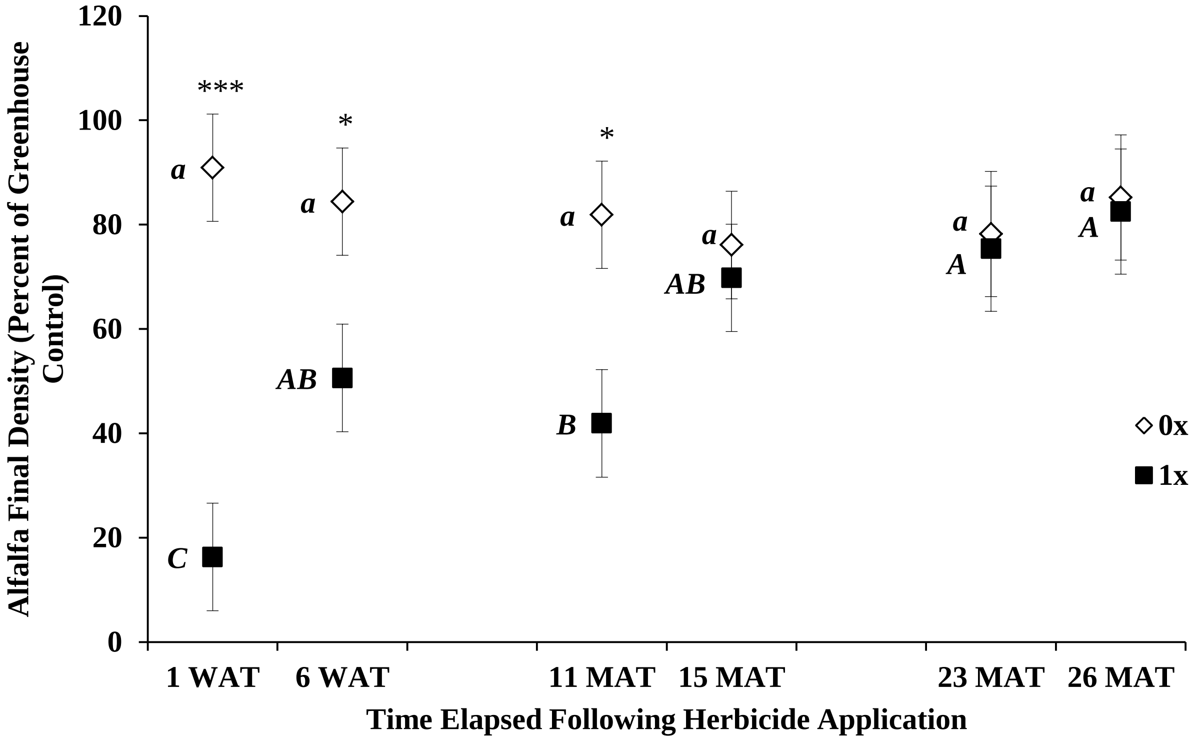

Figure 5.2 Rate by time interaction effects on final alfalfa density (expressed as a percent of greenhouse controls). Within a rate means with different letters differ based on a Tukey HSD test ($P \leq 0.05$), while asterisks indicate differences between rate means within a time (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.0001$). WAT designates 'weeks after treatment', and MAT 'months after treatment'.

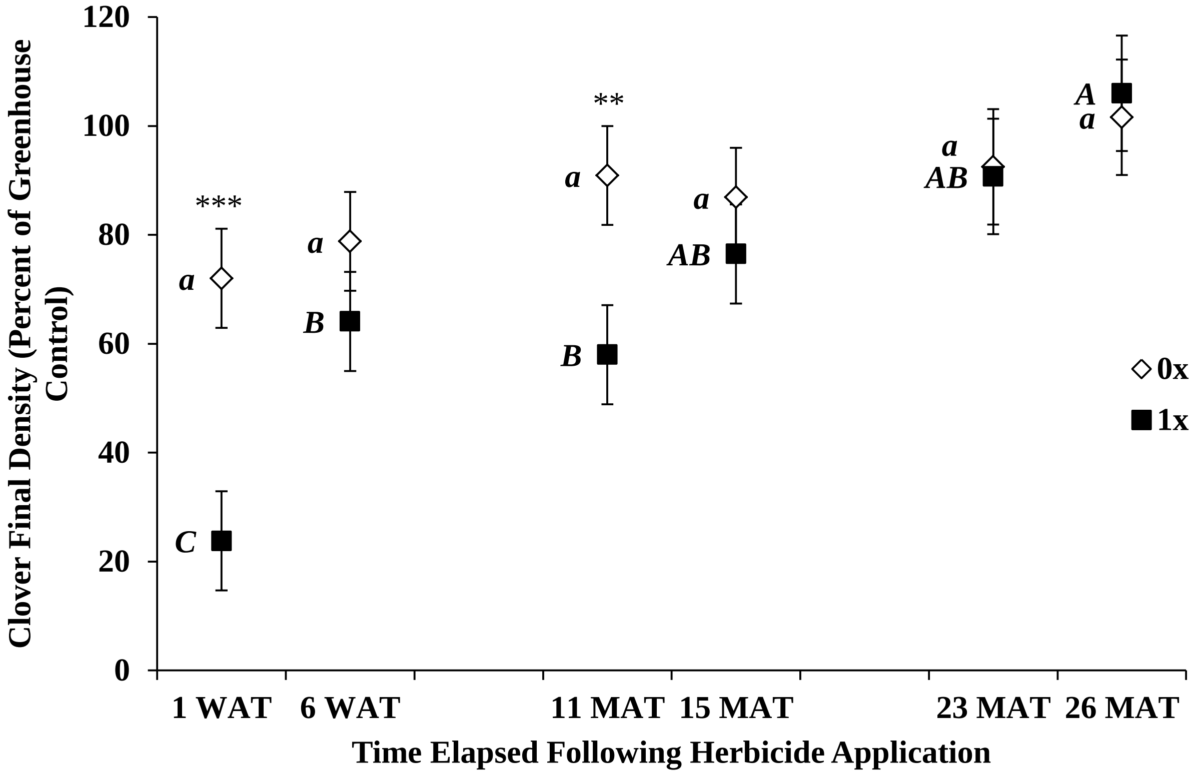

Figure 5.3 Rate by time interaction effects on final clover density (expressed as a percent of greenhouse controls). Within a rate means with different letters differ based on a Tukey HSD test ($P \leq 0.05$), while asterisks indicate differences between rate means within a time (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.0001$). WAT designates 'weeks after treatment', and MAT 'months after treatment'.

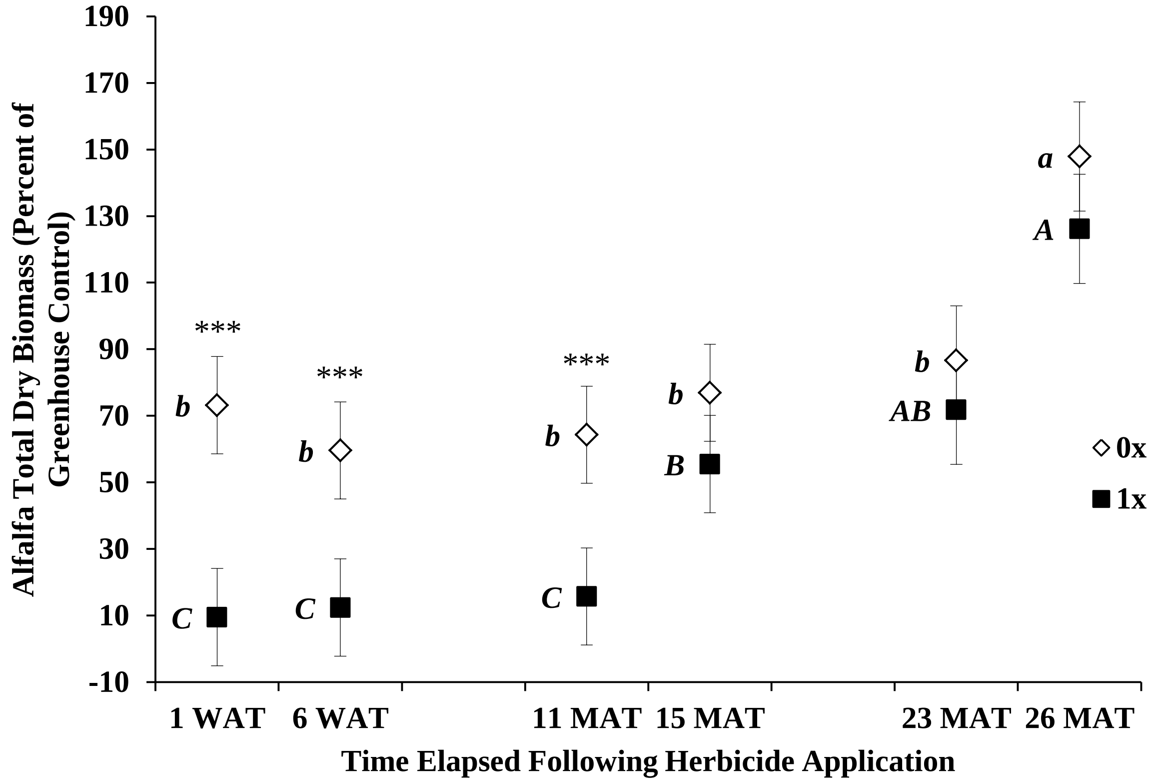

Figure 5.4 Rate by time interaction effects on alfalfa total dry biomass (expressed as a percent of greenhouse controls). Within a rate means with different letters differ based on a Tukey HSD test ($P \leq 0.05$), while asterisks indicate differences between rate means within a time (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.0001$). WAT designates ‘weeks after treatment’, and MAT ‘months after treatment’.

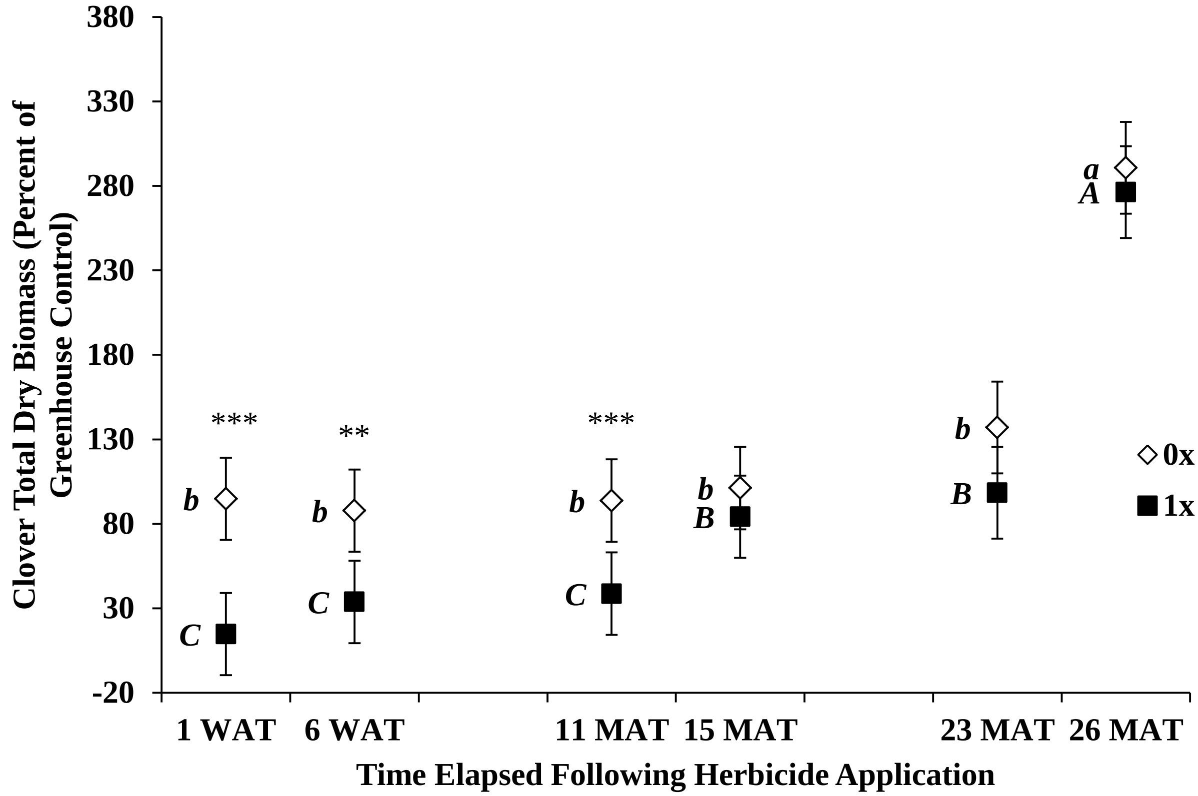

Figure 5.5 Rate by time interaction effects on clover total dry biomass (expressed as a percent of greenhouse control). Within a rate means with different letters differ based on a Tukey HSD test ($P \leq 0.05$), while asterisks indicate differences between rate means within a time (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.0001$). WAT designates 'weeks after treatment', and MAT 'months after treatment'.

6. SYNTHESIS

6.1 Research Summary

The integration of effective weed control and maintenance of beneficial legumes is a significant issue in northern temperate pastures, such as those of western Canada. Striking a balance between these two opposing facets of range and pasture management can be difficult, and ineffective reestablishment of legumes following herbicide application may result in economic losses for producers. Invasive weeds are ubiquitous in the agro-ecosystems of Alberta and may contribute to substantial forage yield losses (Grekul and Bork 2004). As a result herbicides are now a significant input into agricultural systems, contributing 20-30% of the input costs in cropping systems of North America (Derksen et al. 2002).

The negative effects of broadleaf herbicide use include the removal of legumes, which decreases forage productivity and quality (McLeod 2011). As the demand for sustainable forage production and herbicide use increases, legume removal will become increasingly problematic for producers. The intent of this research project was to quantify the withdrawal period of two broadleaf herbicide bioactives, aminocyclopyrachlor (AMCP) and aminopyralid (AMP), in northern temperate pastures of central Alberta, and to investigate the impacts of secondary factors, moisture, light, and defoliation, on legume recovery. Three different experiments, with several associated field sites and greenhouse trials, were used to address these objectives and fully capture the dynamics of legume recovery.

The short-term dose trials (Chapter 3) indicated that below-label herbicide application does not favour legume reestablishment in the short term (over one growing season). Several different rates of application were tested (6.25, 12.5, 25, 50, and 100% of label recommendations) and although better recovery was evident at the lowest rates of application, reestablishment was limited relative to the desired levels of legume reestablishment needed. Moreover, observed legume reestablishment was inadequate to justify the loss of weed control efficacy associated with such low use rates. Bioactive identity did not have any impact on legume reestablishment.

Long-term field trials (Chapter 4) indicated that herbicide application was the main factor determining legume abundance, and in the absence of application additional factors such as mowing and seeding affected legume density and biomass. Following herbicide application clover density recovered relative to 0x controls 24 months after treatment (MAT), while no recovery was seen for alfalfa, even up to 26 MAT. Clover recovery indicates that reseeding of legumes at 24 MAT, the second spring following herbicide application, may lead to clover establishment within these swards, although this recovery is likely to occur only if accompanied by defoliation, such as occurs under mowing, or potentially grazing. Although alfalfa did show the potential for recovery, this response was likely due to a combination of factors, including aggravating influences of vegetative competition from other forage plants, direct stress from mowing/defoliation, and autotoxic affects, in addition to herbicide application, which reduced alfalfa density.

Legume biomass also failed to reach control values over the long-term study period, which was likely due to the natural declines in legume populations seen over time in pastures (Kunelius et al. 1982; Kunelius and Campbell 1984). Despite this, total forage productivity increased, likely a short-term response resulting from compensatory responses to the removal of competition, and potentially exploitation by grass plants of the nitrogen flush associated with decay of legume plants (Groya and Sheaffer 1981; Haystead and Marriott 1979; Sanderson et al. 2005). Dandelion cover was also reduced by herbicide application, but recovered to preapplication levels the following growing season. Again, no difference was found between bioactives in legume density, biomass, or weed cover responses.

Greenhouse trials (Chapter 5) showed quicker recovery than the long-term field trials, with full recovery of alfalfa and clover densities and total legume dry biomass when grown on soils removed 15 MAT from long-term field trials. Again, no difference in legume response to bioactive identity was found. Notably, responses did not differ between clover and alfalfa, indicating these two legumes have the same functional responses to the two bioactives examined.

Differences in withdrawal periods, as evidenced by legume reestablishment, were seen between studies. Longer withdrawal periods were seen in field trials than in corresponding greenhouse bioassay. Bioactive type had indistinguishable effects on legume recovery, forage production, and weed dynamics.

6.2 Management Implications

This research indicates that successful legume reestablishment following herbicide application can be realized in central Alberta, allowing for effective weed control and the restoration of high-quality, productive, grass-legume pastures. Although there were conflicting results regarding withdrawal rates (15 vs. 24 MAT in the bioassay and field trials, respectively), it is safe to say that following a withdrawal period of approximately two growing seasons under average field conditions, producers can safely reseed legumes and expect little to no negative impacts from bioactive soil residues. This difference may have been due to an underestimation of herbicide effects in bioassay trials associated with differences between greenhouse and field conditions. Although legumes will be nearly absent during this withdrawal period, total forage production of the long-term trials indicate that total forage productivity may be maintained, potentially due to compensatory responses (Haystead and Marriott 1979; Groya and Sheaffer

1981; Sanderson et al. 2005). An understanding of withdrawal periods can allow for the development of legume reseeding guidelines, and observed forage productivity following herbicide application may put producers at ease as forage responses were neutral to positive following legume and weed removal.

Effects of secondary factors on legume recovery (moisture, light, and defoliation) in the absence of herbicide application were variable. Light was not a major moderator of legume recovery, but moisture availability was found to be positively correlated with legume plant density, as well as biomass, reaffirming that soil moisture is one of the main factors regulating legume establishment (Taylor et al. 1969; Vough and Marten 1971; Groya and Sheaffer 1981). While moisture is not typically a limiting factor for growth in the Parkland of Alberta, the importance of available moisture for legume establishment and survival emphasizes the importance of seeding at times with abundant moisture, such as spring.

Defoliation is another factor influencing legume recovery, depending on the species. Clover recovery in particular, was highly dependent on defoliation regime. This species is easily outcompeted by grasses due to its short stature and will eventually disappear from pastures devoid of defoliation. If clover is the legume species of interest for reestablishment, it must be seeded into an area subject to periodic defoliation, and will not persist beyond the establishment year if competing vegetation is allowed to overtop and suppress it (Frame 2005). Alfalfa on the other hand, is relatively intolerant to defoliation (Katepa-Mupondwa et al. 2002), and will drop out of areas with frequent and intense defoliation, making it more difficult to establish as an interseeded species, particularly because alfalfa will not establish in areas with competitive vegetative communities (Groya and Sheaffer 1981; Rioux 1994). In the case of this study, alfalfa density was not affected by mowing, with the exception of alfalfa in the short-term study where alfalfa increased in non-mowed plots, indicating that infrequent defoliation does not impact alfalfa reestablishment. This response is likely a result of the suppression of competing vegetation, which may compensate for the stresses on carbohydrate reserves on alfalfa plants imposed by defoliation stresses (Brummer and Moore 2000; Katepa-Mupondwa et al. 2002). These results indicate that white clover recovery will be more successful in areas frequently grazed, while alfalfa establishment is not as reliant on defoliation regime, although persistence will likely be reduced under frequent and intense defoliation (Groya and Sheaffer 1981; Rioux 1994; Katepa-Mupondwa et al. 2002).

Use of herbicides at below-label rates may be cost effective for producers, but the short-term dose trials indicate that legume reestablishment in the short-term is not enhanced by reductions in herbicide rate. This study shows that at least 4 half-lives are required to show increases in legume reestablishment (0.0625x application rate). Although reduced rate applications may have positive effects on legume reestablishment in the long term, producers should not expect to see legume reestablishment during the short-term, and may, depending on weed responses, lead to sub-standard weed control in the long-term.

Weed recovery dynamics are an important aspect of herbicide application, and also contribute to competition against legumes. Recovery of dandelions and legumes in field trials occurred at roughly the same time (22 MAT for dandelion, and 24 MAT for clover), indicating that legumes will likely be subject to competition from weedy species during reestablishment. This study indicated that dandelion abundance decreased drastically in non-mowed treatments, suggesting weed control can be prolonged in areas with infrequent defoliation. As white clover did not persist in non-mowed treatments, areas with persistent weed issues and a well-developed weed seed bank could be seeded to alfalfa and combined with a low intensity, low frequency defoliation regime to promote alfalfa establishment. Alfalfa persists well under infrequent defoliation, and weeds such as dandelion will likely show reduced recovery (or even declines) relative to areas with frequent defoliation.

Awareness of the implications of different management practices on legume recovery, and the intrinsic differences between legume species, should allow for the development of improved legume recovery guidelines for the Parkland region of Alberta. Legume recovery is dependent not only on the degradation of soil herbicide residues, but also the interactions between existing plants in forage swards and the availability of light and soil resources. Management practices should be tailored for the intended use of pastures, with consideration given to the defoliation practices, and type of legume best suited for them. This information should enable producers to make effective, informed decisions regarding legume reintroduction, allowing for productive pasture and rangeland systems in Alberta.

6.3 Future Research

Information obtained during the course of this study has added to our knowledge regarding the withdrawal periods of residual broadleaf herbicides in the northern temperate pastures of western North America. Future research investigating reseeding intervals following herbicide application in field trials may yield more consistent results in regards to legume recovery. Additional studies should also investigate the long-term effects of below-label herbicide use on legume recovery, with an eye on the efficacy of these reduced rates on weedy species. To discern when legume removal by herbicide application is justified, work on the ratio of legume to weedy species must be done to give producers an idea of when herbicide application is the best option for weed control. Moreover, an understanding of the weed densities needed to economically justify the removal of legumes via broadleaf herbicides would be advantageous for producers.

6.4 Literature Cited

Brummer, E. C. and Moore, K. J. 2000. Persistence of perennial cool-season grass and legume cultivars under continuous grazing by beef cattle. Agron. J. **92**: 466-471.

Derksen, D. A., Anderson, R. A., Blackshaw, R. E., Maxwell, B. 2002. Weed dynamics and management strategies for cropping systems in the northern great plains. Agron. J. **94**: 174-183.

Frame, J. 2005. Forage legumes for temperate grasslands. Science Publishers Inc., Enfield, NH, United States of America. 309 p pp.

Grekul, C. W. and Bork, E. W. 2004. Herbage yield losses in perennial pasture due to Canada thistle (C*irsium arvense*). Weed Technol. **18**(3): 784-794.

Groya, F. L. and Sheaffer, C. C. 1981. Establishment of sod-seeded alfalfa at various levels of soil moisture and grass competition. Agron. J. **73**: 560-565.

Haystead, A. and Marriott, C. 1979. Transfer of legume nitrogen to associated grass. Soil Biol. Biochem. **11**: 99-104.

Katepa-Mupondwa, F., Singh, A., Smith Jr., S. R., McCaughey, W. P. 2002. Grazing tolerance of alfalfa (*Medicago* spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (*Bromus riparious* rehm. syn. *B. biebersteinii* roem & schult). Can. J. Plant Sci. **82**: 337-347.

Kunelius, H. T. and Campbell, A. J. 1984. Performance of sod-seeded temperate legumes in grass dominant swards. Can. J. Plant Sci. **64**: 643-650.

Kunelius, H. T., Campbell, A. J., McRae, K. B., Ivany, J. A. 1982. Effects of vegetation suppression and drilling techniques on the establishment and growth of sod-seeded alfalfa and bird's-foot trefoil in grass dominant swards. Can. J. Plant Sci. **62**: 667-675.

McLeod, E. M. 2011. Forage responses to herbicide weed control in grass-legume swards. M.Sc. Thesis, University of Alberta, Edmonton, AB. 60 pp.

Rioux, R. 1994. Direct seeding of alfalfa in grain stubble and bromegrass sod. Can. J. Plant Sci. **74**(4): 773-778.

Sanderson, M. A., Soder, K. J., Muller, L. D., Klement, K. D., Skinner, R. H., Goslee, S. C. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J. **97**: 1465-1471.

Taylor, T. H., Smith, E. M., Templeton Jr., W. C. 1969. Use of minimum tillage and herbicide for establishing legumes in kentucky bluegrass (*Poa pratensis* L.) swards. Agron. J. **61**: 761-766.

Vough, L. R. and Marten, G. C. 1971. Influence of soil moisture and ambient temperature on yield and quality of alfalfa forage. Agron. J. **63**: 40-42.

APPENDICIES

Appendix A: Site Maps

Appendix A.1 Short-term study layout

	401b	402b	403b	404b	405b	406b	407b	408b	409b	410b	411b	412b
Non-mowed	***AMCP***	***AMCP***	***AMP***	***AMP***	***AMCP***	***AMP***	***AMP***	***AMCP***	***AMP***	***AMCP***	***AMP***	***AMCP***
Mowed	***1x***	***0.25x***	***0.0625x***	***0.25x***	***0.125x***	***0.5x***	***0.125x***	***0.0625x***	***Control***	***0.5x***	***1x***	***Control***
	401a	402a	403a	404a	405a	406a	407a	408a	409a	410a	411a	412a

REP 4

	301b	302b	303b	304b	305b	306b	307b	308b	309b	310b	311b	312b
Non-mowed	***AMCP***	***AMP***	***AMP***	***AMCP***	***AMCP***	***AMCP***	***AMCP***	***AMCP***	***AMP***	***AMP***	***AMP***	***AMP***
Mowed	***0.0625x***	***1x***	***Control***	***0.125x***	***0.25x***	***0.5x***	***Control***	***1x***	***0.0625x***	***0.5x***	***0.125x***	***0.25x***
	301a	302a	303a	304a	305a	306a	307a	308a	309a	310a	311a	312a

REP 3

	201b	202b	203b	204b	205b	206b	207b	208b	209b	210b	211b	212b
Mowed	***AMCP***	***AMP***	***AMCP***	***AMP***	***AMP***	***AMCP***	***AMCP***	***AMP***	***AMCP***	***AMP***	***AMCP***	***AMP***
Non-mowed	***Control***	***0.25x***	***0.5x***	***1x***	***0.125x***	***0.0625x***	***1x***	***0.5x***	***0.25x***	***0.0625x***	***0.125x***	***Control***
	201a	202a	203a	204a	205a	206a	207a	208a	209a	210a	211a	212a

REP 2

	101b	102b	103b	104b	105b	106b	107b	108b	109b	110b	111b	112b
Non-mowed	***AMP***	***AMCP***	***AMP***	***AMCP***	***AMP***	***AMP***	***AMCP***	***AMCP***	***AMP***	***AMCP***	***AMCP***	***AMP***
Mowed	***0.0625x***	***Control***	***0.125x***	***0.5x***	***0.5x***	***Control***	***0.125x***	***1x***	***1x***	***0.0625x***	***0.25x***	***0.25x***
	101a	102a	103a	104a	105a	106a	107a	108a	109a	110a	111a	112a

REP 1

Figure A.1 Example of short-term variable dose trial layout and mowing pattern.

Appendix A. 2 Long-term study layout

	AMP Control	AMCP 1x	AMP 1x	AMCP Control
	CLR	ALF	CLR	NS
Mowed	ALF	NS	NS	CLR
	NS	CLR	ALF	ALF
	ALF	CLR	ALF	NS
Non-mowed	NS	ALF	NS	CLR
	CLR	NS	CLR	ALF

REP 3

AMCP 1x	AMP 1x	AMP Control	AMCP Control	
CLR	ALF	ALF	NS	
ALF	CLR	NS	ALF	Mowed
NS	NS	CLR	CLR	
CLR	CLR	NS	NS	
NS	NS	ALF	CLR	Non-mowed
ALF	ALF	CLR	ALF	

REP 4

	AMCP Control	AMP 1x	AMCP 1x	AMP Control
	NS	NS	CLR	CLR
Mowed	ALF	CLR	NS	ALF
	CLR	ALF	ALF	NS
	CLR	CLR	ALF	CLR
Non-mowed	ALF	NS	CLR	ALF
	NS	ALF	NS	NS

REP 1

AMP Control	AMP 1x	AMCP 1x	AMCP Control	
ALF	ALF	CLR	NS	
NS	CLR	ALF	CLR	Non-mowed
CLR	NS	NS	ALF	
CLR	NS	NS	ALF	
NS	ALF	CLR	NS	Mowed
ALF	CLR	ALF	CLR	

REP 2

Figure A.2 Example of long-term trial layout and mowing pattern.

Appendix B: Meteorological Data

Appendix B.1 St. Albert

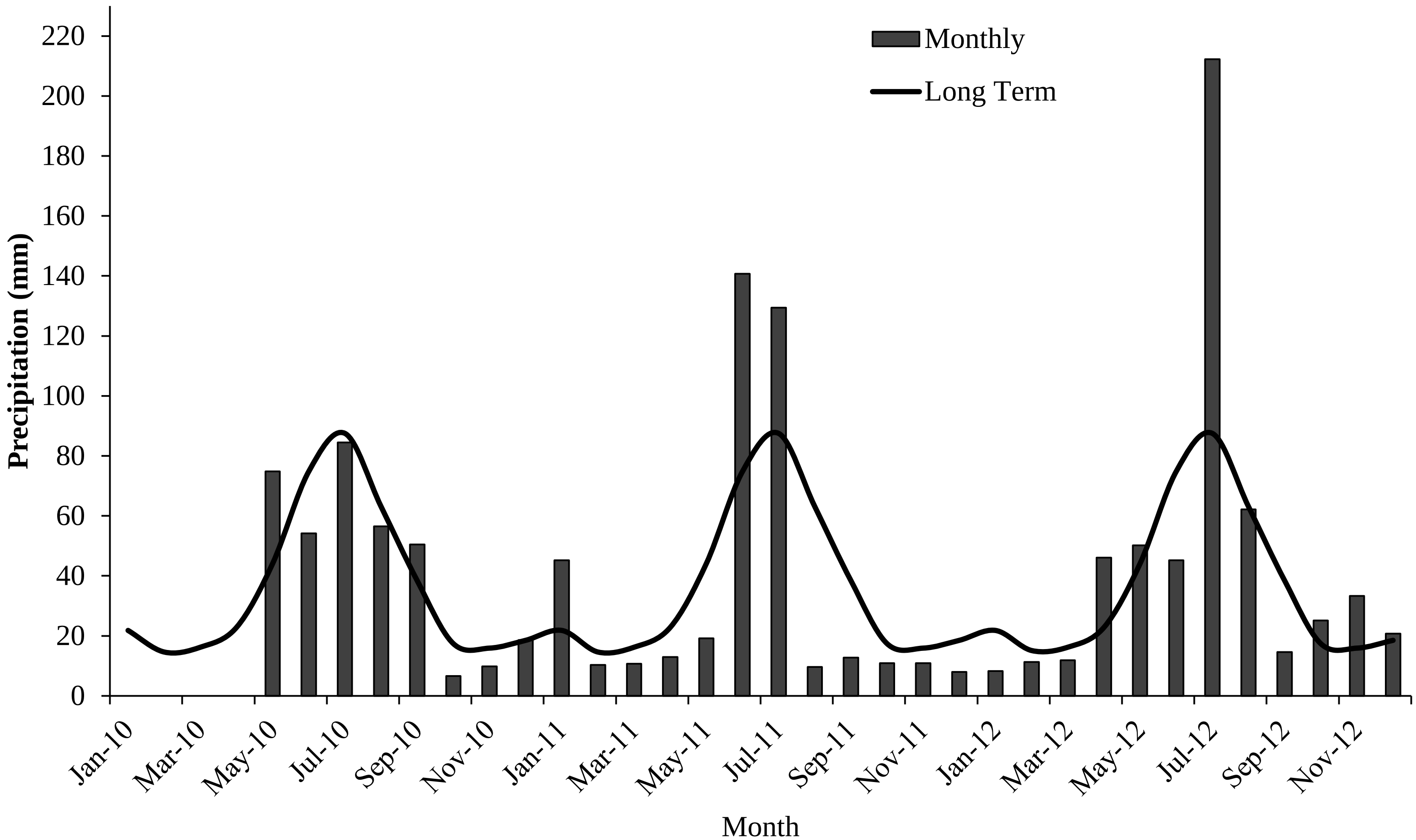

Figure B.1.1 Average monthly and long-term (30 year average) precipitation (mm) at St. Albert study site for 2010-2012. Data were not available for January to April 2010.

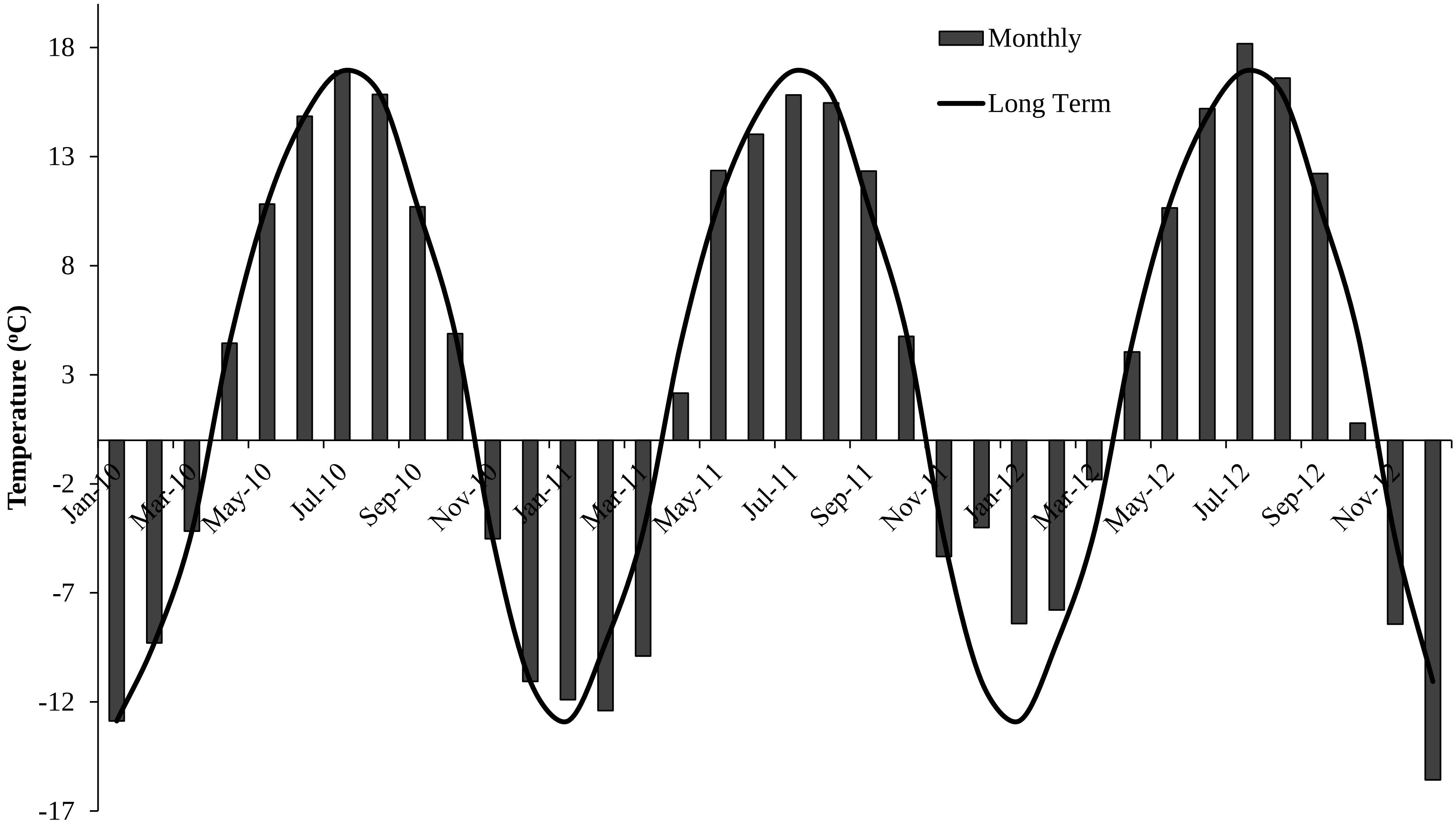

Figure B.1.2 Average monthly and long-term (30 year average) temperature (°C) at St. Albert study site for 2010-2012.

Appendix B.2 Stony Plain

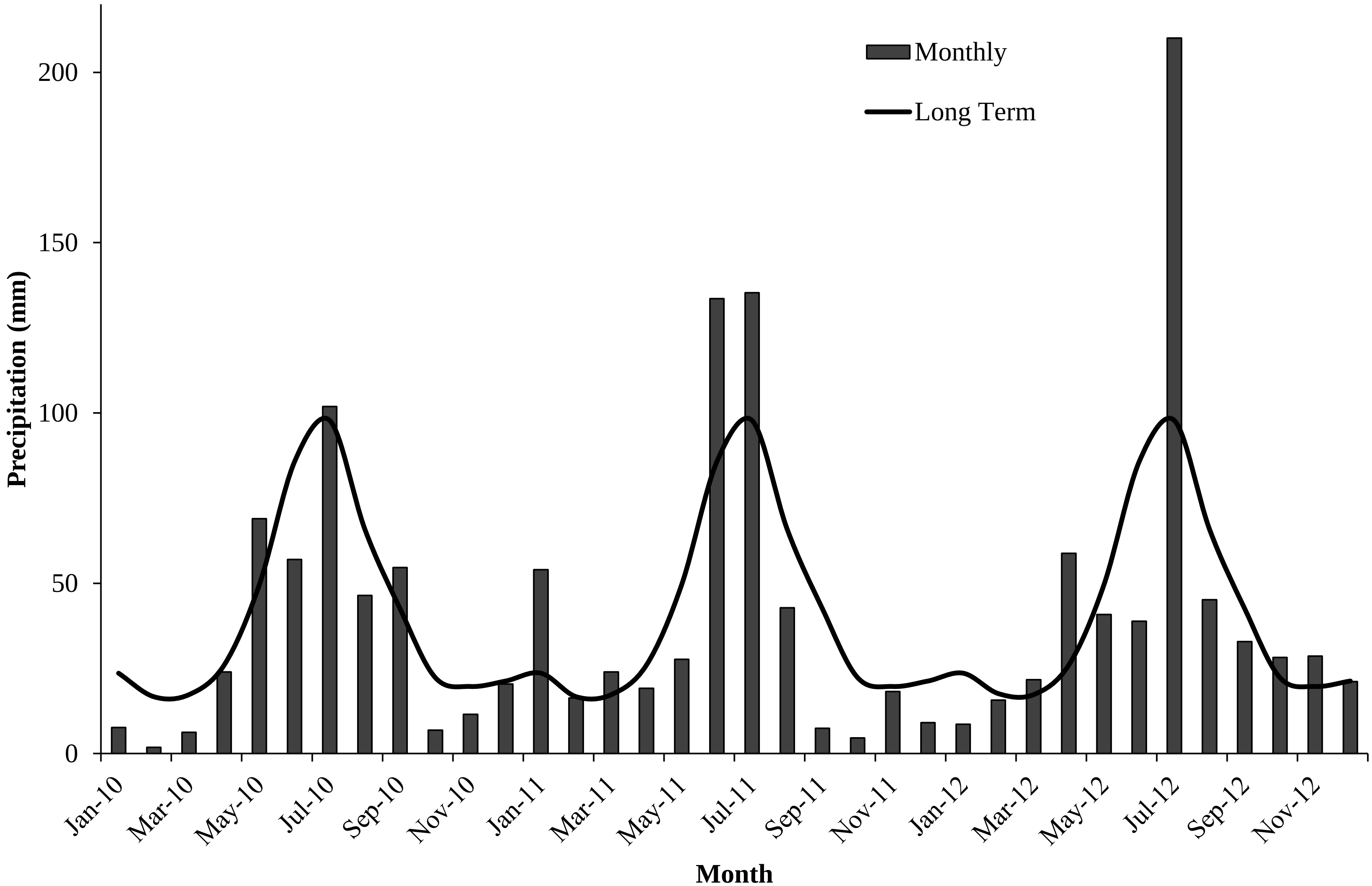

Figure B.2.1 Average monthly and long-term (30 year average) precipitation (mm) at Stony Plain study site for 2010-2012.

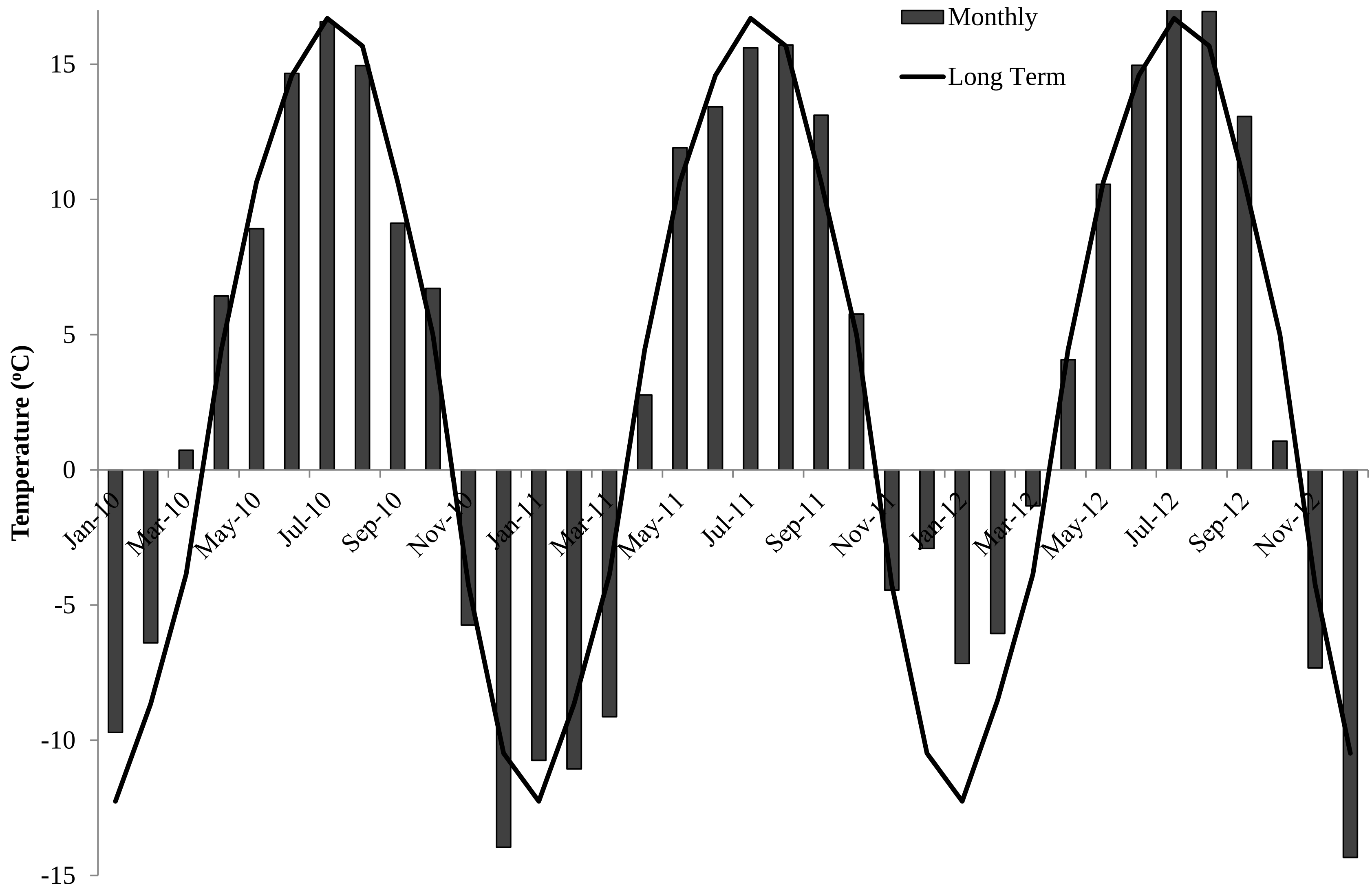

Figure B.2.2 Average monthly and long-term (30 year average) temperature (^{o}C) for Stony Plain study site for 2010-2012.

Appendix B.3 Lamont

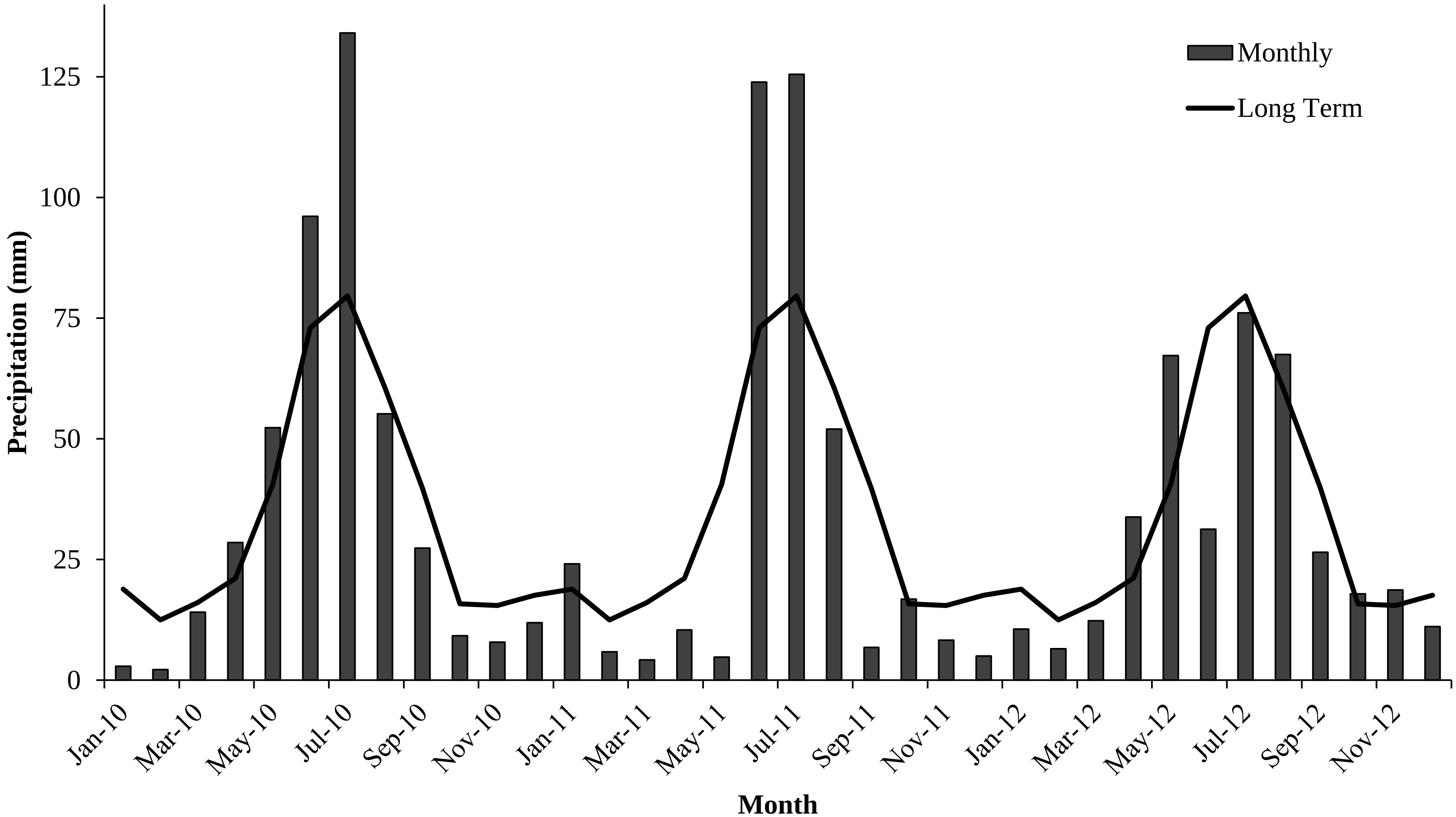

Figure B.3.1 Average monthly and long-term (30 year average) precipitation (mm) data for Lamont study site, 2010-2012.

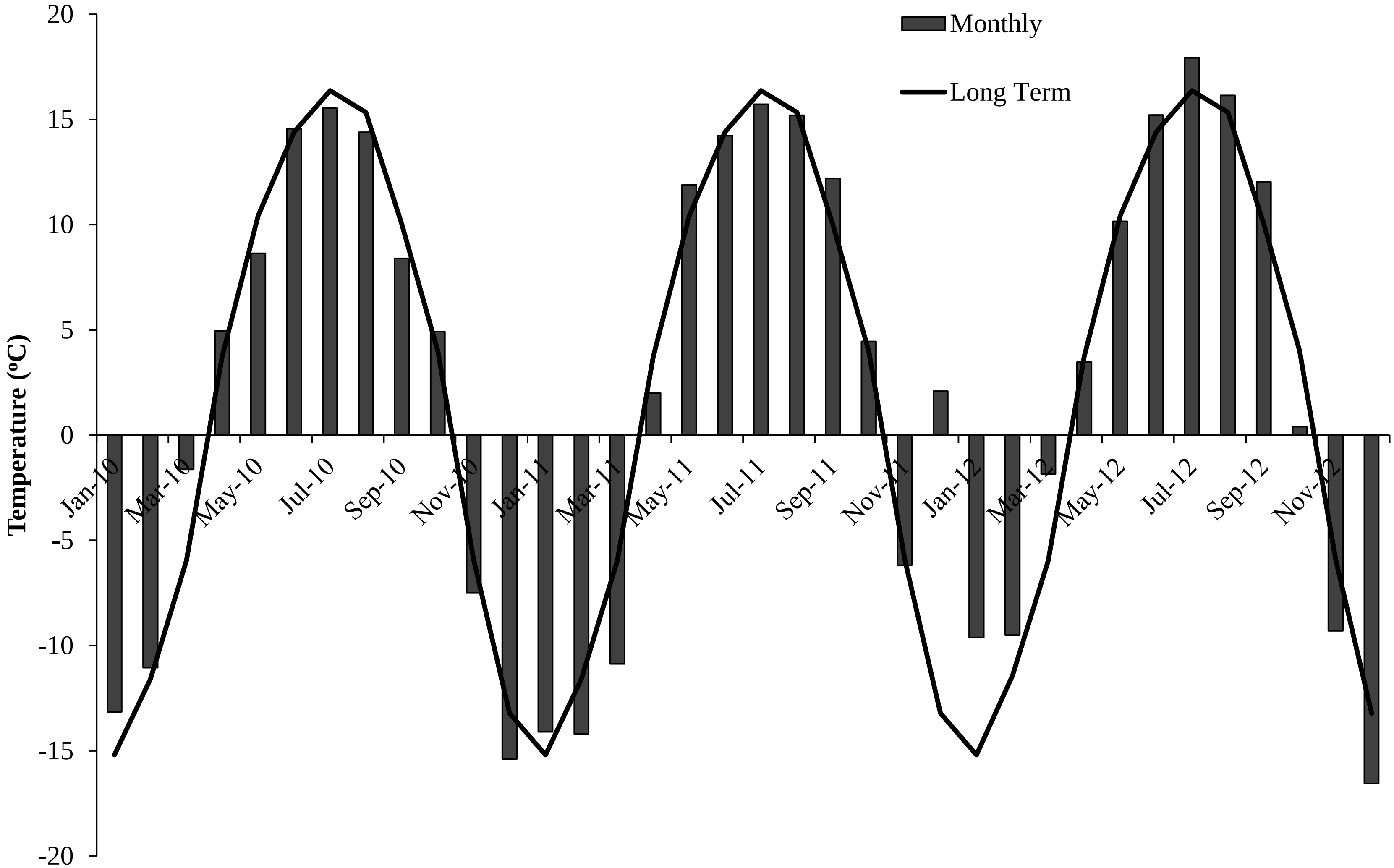

Figure B.3.2 Average monthly and long-term (30 year average) temperature (^{o}C) data for Lamont study site, 2010-2012.

Appendix B.4 Fort Saskatchewan

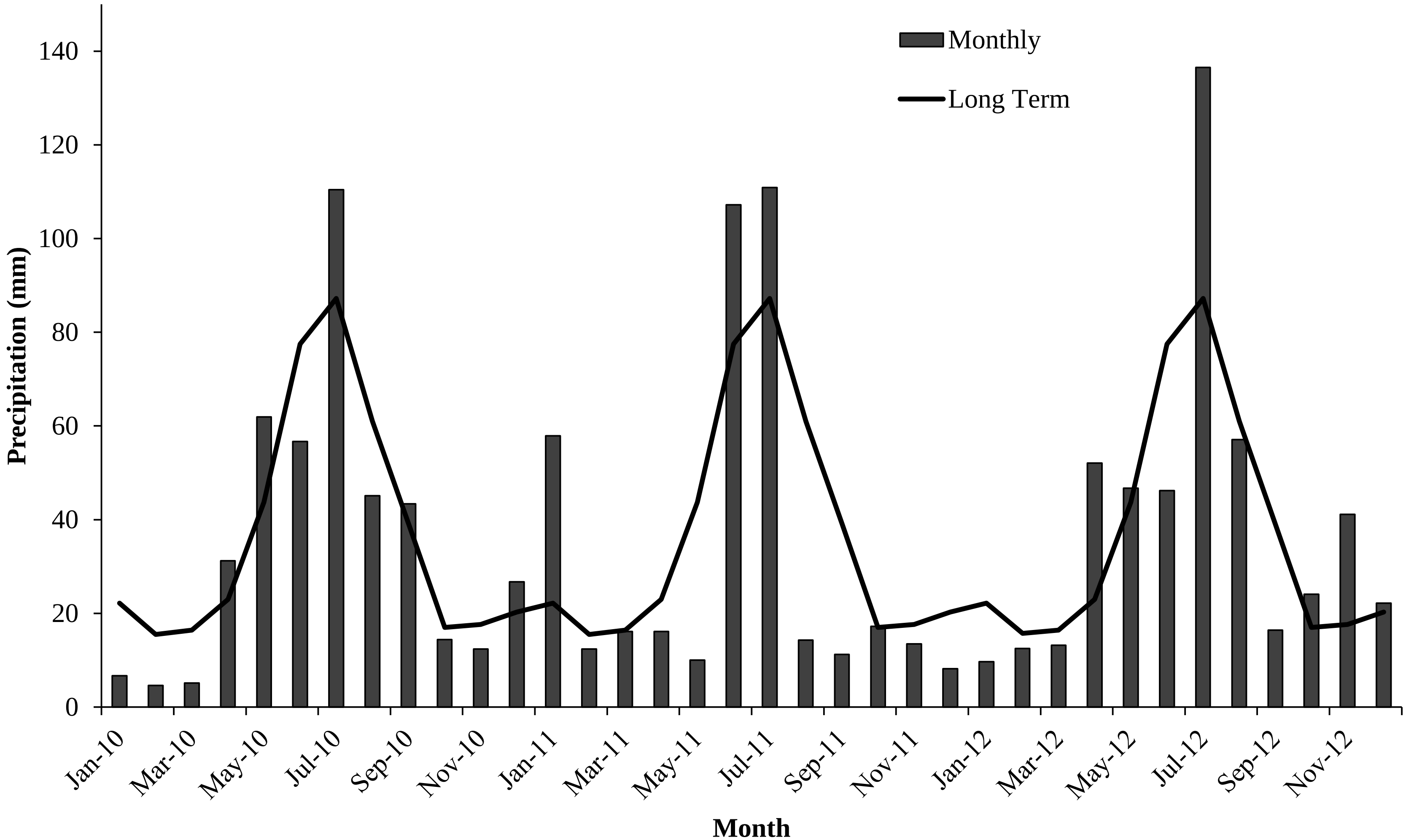

Figure B.4.1 Average monthly and long-term (30 year average) precipitation (mm) data for Fort Saskatchewan study site, 2010-2012.

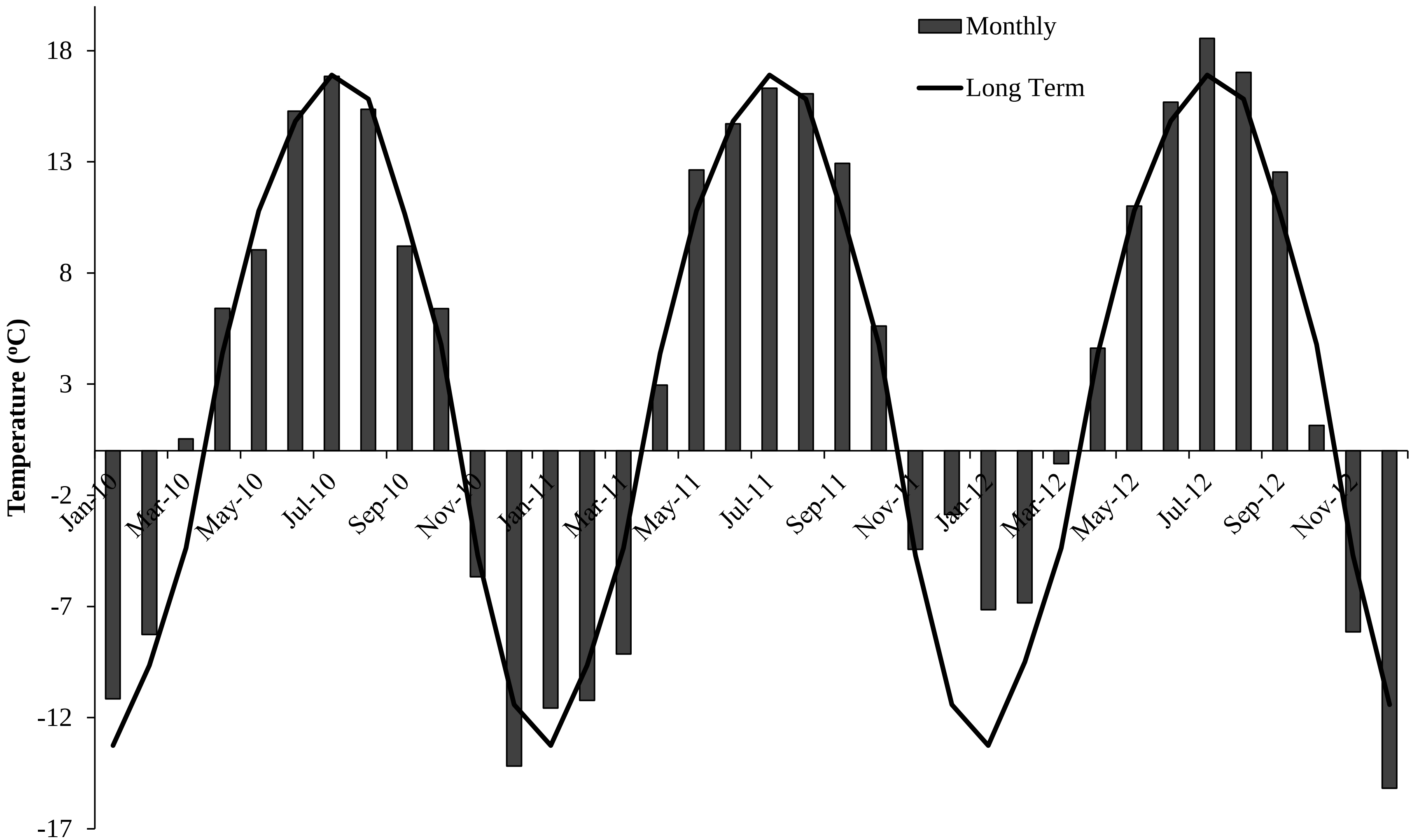

Figure B.4.2 Average monthly and long-term (30 year average) temperature (^{o}C) data for Fort Saskatchewan study site, 2010-2012.

Appendix B.5 Glenpark and Ellerslie

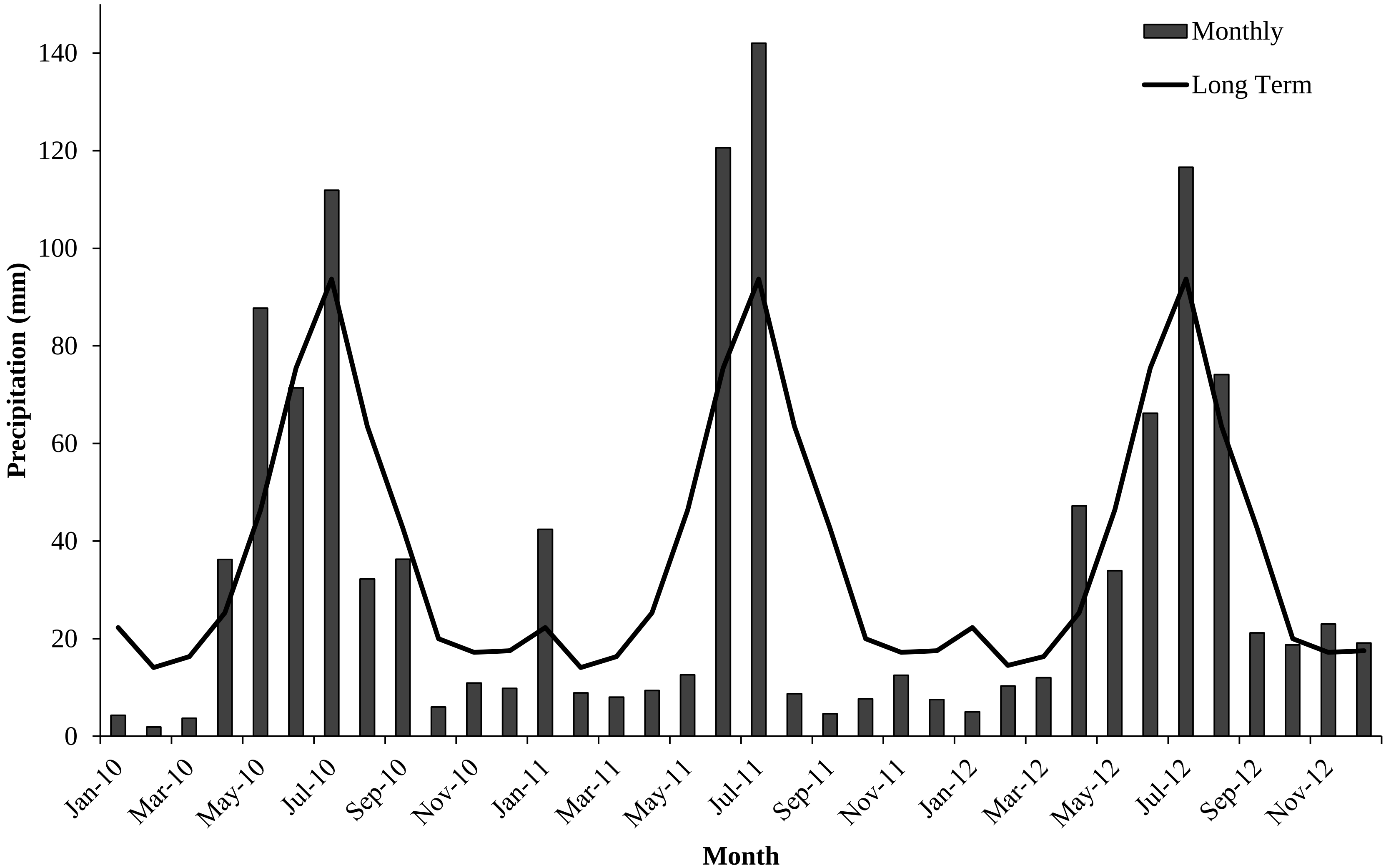

Figure B.5.1 Average monthly and long-term (30 year average) precipitation (mm) for Ellerslie and Glenpark study sites, 2010-2012.

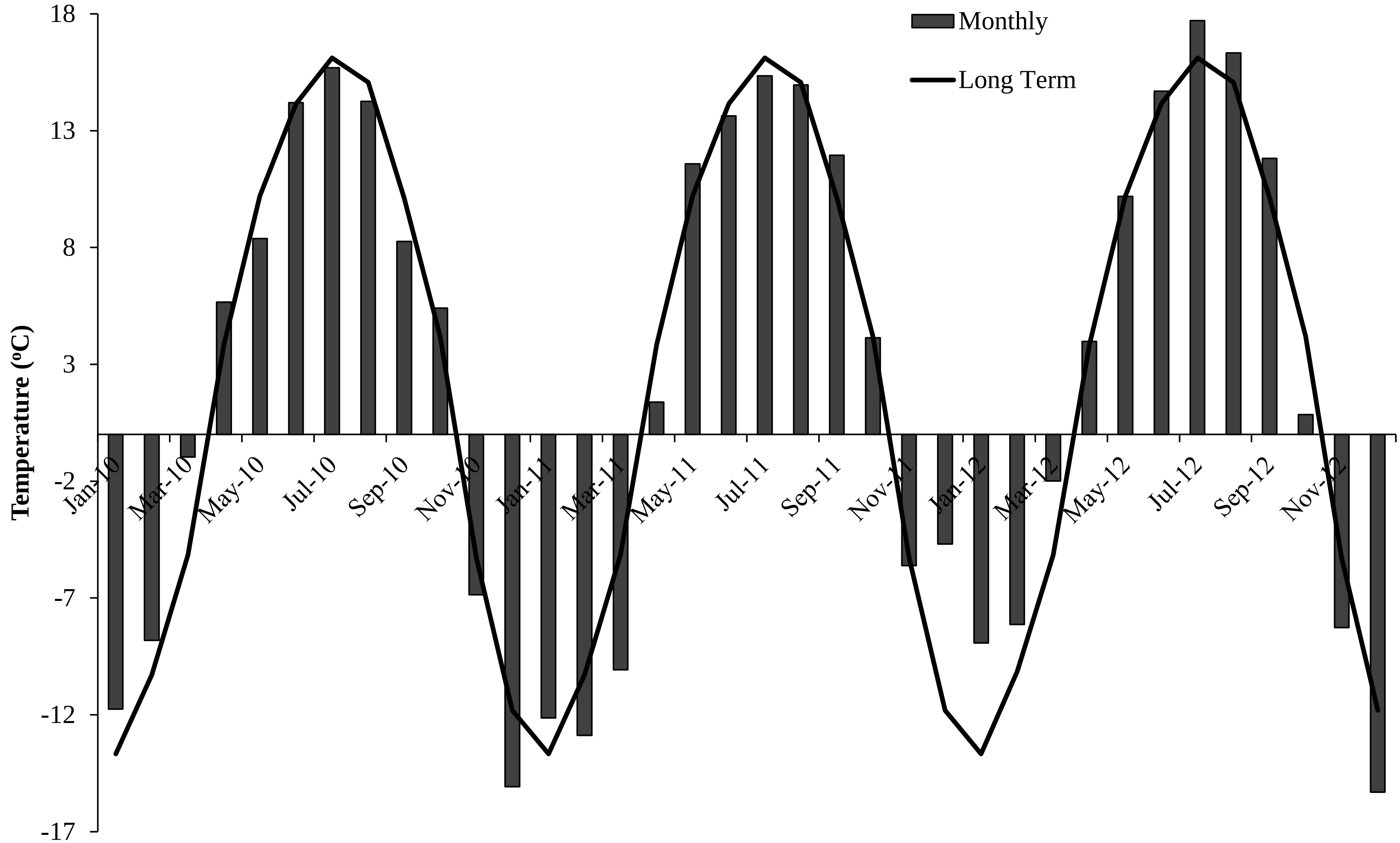

Figure B.5.2 Average monthly and long-term (30 year average) temperature (^{o}C) for Ellerslie and Glenpark study sites, 2010-2012.

Appendix C: Soil Descriptions

Table C.1 Soil description for St. Albert.

Pedon description – Eluviated Black Chernozem

Texture (topsoil/subsoil) – Silty Clay / Clay

Drainage class – Moderate to imperfect

Horizon	Depth (cm)	Description
Ah	0-32	Black (7.5YR 1.7/1 m), brownish black (10YR 2/2 d); clay; structureless, fine, granular; soft, slightly sticky, slightly plastic; plentiful, very fine, vertical roots; gradual, wavy boundary; 28-35 cm thick.
Bm	32-110	Grayish yellow brown (10YR 5/3 m), grayish yellow brown (10YR 4/2 d); clay; amorphous; firm, sticky, plastic; few, fine, vertical roots; 5% medium rounded-subrounded coarse fragments; diffuse, wavy boundary; 65-75cm thick.
Cca	110+	Brownish gray (7.5YR 6/1 m), brownish gray (7.5YR 6/1 d); heavy clay; amorphous; firm, very sticky, very plastic; 5% medium rounded-subrounded coarse fragments.

Table C.2 Soil description for Ellerslie.

Pedon Description – Eluviated Black Chernozem

Texture (topsoil/subsoil) – Loam / Clay

Drainage class – Moderately well to well

Horizon	Depth (cm)	Description
Ah	0-33	Black (10YR 2/1 m), black (10YR 2/1 d); clay loam, weak, fine granular; soft, very friable, very sticky, plastic; plentiful, very fine, vertical roots; diffuse, irregular boundary, 22-38 cm thick.
Bmt	33-58	Brownish black (10YR 3/2 m), brownish black (10YR 3/2 d); clay; weak, fine, subangular blocky; soft, very friable, very sticky, plastic; very few, fine, random roots; 1-2% small rounded-subrounded coarse fragments; gradual, irregular boundary; 18-32 cm thick.
Ckg	58+	Dull yellowish brown (10YR 5/3 m), dull yellowish brown (10YR 4/3 d); heavy clay; many, coarse, distinct brown (10YR 4/6) mottles; amorphous, slightly hard, very sticky, very plastic; 1-5% small rounded-subrounded coarse fragments.

Table C.3 Soil description for Glenpark.

Pedon Description – Gleyed Eluviated Black Chernozem

Texture (topsoil/subsoil) – Silty Clay Loam / Silty Clay

Drainage class – Moderate to imperfect

Horizon	**Depth (cm)**	**Description**
Ah	0-32	Black (7.5YR 1.7/1 m), black (7.5YR 1.7/1 d); silty loam; weak, fine granular; soft, nonsticky, nonplastic; plentiful, fine, vertical roots; gradual, irregular boundary; 25-38 cm thick.
Btjg	32-65	Black (7.5YR 1.7/1 m), black (7.5YR 1.7/1 d); silty clay; few, medium distinct brown (7.5YR 4/4) mottles; weak, fine, subangular blocky; soft, very friable, sticky, plastic; few, very fine, random roots; 5% small rounded-subrounded coarse fragments; diffuse, wavy boundary; 22-43 cm thick.
Ckg	65+	Brownish Gray (7.5YR 6/1 m), brownish gray (7.5YR 6/1 d); heavy clay; many, fine, prominent bright brown (7.5YR 5/8) mottles; amorphous, slightly hard, friable, very sticky, very plastic; 5% medium rounded-subrounded coarse fragments.

Table C.4 Soil Description for Fort Saskatchewan.

Pedon Description – Solodized Solonetz

Texture (topsoil/subsoil) - Silt Loam / Silty Clay

Drainage class – Moderately well to imperfect

Horizon	**Depth (cm)**	**Description**
Ah	0-30	Brownish black (10YR 2/2 m), dark brown (10YR 3/3 d); silty clay loam; structureless, fine, granular; soft, slightly sticky, slightly plastic; plentiful, fine, vertical roots; 1-2% rounded-subrounded coarse fragments; gradual, wavy boundary; 25-35cm thick.
Bm	30-42	Brownish black (10YR 2/3 m), dark brown (10YR 3/5 d); silty clay; amorphous; slightly hard, sticky, plastic; few, fine, vertical roots; 5% rounded-subrounded coarse fragments; gradual, wavy boundary; 25-47cm thick.
Cca	42+	Dull yellowish brown (10YR 5/3 m), dull yellowish brown (10YR 4/3 d); clay; amorphous; firm, sticky, plastic; 5% medium rounded-subrounded coarse fragments.

Table C.5 Soil Description for Lamont.

Pedon Description – Eluviated Black Chernozem

Texture (topsoil/subsoil) - Sandy Loam / Sandy Loam

Drainage class – Moderately well to well

Horizon	Depth (cm)	Description
Ah	0-25	Black (7.5YR 2/1 m), black (7.5YR 1.7/1 d); sandy loam; structureless, fine, granular; soft, non-sticky, nonplastic; plentiful, fine, vertical roots; clear, smooth boundary; 23-27 cm thick.
Bm	25-45	Brownish black (10YR 3/2 m), brownish black (10YR 3/2 d); sandy loam; fine, granular; soft, very friable, non-sticky; 1-2% small rounded-subrounded coarse fragments; clear, smooth boundary; 43-47 cm thick.
Cca	45+	Brownish gray (7.5YR 6/1 m), brownish gray (7.5YR 6/1 d); sandy clay loam; amorphous; slightly hard, friable, very sticky, very plastic; 5% medium rounded-subrounded coarse fragments.

Table C.6 Soil Description for Stony Plain.

Pedon Description – Eluviated Black Chernozem

Texture (topsoil/subsoil) - Sandy Loam / Sandy Clay Loam

Drainage class - Moderately well to well

Horizon	**Depth (cm)**	**Description**
Ah	0-18	Brownish Black (10YR 2/2 m), dark brown (10YR 3/3 d); silty clay; weak, fine granular; very friable, slightly sticky, slightly plastic; plentiful, fine random roots ; clear, wavy boundary; 15-21 cm thick.
Bm	18-32	Brownish black (10YR 2/3 m), dark brown (10YR 3/5 d); sandy clay loam; weak, fine granular; very friable, slightly sticky, slightly plastic; very few, fine random roots; 1-2% small rounded-subrounded coarse fragments; clear, smooth boundary; 10-22 cm thick.
Ck	32+	Yellowish brown (10YR 5/4 m) brown (10YR 4/6 d); sandy clay; amorphous; soft, friable, sticky, plastic; very few, fine, random roots, 1-2% small rounded-subrounded coarse fragments.

Appendix D: Comparison of Herbicide Bioactives.

Table D.1 Aminopyralid and aminocyclopyrachlor properties (Dow AgroSciences 2005, DuPont 2009).

Herbicide	Molecular Formula	Molecular Weight (g/mol)	K_{ow} (pH 7)	K_{oc}	pKa	Half-life (days)
Aminopyralid	$C_6H_4Cl_2N_2O_2$	207.026	-2.87	10.8	2.56	31.5-533.2
Aminocyclopyrachlor	$C_8H_8ClN_3O_2$	213.62	-2.48	28	N/A	22-126