University of Alberta

FESTUCA HALLII (VASEY) PIPER (PLAINS ROUGH FESCUE) AND FESTUCA CAMPESTRIS RYDB (FOOTHILLS ROUGH FESCUE) RESPONSE TO SEED MIX DIVERSITY AND MYCORRHIZAE

by

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DEDICATION

This MSc thesis is dedicated to my grandfather, Fred A. Forster,

who instilled in me a passion for always learning,

and for always reminding me that if you're going to do a job,

do it right the first time.

ABSTRACT

Rough fescue (*Festuca hallii* (Vasey) Piper (plains rough fescue) and *Festuca campestris* Rydb (foothills rough fescue) are long lived perennials that have been difficult to establish on disturbed sites. This research assessed the impact of seed mix diversity and suppression of arbuscular mycorrhizal fungi on fescue establishment. Three research sites were examined in each of the northern fescue and foothills fescue subregions. Fescue seeded alone, a mix of fescue and closely associated species and fescue with a cover crop of *Elymus dahuricus* (dahurian wild rye) were seeded and compared. Mycorrhizae impact was assessed by comparing plots treated with a fungicide (Rovral) to controls. Rough fescue was able to establish by seeding in the field. Fescue monocultures had better fescue establishment than mixes. *Elymus dahuricus* was not a successful cover crop for *Festuca hallii* and was marginal for *Festuca campestris*. Fungicide application did not have any impact on fescue establishment.

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CHAPTER I. INTRODUCTION

1.1 BACKGROUND

Alberta fescue grassland is divided into three ecoregion types. Northern fescue and aspen parkland subregions were historically dominated by *Festuca hallii* (Vasey) Piper (plains rough fescue), montane grasslands were dominated by *Festuca altaica* Trin. (altai fescue) and foothills fescue grasslands were dominated by *Festuca campestris* Rydb. (foothills rough fescue) (Pavlick and Looman 1984).

Fescue grasslands perform important ecological, aesthetic and economical functions. Rough fescue is a large bunch grass often growing over 1 m in height with roots that can exceed 1 m in depth (Looman 1969). This deep rooting characteristic is one factor that led to formation of the characteristic black chernozemic soils of the fescue grasslands. Ecologically, the rough fescue plant growth form aids in preventing weedy species from invading and increases site stability (Looman 1969). Rough fescue productivity is high and contributes to litter formation which helps maintain soil water and infiltration capacity (Naeth et al. 1991a, 1991b, 1990). Economically, fescue grasslands are an important grazing resource. They have higher forage production than any other native grassland in western Canada except tallgrass prairie remnants in Manitoba (Looman 1969). Using these grasslands for winter forage helps protect fescue prairie while reducing the cost of feeding livestock (Willms 1992).

Fescue grasslands have high intrinsic value. Approximately 150 plant species have been recorded for the foothills fescue region and just over 100 plant species have been recorded for the northern fescue region (Moss and Campbell 1947). Fehr (1982) reported 290 species for Rumsey Block; nine were considered rare at the time. The greater number of species in the foothills could be explained by proximity to mountains and other vegetation types not found in the northern fescue region (Moss and Campbell 1947). Bradley et al. (2002) noted two species currently on the Alberta Natural History Information Centre (ANHIC) vascular plant tracking list and over 60 plants on the list could potentially occur within the foothills fescue grasslands.

Fescue grasslands currently comprise approximately 112,000 km² of southern Alberta, with 15 % being northern and foothills fescue and 5 % central parkland and mixed grass (Adams et al. 2003). Of 1,686 grassland sites studied in the Alberta central parkland, only 12.5 % had plains rough fescue communities (Holcroft-Weerstra 2003). Once comprising about 1.5 million ha, foothills fescue grassland is now reduced to about 16.8 % (252,000 ha) of its original size (Adams et al. 2003).

Historically there have been two major disturbances to fescue grassland, semi frequent fires and grazing. Since the turn of the 20th century, two more major disturbances have been added, conventional dryland agriculture and natural resource development and extraction in the form of well sites and pipelines. These disturbances differ from grazing and fire in that they cause a greater degree of soil disturbance. To date there have been no documented examples in western Canada of successful reclamation of fescue grassland (Alberta Wildlife Association 2006).

Rough fescue plant communities are at more risk of conversion to non-native community types than other native grasslands in Alberta. Once disturbed or invaded by non-native species, rough fescue grasslands are less likely to be restored (Alberta Wildlife Association 2006). Looman (1969) documented that *Bromus inermis* Leyss. (smooth brome) and *Medicago falcata* L. Arcang. (yellow alfalfa) could successfully replace the native cover of black soils when seeded. Based on lack of restoration success to date, and given the value of these grasslands, strategies other than seeding disturbances need to be considered if the end goal is a grassland that can resemble undisturbed areas. This research will focus on re-establishment of fescue on well site disturbances.

1.2 ROUGH FESCUE AND ROUGH RESCUE GRASSLANDS

1.2.1 Rough Fescue Grasslands

Foothills fescue grasslands are typically associated with black chernozemic soils on moist sites. Northern rough fescue grassland is associated with black chernozems on moist sites in northern parts of the ecoregion and dark brown chernozems on southern parts, which is typically drier (Moss and Campbell

1947). The dark brown soil zone is approximately the middle of the tension zone between fescue grassland to the north and *Stipa* grassland to the south. Foothills fescue grasslands are also typically associated with black chernozems, but do not have the dark brown association, as precipitation is greater than that of the northern fescue region.

The modal plant community on mesic sites in the northern fescue subregion is *Festuca hallii* associated with *Stipa curtiseta* (A.S. Hitchc.) Barkworth (western porcupine grass) (Moss and Campbell 1947). It changes slightly on drier southern parts as *Bouteloua gracilis* Willd. ex Kunth (blue grama grass) becomes dominant. The modal plant community for foothills fescue is *Festuca campestris* associated with *Danthonia parryi* Scribn. (Parry's oat grass). *Danthonia* appears to be a local dominant of importance in restricted areas, especially on shallow soils of rocky and gravelly slopes. It may be best to regard *Danthonia parryi* as forming an edaphic climax.

Both foothills and northern fescue grasslands are presumed to have formed under co-evolution with grazing by plains bison (Morgan 1980). Bison wintered on fescue prairie and aspen parkland, thus supporting the idea that fescue prairie evolved under a history of dormant season grazing. This is also evidence that these grasslands have evolved under a dormant season disturbance regime.

When comparing climates of fescue grasslands to those of other vegetation types, Weaver (1979) noted that the climate of fescue grasslands is more similar to those of some coniferous forest types than those of other grassland types. This could suggest that other factors besides temperature and precipitation are responsible for maintaining fescue prairies, potentially including wind, snow cover, soil characteristics or fire frequency. Fescue grasslands occur in regions of greater water efficiency than do mixed prairie communities. The availability of water is enhanced by lower temperatures which lead to lower evaporation rates, and slightly higher precipitation in fescue grasslands (Anderson 2006).

1.2.2 Rough Fescue Biology and Ecology

Festuca campestris is a cool season grass adapted to short growing seasons (Anderson 2006). It is a large bunch grass, usually comprised of up to 250 culms,

that rarely has rhizomes (Pavlick and Looman 1984). This growth form suggests that it is adapted to periodic low intensity fires (Aiken and Darbyshire 1990). In undisturbed areas, crown diameters can be 20 to 50 cm (Moss and Campbell 1947). *Festuca hallii* is a cool season grass adapted to short growing seasons (Anderson 2006). It differs from *Festuca campestris* in usually being rhizomatous, and forms smaller (three to five culms) bunches (Pavlick and Looman 1984).

Both fescues are characteristic of climax grasslands (Tirmenstein 2000, Willms and Fraser 1992) and are also present in other successional stages. As long lived perennial species that devote several years to vegetative growth before reproducing via seed, and fit into a K selected classification (Anderson 2006). Both reproduce primarily by seed (Pavlick and Looman 1984) although seed production does not occur often or in a predictable manner. In southern Alberta, Johnston and MacDonald (1967) reported large seed production in 1902, 1952, 1964 and 1966. Both species typically initiate growth immediately following snow melt, start to senesce before the onset of summer drought and are dormant by October (Johnston and MacDonald 1967).

The response of *Festuca campestris* to infection by mycorrhizal fungi may impact plant growth characteristics. These changes could include larger size or production of wide, flat leaves. Aiken and Fedak (1992) describe two plants of *Festuca campestris* in Alberta that were growing close together but were different in size and morphology. The arbuscular mycorrhizal (AM) fungus *Glomus fasiculatus* was found in the roots of the larger individual. Although no evidence has been found for similar effects on *Festuca hallii*, Anderson (2006) stated that infection by mycorrhizae could have similar impacts to growth morphology.

1.2.3 Rough Fescue Establishment

The few attempts to restore *Festuca hallii* plant communities in the parkland and northern fescue subregions have been unsuccessful, mainly due to the difficulty in establishing rough fescue. Gas well sites and pipelines reclaimed in these ecoregions had fair to poor establishment of native species, including rough fescue, from seed mixes and sod salvage (Elsinger 2006, AXYS Environmental Consulting 2003, Petherbridge 2000, Integrated Environments Ltd. 1991). A restoration experiment in the grasslands of central Saskatchewan resulted in the

conclusion that conserving remaining rough fescue prairie rather than restoring it would have greater benefit (Clark 1986).

Grassland restorations are often unsuccessful due to unreliable seed sources, competition from weeds and agronomic species and variation in climate (Desserud 2006, Wilson 2002). Perennial weed invasion is a problem throughout the fescue prairie, which can negatively impact rough fescue re-establishment (Clark 1998). Research preventing or reducing competition from non-native or weed species includes burning, grazing or mowing and applying herbicides. Stromberg and Kephart (1996) reviewed successful restoration techniques to reduce competition for native seedlings, including mowing annuals before their seeds mature. Ewing (2002) concluded that lower weed biomass was associated with greater *Festuca idahoensis* survival.

In 1991, an assessment of revegetation of 14 industrial sites was conducted in Rumsey Block including well sites, pipelines, an access road and a right-of-entry (Integrated Environments Ltd. 1991). These sites varied in age from 4 to 14 years. Results varied from persistence of wheat grasses, such as *Agropyron dasystachyum* (Hook.) Scribn. & J.G. Sm. (northern wheat grass) or *Agropyron smithii* (Rydb.) A. Löve (western wheat grass) from the seed mix, encroachment of *Phleum pratense* L. (timothy) or *Bromus inermis* and natural recovery of rough fescue and other native species. Plant species composition of the majority of disturbed sites was not similar to adjacent native range. A few exceptions occurred where natural recovery resulted in encroachment of *Festuca hallii* and *Stipa curtiseta* on pipelines.

Two studies examined long term (\geq 20 years) restoration success of *Festuca hallii*. Vujnovic (1998) studied species composition after 20 years of grazing or other disturbance in *Festuca hallii* dominated communities in the central parkland. Slogan (1997) studied vegetation dynamics after 23 years in *Festuca hallii* grasslands in Manitoba. No other research studies examined the long term results of revegetation of rough fescue grasslands (Desserud 2006).

Spring seeding is recommended over fall seeding, as in the spring seedbed temperatures are increasing and become more conducive to germination. Temperatures near 15 °C seem to be most favourable for germination of *Festuca hallii* (Grilz 1992). The higher soil water in spring, due to snow melt, also favours germination. Optimal growth and regrowth following defoliation occurs near or below 17 °C for *Festuca hallii*; as temperatures increase above this, growth starts to decline (King et al. 1998).

1.3 MYCORRHIZAL FUNGI

1.3.1 Mycorrhizal Fungi Classification

Mycorrhizal fungi are classified into two main groups, endomycorrhizal and ectomycorrhizal, based on hyphal association with plant roots (Smith and Read 2008). Endomycorrhizal fungi are further divided into three groups, arbuscular mycorrhizae (AM), ericoid mycorrhizae and orchidaceous mycorrhizae.

Endomycorrhizal fungi bodies grow branched in root cortical cells, forming an arbuscule. External structures, hyphae, extend from the root surface several mm into the soil. Ectomycorrhizal fungi form a hartig net, a mycelia complex between root cortical cells and the mantle, and a hyphal network that partially or fully encloses the root. Endomycorrhizae and ectomycorrhizae differ in plant species associations. Endomycorrhizae do not form associations with specific plants; ectomycorrhizae are highly specific in their plant associations. Ectomycorrhizae are commonly associated with woody plant species; arbuscular mycorrhizal fungi occur in herbaceous and woody plants (Gurevitch et al. 2006).

Arbuscular mycorrhizal fungi are the most common mycorrhizal type associated with flowering plants. They are possible major factors in determining interactions between plants, and on a larger scale, vegetation ecosystem functioning (Smith and Read 2008). Arbuscular mycorrhizal fungi require plant hosts to complete their life cycle, but many of these potential hosts plants grow and survive without fungi. Historically the relationship between fungi and plant was considered a mutualism. Asai (1944) first recognized a relationship between development of fungi and plant growth. Recently the relationship has been evaluated on a continuum of interactions, ranging from mutualism to parasitism depending on the partners and the environmental variables (Jones and Smith 2004, Johnson et al. 1997). Fungi require a plant host for a carbon source for energy, and thus use a considerable amount of carbon that is fixed by the plants themselves through the process of photosynthesis.

1.3.2 Carbon Allocation and Use

Arbuscular mycorrhizal fungi associated with plant roots comprise 3 to 20 % of root mass (Douds et al. 2000, Harris and Paul 1987). The values do not include spores or external hyphae which can contribute up to 90 % of fungal biomass (Olsson et al 1999, Sieverding et al. 1989). Fungal biomass is estimated to consume 4 to 20 % of photosynthate (Douds et al. 2000, Eissenstat et al. 1993, Jakobson and Rosendahl 1990, Douds et al. 1988, Koch and Johnson 1984).

Plants use carbon fixed via photosynthesis for above and below ground growth. Carbon used by mycorrhizae can cost the plant which may be offset by the benefit of increased nutrient uptake (Fitter, 1991, Stribley et al. 1980a, 1980b). This could lead to a plant being limited by carbon rather than nutrients. Carbon consumption would be a cost only if it could have been used for increasing biomass or improving fitness (Smith and Read 2008). Koide and Elliott (1989) said cost is the carbon used to support mycorrhizae and the gross benefit is the increase in carbon fixed from mycorrhizal colonization. Colonization may offset the cost to the plant by increasing plant ability to fix carbon dioxide (CO_2) (Schwob et al. 1998, Wright et al. 1998). Under low nutrient conditions that limit growth, the cost of producing fungal hyphae is two orders of magnitude less than for producing roots, so producing hyphae for nutrient acquisition is favoured (Smith and Read 2008). Eissenstat et al. (1993) found that at an equivalent phosphorus status abuscular mycorrhizal associated plants had lower efficiency of carbon production and were less efficient than non-mycorrhizal plants.

1.3.3 Phosphorus Uptake

Arbuscular mycorrhizal infected plant roots are known to be more efficient per unit of length in nutrient uptake than uncolonized roots (Smith and Read 2008). The largest impact of colonization by arbuscular mycorrhizal fungi is an increase in phosphorous uptake. When soil phosphorus availability is low, non mycorrhizal roots may be unable to absorb phosphorus effectively which leads to deficiency. Colonization can increase uptake and help eliminate deficiency.

Arbuscular mycorrhizae infected plant roots have two potential pathways to absorb phosphorus and other nutrients from the soil. Non arbuscular mycorrhizal

infected plants use a direct pathway by absorbing nutrients through root hairs and the root epidermis. Arbuscular mycorrhizal infected plants use a pathway which involves uptake through fungal hyphae in the soil. Indirect evidence shows that the infected roots are more efficient in nutrient uptake than non infected roots (Smith and Read 2008). Sanders and Tinker (1971) calculated that arbuscular mycorrhizal fungi contributed approximately 70 % of the total amount of phosphorus absorbed by infected plants. Rhodes and Gerdemann (1975) showed that fungal hyphae could absorb labelled phosphorus from up to 7 cm away from the roots and fungi with poor hyphal development had limited ability for phosphorus absorption (Smith et al. 2000). Fungal hyphae absorb phosphorus from the soil solution likely as orthophosphate and this is stimulated by the plant through H+ - ATPase activity (Lei et al. 1991).

1.3.4 Water Relations

Arbuscular mycorrhizal fungi colonization affects plant water relations (Auge 2001). When transplanting *Allium cepa* L. (onion) Mosse and Hayman (1971) observed infected plants did not wilt while non infected plants did wilt. Results from studies on *Glycine max* L. Merr. (soya bean) plants by Safir et al. (1971, 1972) showed that infected plants had lower resistances to water transport where most of the difference was due to changes in root resistance. The effects were considered to be due to improved nutrition from colonization as the increases were eliminated with application of a fungicide. Levy and Krikun (1980) found that arbuscular mycorrhizal fungi colonization increased transpiration and stomatal conductance, both during stress and recovery.

1.3.5 Ecological Interactions

Allen and Allen (1984) concluded arbuscular mycorrhizal fungi were likely relatively unimportant in disturbed and early successional ecosystems. However, in low nutrient habitats and late successional stages all plants were colonized. Thus they postulated that arbuscular mycorrhizal fungi colonization may be very important in determining the outcome of plant competition in habitats with high water availability and high nutrient conditions. This may also indicate mixed responses to mycorrhizal fungi at different stages of growth and development.

Plants have different responses to colonization that depend on a number of factors including plant type and environmental factors. An experiment in tall grass prairie showed C4 grasses and perennial forbs were almost always highly responsive to arbuscular mycorrhizal fungi colonization. C3 grasses and forbs were less responsive and less colonized (Wilson and Hartnett, 1998, Bentivenga and Hetrick, 1992, Hetrick et al. 1989, 1991). When species from this system were treated with fungicide (benomyl) in pots the presence of mycorrhizae was reduced. The fungicide treatment reduced the abundance of highly arbuscular mycorrhizal fungi responsive grasses and increases in less responsive grasses (Hartnett and Wilson 1999, Smith et al. 1999, Wilson and Hartnett 1998).

In non-competitive treatments, *Hypericum perforatum* L. (St John's wort) seedlings were more responsive to arbuscular mycorrhizal fungi inoculation than mature plants but both responded positively. In competition positive results were reduced, more in seedlings (Moora and Zobel 1997). Grime et al. (1987) found arbuscular mycorrhizal fungi colonization was beneficial to seedlings germinating in established plant communities. Comparing *Lolium perenne* L. (perennial rye grass) and *Trifolium repens* L. (white clover), *Lolium* became extensively colonized but showed no response, whereas *Trifolium* was able to compete only with *Lolium perenne* when colonized by mycorrhizal fungi (Hall 1978).

Hedlund et al. (2003) observed that plant species richness was loosely positively correlated with arbuscular mycorrhizal fungal biomass. Bever (2002, 2003) proposed a feedback model with both a positive and negative relationship between two plant and two fungal species. He showed in general that the fungus gives the greatest benefit to one plant species but grows best on another plant species, creating a negative feedback loop. No evidence for positive feedback was found. This relationship shows that advantageous relationships between specific plant and fungal species are not advantageous (Smith and Read 2008).

1.3.6 Common Mycelial Networks

The role of common mycelial networks in distributing resources among its linked plants has been discussed but there is no evidence for its significance or what mechanisms are involved (Smith and Read 2008). Grime et al. (1987) provided labelled carbon dioxide ($^{14}CO_2$) to *Festuca ovina* L. (sheep fescue) plants, and

only plants that were mycorrhizal contained radioactive carbon. This suggests there is net movement of carbon between plants (Smith and Read 2008). When studying the interactions between *Festuca idahoensis* Elmer (idaho fescue) and *Centaurea maculosa* Lam. (spotted knapweed), Marler et al. (1999) showed that neither of the species responded positively to inoculation when grown alone in pots. When the two plant species were grown together and inoculated with the fungi, *Centaurea maculosa* had a much greater competitive impact on *Festuca idahoensis* compared to when it was grown without mycorrhizae.

Smith (1980) observed growth depressions using seedlings of all the same age, likely the result of carbon utilization during initial stages of root colonization. If hyphae received carbon from an established plant even for a short time, considerable benefit might be gained (Smith and Read 2008). Further evidence that a colonized plant can support a seedling comes from research in grasslands (Birch 1986, Grime et al. 1987, McGee 1985) where *Centaurea* plants can only be successfully colonized if grown with an arbuscular mycorrhizal fungi infected companion plant to supply the carbon to the developing mycorrhizae.

Arbuscular mycorrhizal fungi are thus likely to play an essential role in determining the formation of later successional plant communities and in plant interactions within those communities. The fungi require a plant host to survive; this host supplies the fungus with carbon in return for various benefits. The benefits to the plant from the mycorrhizae range from increased nutrient uptake and drought resistance to a role in determining invasibility of a particular system.

Seedling plants grown in the presence of arbuscular mycorrhizal fungi are hypothesized to have lower fitness than plants grown without fungi. There is evidence to support the hypothesis that a mature plant supports the fungi while the seedling grows to a size that it can then support the fungi. At a field scale do seedling plants require association with a mature plant? Does this plant need to be of the same species, or is there an interaction between co-dominant species that allow each to co-exist? Does co-dominance help buffer impacts of disturbance? These questions need to be answered to determine the ability of a climax species to establish on a disturbed site without some interference in manipulating soil mycorrhizae or seeding associated species.

1.4 RESEARCH OBJECTIVES

The research objective was to determine if *Festuca hallii* (Vasey) Piper (plains rough fescue) and *Festuca campestris* Rydb. (foothills rough fescue) can be restored by seeding following gas well disturbances. Specific objectives follow.

- To examine the response of *Festuca hallii* and <u>Festuca</u> *campestris* to seeding as a monoculture versus seeding in a mix with closely associated species.
- To examine the response of *Festuca hallii* and *Festuca campestris* to a cover crop of *Elymus dahuricus* Turcz. ex Griseb. (dahurian wild rye).
- To examine the impact of mycorrhizal fungi on *Festuca* growth at seedling and juvenile stages.

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CHAPTER II. FESTUCA HALLII (VASEY) PIPER (PLAINS ROUGH FESCUE) AND FESTUCA CAMPESTRIS RYDB. (FOOTHILLS ROUGH FESCUE) RESPONSE TO SEED MIX DIVERSITY

2.1 INTRODUCTION

A fundamental goal of restoration is re-establishment of plant diversity similar to undisturbed areas (Kline 1997). Although progress has been made, many restorations do not achieve this goal (Middleton et al. 2010). The few attempts to restore *Festuca hallii* plant communities in the parkland and northern fescue subregions of Alberta have been unsuccessful. Gas well sites and pipelines reclaimed in these ecoregions had fair to poor establishment of native species, including rough fescue from seed mixes and sod salvage (Elsinger 2006, AXYS Environmental Consulting 2003, Petherbridge 2000, Integrated Environments Ltd. 1991). A restoration experiment in the grasslands of central Saskatchewan resulted in the conclusion that conserving remaining rough fescue prairie rather than restoring it would have greater benefit (Clark 1986).

Bakker et al. (2003) found seeding was essential for establishing grasses, with almost no native grasses found in unseeded plots. Grassland restorations are often unsuccessful due to unreliable seed sources, competition from weeds and agronomic species and climate (Desserud 2006, Wilson 2002). Perennial weed invasion is a problem throughout the fescue prairie, which can negatively impact rough fescue re-establishment (Clark 1998). Treatments preventing or reducing competition from non-native or weed species include burning, grazing, mowing and applying herbicides. Foster et al. (2007) found early successional species such as annuals and short lived perennials could persist for many years after abandonment. Stromberg and Kephart (1996) reviewed successful restoration techniques to reduce competition for native seedlings, including mowing annuals before their seeds mature. Ewing (2002) concluded that lower weed biomass was associated with greater *Festuca idahoensis* survival.

Native grassland restorations are often slow and unpredictable due to lack of available seed bank propagules (Hutchings and Booth, 1996). To direct plant community development towards late seral pre-disturbance diversity, site

managers attempt to direct the pathway of succession towards the desired plant community (Pakeman et al. 2002). Secondary succession is affected by the initial presence of propagules (Egler 1954). These propagules can either be added by seeding the desired species or transplanting live individuals.

Foster et al. (2007) found that when comparing low and high density seed mixes, that high density mixes resulted in target plant communities that maintained higher diversity and function. This is important when determining diversity of a seed mix for restoration. Lower diversity mixes although less expensive may not be as successful long term in establishing fescue. Use of a nurse or a cover crop may also be useful in establishing fescue. Cover crops may have a positive impact on germination and establishment of seeds by reducing solar radiation, moderating temperature and increasing air humidity (Withgott 2000). The cover crop creates a microhabitat that is potentially more suitable to germination and establishment. *Festuca hallii* has a temperature requirement for germination of approximately 15 °C (Grilz 1992) and having a mechanism of moderating this temperature may lead to an increase in germination and establishment.

2.2 OBJECTIVES AND HYPOTHESES

2.2.1 Objectives

The objective of this research was to determine whether *Festuca hallii* (Vasey) Piper (plains rough fescue) and *Festuca campestris* Rydb. (foothills rough fescue) could be restored by seeding following gas well site disturbances. Specific research objectives were as follows.

- To examine the response of *Festuca hallii* and *Festuca campestris* to seeding as a monoculture versus seeding in a mix with closely associated species.
- To examine the response of *Festuca hallii* and *Festuca campestris* to a cover crop of *Elymus dahuricus* Turcz. ex Griseb. (dahurian wild rye).

2.2.2 Hypotheses

Development of a greater canopy cover and higher density was hypothesized to occur with seeding *Festuca campestris* or *Festuca hallii* and associated species

than seeding *Festuca* alone. Species with higher growth rates could facilitate faster establishment to fill niches that invading plants could occupy and provide protection from sun, wind and other environmental stressors to small, slow growing *Festuca*. This would mimic a facilitation pathway where fast growing colonizing species facilitate establishment of slow growing later successional species. A *Festuca* mix would establish better the first growing season, with more *Festuca* surviving than if seeded alone. Species seeded with *Festuca* could facilitate over winter survival, by providing a microsite of biomass to buffer adverse climate conditions, such as drying winds and blowing snow.

Species seeded with *Festuca* may help reduce invasive species establishment, particularly perennials. Annual or biennial weeds will have less impact on seeded grass establishment than perennial weeds. Due to their life cycle, annual weeds are only competitive during early establishment and lose effect as perennial grasses establish. With time annual species are out competed by perennials, which then form a dominant cover. During initial establishment annual species mimic a cover crop and may facilitate establishment of perennial species.

Festuca seeded with *Elymus dahuricus* was hypothesized to be more successful than *Festuca* seeded alone, in the same way as seed species discussed above. *Elymus* is a short lived species which could provide protection for *Festuca* then die out prior to becoming a competitor to older and larger *Festuca* plants.

Festuca establishment was hypothesized to be inversely related to soil water during the first growing season. Dry conditions could be more conducive to establishment of *Festuca* as it is more tolerant than some perennial plants against which it would need to compete. *Festuca* would not compete with faster growing species for water, as it is more shallow rooted in early growth stages.

2.3 MATERIALS AND METHODS

2.3.1 Festuca Hallii Research Area

The northern fescue subregion is bordered to the north by the central parkland and to the south by mixed grass prairie (Natural Regions Committee 2006). The plains rough fescue research sites occurred within Rumsey Block in east central

Alberta (Figure 3.1). Rumsey Block is the largest remaining tract of aspen parkland in the world (American Wilderness Association 2000). It is divided into two areas, the Rumsey ecological reserve which is 33.5 km² and the Rumsey natural area which is 149 km² in size.

Rumsey Block has a continental climate, with long, cold, dry winters and short, moderately warm summers (Fehr 1982). Average mean annual temperature is 2.7 °C, ranging from -14.3 °C in winter to 17.2 °C in summer. Growing degree days average 1,490 (Natural Regions Committee 2006). Precipitation averages 384.6 mm annually with 286.8 mm during the April to August growing season.

Rumsey Block is characterized by symmetrical knob and kettle terrain with the occasional depressed center (Stalker 1972). Surface drainage is poor and internal. Many sloughs are dry by mid-summer because of low precipitation; standing water either evaporates or seeps into the ground (Fehr 1982).

Soils are predominantly dark brown chernozems, at the northern edge of the dark brown soil zone. Parent material is glacial till over Paskapoo and Edmonton formations (Bowser et al. 1951). The principle soil is Hughenden loam developed under native grass cover on well drained sites (Fehr 1982). On level areas, soil is Halkirk loam with a solodized solonetz B horizon. Due to topography, depth and development of organic horizons varies, grading to gleysols in depressions.

Undisturbed south facing grassland sites are dominated by *Festuca hallii -Stipa curtiseta* (Hitchc.) (western porcupine grass) associations grading into sedge wetlands in the kettles. Moister north facing slopes contain *Symphoricarpos occidentalis* Hook (buck brush) and *Populus tremuloides* Michx. (trembling aspen) groves. Moist grasslands favour *Festuca hallii* dominance, with other species such as *Stipa curtiseta*, *Helictotrichon hookerii* (Scribn.) Henr. (hookers oat grass), *Koeleria macrantha* (Ledeb.) J.A. Schultes) (june grass) and perennial forbs. Drier sites and grazing of moist sites favour sub-dominant species with *Festuca hallii* present but with a reduced cover.

2.3.2 Festuca Campestris Research Area

The foothills fescue subregion is bordered to the north by central parkland and northern fescue subregions, to the east by mixed prairie and to the west by

foothills parkland (Figure 2.1). Topography is rolling hummocky in the south and west, with undulating plains in the north and east (Natural Regions Committee 2006). Open water and wetlands are uncommon in hillier terrain.

The subregion is characterized by a continental climate (Natural Regions Committee 2006). It has the highest precipitation, warmest summers and shortest growing season of the Alberta grassland subregions. Average mean annual temperature is 3.9 °C, from -9.7 °C in winter to 16.3 °C in summer. Growing degree days average 1,388. Average annual precipitation is 469.6 mm with 333.1 mm falling during the April to August growing season.

Parent material is predominantly glacial till. Soils under fescue grassland are dominated by variants of black chernozems. Drainage in the northern parts is into the South Saskatchewan River system (Adams et al. 2003).

Festuca campestris, Festuca idahoensis Elmer (idaho fescue), *Carex* sp. (sedges) and *Agropyron smithii* (Rydb.) A. Löve (western wheat grass) dominate the southern half of the subregion (Natural Regions Committee 2006). In the northern half, *Festuca campestris* and *Danthonia parryi* Scribn. (parry's oat grass) dominate. *Lupinus argenteus* Pursh (silvery lupine), *Geum triflorum* Pursh (three flowered aven), *Geranium viscossisimum* Fisch. & C.A. Mey. ex C.A. Mey. (sticky purple geranium), *Artemisia frigida* Willd (pasture sage) and *Thermopsis rhombifolia* (Nutt. ex Pursh) Nutt. ex Richardson (golden bean) are common forbs. Shrubs such as *Elaeagnus commutata* Bernh. ex Rydb. (silver berry), *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem. (saskatoon), *Rosa acicularis* Lindl. (prickly rose) and *Ceanothus cuneatus* (Hook.) Nutt. (buck brush) occur on moist sites, while shrubs such *as Potentilla fruticosa* L. (shrubby cinquefoil) are present but most abundant on areas that are at least moderate grazed.

2.3.3 Research Site Locations

Three natural gas well sites in each of foothills rough fescue and plains rough fescue grassland were selected for research. Well sites were either drilled and abandoned or drilled and producing then abandoned. All were drilled and operated with standard industry procedures. The foothills research sites were located on Compton Petroleum well sites at 4-12-14-30 W4M, 4-4-14-29 W4M

and 3-33-8-2 W5M. The plains rough fescue sites were located in Rumsey Block at MSL 860346 (Canadian Natural Resources Ltd.), MSL 880398 (Husky Energy) and MSL 852250 (Trident Resources).

2.3.4 Experimental Design and Treatments

Research plots on each site were arranged in a randomized grid pattern (Figure 2.2). Each plot was 2 m x 2 m in size, separated by a 50 cm buffer. Plots were placed in a flat area with lowest cover of non-native species. Each of the following treatments was replicated four times on each site.

- Festuca hallii or Festuca campestris seeded alone at a rate of 15 kg ha⁻¹.
- Festuca hallii or Festuca campestris seeded with three common associated species at a rate of 15 kg ha⁻¹. The mixes were weight based. Festuca hallii 30 % with Stipa curtiseta 30 %, Koeleria macrantha 20 % and Helictotrichon hookeri 20 %. Festuca campestris 30 % with Danthonia parryi 30 %, Koeleria macrantha 20 % and Festuca idahoensis 20 %.
- *Festuca hallii* or *campestris* seeded at a rate of 15 kg ha⁻¹ with *Elymus dahuricus* Turcz ex Giseb. (dahurian rye) at a rate of 1.1 kg ha⁻¹.

2.3.5 Research Site Establishment

Research plots were established in summer 2008. *Festuca campestris* sites were established on June 26 and 27, *Festuca hallii* sites on July 3. *Festuca hallii* sites were completed in one day, including tilling and seeding. *Festuca campestris* sites were tilled and packed the first day then seeded the following day.

Festuca campestris plots were tilled for a concurrent research project then rototilled for this research project to a depth of approximately 20 cm using a front mount tine rototiller, across the plots in one direction. Site 2 had numerous small rocks, but not enough of them to impact tilling effectiveness. Site 3 had numerous small rocks which reduced tilling depth relative to the other sites. Each site was tilled as deep as possible to destroy growing vegetation and prepare an adequate seedbed. After tilling, the seedbed was too loose to seed directly. Thus each tilled row was packed with a home built cement roller until the soil was compressed to a depth of approximately 1.3 to 1.9 cm.

Plot establishment on *Festuca hallii* sites was similar to that on *Festuca campestris* sites, although it required an additional step prior to tilling. Litter and live plant biomass were removed to create a uniform seedbed to resemble conditions of bare soil on the foothills sites. Live material was cut using a weed eater and dead material raked off the plots. Heavy soil compaction required use of a rear mount Troy Bilt tiller. Sites were tilled twice, perpendicular to each other. Site 1 was highly compacted and could not be tilled to more than a depth of 2.5 to 4 cm. *Mellilotus officinalis* L. (sweet clover) was present on this site and even after multiple passes there was still evidence of stems and roots. Sites 2 and 3 were tilled to the maximum depth of the tiller, approximately 20 cm.

Seed was weighed as individual plant species in the laboratory and placed in small seed packets. In the field individual seed packets were mixed into one bag to form a homogeneous seed mix. The one exception was *Danthonia parryi* which was hand broadcasted separately on to each plot due to the hair on the lemma making it hard to mix with other species *Elymus dahuricus* seeds were hand dropped over the plot. *Poa juncifolia* Scribn. (sandberg bluegrass) was seeded as a cover crop in the area surrounding the research site.

Wind was a problem during seeding of the foothills sites which was dealt with by holding the seed close to the ground and using the wind as a dispersal tool. The foothills sites were seeded on June 27, 2008 and plains sites on July 3, 2008. After seeding, a lawn rake was used on each plot to simulate harrowing.

Sites were all fenced to prevent grazing. Weed management was not practiced on any of the sites during the study period. Weed control was considered but not deemed a good option due to the differences between sites and herbicide application did not fit with the goal of keeping site management to a minimum as would be done in a reclamation scenario. However, site 2 was erroneously spot sprayed by Alberta Innovates staff, targeting *Bromus inermis*.

2.3.6 Sampling and Measurement Methods

2.3.6.1 Soil water measurements

Volumetric soil water measurements were taken at the center of each research plot using a Thetaprobe ML2x[®] prior to tilling during plot establishment. In 2008

measurements were taken for foothills sites June 26, July 29 to 30, August 26 to 27 and September 25 to 26, 2008 and for plains sites on July 2, August 28 and October 2 to 3. Measurements were not taken in late July at the plains sites due to a problem with the theta probe. Measurements during the 2009 field season were on May 27 to 30, June 24 to 25, July 29 to 31 and August 27 to 28 at the foothills sites and on May 29 to 30, June 23, July 28 and August 24 to 26 at the plains sites. One measurement was taken at the center of each plot at each site, to a depth of 5 cm.

2.3.6.2 Vegetation measurements

Canopy cover, density and height were assessed in a 1 m² quadrat in the center of each plot in September 2008 and 2009. This m² area was subdivided into ten 20 x 50 cm quadrats. Three of the 20 x 50 cm areas were randomly chosen for sampling in each plot.

Canopy cover and plant density were visually assessed by species. If unidentifiable, the plant was recorded as a seedling. Height of the tallest and shortest plants of each species was measured and a visual average height for each species was estimated. Height was measured from the soil surface to the tallest part of the plant; if the plant had a seed head, it was deemed the tallest part of the plant. For species with a prostrate growth form that were not flowering, maximum height was measured as the longest leaf. Height of three (if available) *Festuca hallii* and three *Festuca campestris* plants in each 20 x 50 cm area was measured in plains and foothills sites, respectively. Density for species that reproduce vegetatively through tillering was recorded as one plant if a number of stems were bunched close together; if the source of stems could not be determined, stems were considered to be separate plants.

Tiller production of individual *Festuca hallii* or *Festuca campestris* plants was determined on three plants in each plot at each site in May and September 2009. Plants were marked with painted nails for accuracy of location. For each plant, tillers were counted and plant height measured. The difference between the May and September tiller counts was considered growth rate of the individual plants.

In September 2009 above ground biomass of each grass species was sampled; above ground biomass of forb species was collected as a whole. Plants were

clipped as close to the ground as possible and biomass stored in brown paper bags until dried. Seeded species were collected from the entire 1 m^2 area within the center of each plot. All other species were collected from a 50 cm x 50 cm area within the 1 m^2 area representative of the species composition. Litter on the soil surface, but not standing litter, was collected from the 50 cm by 50 cm area by hand raking. Biomass from *Festuca campestris* and *hallii* plants that were previously assessed for growth rate determination was collected separately from the rest of the *Festuca* plants in the plots. If the *Festuca* plant for growth rate determination was inside the 1 m^2 sampling area, its biomass was added to the total biomass of the *Festuca* for that sampling area.

2.3.7 Statistical Analyses

Data were initially analyzed with a two way analysis of variance (ANOVA) using SAS. Data were not normally distributed or homogenous as determined by the Shappiro Wilk test (Shappiro and Wilk 1965), and not significant. Based on the questions being asked it was determined that correlation analysis should be used for a further analysis. With the non normal data, Spearman correlation was used. Fescue response variables, biomass, maximum height and tiller production were compared to vegetation response variables of biomass, cover, density and height. Fescue and mix treatments were analyzed separately to keep seed mix diversity effects separate. Data were presented as means of the raw data with the associated standard deviations.

2.4 RESULTS AND DISCUSSION

2.4.1 Festuca Hallii Sites

2.4.1.1 Seeded species

Festuca hallii successfully established from seed (Table 2.1). Its biomass was low relative to total biomass on all sites and treatments. *Festuca hallii* biomass, maximum height, density and initial and final tiller numbers were generally highest in fescue alone treatments and lowest in mix treatments, varying among sites. Minimum and average height data were not presented due to the small

number of *Festuca* plants in each quadrat, with values thus equal to the maximum height presented. Cover data were not presented as there was never more than a trace due to the small stature of the *Festuca* plants.

There have been few studies assessing success of establishing rough fescue from seed. In those that have been conducted, researchers found it mostly unsuccessful. Petherbridge (2000) using sod salvage and Elsinger (2009) assessing reclamation seed mixes found fair to poor establishment of rough fescue on well sites and pipelines. Reclamation seed mixes often include some fast establishing species to minimize soil erosion and facilitate ground cover establishment. These fast establishing species are thought to provide cover and aid in establishment of slower growing climax species (Desserud 2011). However, Desserud (2010) and Elsinger (2009) found that even though *Festuca hallii* was included in reclamation seed mixes, little fescue was found during vegetation assessments.

Seeding rates differences must be considered when evaluating the performance of *Festuca hallii* in the mix treatment relative to that in the fescue alone treatment. *Festuca hallii* was seeded at a rate of 15 kg ha⁻¹ in the fescue alone treatment and at 4.5 kg ha⁻¹ in the mix treatment. Thus fescue biomass, density and cover are expected to be lower in the mix than the fescue alone treatments, making these results even more remarkable. Plant height and tiller production, as determined by subtracting May tiller number from September tiller number, may be more readily separated from effects of seeding rate (Table 2.1). Tiller production and plant height were consistently lowest in the mix treatment, suggesting *Festuca* may establish better when seeded alone. Since total cover and total biomass were generally higher in the mix than in the fescue alone treatment, competition may have been a factor in reduced fescue success. This competition would have been mainly from non-seeded species as the only seeded species other than *Festuca* that established was *Koeleria macrantha*.

Koeleria macrantha biomass and cover were low, although its density was second only to *Taraxacum officinale* L. (dandelion) (Table 2.2). It was higher in every measured category, other than height compared to *Festuca* and shorter than any of the non-seeded species measured. *Elymus dahuricus* was not effective as a cover crop (Table 2.3). It had low biomass and cover and was

highly variable height among sites. Although *Festuca* performed better with *Elymus* than in mixed treatments, fescue alone treatments were better for *Festuca* than *Elymus* treatments.

2.4.1.2 Non-seeded species

Biomass of non-seeded species was highly variable among sites and treatments (Table 2.4). Biomass was dominated by forbs at site 1 and *Agropyron trachycaulum* (Link) Malte (slender wheat grass) at sites 2 and 3. Although *Agropyron trachycaulum* was not seeded it often dominates disturbed sites (Desserud 2011). *Bromus inermis* L. (smooth brome) was only present on site 2, and with the exception of one treatment, formed a small portion of the biomass. *Poa pratensis* L. (kentucky blue grass) was the only non-seeded grass species found at all three sites. Its biomass was generally small and variable among treatments and sites. It occurred in sufficient amounts to theoretically be a competitor for *Festuca* as the plant community developed. Forb biomass occurred only in sites 1 and 2, being very high in site 1. Site 1 was more compacted (determined by observation) than the other two sites and may have been easier for forbs than grasses to colonize.

Non-seeded vegetation cover on all sites was highly variable among treatments and sites (Tables 2.5 and 2.6). Grass cover was low on most sites and treatments with *Agropyron trachycaulum* and *Poa pratensis* contributing the most to total cover (Table 2.5). Forbs typically had low cover except at site 1 (Table 2.6). *Crepis* tectorum L. (hawk's beard), *Melilotus officinalis* L. (Lam) (sweet clover) and *Taraxacum officinale* were the three dominant cover species at site 1, while *Cirsium arvense* L. (canada thistle) was the dominant forb on sites 2 and 3. Non-seeded plant height was considerably variable among sites and treatments (Tables 2.7 and 2.8).

Density of all species was low and highly variable among sites and treatments with no apparent trends (Tables 2.9 and 2.10). Density of *Agropyron repens* L. (quack grass), *Bromus inermis* and *Poa pratensis* was usually highest in the fescue treatment (Table 2.9). *Helictotrichon hookeri* was only present in the fescue treatment on site 1, likely from the fescue seed lot. *Poa pratensis* was not found in any of the mix plots but was found in some fescue plots. Although
fescue wasn't present in all of the mix plots, *Koeleria macrantha* was present in all mix plots. *Crepis tectorum,* in almost all cases when present, had the highest density (Table 2.10). This is mainly related to its growth habit, as a tap rooted single stem plant. *Melilotus* density was high on site 1.

2.4.2 Festuca Campestris Sites

2.4.2.1 Seeded species

The foothills sites had some major challenges that may have impacted revegetation results. Neither the fescue nor seed mix treatments performed well. Similar to the plains sites non-seeded species appeared to play a role on each site and may have impacted the ability of seeded species to successfully establish. Compton Livingstone 13-33-8-2 W5M (Foothills 3) was removed from the experiment because excessive cattle grazing accidentally occurred during summer 2009.

Festuca campestris biomass was highest in the fescue alone treatment at all three sites, especially notable at site 1 (Table 2.11). Plant height, density and tiller production were highly variable among treatments and sites. Plant height and density was generally highest with the *Elymus dahuricus* cover crop.

Other seeded species established included *Koeleria macrantha* and *Festuca idahoensis* (Tables 2.12 and 2.13). At both sites, the mix treatment had nearly twice the biomass as the fescue treatment, a direct result of high *Festuca idahoensis* and *Koeleria macrantha* biomass. Cover, density and height of these two species followed trends similar to that for biomass. *Koeleria macrantha* contributed a large amount to total biomass compared to non-seeded species; *Festuca idahoensis* also did but only at site 1. Average cover of both species was higher than any non-seeded grass species. On both sites the density of *Koeleria macrantha* and *Festuca idahoensis* was higher than any other species.

Danthonia parryi was seeded but not found on any of the plots. The seed is quite hairy on all parts of the lemma, and this hair may have prevented adequate seed to soil contact even after the plots were raked.

Elymus dahuricus established well on both sites, with considerably higher biomass, cover and height at site 1 (Table 2.14). It appeared to have a positive

impact on some *Festuca campestris* variables, especially density. Given that total biomass was at least three times higher in the *Elymus* treatment than the fescue treatment, competition for resources would likely account for lower *Festuca campestris* performance with *Elymus*. Given that *Elymus dahuricus* is relatively short lived, this competition may be outweighed by the positive effect of a cover crop. The positive effect on fescue density might be related to microsite creation and its impacts on germination that any variable measured related to the *Elymus*.

2.4.2.2 Non-seeded species

Biomass of non-seeded species on fescue and mix treatments at both sites was mainly forbs (Table 2.15). Forb biomass was consistently higher in fescue treatments and grasses had no consistent trend between sites or treatments.

Vegetation cover varied among treatments and between sites (Table 2.16). At both sites lowest cover was in the fescue treatment. Site 1 cover was mainly nonseeded grass species, while site 2 cover was mostly *Artemisia frigida* L. (fringed sage), with *Koeleria macrantha* being the second highest in individual cover. The other species present were highly variable and with the exception of *Agropyron trachycaulum* and *Poa juncifolia* were only present on one site.

The Artemisia frigida dominance on site 2 is somewhat different than the *Thlaspi* arvense L. (stink weed) dominance at site 1 in that Artemisia frigida is a perennial while *Thlaspi arvense* is a winter annual. The perennial cover may have had a greater negative impact on seeded species because of its longer life cycle. This may have led to increased competition throughout the growing season. The winter annual growth form may have benefitted seeded species. Winter annuals senesce earlier in the growing season limiting the competition period while still providing some protection for seedlings. *Poa juncifolia* was used as a cover crop surrounding the research plot, and drift during seeding most likely occurred.

Non-seeded grass and forb density and height varied with treatment and site (Tables 2.17 and 2.18). Values were relatively low, with no discernible trends.

2.4.3 Relationships

Numerous positive and negative correlations were found among the measured variables, however, there were no strong discernible trends for either fescue or

mixed treatments (Tables 2.19, 2.20, 2.21, 2.22). Some correlations were only significant at one or two of the sites.

As a slow establishing species, *Festuca hallii* would likely be affected by a combination of competition from non-native species and low soil water during early establishment. *Festuca hallii* and *Koeleria macrantha* biomass were positively correlated on all sites. This suggests that seeding *Festuca hallii* with associated native species may increase its growth during the first growing season. Considering other measured variables, *Koeleria macrantha* could be used as a native cover crop species for establishing *Festuca hallii*. These correlations contradict the fact that *Festuca hallii* growth variables were higher when *Festuca hallii* was seeded alone. This positive correlation could indicate that *Koeleria macrantha* was able to provide a microsite suitable for increased success of fescue growth, or the conditions that favoured *Koeleria macrantha* also favoured *Festuca hallii*.

Desserud (2011) found *Festuca hallii* did best growing next to other *Festuca hallii* plants or forbs. In our study, correlations between perennial forbs and *Festuca hallii* growth were mixed. Forb cover was negatively correlated with fescue growth while forb height had a positive correlation. Perennial forbs, especially those such as *Taraxacum officinale*, have large leaves which could limit light penetration to the soil surface, but leaves are low to the ground. A plant such as *Crepis tectorum* has a much smaller leaf area but it has an upright growth form.

The low cover and presence of undesirable non-native or pioneer type species on most sites is not unexpected in the early establishment stage of fescue grassland. Over time this cover is expected to increase, but with species such as *Bromus inermis* and *Cirsium arvense* present, non-native species might persist or expand and prevent establishment of other native species. *Crepis tectorum* cover was positively correlated with *Festuca hallii* and *Koeleria macrantha* density. At site 1, *Crepis tectorum* may have acted differently than the other nonnative forb species. Given its single stem and tap root growth form, it may have benefitted establishment of native species by acting as a nurse crop.

The high presence of non-seeded species suggest site preparation was not appropriate for their control and different control measures should be evaluated. This is expected for seed bank rich sites, particularly those that are overgrazed

or otherwise disturbed, facilitating a larger seed bank of non-native species. Canopy cover of more rapidly growing non-seeded species may have limited light penetration to the soil surface; as the seeded species were typically among the shortest plants this may have been a limitation. The canopy cover may also have been a limitation to precipitation reaching the soil.

Three species that are of some concern to seeded species growth are *Agropyron trachycaulum*, *Bromus inermis* and *Melilotus officinalis*. These species are considered highly competitive. *Taraxacum officinale* was present on at least one plot on all three sites, which is not unexpected as it is an indicator of unhealthy communities (Tannas 2004).

Lack of *Stipa curtiseta* establishment is difficult to explain. There are limited data on its establishment and it is thought to require two growing seasons to establish (Pahl and Smreciu 1999). Since it is a large seed compared to *Koeleria macrantha* and *Festuca hallii*, broadcast seeding may not have been the most effective seeding method as it may have insufficient seed to soil contact. The low establishment of *Helictotrichon hookeri* is also difficult to explain. It too has little data on establishment. The seed was wild harvested from a different location and may not have been adapted to the site conditions seeded into.

Elymus dahuricus was not a successful cover crop for *Festuca hallii* and was marginal for *Festuca campestris*. This was based on the biomass data for *Elymus dahuricus*. It was low on the plains fescue sites, and although higher on the foothills sites the density was still low.

Presence of winter annual forb species may aid in establishment of *Festuca campestris*. Although no data were shown for winter annuals, foothills site one had a higher proportion of their cover during the growing season. Fescue biomass and tiller production was higher on site one when compared to site two. Thus there may be a relationship to explore in future research to determine if this relationship can be quantified.

2.4.4 Soil Water

Volumetric soil water was variable among sites but showed a general trend in both 2008 and 2009 (only 2009 data shown due to very little differences among

years) of increasing from May to June, decreasing into July, increasing in August and decreasing from August to September (Table 2.23). This is to be expected, as Alberta is subject to the plains precipitation pattern where the majority of precipitation falls in late spring and early summer. There were no specific treatment effects evident and thus no interpretations of the results of this research as related to soil water content.

2.4.5 Reclamation Applications

This study demonstrates that fescue plants can be grown and established from seed in the field. Similar work by Desserud (2011) also showed that *Festuca hallii* could be grown from seed in the field. Although plants were generally small in size at the start of the 2009 growing season, growth over four months between measurements suggests the plants were productive and healthy. Although these sites would likely not meet reclamation criteria due to the low ground cover, there is potential for fescue to persist and develop. These results contradict those of Tannas (2010) and Sheley et al. (2006) who found that *Festuca campestris* establishment from seed in the field was very poor. Grilz (1994) found that germination requirements for *Festuca hallii* were even narrower than those for *Festuca campestris*, suggesting establishment may have potential to be more difficult. However, this was not supported by our results.

This study has shown that *Festuca hallii* and *campestris* are very slow to establish, which is similar to Wilson and Johnston (1969). This could mean that timelines for reclamation success need to be adjusted accordingly. The slow growth rate could indicate poor competitiveness while juvenile and leave it poorly adapted to adverse growing conditions found on disturbed sites (Bailey 1972). Fescue is inherently slow growing and this should be taken into consideration when evaluating it in a reclamation scenario. Even with evidence that establishment from seed is possible it may not be enough to restore rough fescue to predisturbance levels. Seeding may need to be accompanied by other methods of revegetation to be completely successful. Establishment may be further impacted by seeding time and rate. Although these factors were not directly compared, seeding rate may have been a factor in the greater success of the fescue alone treatment.

2.5 CONCLUSIONS

- Festuca hallii and Festuca campestris can establish from seed.
- The slow rate of establishment of *Festuca hallii* and *Festuca campestris* means reclamation success determination timelines need to be extended relative to those for non-native species.
- *Koeleria macrantha* and *Festuca idahoensis* are native species that are able to establish well from seed.
- *Elymus dahuricus* was not a successful cover crop for *Festuca hallii* and was marginal for *Festuca campestris*.
- The presence of winter annual forb species may aid in establishment of *Festuca campestris*.

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Figure 2.1 Research site locations.

Fescue	Fescue Fungicide	Mix	Wild Rye
Mix Fungicide	Wild Rye	Mix Fungicide	Fescue Fungicide
Fescue Fungicide	Mix	Fescue	Wild Rye
Mix Fungicide	Fescue Fungicide	Mix	Fescue
Mix	Fescue	Mix Fungicide	Wild Rye

Figure 2.2 Research plot layout. (Note fungicide treatments are part of the research discussed in Chapter 3).

Site	Treatment	Biomass	Maximum	Density	Tiller	May Tiller	Tiller Number
		(g m⁻²)	Height (cm)	(plants m⁻²)	Production	Number	September
1	Fescue	0.8 (0.8)	2.8 (3.3)	12.5 (20.5)	9.3 (10.9)	4.7 (2.9)	17.4 (15.1)
1	Mix	0.0 (0.1)	2.4 (2.9)	40.0 (6.2)	1.7 (1.5)	3.7 (3.5)	4.3 (1.5)
1	Elymus	0.8 (0.9)	8.5 (12.7)	8.3 (10.9)	6.8 (4.5)	1.9 (1.6)	9.6 (9.1)
2	Fescue	1.6 (1.6)	12.1 (11.1)	20.8 (14.9)	11.8 (8.7)	5.0 (2.7)	17.9 (11.2)
2	Mix	0.4 (0.3)	5.3 (5.5)	8.0 (8.5)	8.8 (2.5)	6.8 (5.4)	13.8 (11.6)
2	Elymus	0.3 (0.4)	4.0 (5.7)	1.5 (2.1)	12.0	3.6 (3.4)	16.0 (7.1)
3	Fescue	1.9 (2.2)	14.5 (5.2)	14.3 (5.1)	5.8 (3.8)	5.3 (2.4)	10.9 (8.5)
3	Mix	0.5 (0.4)	6.8 (13.5)	0.8 (1.5)	4.1 (2.5)	3.7 (2.0)	8.3 (5.7)
3	Elymus	0.8 (0.9)	8.1 (6.1)	11.8 (17.5)	4.2 (3.8)	4.4 (3.7)	8.6 (6.6)
Mean	Fescue	1.4 (1.6)	9.8 (8.4)	15.8 (14.0)	9.0 (8.0)	4.9 (2.6)	15.0 (11.5)
Mean	Mix	0.3 (0.3)	4.8 (8.2)	3.9 (3.9)	4.3 (3.4)	4.7 (4.1)	8.8 (7.2)
Mean	Elymus	0.7 (0.8)	7.5 (8.5)	8.3 (8.3)	6.2 (4.4)	3.3 (3.1)	9.6 (7.7)

Table 2.1 Festuca hallii growth variables at plains sites in 2009

Site	Treatment	Biomass (g m ⁻²)	Cover (%)	Height (cm)	Density (plants m ⁻²)
1	Fescue	-	-	-	-
1	Mix	1.5 (1.7)	4.8 (6.8)	8.3 (1.2)	3.8 (1.5)
1	Elymus	0.3 (0.5)	1.4 (2.9)	1.8 (3.5)	0.3 (0.5)
2	Fescue	0.1 (0.1)	-	-	-
2	Mix	6.5 (4.6)	2.4 (1.0)	8.9 (2.0)	6.0 (6.1)
2	Elymus	-	-	-	-
3	Fescue	-	-	-	-
3	Mix	4.2 (2.5)	2.0 (2.0)	10.4 (3.2)	2.9 (1.6)
3	Elymus	-	0.1 (0.2)	1.0 (2.0)	0.1 (0.2)
Mean	Fescue	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean	Mix	3.9 (3.4)	3.1 (4.1)	9.2 (2.3)	4.0 (4.0)
Mean	Elymus	0.1 (0.3)	0.6 (1.8)	1.1 (2.4)	0.1 (0.1)

Table 2.2 Koeleria macrantha growth variables at plains sites in 2009

Table 2.3 *Elymus dahuricus* growth variables at plains sites in 2009

Site	Treatment	Biomass	Cover	Height	Density
		(g m⁻²)	(%)	(cm)	(plants m ⁻²)
1	Elymus	9.2 (14.7)	4.5 (8.1)	15.8 (13.6)	0.6 (0.3)
2	Elymus	8.8 (11.7)	7.2 (8.7)	104.5 (14.8)	0.3 (0.0)
3	Elymus	3.4 (3.8)	-	-	0.15 (0.17)
Mean	Elymus	7.1 (10.4)	3.2 (6.3)	27.2 (42.4)	0.4 (0.3)

Site	Treatment	Agropyron	Bromus	Poa	Forbs	Total
		trachycaulum	inermis	pratensis		Biomass
1	Fescue	-	-	0.4 (0.6)	154.9 (178.3)	161.4 (184.1)
1	Mix	-	-	-	84.4 (36.6)	91.4 (48.8)
1	Elymus	-	-	0.6 (1.1)	68.2 (58.7)	82.9 (65.2)
2	Fescue	80.5 (86.7)	57.2 (64.3)	13.7 (20.7)	15.2 (22.7)	126.8 (91.2)
2	Mix	171.5 (81.4)	17.7 (17.9)	4.9 (5.5)	8.8 (8.0)	260.5 (181.3)
2	Elymus	70.3 (60.4)	2.1 (2.9)	72.5 (62.5)	28.8 (38.0)	305.3 (19.5)
3	Fescue	80.3 (40.1)	-	31.1 (21.2)	-	190.1 (30.7)
3	Mix	72.1 (12.7)	-	1.4 (1.7)	-	197.8 (41.6)
3	Elymus	139.6 (87.9)	-	2.9 (5.7)	-	190.0 (53.4)
Mean	Fescue	51.1 (60.2)	15.6 (39.3)	16.7 (20.7)	51.0 (111.2)	159.2 (101.8)
Mean	Mix	73.0 (80.1)	4.8 (11.5)	1.8 (3.3)	33.1 (45.6)	176.2 (114.0)
Mean	Elymus	69.9 (85.5)	0.4 (1.3)	15.9 (36.5)	33.1 (48.5)	170.3 (100.1)

Table 2.4 Non-seeded grass and forb biomass (g m^{-2}) at plains sites in 2009

Site	Treatment	Aaropvron	Bromus	Poa	Stipa	Total
		trachycaulum	inermis	pratensis	viridula	Cover
1	Fescue	-	-	-	-	20.6 (22.3)
1	Mix	-	-	-	-	29.7 (33.6)
1	Elymus	-	-	-	-	34.8 (30.3)
2	Fescue	2.5 (2.9)	5.0 (2.3)	5.3 (5.5)	-	27.1 (13.4)
2	Mix	6.7 (2.9)	1.9 (1.4)	0.1 (0.2)	-	16.0 (2.8)
2	Elymus	12.7 (5.7)	2.9 (4.0)	14.0 (18.0)	-	50.0 (33.9)
3	Fescue	7.3 (4.1)	-	1.8 (1.7)	0.2 (0.2)	15.1 (4.9)
3	Mix	4.1 (0.9)	-	0.5 (0.6)	0.3 (0.3)	16.7 (2.1)
3	Elymus	10.8 (4.5)	-	1.7 (1.3)	1.2 (0.9)	18.3 (5.8)
Mean	Fescue	3.3 (4.1)	1.7 (2.7)	2.4 (3.8)	0.0 (0.0)	20.9 (14.8)
Mean	Mix	3.3 (3.2)	0.5 (1.1)	0.2 (0.4)	0.0 (0.0)	21.1 (19.6)
Mean	Elymus	6.8 (6.7)	0.6 (1.8)	3.5 (8.2)	0.0 (0.0)	31.2 (24.6)

Table 2.5 Non-seeded grass cover (%) at plains sites in 2009

Site	Treatment	Cirsium	Crepis	Melilotus	Taraxacum	Total
		arvense	tectorum	officinalis	officinale	Cover
1	Fescue	-	3.5 (2.3)	5.8 (7.4)	5.8 (4.9)	20.6 (22.3)
1	Mix	-	6.9 (5.5)	8.2 (10.3)	3.2 (2.5)	29.7 (33.6)
1	Elymus	-	8.2 (6.4)	8.3 (7.2)	5.8 (2.8)	34.8 (30.3)
2	Fescue	0.8 (1.7)	-	-	-	27.1 (13.4)
2	Mix	0.8 (1.1)	-	-	-	16.0 (2.8)
2	Elymus	-	-	-	-	50.0 (33.9)
3	Fescue	0.8 (1.5)	-	-	-	15.1 (4.9)
3	Mix	4.3 (3.4)	-	-	-	16.7 (2.1)
3	Elymus	0.4 (0.9)	-	-	-	18.3 (5.8)
Mean	Fescue	0.5 (1.2)	1.2 (2.1)	1.9 (4.8)	2.0 (3.8)	20.9 (14.8)
Mean	Mix	1.8 (2.8)	2.3 (4.5)	2.7 (6.7)	1.1 (2.0)	21.1 (19.6)
Mean	Elymus	0.2 (0.5)	3.3 (5.6)	3.3 (6.0)	2.8 (3.1)	31.2 (24.6)

Table 2.6 Non-seeded forb cover (%) at plains sites in 2009

Site	Treatment	Agropyron	Bromus	Poa	Stipa
		trachycaulum	inermis	pratensis	viridula
1	Fescue	-	9.2 (6.0)	-	-
1	Mix	-	6.1 (7.4)	-	-
1	Elymus	-	5.0 (8.7)	-	-
2	Fescue	48.3 (33.7)	45.2 (15.9)	33.7 (19.0)	-
2	Mix	71.4 (20.2)	40.1 (26.9)	13.8 (11.9)	-
2	Elymus	80.0 (4.9)	22.8 (32.2)	47.6 (5.8)	-
3	Fescue	50.2 (7.0)	-	26.9 (11.9)	16.5 (21.4)
3	Mix	56.2 (16.6)	-	20.0 (15.5)	27.0 (34.8)
3	Elymus	58.6 (4.4)	-	32.0 (9.4)	53.6 (14.0)
Mean	Fescue	32.8 (30.2)	18.1 (22.3)	20.7 (18.7)	8.3 (14.1)
Mean	Mix	39.9 (34.7)	13.9 (22.8)	11.0 (13.5)	9.0 (22.5)
Mean	Elymus	39.5 (35.1)	6.1 (14.7)	23.9 (19.6)	22.7 (28.0)
1/1					

Table 2.7 Non-seeded grass height (cm) at plains sites in 2009

Site	Treatment	Cirsium	Crepis	Melilotus	Taraxacum
		arvense	tectorum	officinalus	officinale
1	Fescue	-	8.2 (6.0)	43.2 (11.9)	9.3 (4.5)
1	Mix	-	7.8 (9.3)	42.7 (15.6)	7.5 (4.8)
1	Elymus	-	6.4 (7.2)	43.2 (9.8)	6.9 (6.5)
2	Fescue	12.3 (24.5)	-	-	-
2	Mix	15.2 (18.7)	-	-	-
2	Elymus	-	-	-	-
3	Fescue	4.5 (9.0)	-	-	-
3	Mix	27.0 (18.2)	-	-	-
3	Elymus	5.0 (10.0)	-	-	-
Mean	Fescue	5.6 (14.6)	1.2 (2.1)	1.9 (4.8)	1.9 (3.8)
Mean	Mix	13.9 (17.7)	2.3 (4.5)	2.7 (6.7)	1.1 (2.1)
Mean	Elymus	2.0 (6.3)	3.3 (5.6)	3.3 (6.0)	2.3 (3.4)

Table 2.8 Non-seeded forb height (cm) at plains sites in 2009

Site	Treatment	Agropyron	Agropyron	Agropyron	Bromus	Helictotrichon	Poa	Poa	Stipa
		dasystachum	repens	trachycaulum	inermis	hookeri	palustris	pratensis	viridula
1	Fescue	2.5 (3.3)	-	-	-	1.8 (3.5)	-	-	-
1	Mix	22.5 (43.0)	-	-	-	0.8 (1.5)	-	-	-
1	Elymus	6.5 (6.0)	-	-	-	0.8 (1.5)	-	-	-
2	Fescue	-	-	14.8 (19.6)	41.8	-	-	21.8 (11.5)	-
					(40.5)				
2	Mix	-	-	30.3 (15.3)	18.7	-	-	4.3 (5.1)	-
					(21.4)				
2	Elymus	-	-	8.5 (2.1)	5.0 (7.1)	-	-	10.0 (4.2)	-
3	Fescue	-	43.5 (41.6)	27.5 (16.7)	0.8 (1.5)	-	-	21.0 (18.9)	1.5 (1.7)
3	Mix	1.5 (1.7)	27.5 (26.4)	17.5 (7.6)	0.8 (1.5)	-	0.8 (1.5)	6.8 (5.3)	1.5 (1.7)
3	Elymus	0.8 (1.5)	16.8 (33.5)	38.3 (12.8)	5.0 (6.3)	-	4.3 (5.1)	18.5 (7.0)	3.3 (2.9)
Mean	Fescue	0.8 (2.1)	14.1 (17.9)	14.1 (17.9)	14.2	0.6 (2.0)	0.0 (0.0)	14.3 (15.6)	0.5 (1.2)
					(29.4)				
Mean	Mix	8.7 (8.7)	14.6 (14.6)	14.6 (14.6)	5.4 (5.4)	0.3 (0.3)	0.3 (0.3)	3.6 (3.6)	0.5 (0.5)
Mean	Elymus	2.9 (2.9)	6.7 (6.7)	17.0 (17.0)	3.0 (3.0)	0.3 (0.3)	1.7 (1.7)	9.4 (9.4)	1.3 (1.3)

Table 2.9 Non-seeded grass density (plants m^{-2}) at plains sites in 2009

Site	Treatment	Achillea	Androsace	Cirsium	Crepis	Melilotus	Taraxacum
		millefolium	septentrionalis	arvense	tectorum	officinalus	officinale
1	Fescue	-	-	-	91.0 (66.6)	11.8 (8.1)	39.3 (53.2)
1	Mix	-	-	-	85.8 (69.1)	32.8 (27.8)	11.8 (10.6)
1	Elymus	-	-	-	144.3 (98.9)	31.8 (10.2)	9.3 (2.9)
2	Fescue	-	-	-	54.0 (97.5)	-	23.3 (39.9)
2	Mix	-	-	-	7.7 (13.3)	-	198.0 (185.2)
2	Elymus	-	-	-	5.0 (2.8)	-	1.5 (2.1)
3	Fescue	3.3 (4.7)	5.8 (7.6)	1.8 (3.5)	-	-	9.3 (18.5)
3	Mix	7.5 (10.8)	2.5 (3.3)	23.3 (22.0)	-	-	5.8 (7.6)
3	Elymus	5.8 (6.8)	8.5 (12.8)	0.8 (1.5)	-	-	10.0 (20.0)
Mean	Fescue	1.1 (2.9)	1.9 (4.9)	0.6 (2.0)	48.3 (73.0)	3.9 (7.2)	23.9 (38.3)
Mean	Mix	2.7 (2.7)	0.9 (0.9)	8.5 (8.5)	33.3 (33.3)	11.9 (11.9)	60.4 (60.4)
Mean	Elymus	2.3 (2.3)	3.4 (3.4)	0.3 (0.3)	58.7 (58.7)	12.7 (12.7)	8.0 (8.0)
Value		بامعام متعقم اممت	معسيا مناحمة مسماكمات	leata			

Table 2.10 Non-seeded forb density (plants m⁻²) at plains sites in 2009

Site	Treatment	Biomass	Maximum	Density	Tiller	Tiller	Tiller
		(g m ⁻²)	Height	(plants m ⁻²)	Production	Number	Number
			(cm)			May	September
1	Fescue	5.2 (3.8)	0.0 (0.0)	-	24.4 (9.2)	5.7 (4.3)	28.6 (8.3)
1	Mix	1.6 (1.9)	3.8 (7.5)	0.8 (1.5)	30.0 (14.1)	5.3 (2.5)	34.5 (13.4)
1	Elymus	1.8 (1.5)	6.3 (7.3)	3.3 (4.7)	16.2 (8.3)	4.8 (4.4)	19.4 (10.2)
2	Fescue	1.6 (0.6)	4.5 (8.9)	4.3 (8.5)	15.5 (2.1)	5.7 (5.7)	20.6 (20.6)
2	Mix	-	0.9 (1.8)	1.8 (3.5)	3.0 ¹	9.3 (9.3)	$4.0 (4.0)^2$
2	Elymus	0.2 (0.3)	4.5 (9.0)	4.3 (8.5)	17.0 ¹	5.2 (5.2)	21.0 (21.0)
Mean	Fescue	3.4 (3.2)	2.2 (6.3)	2.1 (6.0)	5.7 (3.8)	24.6 (8.2)	19.8 (8.5)
Mean	Mix	0.8 (1.5)	3.1 (5.2)	1.3 (2.5)	7.3 (20.0)	24.3 (20.0)	21 (18.5)
Mean	Elymus	1.0 (1.3)	5.4 (7.7)	3.8 (6.4)	5 (3.5)	19.9 (8.4)	16.4 (6.8)

Table 2.11 Festuca campestris growth variables at foothills sites in 2009

¹Only one value ²Only one plant survived Values are means and standard deviations in brackets

meannent	Biomass	Cover	Density	Height
	(g m⁻²)	(%)	(plants m ⁻²)	(cm)
Fescue	-	0.8 (1.7)	0.8 (1.5)	2.0 (4.0)
Mix	42.9 (16.3)	8.3 (5.8)	16.8 (9.7)	14.0 (4.2)
Elymus	-	1.3 (2.5)	0.8 (1.5)	2.5 (5.0)
Fescue	-	-	-	-
Mix	39.5 (6.6)	19.2 (15.1)	63.3 (66.3)	6.7 (4.5)
Elymus	0.0 (0.0)	-	-	-
Fescue	0.0 (0.0)	0.4 (1.2)	0.4 (1.1)	1.0 (2.8)
Mix	41.2 (11.6)	13.7 (12.1)	40.0 (50.4)	10.4 (5.6)
Elymus	0.0 (0.0)	0.6 (1.8)	0.4 (1.1)	1.3 (3.5)
	Fescue Mix Elymus Fescue Mix Elymus Fescue Mix Elymus	(g m ⁻²) Fescue - Mix 42.9 (16.3) Elymus - Fescue - Mix 39.5 (6.6) Elymus 0.0 (0.0) Fescue 0.0 (0.0) Mix 41.2 (11.6) Elymus 0.0 (0.0)	$\begin{array}{c ccccc} (g\ m^{-2}) & (\%) \\ \hline \\ $	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table 2.12 Koeleria macrantha growth variables at foothills sites in 2009

Table 2.13 Festuca idahoensis growth variables at foothills sites in 2009

Site	Treatment	nent Biomass Cove		Density	Height
		(g m ⁻²)	(%)	(plants m ⁻²)	(cm)
1	Fescue	0.5 (1.0)	3.2 (4.0)	0.5 (0.6)	5.8 (8.0)
1	Mix	20.0 (8.3)	8.8 (7.1)	2.8 (1.7)	11.8 (3.3)
1	Elymus	0.6 (1.2)	2.4 (3.2)	0.5 (0.6)	6.1 (7.2)
2	Fescue	0.8 (0.9)	5.0 (3.6)	1.3 (0.3)	11.2 (6.0)
2	Mix	1.9 (0.8)	6.4 (6.2)	3.0 (2.8)	7.7 (2.2)
2	Elymus	0.8 (1.1)	2.1 (2.5)	1.2 (1.6)	5.5 (6.4)
Mean	Fescue	0.6 (0.9)	4.1 (3.6)	0.9 (0.6)	8.5 (7.2)
Mean	Mix	11.0 (11.1)	7.6 (6.3)	2.9 (2.2)	9.7 (3.4)
Mean	Elymus	0.7 (1.1)	2.2 (2.6)	0.8 (1.1)	5.8 (6.3)

Values are means and standard deviations in brackets

Table 2.14 Elymus dahuricus growth variables at foothills sites in 2009

Site	Treatment	Biomass	Cover	Height	
		(g m⁻²)	(%)	(cm)	
1	Elymus	288.1 (277.4)	21.1 (17.3)	91.4 (24.5)	
2	Elymus	113.0 (76.0)	11.7 (10.4)	57.5 (38.5)	
Mean	Elymus	200.6 (210.3)	16.4 (14.1)	74.5 (34.9)	

		-				
Site	Treatment	Hordeum	Poa	Poa	Forbs	Total
		jubatum	juncifolia	pratensis		Biomass
1	Fescue	28.0 (33.1)	-	30.3 (32.9)	100.3 (192.5)	159.2 (154.3)
1	Mix	-	-	44.3 (44.7)	1.4 (3.1)	108.8 (44.7)
1	Elymus	34.6 (69.2)	-	0.6 (1.3)	50.9 (101.8)	374.9 (199.5)
2	Fescue	-	1.2 (3.9)	2.1 (3.9)	163.1 (72.9)	167.1 (73.2)
2	Mix	-	4.5 (6.8)	-	122.9 (70.8)	168.8 (63.4)
2	Elymus	-	4.4 (8.7)	2.4 (4.7)	128.5 (134.0)	249.0 (142.9)
Mean	Fescue	14.0 (26.3)	0.6 (1.7)	16.2 (26.4)	131.7 (138.9)	163.1 (111.9)
Mean	Mix	-	2.2 (5.1)	22.2 (34.5)	62.2 (79.7)	138.8 (60.1)
Mean	Elymus	17.3 (48.9)	2.2 (6.2)	1.5 (3.3)	89.7 (117.7)	311.9 (174.2)
<u> </u>						

Table 2.15 Non-seeded grass and forb biomass (g m^{-2}) at foothills sites in 2009

		-					
Site	Treatment	Agropyron	Hordeum	Poa	Poa	Artemisia	Total
		trachycaulum	jubatum	juncifolia	pratensis	frigida	Cover
1	Fescue	-	7.5 (9.6)	6.9 (8.1)	6.9 (8.1)	-	27.7 (7.8)
1	Mix	6.7 (11.2)	1.8 (3.3)	6.3 (6.3)	6.3 (6.3)	-	38.1 (21.2)
1	Elymus	-	16.8 (33.3)	2.2 (1.4)	2.2 (1.4)	-	44.5 (18.3)
2	Fescue	5.8 (6.9)	-	1.0 (1.6)	-	27.5 (15.0)	39.7 (24.5)
2	Mix	1.3 (1.9)	-	0.3 (0.7)	-	24.9 (24.6)	52.5 (34.8)
2	Elymus	2.1 (4.2)	-	2.1 (3.2)	-	19.9 (21.4)	40.9 (29.0)
Mean	Fescue	2.9 (5.5)	3.8 (7.4)	0.5 (1.2)	3.5 (6.4)	13.8 (17.7)	33.7 (18.0)
Mean	Mix	4.0 (8.0)	10.5 (13.7)	0.2 (0.5)	3.1 (5.3)	12.4 (20.9)	18.0 (27.7)
Mean	Elymus	1.0 (2.9)	8.4 (23.6)	1.1 (2.4)	1.1 (1.4)	10.0 (17.6)	42.7 (22.5)

Table 2.16 Non-seeded grass and forb cover (%) at foothills sites in 2009

Site	Treatment	Agropyron	Hordeum	Poa	Poa	Artemisia frigida
		trachycaulum	jubatum	juncifolia	pratensis	
1	Fescue	-	2.5 (3.3)	-	11.8 (13.9)	-
1	Mix	-	2.5 (3.3)	-	30.3 (28.8)	-
1	Elymus	-	6.8 (9.4)	-	6.5 (4.7)	-
2	Fescue	2.5 (3.3)	-	2.5 (3.3)	0.8 (1.5)	31.8 (13.8)
2	Mix	5.8 (6.8)	-	3.3 (6.5)	-	26.8 (9.4)
2	Elymus	1.2 (3.5)	-	1.5 (1.7)	-	12.5 (11.2)
Mean	Fescue	1.3 (2.5)	1.3 (2.5)	1.3 (2.5)	6.3 (10.9)	15.9 (19.2)
Mean	Mix	2.9 (5.4)	1.3 (2.5)	1.6 (4.6)	15.1 (24.8)	13.4 (15.6)
Mean	Elymus	0.9 (2.5)	3.4 (7.2)	0.8 (1.4)	3.3 (4.7)	6.3 (9.9)

Table 2.17 Non-seeded grass and forb density (plants m^{-2}) at foothills sites in 2009

Site	Treatment	Agropyron	Hordeum	Poa	Poa	Artemisia
		trachycaulum	jubatum	juncifolia	pratensis	frigida
1	Fescue	-	19.5 (22.5)	-	13.5 (15.6)	-
1	Mix	33.3 (38.4)	7.5 (11.4)	-	22.8 (15.5)	-
1	Elymus	-	15.8 (26.4)	-	37.5 (16.3)	-
2	Fescue	25.3 (30.6)	-	6.3 (7.2)	-	36.3 (1.1)
2	Mix	9.5 (12.8)	-	3.3 (6.5)	-	23.3 (13.2)
2	Elymus	5.0 (10.0)	-	18.0 (21.4)	-	16.4 (19.0)
Mean	Fescue	12.6 (24.2)	9.8 (18.1)	3.1 (5.8)	6.8 (12.5)	18.1 (19.4)
Mean	Mix	21.4 (29.4)	3.8 (8.4)	1.6 (4.6)	11.4 (15.8)	11.6 (15.1)
Mean	Elymus	2.5 (7.1)	7.9 (19.2)	9.0 (17.0)	18.8 (22.7)	8.2 (15.2)

Table 2.18 Non-seeded grass and forb height (cm) at foothills sites in 2009

Site	Variable	Parameter 1	Parameter 2	+ or -	Correlation Coefficient	Value
1	Biomass	Forb	Total biomass	+	0.92	0.0030
1	Biomass	Forb	Fescue tiller	-	0.65	0.1200
2	Biomass	Agropyron trachycaulum	Fescue tiller	+	0.82	0.0900
2	Biomass	Poa pratensis	Fescue tiller	-	0.87	0.0500
2	Biomass	Bromus inermis	Poa pratensis biomass	+	0.81	0.0500
3	Biomass	Total	Fescue height	+	0.71	0.0500
3	Biomass	Poa pratensis	Fescue height	+	0.69	0.0600
1	Cover	<i>Melilotus</i> spp	Fescue tiller	-	0.95	0.0003
2	Cover	Total	Fescue height	-	0.81	0.0300
2	Cover	Bromus inermis	Cirsium arvense cover	-	0.86	0.0100
2	Cover	Agropyron trachycaulum	Poa pratensis cover	-	0.79	0.0400
3	Cover	Stipa viridula	Fescue height	-	0.80	0.0200
3	Cover	Stipa viridula	Fescue biomass	-	0.80	0.0200
1	Density	Crepis tectorum	Fescue biomass	+	0.72	0.0400
1	Density	Festuca	Fescue height	+	0.73	0.0400
1	Density	<i>Melilotus</i> spp	Fescue height	+	0.66	0.0800
2	Density	Bromus inermis	Fescue density	+	0.75	0.0500
2	Density	Bromus inermis	Poa pratensis	+	0.77	0.0400
2	Density	Crepis tectorum	<i>Taraxacum</i> officinale density	-	0.75	0.0500
2	Density	Crepic tectorum	Fescue biomass	+	0.87	0.0500
3	Density	Agropyron dasystachyum	Fescue biomass	-	0.76	0.0300
3	Density	Stipa viridula	Fescue height	-	0.77	0.0200
3	Density	Cirsium arvense	Fescue tiller	+	0.73	0.0400
1	Height	<i>Melilotus</i> spp	Fescue tiller	-	0.95	0.0003
1	Height	Crepis tectorum	Fescue height	-	0.70	0.0500
2	Height	Poa pratensis	Fescue biomass	-	0.80	0.1000
2	Height	Bromus inermis	Fescue height	+	0.85	0.0200
3	Height	Cirsium arvense	Fescue tiller	+	0.76	0.0300
3	Height	Cirsium arvense	<i>Stipa viridula</i> height	+	0.69	0.0600
3	Height	Agropyron trachycaulum	<i>Poa pratensis</i> height	-	0.71	0.0500

Table 2.19 Correlations for fescue treatments at plains sites in 2009

Site	Variable	Parameter 1	Parameter 2	+ or -	Correlation Coefficient	Value
1	Biomass	Forb	Total biomass	+	1.00	0.0001
2	Biomass	Koeleria macrantha	Fescue biomass	+	0.80	0.1000
2	Biomass	Agropyron trachycaulum	Total biomass	+	0.80	0.1000
3	Biomass	Koeleria macrantha	Total biomass	-	0.69	0.0600
3	Biomass	Koeleria macrantha	Fescue biomass	+	0.71	0.0700
1	Cover	Koeleria macrantha	Fescue biomass	+	0.78	0.0200
1	Cover	Crepis tectorum	Fescue height	-	0.73	0.0400
1	Cover	Taraxacum officinale	Fescue tiller	-	0.79	0.0600
2	Cover	Agropyron trachycaulum	Total biomass	+	0.87	0.0500
2	Cover	Koeleria macrantha	Poa pratensis cover	+	0.76	0.1300
2	Cover	Koeleria macrantha	Fescue height	+	0.89	0.0400
2	Cover	Koeleria macrantha	Fescue biomass	+	0.87	0.0500
2	Cover	Cirsium arvense	Koeleria macrantha cover	-	0.94	0.0100
2	Cover	Cirsium arvense	Fescue height	-	0.95	0.0100
3	Cover	Poa pratensis	Stipa viridula cover	-	0.72	0.2000
3	Cover	Cirsium arvense	Total biomass	+	0.79	0.2000
3	Cover	Cirsium arvense	Fescue tiller	-	0.68	0.0700
1	Density	Taraxacum officinale	Fescue height	+	0.77	0.0200
1	Density	Helictotrichon hookeri	Fescue biomass	+	0.66	0.0800
1	Density	Koeleria macrantha	<i>Taraxacum</i> officinale density	-	0.86	0.0060
2	Density	Crepis tectorum	Fescue	+	1.00	0.0001
2	Density	Crepis tectorum	<i>Koeleria macrantha</i> density	+	1.00	0.0001
2	Density	Crepis tectorum	Fescue height	+	1.00	0.0001
2	Density	Festuca	<i>Koeleria macrantha</i> density	+	1.00	0.0001
2	Density	Agropyron trachycaulum	Fescue	+	0.95	0.0500
2	Density	Bromus inermis	Fescue	+	0.83	0.1700
2	Density	Festuca	Fescue height	+	1.00	0.0001

Table 2.20 Correlations for mixed treatments at plains sites in 2009

Site	Variable	Parameter 1	Parameter 2	+ or -	Correlation Coefficient	Value
2	Density	Agropyron trachycaulum	Koeleria macrantha density	+	0.95	0.0500
2	Density	Agropyron trachycaulum	<i>Taraxacum</i> officinale density	-	0.80	0.2000
2	Density	Bromus inermis	Koeleria macrantha density	+	0.83	0.1700
2	Density	Poa pratensis	Fescue biomass	+	1.00	0.0001
3	Density	Achillea millefolium	Fescue tiller	+	0.86	0.0070
3	Density	Koeleria macrantha	Fescue tiller	+	0.70	0.0500
3	Density	Bromus inermis	Fescue tiller	-	0.76	0.0300
3	Density	Cirsium arvense	Fescue tiller	-	0.80	0.0100
3	Density	Festuca	Agropyron trachycaulum density	+	0.84	0.0090
3	Density	Festuca	Fescue height	+	0.90	0.0020
3	Density	Bromus inermis	<i>Koeleria macrantha</i> density	-	0.78	0.0200
3	Density	Cirsium arvense	Koeleria macrantha density	-	0.68	0.0600
3	Density	Bromus inermis	<i>Cirsium arvense</i> density	+	0.74	0.0400
3	Density	Cirsium arvense	<i>Taraxacum</i> officinale density	-	0.83	0.0100
1	Height	Taraxacum officinale	Fescue tiller	-	0.79	0.0600
1	Height	Koeleria macrantha	Fescue biomass	+	0.78	0.0200
1	Height	Cirsium arvense	Fescue height	-	0.73	0.0400
2	Height	Cirsium arvense	Fescue height	-	0.79	0.1100
2	Height	Poa pratensis	<i>Koeleria macrantha</i> density	-	0.87	0.0500
3	Height	Cirsium arvense	Fescue tiller	-	0.68	0.0700
3	Height	Koeleria macrantha	Fescue height	-	0.65	0.0800
3	Height	Poa pratensis	<i>Stipa viridula</i> density	-	0.71	0.0500

Table 2.20 Correlations for mixed treatments at plains sites in 2009 (continued)

 Site	Variable	Parameter 1	Parameter 2	+ or -	Correlation Coefficient	Value
 1	Biomass	Poa pratensis	Fescue tiller biomass	-	1.00	0.0001
1	Biomass	Total	Fescue tiller biomass	-	0.80	0.2000
1	Biomass	Forb	Fescue tiller biomass	+	0.77	0.2300
1	Biomass	Poa pratensis	Festuca idahoensis biomass	+	0.68	0.0600
1	Biomass	Poa pratensis	Forb biomass	-	0.87	0.0050
2	Biomass	Forb	Fescue tiller biomass	-	0.80	0.1000
2	Biomass	Poa pratensis	Fescue biomass	+	0.76	0.0300
2	Biomass	Poa juncifolia	Fescue height	+	0.90	0.0020
1	Cover	Poa pratensis	Fescue tiller	-	1.00	0.0001
1	Cover	Total	Fescue tiller	+	0.80	0.2000
1	Cover	Koeleria macrantha	Fescue tiller	+	0.77	0.2300
1	Cover	Total	Fescue biomass	+	0.67	0.0700
1	Cover	Total	Fescue height	+	0.79	0.0200
1	Density	Festuca	Fescue height	+	0.97	0.0001
1	Density	Festuca	Fescue tiller	-	0.77	0.2200
1	Density	Koeleria macrantha	Fescue tiller	+	0.77	0.2300
1	Height	Festuca	Fescue tiller	-	0.77	0.2300
1	Height	Poa pratensis	Fescue tiller	-	0.80	0.2000
1	Height	Festuca idahoensis	Fescue tiller	+	0.80	0.2000
1	Height	Koeleria macrantha	Fescue tiller	+	0.77	0.2300
2	Height	Poa juncifolia	Fescue biomass	+	0.81	0.0100
 2	Height	Artemisia frigida	Fescue tiller	-	0.82	0.0100

Table 2.21 Correlations for fescue treatments at foothills sites in 2009

Site	Variable	Parameter 1	Parameter 2	+ or -	Correlation Coefficient	Value
1	Biomass	Festuca idahoensis	Fescue biomass	+	0.71	0.0700
1	Biomass	Koeleria macrantha	Total biomass	+	0.79	0.0400
1	Biomass	Poa pratensis	Total biomass	+	0.68	0.0900
2	Biomass	Festuca idahoensis	Forbs biomass	-	0.79	0.0200
2	Biomass	Festuca idahoensis	Koeleria macrantha biomass Koeleria	+	0.93	0.0009
2	Biomass	Forbs	<i>macrantha</i> biomass	-	0.88	0.0040
1	Cover	Hordeum jubatum	Koeleria macrantha cover	-	0.74	0.0600
1	Cover	Hordeum jubatum	Poa pratensis cover	-	0.80	0.0300
1	Cover	Koeleria macrantha	Festuca idahoensis cover	+	0.79	0.0300
2	Cover	Festuca idahoensis	Artemisia frigida cover	+	0.75	0.0300
1	Density	Festuca	Fescue height	+	1.00	0.0001
1	Density	Festuca idahoensis	Poa pratensis density	+	0.93	0.0030
1	Density	Festuca idahoensis	Koeleria macrantha density	+	0.85	0.0100
1	Density	Festuca idahoensis	<i>Hordeum jubatum</i> density	-	0.86	0.0100
1	Density	Koeleria macrantha	Hordeum jubatum density	-	0.81	0.0300
1	Density	Koeleria macrantha	Poa pratensis density	+	0.85	0.0200
1	Height	Festuca idahoensis	Poa pratensis height	+	0.90	0.0060
1	Height	Koeleria macrantha	Poa pratensis height	+	0.94	0.0020
1	Height	Festuca idahoensis	<i>Koeleria macrantha</i> height	+	0.96	0.0005
1	Height	Agropyron trachycaulum	Poa juncifolia height	+	0.73	0.0400

Table 2.22 Correlations for mixed treatments at foothills sites in 2009

Month	Treatment	R1	R2	R3	F1	F2	F3
May	Fescue	12.8	9.2	11.2	19.9	15.7	20.5
-		(2.6)	(2.6)	(2.2)	(2.4)	(2.0)	(2.4)
	Mix	11.6	9.6	13.2	22.4	12.0	19.8
		(3.8)	(1.6)	(4.0)	(2.2)	(1.6)	(3.5)
	Wild Rye	11.7	10.2	13.3	18.2	15.2	19.9
		(3.3)	(1.6)	(1.3)	(2.4)	(0.6)	(1.9)
June	Fescue	23.0	22.0	17.4	22.4	11.5	27.2
		(0.3)	(3.5)	(1.8)	(2.3)	(1.0)	(3.0)
	Mix	24.0	21.1	18.1	19.4	11.0	29.5
		(0.7)	(1.1)	(2.4)	(1.8)	(2.8)	(1.3)
	Wild Rye	23.3	22.9	18.0	20.9	11.8	24.4
		(2.5)	(2.0)	(1.1)	(3.0)	(0.9)	(3.5)
July	Fescue	10.5	9.4	11.2	15.3	11.9	35.7
		(2.4)	(1.4)	(2.2)	(3.6)	(2.1)	(4.7)
	Mix	10.6	7.3	9.7	15.5	12.4	35.2
		(2.7)	(0.8)	(1.1)	(2.0)	(1.0)	(5.1)
	Wild Rye	10.8	10.1	12.9	14.4	9.6	36.5
		(2.4)	(1.4)	(2.2)	(2.5)	(3.0)	(2.7)
August	Fescue	18.0	16.8	17.1	18.6	6.5	19.7
		(4.2)	(3.5)	(2.3)	(4.1)	(0.8)	(4.4)
	Mix	17.4	15.2	16.9	19.3	6.3	16.8
		(4.7)	(1.3)	(2.6)	(1.8)	(1.1)	(2.6)
	Wild Rye	16.6	17.8	17.3	14.1	6.5	19.2
		(0.7)	(2.8)	(3.3)	(2.0)	(1.1)	(3.4)
September	Fescue	9.8	10.9	11.7	11.7	4.0	-
		(1.6)	(2.8)	(2.8)	(4.7)	(0.8)	
	Mix	9.9	10.5	11.4	12.4	3.7	-
		(2.3)	(0.8)	(2.4)	(3.6)	(0.6)	
	Wild Rye	9.7	11.4	13.2	9.9	2.9	-
		(1.2)	(2.2)	(6.4)	(2.0)	(1.0)	

Table 2.23 Volumetric soil water (%) at 0-5 cm depth in 2009

R = plains sites F = foothills sites

CHAPTER III. MYCORRHIZAE IMPACTS ON *FESTUCA HALLII* (VASEY) PIPER (PLAINS ROUGH FESCUE) AND *FESTUCA CAMPESTRIS* RYDB. (FOOTHILLS ROUGH FESCUE)

3.1 INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are generally regarded as required by many vascular plants for uptake of phosphates and other nutrients from soil (Molina et al. 1978). This benefit is particularly important for plants in marginal habitats (Allen et al. 1981). The association between plant and fungi is generally regarded as mutualistic with benefits usually limited to individual plants (Fitter 1991). Recent research has focused on the impact of AMF on plant interactions in a community, by evaluating interactions between plants and how AMF may be associated with them (Hartnett et al. 1993). This role may be most pronounced in disturbed site restoration where natural succession is occurring (Smith et al. 1998). Smith and Read (2008) found seedlings growing in a community with AMF colonization may be linked to other plants by a common mycelial network and acquire inorganic nutrients without supplying carbon required to support AMF.

Bever (2003, 2002) proposed a feedback model with both a positive and negative relationship between two plant and two fungal species. He showed in general that the fungus gives greatest benefit to one plant species but grows best on another plant species, creating a negative feedback loop. No evidence for positive feedback was found. This shows relationships between specific plant and fungal species are not always advantageous (Smith and Read 2008).

Oil and gas disturbances typically involve soil disturbance; topsoil is usually stripped and stored before replacement. AMF in this soil may be affected by storage time, with AMF viability decreasing the longer soil is stored (Gould and Liberta 1981, Rives et al 1980). *Festuca hallii* and *campestris* have multiple AMF species colonize their roots including *Glomus fasciculatus* (Thaxter) Gerd. & Trappe, *Glomus macrocarpus* var. *Macrocarpus* Tul. and Tul. and *Glomus scrobiculata* Trappe (Molina et al. 1978). Knowing rough fescue can be colonized by AMF and that there may be an association between AMF and plant response, reduction in AMF by disturbances may impact restoration of fescue.

3.2 OBJECTIVES AND HYPOTHESES

3.2.1 Objectives

The objective of this research was to determine whether *Festuca hallii* (Vasey) Piper (plains rough fescue) and *Festuca campestris* Rydb. (foothills rough fescue) will be impacted by mycorrhizal fungi at young seedling and early juvenile stages.

3.2.2 Hypotheses

- *Festuca* plants infected with mycorrhizae will be smaller in size than those without mycorrhizae infections, with reduced height and tillering due to the increased carbon demand from mycorrhizae which will limit resources (carbon) the *Festuca* plant has to allocate to growth.
- Festuca plants infected with mycorrhizae will have less root biomass than those without mycorrhizae infections. Mycorrhizae will increase the depletion zone for plant nutrients; the increased surface area for nutrient uptake will result in the plant not having to put resources into root growth for nutrient and water uptake.

3.3 MATERIALS AND METHODS

3.3.1 Festuca Hallii Research Area

The northern fescue subregion is bordered to the north by the central parkland and to the south by mixed grass prairie (Natural Regions Committee 2006). The plains rough fescue research sites occurred within Rumsey Block in east central Alberta (Figure 3.1). Rumsey Block is the largest remaining tract of aspen parkland in the world (American Wilderness Association 2000). It is divided into two areas, the Rumsey ecological reserve which is 33.5 km² and the Rumsey natural area which is 149 km² in size.

Rumsey Block has a continental climate, with long, cold, dry winters and short, moderately warm summers (Fehr 1982). Average mean annual temperature is 2.7 °C, ranging from -14.3 °C in winter to 17.2 °C in summer. Growing degree

days average 1,490 (Natural Regions Committee 2006). Precipitation averages 384.6 mm annually with 286.8 mm during the April to August growing season.

Rumsey Block is characterized by symmetrical knob and kettle terrain with the occasional depressed center (Stalker 1972). Surface drainage is poor and internal. Many sloughs are dry by mid-summer because of low precipitation; standing water either evaporates or seeps into the ground (Fehr 1982).

Soils are predominantly dark brown chernozems, at the northern edge of the dark brown soil zone. Parent material is glacial till over Paskapoo and Edmonton formations (Bowser et al. 1951). The principle soil is Hughenden loam developed under native grass cover on well drained sites (Fehr 1982). On level areas, soil is Halkirk loam with a solodized solonetz B horizon. Due to topography, depth and development of organic horizons varies, grading to gleysols in depressions.

Undisturbed south facing grassland sites are dominated by *Festuca hallii -Stipa curtiseta* (Hitchc.) (western porcupine grass) associations grading into sedge wetlands in kettles. On moister north facing slopes *Symphoricarpos occidentalis* Hook (buck brush) and *Populus tremuloides* Michx. (trembling aspen) groves occur. Moist grasslands favour *Festuca hallii* dominance, with other species such as *Stipa curtiseta*, *Helictotrichon hookerii* (Scribn.) Henr. (hookers oat grass), *Koeleria macrantha* (Ledeb.) J.A. Schultes) (june grass) and perennial forbs. Drier sites and grazing of moist sites favour sub-dominant species with *Festuca hallii* present but with a reduced cover.

3.3.2 Festuca Campestris Research Area

The foothills fescue subregion is bordered to the north by central parkland and northern fescue subregions, to the east by mixed prairie and to the west by foothills parkland. Topography is rolling hummocky in the south and west, with undulating plains in the north and east (Natural Regions Committee 2006). Open water and wetlands are uncommon in hillier terrain.

The subregion is characterized by a continental climate (Natural Regions Committee 2006). It has the highest precipitation, warmest summers and shortest growing season of the Alberta grassland subregions. Average mean annual temperature is 3.9 °C, from -9.7 °C in winter to 16.3 °C in summer. Growing degree days average 1,388. Average annual precipitation is 469.6 mm with 333.1 mm falling during the April to August growing season.

Parent material is predominantly glacial till. Soils under fescue grassland are dominated by variants of black chernozems. Drainage in the northern parts is into the South Saskatchewan river system (Adams et al. 2003).

Festuca campestris, Festuca idahoensis Elmer (idaho fescue), *Carex* sp. (sedges) and *Agropyron smithii* (Rydb.) A. Löve (western wheat grass) dominate the southern half of the subregion (Natural Regions Committee 2006). In the northern half, *Festuca campestris* and *Danthonia parryi* Scribn. (parry's oat grass) dominate. *Lupinus argenteus* Pursh (silvery lupine), *Geum triflorum* Pursh (three flowered aven), *Geranium viscossisimum* Fisch. & C.A. Mey. ex C.A. Mey. (sticky purple geranium), *Artemisia frigida* Willd (pasture sage) and *Thermopsis rhombifolia* (Nutt. ex Pursh) Nutt. ex Richardson (golden bean) are common forbs. Shrubs such as *Elaeagnus commutata* Bernh. ex Rydb. (silver berry), *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem. (saskatoon), *Rosa acicularis* Lindl. (prickly rose) and *Ceanothus cuneatus* (Hook.) Nutt. (buck brush) occur on moist sites, while shrubs such *as Potentilla fruticosa* L. (shrubby cinquefoil) are present but most abundant on areas that are at least moderate grazed.

3.3.3 Research Site Locations

Three natural gas well sites in each of foothills rough fescue and plains rough fescue grassland were selected for research. Well sites were either drilled and abandoned or drilled and producing then abandoned. All were drilled and operated with standard industry procedures. The foothills research sites were located on Compton Petroleum well sites at 4-12-14-30 W4M, 4-4-14-29 W4M and 3-33-8-2 W5M. The plains rough fescue sites were located in Rumsey Block at MSL 860346 (Canadian Natural Resources Ltd.), MSL 880398 (Husky Energy) and MSL 852250 (Trident Resources).

3.3.4 Experimental Design and Treatments

Research plots on each site were arranged in a randomized grid pattern in a 4×5 arrangement. Each plot was 2 m x 2 m in size, separated by a 50 cm buffer.

Plots were placed in a flat area with low cover of non-native species. Each of the following treatments was replicated four times on each site.

- Festuca hallii or Festuca campestris seeded alone at a rate of 15 kg ha⁻¹.
- *Festuca hallii* or *Festuca campestris* seeded alone at a rate of 15 kg ha⁻¹, with a fungicide (Rovral) application.
- Festuca hallii or Festuca campestris seeded with a mix of three common associated species at a rate of 15 kg ha⁻¹. The mixes were weight based. Festuca hallii 30 % with Stipa curtiseta 30 %, Koeleria macrantha 20 % and Helictotrichon hookeri 20 %. Festuca campestris 30 % with Danthonia parryi 30 %, Koeleria macrantha 20 % and Festuca idahoensis 20 %.
- Festuca hallii or Festuca campestris seeded with a mix of three common associated species at a rate of 15 kg ha⁻¹. The mixes were weight based. Festuca hallii 30 % with Stipa curtiseta 30 %, Koeleria macrantha 20 % and Helictotrichon hookeri 20 %. Festuca campestris 30 % with Danthonia parryi 30 %, Koeleria macrantha 20 % and Festuca idahoensis 20 %, with a fungicide (Rovral) application.

3.3.5 Research Site Establishment

Research plots were established in summer 2008. *Festuca campestris* sites were established on June 26 and 27, *Festuca hallii* sites on July 3. *Festuca hallii* sites were completed in one day, including tilling and seeding. *Festuca campestris* sites were tilled and packed the first day then seeded the following day.

Festuca campestris plots were tilled for a concurrent research project then rototilled for this research project to a depth of approximately 20 cm using a front mount tine rototiller, across the plots in one direction. Site two had numerous small rocks, but not enough of them to impact tilling effectiveness. Site three had numerous small rocks which reduced tilling depth relative to the other sites. Each site was tilled as deep as possible to destroy growing vegetation and prepare an adequate seedbed. After tilling, the seedbed was too loose to seed directly. Thus each tilled row was packed with a home built cement roller until the soil was compressed to a depth of approximately 1.3 to 1.9 cm.

Plot establishment on *Festuca hallii* sites was similar to that on *Festuca campestris* sites, although they required an additional step prior to tilling. Litter

and live plant biomass were removed to create a uniform seedbed to resemble conditions of bare soil on the foothills sites. Live material was cut using a weed eater and dead material was raked off the plots. Heavy soil compaction required use of a rear mount Troy Bilt tiller. Sites were tilled twice, perpendicular to each other. Site 1 was highly compacted and could not be tilled to more than a depth of 2.5 to 4 cm. *Mellilotus officinalis* L. (sweet clover) was present on this site and even after multiple passes there was still evidence of stems and roots. Sites 2 and 3 were tilled to the maximum depth of the tiller, approximately 20 cm.

Seed was weighed as individual plant species in the laboratory and placed in small seed packets. In the field individual seed packets were mixed into one bag to form a homogeneous seed mix. The exception was *Danthonia parryi* which was hand broadcasted separately on to each plot due to the hair on the lemma making it hard to mix with other species; *Elymus dahuricus* seeds were hand dropped over the plot. *Poa juncifolia* Scribn. (sandberg bluegrass) was seeded as a cover crop in the area surrounding the research site.

Wind was a problem during seeding of the foothills sites which was dealt with by holding the seed close to the ground and using the wind as a dispersal tool. Seeding was done on foothills sites on June 27, 2008 and on plains sites on July 3, 2008. After seeding, a lawn rake was used on each plot to simulate harrowing.

Sites were fenced to prevent grazing. Weed management was not practiced on any of the sites during the study period. Weed control was considered but not deemed a good option due to differences between sites, and herbicide application did not fit with the goal of keeping site management to a minimum as would be done in a reclamation scenario. However, site 2 was erroneously spot sprayed by Alberta Innovates staff, targeting *Bromus inermis*.

Fungicide (Rovral) was applied at 1.56 L ha ⁻¹ using a back pack sprayer with single spray nozzle. 2008 application occurred on June 26, July 29 to 30, August 26 to 27 and September 25 to 26 for the foothills sites and for plains sites on July 2, August 28 and October 2 to 3. Application during the 2009 field season was on May 27 to 30, June 24 to 25, July 29 to 31 and August 27 to 28 at the foothills sites and on May 29 to 30, June 23, July 28 and August 24 to 26 at the plains sites. Fungicide was measured in a 25 ml graduated cylinder and mixed with water in the sprayer tank. Amount of water used was 4 L per plot to provide
sufficient coverage. Each plot was sprayed individually in a horseshoe pattern, starting with the outside perimeter and working backwards to evenly apply the fungicide and prevent tracking of sprayed soil. The procedure for the second and third applications of fungicide differed slightly from the initial application. A backpack sprayer with a four nozzle boom was used to reduce the time required to spray each site and get a more even distribution of fungicide.

3.3.6 Sampling and Measurement Methods

3.3.6.1 Soil water measurements

Volumetric soil water measurements were taken at the center of each research plot using a Thetaprobe $ML2x^{(B)}$ prior to tilling during plot establishment. Measurements were taken during 2008 for foothills sites June 26, July 29 to 30, August 26 to 27 and September 25 to 26 and for plains sites on July 2, August 28 and October 2 to 3. Measurements were not taken in late July at the plains sites due to a problem with the theta probe. Measurements during the 2009 field season were on May 27 to 30, June 24 to 25, July 29 to 31 and August 27 to 28 at the foothills sites and on May 29 to 30, June 23, July 28 and August 24 to 26 at the plains sites. One measurement was taken at the center of each plot at each site, to a depth of 5 cm.

3.3.6.2 Vegetation measurements

Canopy cover, plant density and plant height were assessed in a 1 m² quadrat in the center of each plot in September 2008 and 2009. This m² area was subdivided into ten 20 x 50 cm quadrats. Three of the 20 x 50 cm areas were randomly chosen for sampling in each plot.

Canopy cover and density were visually assessed by species. If unidentifiable, the plant was recorded as a seedling. Height of the tallest and shortest plants of each species was measured and a visual average height for each species was estimated. Height was measured from the soil surface to the tallest part of the plant; if the plant had a seed head, it was deemed the tallest part of the plant. For species with a prostrate growth form that were not flowering, maximum height was measured as the longest leaf. Height of three (if available) *Festuca hallii* and three *Festuca campestris* plants in each 20 x 50 cm area was measured in plains and foothills sites, respectively. Density for species that reproduce vegetatively through tillering was recorded as one plant if a number of stems were bunched close together; if the source of stems could not be determined, stems were considered to be separate plants.

Tiller production of individual *Festuca hallii* or *Festuca campestris* plants was determined on three plants in each plot at each site in May and September 2009. Plant locations were marked with painted nails. For each plant, tillers were counted and plant height measured. The difference between the May and September tiller counts was considered growth rate of the individual plants.

In September 2009 above ground biomass of each grass species was sampled; above ground biomass of forb species was collected as a whole. Plants were clipped as close to the ground as possible and biomass was stored in brown paper bags until dried. Seeded species were collected from the entire 1 m^2 area within the center of each plot. All other species were collected from a 50 cm x 50 cm area within the 1 m^2 area representative of the species composition. Litter on the soil surface, but not standing litter, was collected from the 50 cm by 50 cm area by hand raking. Biomass from *Festuca campestris* and *hallii* plants that were previously assessed for growth rate determination was collected separately from the rest of the *Festuca* plants in the plots. If the *Festuca* plant for growth rate determination was inside the 1 m^2 sampling area, its biomass was added to the total biomass of the *Festuca* for that sampling area.

3.3.6.3 Glucosamine Assay

Assessment of AMF colonization of *Festuca campestris* and *Festuca hallii* roots was done using a glucosamine assay. The assay is based on the fact that chitin, a polymer of N-acetyl- β -Dglucosamine, is a component of the cell wall of the majority of fungi and is not found in plant cells (Nilsson and Bjurman 1998). Glucosamine indicates the presence of chitin and may be used to determine AMF biomass (Appuhn and Joergensen 2006, Appuhn et al. 2004, Ekblad and Nälsholm 1996, Sylvia 1994, Vignon et al. 1986).

Roots from the field collected samples were cut by hand from the crown and washed by hand with water to remove any soil and other foreign material.

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Washed roots were oven dried at 96 °C for 48 hours (Nilsson and Bjurman 1998). Dried roots were ground using a mortar and pestle to achieve a particle size of < 0.5 mm, any material larger than 0.5 mm was removed using a sieve.

A modified version of the glucosamine assay used by Nilsson and Bjurman (1998) was followed. A maximum of 200 mg of ground root material from each sample was placed in clean test tubes with three wraps of Teflon tape on the threads. Exact weight of the sample was determined by weighing an empty test tube and then weighing the same test tube with the ground root sample. To the samples weighing between 100 and 200 mg, 5 ml of 6 N HCL was added (first dilution). Test tube caps were tightened and the samples were hydrolyzed in an oven at 96 °C for 48 hours and then cooled to ambient temperature. Five ml of de-ionized water was added to the samples (second dilution).

Two ml of hydrolysate was removed from the hydrolyzed samples, carefully avoiding solid root particles, and placed in clean test tubes. These samples were evaporated in a 50 °C water bath. Evaporation was assisted by compressed air injection using a pasteur pipette. Evaporated precipitate was rehydrated by adding one ml of de-ionized water immediately after evaporation was complete to ensure particles did not stick to the test tube, 4 ml of de-ionized water was then added to complete the 5 ml dilution (third dilution). One ml was then extracted and placed in teflon wrapped test tubes.

A glucosamine standard is needed to compare to field samples. The standard was prepared with 50 µg/ml glucosamine: one with 100% glucosamine, one with 100% distilled water and five with graduated dilutions. To 1 ml samples and glucosamine standards, 0.25 ml of 4 % acetylacetone solution (4 % by volume acetylacetone in 1.25 N sodium carbonate) was added. Test tubes were tightly capped and bathed in a 100 °C water bath for 1 hour, then cooled to ambient temperature in a cool water bath. Two ml of ethanol was added to each sample, which were then shaken with an agitator for 5 seconds to dissolve the precipitate. Then, 0.25 ml of Ehrlich reagent (1.6 g of N-N-dimethyl-Paminobenzaldehyde in 60 ml of 1:1 mixture of ethanol and concentrated HCl) was added and shaken with an agitator for 5 seconds (Nilsson and Bjurman 1998).

These samples were then placed in a Spectronic-2 spectrophotometer. Readings of absorbance were taken at A530 nanometers, de-ionized water sample to zero

the spectrometer. Readings were compared to a standard curve made from the glucosamine standard and used to calculate the amount of glucosamine per dry gram of root, carefully incorporating all dilutions into the calculation.

3.3.7 Statistical Analyses

Mycorrhizae measured in the form of glucosamine was compared with Spearman correlations against fescue growth variables of density, tiller production and biomass. Soil water was also compared against glucosamine. Data were not separated by treatment as it was determined that the fungicide was not effective in reducing mycorrhizae colonization on the roots (Table 3.1). Treatment was therefore removed and it was a comparison of glucosamine levels to growth variables.

3.4 RESULTS AND DISCUSSION

Glucosamine varied numerically with site and treatment, with no clearly defined trends (Table 3.1). At foothills site 2, glucosamine was positively correlated with soil water at 0.95 (0.05) and at site 3, glucosamine was negatively correlated with fescue biomass at -1.0 (0.0001). At plains sites, significance was again limited. At site 2, soil water was negatively correlated with glucosamine at -1.0 (0.0001). At site 3 soil water and fescue density were both positively correlated with glucosamine at 0.8 (0.2).

There are a number of reasons why the fungicide may not have worked. Application may not have been as effective as desired. The goal was to apply the fungicide to soil. Cover of unseeded species on all sites may have acted as a barrier to soil contact during spraying. Fungicide that was sprayed that contacted any plants may not have reached the soil at all. Carrier volume could have had an effect. There may have not been enough water applied to get adequate movement into the soil profile, which would have prevented reduction of mycorrhizae. Since the amount of fungicide was being applied to a controlled area, the carrier volume could have been increased. The rototilling may have pulverized the fungal mycelium, making it unable to colonize fescue roots at the time of seeding.

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Environmental characteristics may have played a role in the fungicide being ineffective in this research. Fungicide was applied during the day and was always applied on days with full sun. These warm conditions may have evaporated the fungicide before it was able to infiltrate and penetrate into the soil profile. Soil pH may have been a factor as Rovral[™] has specific pH requirements that may not have been met. Desserud (2011) showed that even under optimum pH conditions Rovral[™] was still not effective in reducing soil mycorrhizae. This would suggest that the choice of Rovral as the fungicide to use may not have been the best choice. In future work, a different fungicide or different application rate should be explored.

The correlation results at the foothills sites confirm one hypothesis presented and disprove one hypothesis in the literature. The significant negative correlation between glucosamine present on fescue roots and fescue biomass may suggest that presence of mycorrhizae is a limitation to fescue growth during early stages of fescue establishment and growth. Smith (1980) found that growth depressions at seedling stages when all plants were of the same age. Although this may not always be the case as there was no correlation between mycorrhizae and height or tiller production.

The positive correlation between glucosamine and soil water (Table 3.2) does not confirm the hypothesis that mycorrhizae is a drought tolerance mechanism for plants. If this was the case, then lower soil water would have theoretically produced higher mycorrhizae on the fescue roots to compensate for less water. The drought tolerance mechanism may be a more important factor on mature plants with well developed root systems and well developed relationships with mycorrhizae. At a juvenile stage the fescue roots and associated mycorrhizae may not be developed enough to produce any benefit to growth such as increased nutrient uptake or drought tolerance.

At the plains sites there was both positive and negative relationships between soil water and glucosamine. Differences may have been site specific with soil properties of each site. The positive correlation of glucosamine with fescue density is somewhat different than what was expected. Density however is not a fitness characteristic but more of a result of seeding techniques and environmental factors.

3.5 CONCLUSIONS

Based on the limited results with the glucosamine test conclusions are difficult to draw. From the correlation and the glucosamine test, it appears that there is no relationship between mycorrhizae on rough fescue roots and growth response variables. However, many unknowns preclude this as a conclusion.

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Treatment	Location										
	Foothills 1	Foothills 2	Foothills 3	Foothills	Plains 1	Plains 2	Plains 3	Plains			
Fescue	2.1 (0.7)	2.6 (2.0)	1.3 (1.1)	2.0 (0.7)	1.0 (0.3)	1.5 (1.2)	1.1 (0.5)	1.2 (0.4)			
Fescue Fungicide	1.8 (1.5)	1.9 (1.1)	0.0 (0) ¹	1.2 (0.8)	1.7 (0) ¹	0.6 (0) ¹	1.8 (1.8)	1.4 (0.9)			
Mix	1.5 (0) ¹	1.9 (0) ¹	1.7 (1.0)	1.7 (0.6)	3.0 (0) ¹	1.8 (0.7)	0.9 (0.5)	1.9 (0.1)			
Mix Fungicide	2.7 (0) ¹	2.0 (0) ¹	1.2 (0) ¹	2.0 (0) ¹	0.5 (0) ¹	0.9 (0) ¹	1.8 (1.7)	1.1 (1.0)			
Wild Rye	1.6 (0.7)	1.0 (0.1)	1.5 (0.2)	1.4 (0.2)	0.0 (0) ¹	0.0 (0) ¹	0.6 (0.2)	0.2 (0.2)			

Table 3.1 Glucosamine values (mg g⁻¹) for all sites

1 only one value for these sites Values are means and standard deviations in brackets

Month	Trootmont	Plains	Plains Site	Plains	Foothills	Foothills	Foothills
	mealment	Site 1	2	Site 3	Site 1	Site 2	Site 3
Мау	Fescue	10.5 (2.7)	11.3 (0.7)	12.0 (1.3)	19.0 (3.6)	14.2 (1.6)	20.8 (2.3)
	Mix	12.6 (1.5)	10.8 (2.7)	13.6 (2.1)	19.4 (1.2)	13.7 (1.6)	20.0 (3.1)
June	Fescue	23.9 (1.7)	23.1 (3.5)	21.5 (4.2)	20.1 (3.4)	11.2 (1.9)	25.1 (5.5)
	Mix	24.3 (0.4)	21.3 (2.7)	18.1 (1.6)	19.7 (3.0)	11.3 (0.9)	29.0 (3.5)
July	Fescue	9.9 (3.0)	10.5 (3.3)	12.4 (2.9)	14.3 (2.4)	10.4 (1.8)	38.9 (2.0)
	Mix	9.3 (2.8)	10.9 (1.8)	10.6 (2.6)	17.0 (0.8)	11.5 (2.1)	38.9 (2.5)
August	Fescue	17.5 (3.8)	14.7 (2.6)	19.8 (1.3)	18.0 (4.5)	7.1 (1.7)	17.5 (3.1)
	Mix	16.9 (1.8)	17.8 (3.9)	19.7 (2.1)	18.8 (1.8)	7.1 (0.8)	16.9 (3.0)
September	Fescue	8.4 (1.2)	9.9 (1.8)	13.9 (2.4)	9.9 (3.0)	4.0 (1.4)	-
	Mix	10.6 (2.3)	8.6 (2.1)	12.4 (1.8)	14.2 (2.6)	3.8 (0.4)	-

Table 3.2 Volumetric soil water (%) in 2009

Values are means and standard deviations in brackets

CHAPTER IV. RESEARCH SUMMARY AND APPLICATIONS

4.1 RESEARCH SUMMARY

Both *Festuca hallii* and *Festuca campestris* grasslands are susceptible to disturbance. In this research the disturbance was resource extraction related. Rough fescue provides many ecological and economic benefits so successful re-establishment of rough fescue on these areas is critical.

This research was designed to address two main objectives. The first was to determine if rough fescue establishes better as a monoculture or within a mix of closely associated species. The second was to determine the impact of arbuscular mycorrhizae on the establishment of rough fescue.

This research showed that *Festuca hallii* and *Festuca campestris* can establish from seed in the field. The low rate of establishment of both *Festuca hallii* and *Festuca campestris* means reclamation success determination timelines need to be extended relative to those for non-native species. *Koeleria macrantha* and *Festuca idahoensis* were able to establish well from seed. *Elymus dahuricus* was not a successful cover crop for *Festuca hallii* and was marginal for *Festuca campestris*. The presence of winter annual forb species may aid in establishment of *Festuca campestris*.

4.2 RECLAMATION APPLICATIONS

This research shows that seeding rough fescue can be successful. The method of applying a fungicide to the soil to reduce mycorrhizae was not conclusive. To ensure the long term success of rough fescue reclamation, other methods may need to be used in conjunction with seeding.

4.3 RESEARCH LIMITATIONS

A number of things could have been done differently during the field work and project establishment to clarify the results.

- Although weeds are part of reclamation, and are practical to deal with, doing the research in a weed free environment could have eliminated some of the competing factors.
- Site characteristics were variable. Controlling site preparation would have led to better control conditions between sites, and may have led to better fescue growth.
- More research into the effectiveness of the fungicide used and other fungicides may have influenced the results but the choice was limited by time.
- Both Festuca campestris and Festuca hallii are slow growing species, which may have been overlooked or underestimated for the short field duration of the project. There may not have been enough establishment time to get quality growth data. The plants may have needed one more growing season before destructive sampling.
- Although field rough fescue research is important, with the short research periods of an MSc, greenhouse work may have been more applicable.

4.4 FUTURE RESEARCH

Given the limited knowledge about restoration of *Festuca hallii* and *Festuca campestris* grasslands, previous to this study and the research by Desserud (2011) and Tannas (2011), there are still a number of questions that need to be answered by future research.

- This study was unsuccessful in effectively reducing mycorrhizae on roots of Festuca hallii and Festuca campestris, but as per the greenhouse work of Desserud (2011) Festuca hallii did respond positively to reductions in mycorrhizae. The potential for mycorrhizae to negatively impact rough fescue growth needs to be addressed further in the field. Use of a different fungicide and/or a different application method may be used to achieve more effective mycorrhizae reduction.
- The research should be conducted over a longer time period.
- The impact of non-seeded species needs to be addressed. Tannas (2011) examined the impact of *Poa pratensis* but there may be other species that impact establishment success of fescue.

- The use of nurse plants could be explored to determine whether a mature rough fescue plant near a seedling plant can reduce the impact of mycorrhizae on that plant.
- Cover crops and associated species need further investigation. *Elymus dahuricus* may not have been an appropriate cover crop and fescue did not appear to have any short term benefit from it. *Thalapsi arvense* did appear to have a positive effect on fescue growth. Thus a short lived forb may be more desirable for a cover crop.
- The effect that associated species have on fescue success needs to be examined in greater depth. With the limited success of the species chosen for this project, more needs to be learned about the associated species or the species selection needs to be modified.
- Seeding methods and seeding times should be studied. Seeding for this study occurred in late June and early July, which is not regarded as optimum seeding times for native species. Seeding in the spring versus the fall could be explored. Seeding method could be evaluated, such as comparing broadcast seeding to drill seeding.