



**STRATEGIES FOR EVALUATING THE EFFECTIVENESS OF RECLAMATION SUCCESSES ON NATIVE
LANDSCAPES - PROJECT #911651**

Final Report

Prepared for

Petroleum Technology Alliance Canada (PTAC)

Prepared by

By Jay Woosaree

Alberta Innovates – Technology Futures

February 2012

Executive Summary

This study examined three pervasive issues that impacted native ecosystems.

- Numerous land use practices including oil and gas activities have led to the modification of native grasslands both the within the Northern Fescue Region and the Foothills Fescue Region. Is it possible to eradicate non-native species from well leases and pipelines that were previously seeded to non-native forages, and what is the potential for invasive non-native species to convert rough fescue grasslands to non-native communities and can they be restored?
- Can specific salt and hydrocarbon tolerant plant species be used to remediate areas affected by pipeline breaks or produced salt water and hydrocarbon spills?
- A critical part to reclamation is to have appropriate plant materials. Does industry have access to native species? What species are needed and how are they efficiently brought into commercial production?

Non-native invasive species such as *Bromus inermis* (Smooth brome grass), *Agropyron pectiniforme* (Crested wheatgrass), *Phleum pratense* (Timothy), *Cirsium arvense* (Canada thistle) can be eradicated from disturbed sites that were previously seeded to non-native forages. Properly timed and applied herbicide applications followed by mowing and followed by seeding with appropriate native species may provide an effective solution in restoring these grasslands. Once seeded to native species, the successional pathways and the influence of non-native species on the area remains uncertain due to other anthropogenic activities on the landscapes.

At the Rumsey Parkland sites, a number of native grasses and forbs are now growing on the sites, however percent cover provided by *Festuca halli* represents a small percentage.

At the Longview sites, fewer establishments has occurred. Climate and soils play a major role in reclamation success. A dry spell after seeding followed by periods of high precipitation and a rich organic soil proved more favourable to the non-native species. Nutrient and soil microbes were abundant within the soil. Can foothill grasslands be restored? The sites were re-seeded in fall of 2011 and we anticipate establishment in the coming season.

In native species propagation research; we had good establishment of many species such as *Elymus innovatus* (hairy wild rye), *Oryzopsis asperifolia* (White grained mountain rice grass), *Astragalus canadensis* (Canada milk vetch), *Stipa richardsonii* (Richardson's needle grass), *Deschampsia caespitosa*

(tufted hair grass), *Bouteloua gracilis* (blue grama), *Danthonia intermedia* (timber oat grass), *Festuca campestris* (foothills rough fescue), *Festuca hallii* (plains rough fescue) and *Festuca saximontana* (Rocky Mountain fescue) – Parkland ecotype,

Others such as *Helictotrichon hookeri* (Hooker's oat grass), *Puccinellia nuttalliana* (Nuttall's alkali grass), *Hesperostipa comata* (needle and thread grass), *Lathyrus venosus* (pea vine), and *Vicia americana* (American vetch) proved to be more difficult either due to poor germination or to insects and wildlife issues in the field.

Shrubby and forb species differed considerably in their ability to germinate, while some species proved easy to grow. *Viburnum opulus* (low bush cranberry), *Arctostaphylos uva-ursi* (bearberry) and *Shepherdia canadensis* (buffalo berry) are thriving after transplanting within a grassy field at Vegreville. The inclusion of forbs and shrubby species into reclamation practices help to improve the structural layers within the site and can accelerate recovery of the site to its former biological processes and intrinsic value.

Results collected from sites affected by salt spill or pipeline break showed that beneficial micro-organisms do occur naturally in contaminated soils. The use of specific salt and hydrocarbon tolerant plant species showed tolerance to F2, F3 and F4 hydrocarbons. Organic amendments such as manure followed by aeration of the site seem to benefit plant growth at the Husky Energy Sites. Forage growth is improving on the sites. At the Zapata site, no amendments were used, and plants are making slow ingress towards the most severely affected areas of the site. This may have potential for deviating from the old practice of land farming. The recovery process is slow and can be expected to take more than 10 years.

In conclusion, these sites need to be monitored to ensure they settle into the desired trajectories, along with management practices (selective application of herbicide, mowing, grazing), otherwise the whole effort will result in an ecosystem very different from the one intended.

Acknowledgements

I am grateful for financial assistance from the Canadian Association of Petroleum Producers through the Alberta Upstream Petroleum Research Fund, Alberta Sustainable Resource Development, Talisman Energy, Husky Energy, Trident Energy and Canadian Natural Resources Limited.

My thanks to the following individuals are greatly appreciated: Marshall McKenzie, Jian Yang, Courtney Belanger, Tania McDonald and Sandy Sissons (formerly from ASRD - Wainwright) for their technical expertise; Barry Cole (ASRD Red Deer) for providing leadership and guidance on reclamation challenges in the Rumsey Natural Areas; Chris Chattaway and Mac Blade for providing sites for foothills fescue restoration research; and to Terry Ermel for coordinating access and permits to the Husky Energy sites in Wainwright.

Finally, I am indebted to Pat Porter of Public Lands, Alberta Sustainable Resources Development, Wainwright for assistance with seed collection of many of the prairie plant species and for input into the project.

Table of Contents

Executive Summary..... 2

Acknowledgements..... 4

List of Tables 7

List of Figures 7

1.0 Introduction 10

2.0 Plains Rough Fescue Restoration 11

 2.1 Introduction 11

 2.2 Methodology..... 13

 2.2.1 Site Characteristics 13

 2.3 Results..... 16

 2.4 Discussion 22

 2.5 Implications..... 25

 2.6 Conclusion..... 28

 2.7 References 29

3.0 Foothills Fescue Grassland Restoration 30

 3.1 Introduction 30

 3.2 Methodology..... 31

 3.3 Results..... 32

 3.3.1 Control of Invasive Species..... 32

 3.3.2 Soil Nutrient Analysis..... 35

 3.3.3 Plant Survey within the Non-native Forage Area near the Pipeline Right of Way 36

 3.4 Discussion 36

 3.5 Conclusion..... 37

 3.6 References 38

4.0 Revegetation of Sites Affected by Salt and Hydrocarbon/Salt Contamination 39

4.1	Introduction	39
4.2	Methodology.....	39
4.2.1	Greenhouse Studies	39
4.2.2	Field Studies	40
4.2.3	Microbial Growth Studies.....	41
4.3	Results.....	42
4.3.1	Greenhouse Studies	42
4.3.2	Field Studies	46
4.3.3	Microbial Studies.....	56
4.4	Discussion	59
4.5	Conclusion.....	61
4.6	References	61
5.0	Native Species Research	63
5.1	Introduction	63
5.2	Methodology.....	63
5.3	Results.....	64
5.3.1	Seed Germination.....	64
5.3.2	Field Production	67
5.4	Conclusion.....	72

List of Tables

Table 1. Precipitation ¹ from 2008 to 2011 growing season.	23
Table 2. Nutrient status at each site, within each treatment.....	35
Table 3. Concentration of hydrocarbon contaminants in soil before and after planting and with the addition of humates.....	45
Table 4. Hydrocarbon fractions from composite 1 m core sample at site 1.	48
Table 5. Biomass production collected from 3 composite samples at the phytoremediated site 1 and 2, in 2010.	50
Table 6. Analyte measured near the root zone of plants growing on site compared to bare areas on the site.....	55
Table 7. Enumeration of bacteria from soil samples collected at the hydrocarbon contaminated sites in Alberta 2008.....	57
Table 8. Morphology and identification of bacteria from soil samples.....	59
Table 9. Germination evaluation of candidate species for revegetation in forested lands.	64

List of Figures

Figure 1. Topography and rough map of site.	13
Figure 2. Rough map with topography of study area.....	14
Figure 3. Topography and rough map of site.	14
Figure 4. Effectiveness of fall herbicide application on a well pad site that was previously seeded to <i>Bromus inermis</i>	16
Figure 5. Relative plant cover by functional groups at site 1, CNRL.....	17
Figure 6. View of seeded plot in 2011, Site 1: 6-25-33-19 W4 (Canadian Natural Resources Limited).	18
Figure 7. Plant cover of functional groups of species at site 2, Crescent Energy.....	19
Figure 8. Reoccurrence of <i>Bromus inermis</i> at site 2.	20

Figure 9. Plant cover of functional groups of species at site 3, Husky Energy.	21
Figure 10. Site # 3 showing vegetation cover dominated by <i>Bromus inermis</i>	21
Figure 11. Occurrence of native species in the following season, after fall application of herbicide.	22
Figure 12. Survival of <i>Festuca hallii</i> after a fall application of Roundup herbicide in 2008.	24
Figure 13. Before (2007) and after (2011) picture showing vegetation transition from seeded forages (left) to a more native plant community (right).	26
Figure 14. Before (2007) and after picture (2011) showing vegetation transition from seeded forages (left) to a more native plant community (right).	27
Figure 15. Change from forage dominated (left) to predominantly native (right).....	28
Figure 16. Effectiveness of tillage, herbicide application and Basamid on the control of non-native invasive species in fescue grassland.	33
Figure 17. Re-occurrence of invasive weeds on the study plots.	34
Figure 18. Non-native forage invasion into fescue grassland in the foothills fescue grassland.....	37
Figure 19. Growth of <i>Puccinellia nuttalliana</i> (Nuttall’s alkali grass) in a control soil with peat amendment (Left), the Chauvin contaminated soil plus humates (Centre), contaminated Provost soil plus peat (right).	44
Figure 20. Emergence of seeding native species on a pipeline break.	46
Figure 21. Growth of seeded native species at site 1.....	47
Figure 22. Plant cover provided by seeded native species at the Husky Energy site 2.	49
Figure 23. Plant cover at Husky Energy site 2.....	51
Figure 24. Site impacted by hydrocarbon spill from a pipeline break in a sandy soil, showing thriving <i>Achnatherum hymenoides</i> by year 2.	52
Figure 25. Growth of <i>Elymus Canadensis</i> , <i>Achnatherum hymenoides</i> and colonization by other native species (<i>Artemesia frigida</i> and <i>Carex</i> sp) at a petroleum hydrocarbon contaminated site in a sandy soil by year 3.....	53
Figure 26. Aeration of the soil to enhance degradation of hydrocarbon.....	54

Figure 27. Morphology of bacterial colonies on nutrient agar, (A) soil sample from Zapata site, (B) soil sample from Husky site 2, (C) soil from Zapata site control and (D) back view of plate containing soil from Zapata site. 58

Figure 28. *Viburnum opulus* thriving within a *Bromus inermis* field. 69

Figure 29. Transplanting *Arctostaphylos uva-ursi* in the field. 70

Figure 30. Production of a *Bouteloua gracillis* source identified variety at Vegreville. 71

Figure 31. *Festuca hallii* production at Vegreville in 2009-11. 71

1.0 Introduction

Native landscapes such as Alberta's Foothills, Parkland and Boreal regions play a critical role in sustainable land use. These plant communities provide habitats and movement corridors for many wildlife species and important forages for grazing livestock. Following resource development, these sites are required by law (Alberta Environmental Protection and Enhancement Act [EPEA]) to be reclaimed to an equivalent land capability, meaning the disturbed area must evolve into an area similar to its pre-disturbance state to ensure long term ecosystem goods and services.

Ecological thresholds from multiple activities such as oil & gas, other resource industries, ranching and human recreational activities on sensitive landscapes may have been exceeded and altered plant communities have led to a risk of reduced ecosystem performance and productivity (Gramineae services ltd 2007). Sensitive landscapes are defined as lands that support unusually high diversity of plant and/or animal communities or sustain rare or endangered habitats; contain critical habitat of limited range, providing breeding, shelter, or feeding sites for wildlife (Environment Canada, 2010). The challenge with reclaiming these lands is confounded with invasive non-native species encroachment into native plant communities which represents one of the biggest issues affecting grassland integrity. Today, the invasion resulting from previous practices of seeding non-native invasive species for forage crops, and effects from linear disturbances such as transportation corridors, access roads and pipelines is well recognized.

Alberta's new reclamation criteria (2010 Reclamation Criteria) will be used to determine reclamation success and acquire a reclamation certificate. This means working towards a restoration trajectory; which is gauged by indicators of successful reclamation that lead sites to equivalent land capability provided prior to industrial disturbance. Achieving reclamation success requires appropriate site preparation and strategies of plant reintroduction. In the past 20-30 years revegetation practices in Alberta's upstream oil and gas have evolved from using tame forages to provide site stabilisation to using ecologically appropriate native seed mixes. Revegetation success is often hampered by previous practices of using non-native species (Gramineae Services 2007) and a lack of commercially available key grass species (*Festuca campestris*, *Festuca hallii*, *Stipa curtisetata*, etc.). The information gap on these species growth and establishment present a barrier to successful reclamation.

Additionally, reclamation is often complicated by pipeline breaks and surface spills at wellheads or battery sites which may contribute to local soil contamination and salinization. Hydrocarbon and salinity

related water contamination is common throughout the province. The presence of soluble salts affects plant growth by limiting their water uptake. Better knowledge of plant performance may provide options to wholesale soil removal and land filling. Information on eradication of non-native invasive species and strategies to revegetate these disturbed sites will enable resource extraction companies to reclaim environmentally sensitive areas to sound ecological function.

In this project, we focused on four areas of importance in addressing reclamation success in sensitive landscapes. These included evaluating strategies of restoration for fescue grassland in both the Northern fescue region and the Foothills fescue region, re-introduction of plants in soils impacted by petroleum hydrocarbons and salts as well as targeting key species for inclusion in seed mixes for reclamation. Therefore, the overall goal of this project was to determine practical methods for re-introduction of plants on sites disturbed by oil and gas extraction on native landscapes, focusing mainly on:

- The management of non-native forages and re-establishment of native vegetation in both the plains and the foothills fescue grassland
- On sites affected by hydro-carbon contamination
- Identifying key species for reclamation
- Evaluating the germination and propagation of potential native species for reclamation.

2.0 Plains Rough Fescue Restoration

2.1 Introduction

Festuca hallii is a perennial bunch grass which requires 2-3 years to establish, grows slowly and produces seed erratically. Restoring *Festuca hallii* grassland remains a contentious issue to the oil and gas industry, given that less than 5% *Festuca hallii* prairie remains in the province. An Environment Canada (2005) survey showed 34% of grassland sites in the central parkland were dominantly non-native communities and invasive non-native plants were found in 42% of Plains rough fescue communities. Restoring fescue grassland has been a “trial and error” process. In a former study, Alberta Innovates - Technology Futures has had some success in the revegetation of fescue grassland (Woosaree and James 2007). Whether it is possible to reclaim and restore industrial disturbances that have been previously seeded to non-native invasive species to healthy, sustainable native plant communities remains to be

answered. Additionally, if successful, it would help the oil and gas industries in acquiring their reclamation certificate.

To demonstrate the ability to restore fescue grassland, the Rumsey Parkland was selected for this study. The Rumsey Parkland and Rumsey Ecological Reserve together contained the largest tract (183 km²) of plains rough fescue grassland in the world (World Wildlife Fund Canada 1989). The oil and gas industry has a long history in the Rumsey Parkland dating back to the 1950's. Prior to Alberta having any reclamation guidelines, newly drilled sites were seeded to *Festuca ovina* L. var. *duriuscula* (hard fescue), *Agropyron pectiniforme* (L.) Gaertn. (Crested wheatgrass) and *Bromus inermis* Leyss. spp. *inermis* (smooth brome grass). Today, many of these non-native species persist in open grasslands, beneath aspen groves, along roads and near hay dispersal sites (Holcroft and Weerstra 2001). The cumulative effects of oil and gas and other activities such as ranching have influenced the basic ecological integrity of the Rumsey Parkland.

Recognizing the ecological significance of fescue grasslands, the ASRD felt that it was necessary to develop some prescriptive methods for oil well sites where past reclamation practices have compromised the integrity of the Rumsey Parkland. To maintain the natural values of the area while utilizing it primarily for grazing, oil and gas production and undeveloped recreation remains important.

It is believed that properly timed and applied herbicides and mowing can be effective in controlling non-native species. For example, stands of *Agropyron pectiniforme* produce some 100-410 seeds per square metre (Saskatchewan Water shed Authority Fact Sheet) and *Bromus inermis* has both prolific rhizomes and seeds. Additionally, both these species have a high germination rate over a broad range of temperature and moisture stress levels.

The objective of the study was to develop a prescriptive method for the eradication of non-native forages on previously reclaimed oil wellsites in the Rumsey Parkland and to rehabilitate these sites to plains rough fescue grasslands.

Specific objectives include:

- Evaluate the use of tillage, in combination with chemical treatments, to reduce the non-native invasive species.
- Re-establishment of native *Festuca hallii* (plains rough fescue) plant communities;

- Monitor the rehabilitated sites for a minimum period of five years to determine successional trajectory.

2.2 Methodology

In the fall of 2006 (3rd week of October), we identified three post-reclaimed oil well sites in the Rumsey Parkland that were previously seeded *Bromus inermis*, *Agropyron pectiniforme* and *Melilotus officinalis* (sweet clover). In October 2006, all three sites received an application of 300 ml/acre of glyphosate (Roundup®).

Herbicide application was continued in the spring and summer of 2007. During the growing season, herbicides (Round-up and Curtail M were selectively applied to further diminish the seed bank potential of these non-native species.

2.2.1 Site Characteristics

These sites are characterized by a “knob and kettle” topography and have dark brown Chernozemic, loamy soils as illustrated by Figure 1-3. Surrounding vegetation as recorded from ASRD benchmark enclosures is comprised mainly of *Festuca hallii* (41%), *Stipa curtisetata* -12% (Western Porcupine Grass), several native species, and a smaller content of *Poa compressa* -0.5% (Canada Bluegrass) and *Poa pratensis* – 0.4% (Kentucky bluegrass).

Site 1: 6-25-33-19 W4

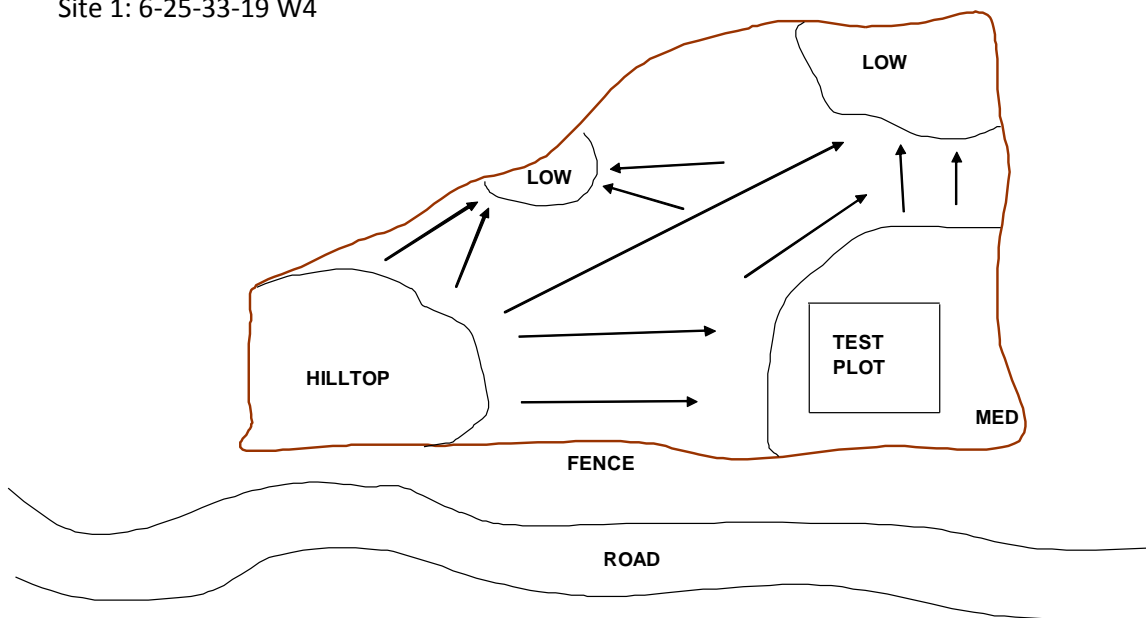


Figure 1. Topography and rough map of site.

Site 1 is dominated by *Cersium arvense* (Canadian Thistle), *Achillea millefolium* (Common Yarrow), *Capsella bursa-pastoris* (Shepherd's Purse), *Bromus inermis* (Smooth Brome grass), *Taraxacum officinale* (Common Dandelion), *Vicia americana* (American Vetch), *Sonchus arvensis* (Sow Thistle) and *Melilotus officinalis*.

Site 2: 16-13-33-19 W4



Figure 2. Rough map with topography of study area.

Site 3: 8-21-33-19 W

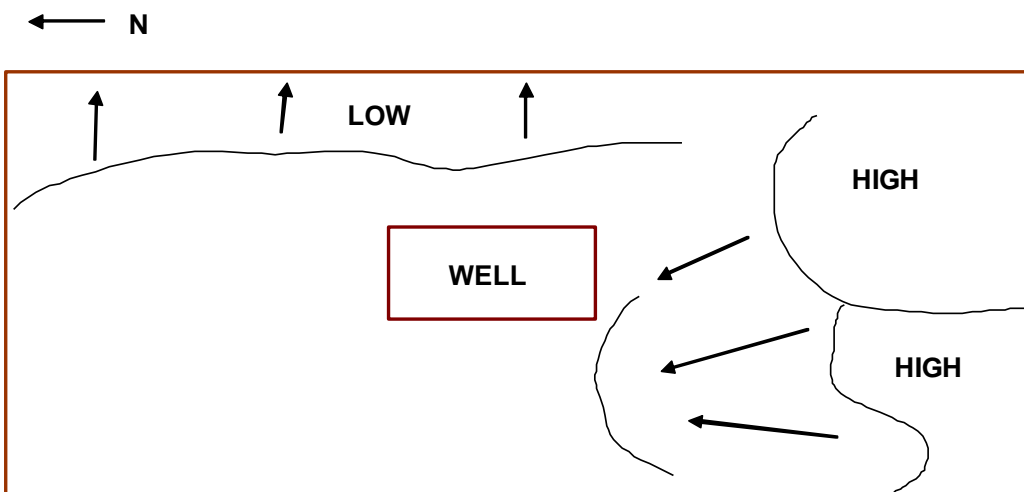


Figure 3. Topography and rough map of site.

Site 2 and 3 are comprised of *Cersium arvense*, *Achillea millefolium*, *Capsella bursa-pastoris*, *Bromus inermis*, *Taraxacum officinale*, *Vicia americana*, *Sonchus arvensis*, *Melilotus officinalis*, *Festuca hallii*, *Artemisia ludoviciana*, *Artemisia frigida* (Pasture sage)

Plant taxonomy follows USDA PLANTS Database (<http://plants.usda.gov>). All three sites received an application of 1830 ml/ha of glyphosate (Round-up) on August 20, 2008.

Due to limited seed availability and uncertainty of the complete elimination of the non-native species from the sites, only half of each site was seeded. The remaining half of each site was left to determine if further applications of Roundup® would be necessary. The non-appearance of non-native forages at the site led to the remaining half of Site 1 and 2 being seeded on June 24th (Site 3 was seeded on October 1st, 2009). A Fabro plot-seeder with double disk openers and rubber packer wheels was used to conduct all seeding. Site 1 and 2 were seeded at a rate of 10 kg/ha. Seeding rates were calculated based on desired number of pure live seed (PLS) per unit area. Seed mix composition was adjusted for seed weight and percent seed viability/purity. A low seeding rate was preferred to facilitate natural recruitment of native species to the site. The heavy re-occurrence of smooth brome grass convinced us to use a heavier seeding rate (20kg/ha) on site 3.

The seed mix consisted of:

<i>Achillea millefolium</i>	03.0%
<i>Agropyron dasystachyum</i>	00.5%
<i>Agropyron trachycaulum</i>	01.0%
<i>Bouteloua gracilis</i>	15.0%
<i>Carex</i> spp	01.0%
<i>Festuca hallii</i>	54.0%
<i>Helictotrichon hookerii</i>	01.5%
<i>Koleria macrantha</i>	15.0%
<i>Solidago missouriensis</i>	02.0%
<i>Stipa curtiseta</i>	02.0%
<i>Stipa viridula</i>	<u>05.0%</u>
Total	100%

In 2009 data collection consisted of 12 sampling points, equidistant along a transect line (Four Daubenmire quadrats per transect) to determine total plant cover, plant cover by functional groups and bare ground. Plots were maintained and herbicides Round-up and Curtail-M were selectively used to control non-native forage and weed species. In 2011, sites were assessed using the 2010 Reclamation Criteria.

2.3 Results

Site 1 – CNRL Site

Fall application of Roundup® herbicide in 2006 severely reduced growth of *Bromus inermis*, *Cirsium arvense* and *Agropyron pectiniforme* within the plot (Figure 4). However, *Bromus inermis* appeared to be persistent and during 2007 and 2008, Roundup was sporadically applied to control emerging plants.



Figure 4. Effectiveness of fall herbicide application on a well pad site that was previously seeded to *Bromus inermis*.

Rumsey Site 1 Community Composition by Plant Group

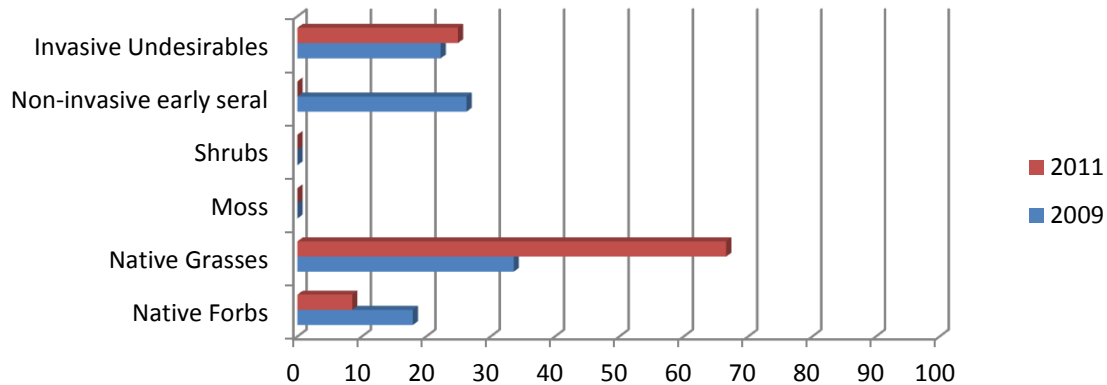


Figure 5. Relative plant cover by functional groups at site 1, CNRL.

By 2011, native grass cover increases to 67% and undesirable species decrease to 25% (Figure 5). Average total cover by species was 85%, with 15% bareground. More native species were found to be occurring on the site in 2011. Average cover by *Melilotus officianalis* is approximately 6% and was found in 42% of the quadrats. *Bromus inermis* was found in 17 % of the quadrats and had an average cover of 7%. Plant cover provided by *Agropyron pectiniforme* accounts for 5% and was found in 8% of the quadrat. Undesirable invasive species increased slightly (3%) in 2011 (Figure 5).

Native species found on the site include *Vicia americana* (American Vetch), *Artemisia ludoviciana* (Prairie Sage), *Aster laevis* (Smooth Blue Aster), *Achillea millefolium* (Yarrow), *Solidago rigida* (Rigid Goldenrod), *Sisyrinchium montanum* (Blue eyed grass), *Androsace septentrionalis* (Fairy Candelabra), *Artemisia frigida* (Pasture Sagewort), *Agrostis scabra* (Hair/Tickle grass), *Pascopyron smithii* (western wheatgrass), *Elymus trachycaulus* (Slender wheatgrass), *Festuca saximontana* (Rocky mountain fescue), *Koeleria macrantha* (June grass), *Bouteloua gracilis* (Blue grama), *Nassella viridula* (Green needle grass), *Agropyron dasystachyum* (Northern Wheat) and *Hordeum jubatum* (Foxtail Barley) [Figure 6]. Although *Festuca hallii* comprised 54% of the seed mix, it has not shown significantly in the plots as yet. Year 2009 was a dry year and there were no seeds to be wild harvested in Rumsey Parkland. Year 2011 was a better seed producing year and could potentially ingress into the site.

Many weedy species continued to grow on the site. These include: *Descurainia sophia* (Flixweed), *Lepidium densiflorum* (Peppergrass), *Thlapsi arvense* (pennycress/stinkweed), *Lactuca pulchella* (Common blue lettuce), *Crepis tectorum* (Hawk's Beard), *Melilotus officianalis* (Yellow sweet clover), *Taraxacum officinale* (Dandelion), *Erigeron canadensis* (Canada fleabane), and *Bromus inermis* (smooth brome grass). *Agropyron pectiniforme* (Crested Wheatgrass) and *Bromus inermis* are of concern as they represent a threat to the native vegetation.



Figure 6. View of seeded plot in 2011, Site 1: 6-25-33-19 W4 (Canadian Natural Resources Limited).

Site2 –Crescent Energy Site

Native species recorded at site 2 include: *Artemisia ludoviciana* (Prairie Sage), *Aster laevis* (Smooth Blue Aster), *Achillea millefolium* (Yarrow), *Solidago rigida* (Rigid Goldenrod), *Sisyrinchium montanum* (Blue eyed grass), *Artemisia frigida* (Pasture Sagewort), *Pascopyron smithii* (western wheatgrass), *Elymus trachycaulus* (Slender wheatgrass), *Koeleria macrantha* (June grass), *Bouteloua gracilis* (Blue grama), *Deschampsia caespitosa* (Tufted hair grass), *Poa palustris* (fowl bluegrass) and *Festuca hallii* (plains rough fescue). *Festuca hallii*, the species of interest averages 10% in plant cover in 2011, from 5% in

2009 (Figure 7). *Festuca hallii* was found in 58% of one quadrat. Relative cover provided by undesirable weeds species increases by 12% (Figure 7) in 2011.

Rumsey Site 2 Community Composition by Plant Group

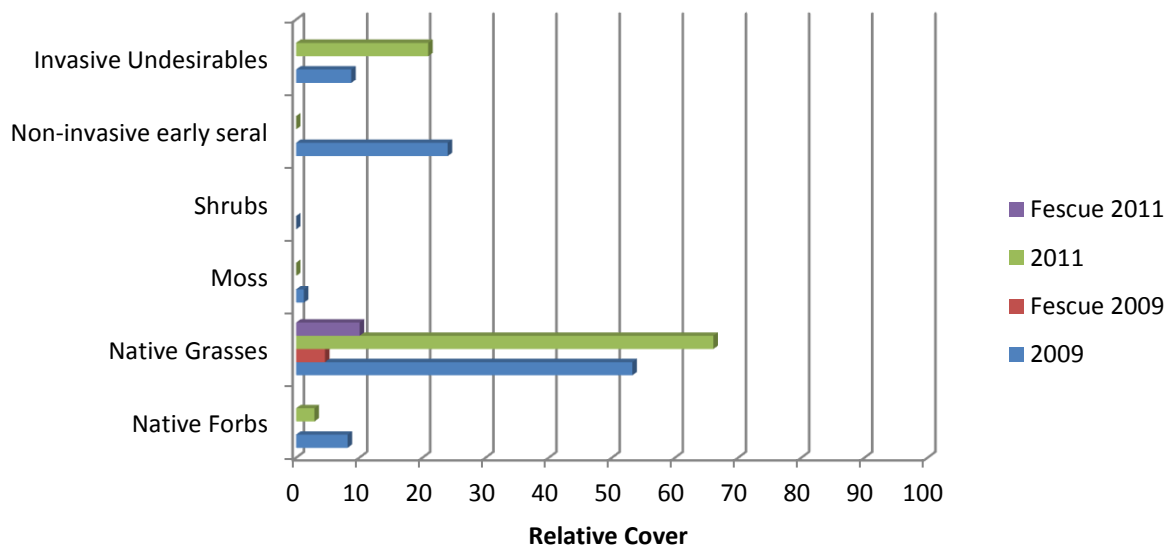


Figure 7. Plant cover of functional groups of species at site 2, Crescent Energy.

The invasive undesirable species were comprised mostly of *Bromus inermis*, which averaged 21% (Figure 7) cover, and was found in nine, transects, ranging from 5-30% cover by 2011. Most of the *Bromus inermis* are re-emerging from underground rhizomes and seeds (Figure 8). Native forbs decreased considerably (3%) in 2011 compared to 8% in 2009. *Agropyron pectiniforme* accounted for nearly one percent of the plant cover.

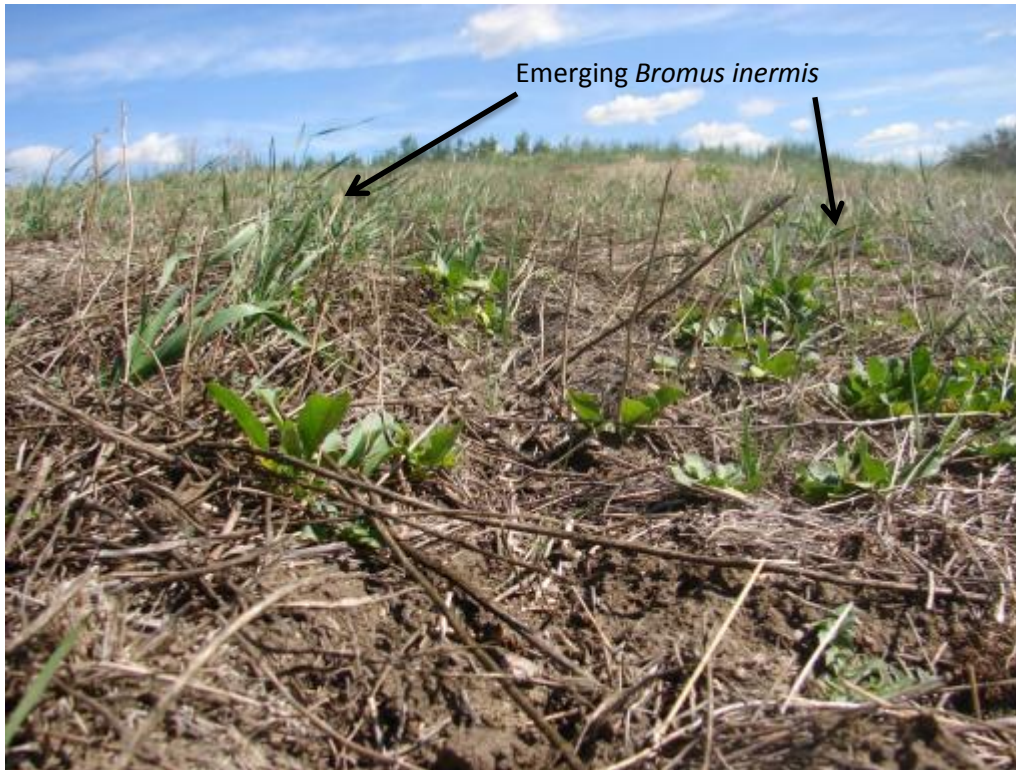


Figure 8. Reoccurrence of *Bromus inermis* at site 2.

Site 3 – Husky Energy Site

This site was more affected by *Bromus inermis* compared to the previous two sites. The herbicide; Roundup®, was selectively applied twice through the growing season prior to seeding on June 24, 2009. In 2010, Roundup® selective applications continued to ensure continued success in controlling the *Bromus inermis*.

Native species occupying the site include *Vicia americana* (American Vetch), *Artemisia ludoviciana* (Prairie Sage), *Aster laevis* (Smooth Blue Aster), *Achillea millefolium* (Yarrow), *Solidago rigida* (Rigid Goldenrod), *Sisyrinchium montanum* (Blue eyed grass), *Androsace septentrionalis* (Fairy Candelabra), *Artemisia frigida* (Pasture Sagewort), *Agrostis scabra* (Hair/Tickle grass), *Pascopyron smithii* (western wheatgrass), *Elymus trachycaulus* (Slender wheatgrass), *Festuca saximontana* (Rocky mountain fescue), *Koeleria macrantha* (June grass), *Bouteloua gracilis* (Blue grama), *Nassella viridula* (Green needle grass), *Agropyron dasystachyum* (Northern Wheat) and *Hordeum jubatum* (Foxtail Barley) [Figure 6].

Rumsey Site 3 Community Composition by Plant Group

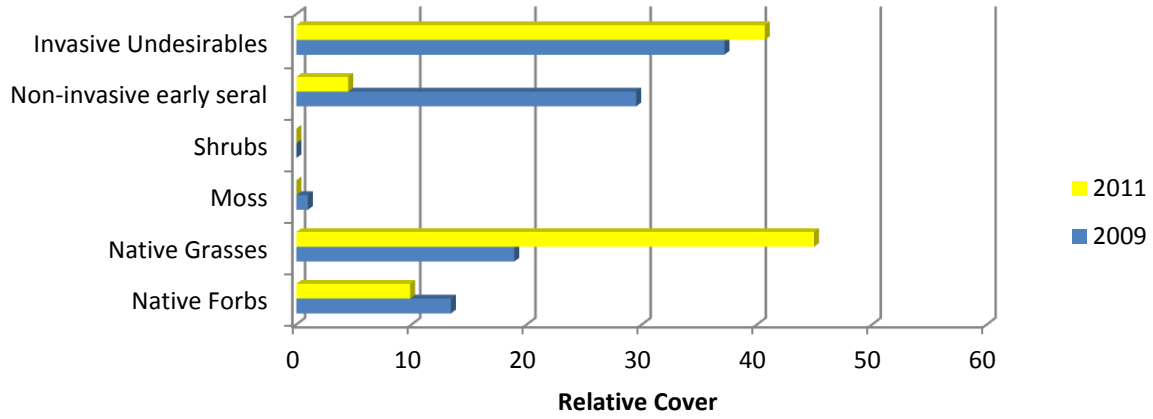


Figure 9. Plant cover of functional groups of species at site 3, Husky Energy.

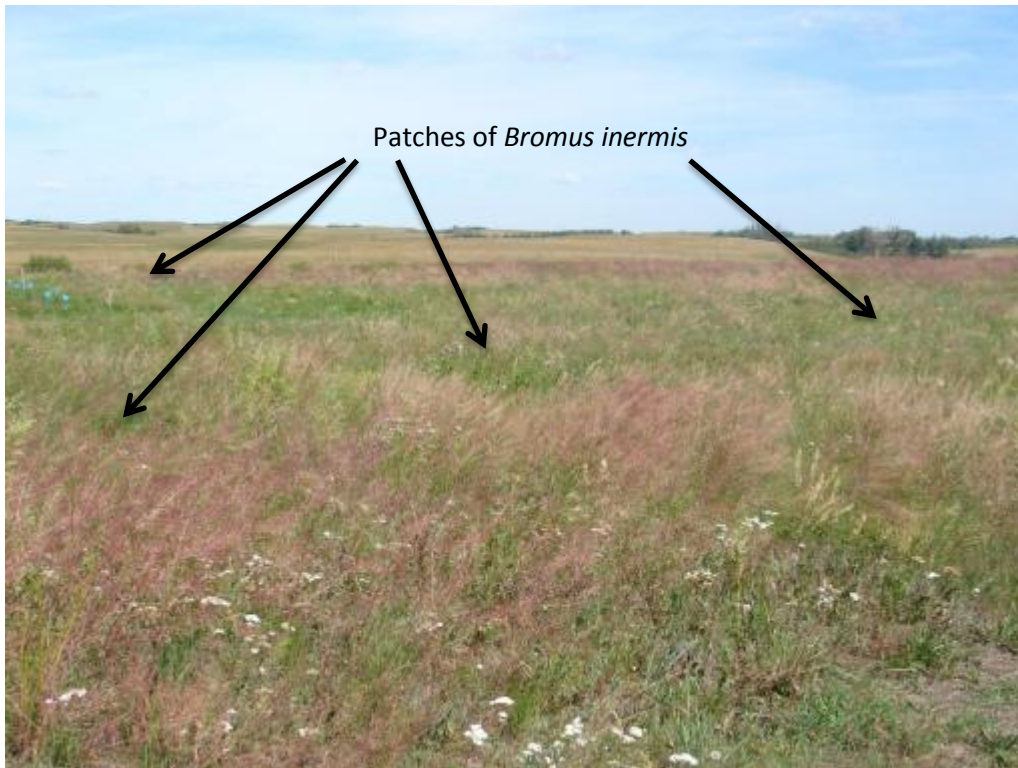


Figure 10. Site # 3 showing vegetation cover dominated by *Bromus inermis*.

In 2011, undesirable invasive species were slightly reduced from 37% to 30% (Figure 9).

Weeds and non-native undesirable species comprised 41% of cover in 2011 and consisted of *Descurainia sophia*, *Brassicaceae* spp (mustard), *Capsella bursa-pastoris*, *Bromus inermis*, *Lappula squarrosa* (blue bur), *Cersium arvense*, *Lepidium densiflorum* and *Erigeron canadensis* (horse weed or Canada flea bean). Only a few live tussocks of *Agropyron pectiniforme* were observed. Roundup® appears to have eliminated most of the *Agropyron pectiniforme*. Native grasses provided 41% of the plant cover.

2.4 Discussion

Despite uniform emergence of seeded native grasses observed at all sites, invasiveness and re-occurrences by non-native grasses, mostly *Bromus inermis* remains a concern. Site 2 appears to have the best success with approximately 10% of the desired *Festuca hallii* occurring on the sites. Site 1 and 3 had *Festuca hallii* on sites but none were recorded within the transects.

Application of glyphosate works best in late fall (middle to last week of October) for control of non-native forages. Many of the native species were dormant and survived the herbicide application (Figure 11).



Figure 11. Occurrence of native species in the following season, after fall application of herbicide.

Visual impact of the seeded trials seems to be disappointing in the early years. Invasive and undesirable species remain a concern by the 3rd year after seeding. Percent cover provided by non-native invasive species is 17% for site 1, 16% for site 2 and 35% for site 3. Seeding rate influences competition between seeded plants and weeds. While a seeding rate of 10 kg/ha would have been ideal for seeding in a totally weed free site, a seeding rate of 25-30 kg/ha may have been desirable in this scenario (Stevenson et al. 1995). Having a high density of seeded natives would provide better competition to *Bromus inermis* and restrict plants from re-occurring on the sites. A low seeding rate was selected for this study as it may facilitate recruitment from adjacent native areas into the disturbed sites (Tilman 1997). Recruitment is defined as the survival of native seedlings in a destined habitat, for a period in which they may survive to complete a life-cycle (adapted from Menge and Sutherland, 1987).

At all three sites, there had been a number of naturally occurring native species such as *Vicia americana*, *Artemisia ludoviciana*, *Aster laevis*, *Achillea millefolium*, *Solidago rigida*, *Sisyrinchium montanum*, *Androsace septentrionalis*, *Artemisia frigida* and *Agrostis scabra*. They have been overshadowed by the weedy species such as *Descurainia Sophia*, *Lepidium densiflorum*, *Thlapsi arvense*, *Lactuca pulchella*, *Crepis tectorum* and *Melilotus officianalis*.

The annual weedy species are not a prime concern as they tend to diminish as the grasses become more prevalent. In the early years of establishment, mowing was conducted to keep the weed population from spreading within the site and open up the plant canopy. Seed dispersal from invasive forage species can move on average 2.4 m to a maximum of 20 m per year (Johnson 2011). Therefore, it becomes equally important to adopt control outside the boundary of these plots.

During 2010 and 2011, the Rumsey region received an unprecedented amount of rain (Table 1), which aided a flush of weeds and *Bromus inermis*.

Table 1. Precipitation¹ from 2008 to 2011 growing season.

	April	May	June	July	August	September
2008	32.1	38.6	56.5	47.3	102.4	12.1
2009	10.2	18.2	17.5	46.2	68.5	16.9
2010	60.9	68.5	110.9	139.1	44.2	41.2
2011	13.4	36.8	81.3	95.3	20.7	1.5
Normals	23.3	54.5	87.1	88.3	64.2	50.9

Note.¹ http://www.climate.weatheroffice.gc.ca/climate_normals/index_e.html

Collected from Stettler north, the only nearby weather station.

Bromus inermis is an aggressive invader of fescue prairie; it will germinate and grow in a wide range of temperatures, light, and moisture conditions (Grittz et al. 1994). *Festuca hallii* establishment remains low within the site. Site 2 had 10-25% of *Festuca hallii* plants occurring within 58% of the transects as that site originally had more fescue plants. Many of these plants survived the fall application of the herbicide Roundup (Figure 12). Sites 1 and 3 had no *Festuca hallii* plants even before the sites became part of the study.



Figure 12. Survival of *Festuca hallii* after a fall application of Roundup herbicide in 2008.

The area within the three sites used by the University of Alberta for their research had no control measures against invasive/non-native species carried out until after their study was abandoned in 2010. As a result, these areas are greatly influenced by *Bromus inermis*, *Melilotus officinalis*, *Crepis tectorum* (Narrow-Leaved Hawk's beard), *Sonchus arvensis* (spiny annual sow thistle) and *Thlaspi arvense*, and *Medicago sativa* (alfalfa). Weed control measures should be implemented.

2.5 Implications

In 2011, the Government of Alberta introduced the new reclamation criteria by which reclamation success will be gauged and certificates acquired. The "Record of Observation Tool" (RoO) was used to gain an understanding of reclamation success as to how these sites fared.

Site 1 had the most *Bromus inermis* plants at the beginning of the study. At present there is still a number of *Bromus inermis* and *Cirsium arvense* plants on the perimeter of the site.

When conducting the detailed site assessment (DSA), this site failed mostly due to problem weeds. The control had an average rating of 8.3 and the site had a rating of 18. *Melilotus officinale*, *Bromus inermis* and *Elymus repens* (Quack grass) accounted for problem weeds with an average rating of 8.3, 9.7 and 5.6 respectively. Additionally, the site also failed on structural layers, with the control having a rating of 2.7 compared to a rating of 1.6 for the test site, (failed by 0.1 as a drop of 1 is allowed; could easily be passed with another assessment and professional judgement or another years growth and infill). The site passed on cover values. Litter quantity values are 86.5 kg/ha for the control compared to 99.5 kg/ha for the study area. Litter quality was also good, showing excellent decomposition. With continued control of the non-native invasive species, mainly *Bromus inermis*, this site can be expected to pass the reclamation criteria in approximately two years (Figure 13).



Figure 13. Before (2007) and after (2011) picture showing vegetation transition from seeded forages (left) to a more native plant community (right).

The area surrounding site 2 (Figure 14) remains relatively intact, except for the southern side within the aspen (*Populus tremuloides*) stand where available moisture has allowed *Bromus inermis* to dominate the stand. The site lies on a large hill from top to bottom so during the DSA, it was stratified into upper, middle, and lower areas and five sampling points were taken within each area and their respective controls. This site had good plant cover and good growth. *Festuca hallii* were present on the site prior to the site being treated with herbicides. Many of the *Festuca hallii* plants survived the herbicide application and have contributed to the diversity onsite. The lower area of the site failed on topsoil depth, problem weeds (*Bromus inermis* and *Taraxacum officinale* (Dandelion)). The middle area failed on consistence but passed on structure so passed on soils. The lower subsoil failed texture. There was clay onsite compared to loam on control site. The vegetation onsite showed good growth, good litter quantity and quality trending towards the right trajectory despite soil failure.

Vegetation failed on structural layers with the control having a rating of 3.6 compared to a rating of 2.0 for the site. For problem weeds the control had none compared to an average of 18.5 % for the test site. The upper area of the site also failed on structural layers with the control having a score of 3.0 compared to 1.8 for the site. Problem weeds such as *Bromus inermis*, *Elymus repens* and *Tragopogon dubius* (goat's beard) had an average cover of 22% compared to none for the control. Litter quantity recorded was 385 kg\ha for the control and 640 kg\ha for the study site. Control had 4% bareground and the study site had 7%.

Both access road sampling points failed on vegetative cover due to a higher presence of type 4 increasers within the transects compared to the control. When a site fails due to type 4 increasers, additional sampling points could have improved the average cover, resulting in the access road passing the criteria.



Figure 14. Before (2007) and after picture (2011) showing vegetation transition from seeded forages (left) to a more native plant community (right).

The surrounding areas at site 3 had the most intact native grassland of all three sites. The test site however had issue with (*Cirsium arvense*). The site currently has the most annual weeds and early colonizers of the three sites. In some areas, seedling establishment was poor. The DSA with the 2010 criteria resulted in failures on problem weeds such as *Bromus inermis*, *Elymus repens* (control = 0, test site = 18.3. Noxious weeds with (*Cirsium arvense*) within the control had a rating of 0 compared to 5 for the study site. Structural layers failed by a slim margin (0.1, a rating drop of 1 is allowed) with the control having a 2.7 compared to 1.6 for the study site. Access road also failed on vegetative cover mostly due to grazing response. The control had 0 for bareground compared to 8.9% for the study site. The site passed for “Litter”, as the control had 372 kg\ha while the study site had 201.6 kg\ha (site must be 15% control).

Many new seedling species are occurring on the site. One season of spot herbicide application may eliminate all the noxious weeds. The structural and vegetative cover should be able to pass as more infill species and later seral species get established onsite (Figure 15).



Figure 15. Change from forage dominated (left) to predominantly native (right).

The ASRD should consider removing the fences surrounding these plots. This would facilitate grazing, providing some control of non-native forages that can lead to a seral community.

2.6 Conclusion

Two years after seeding, these sites have undergone a transition from non-native forages to more native species. The first burn off with Roundup® was very successful and provided good control of many of the non-native forages. Curtail M herbicide was also used and provided effective control against *Cirsium arvense*. There are now many native species growing on site. However, a cool summer and unusual precipitation in the last two years (2010 and 2011) may have contributed to prolific invasion and re-occurrence of *Bromus inermis* and to a lesser extent, *Cirsium arvense*. A treatment that was not part of this study would be to till up the lease areas after the first burn off, then continue with two more burn offs to deplete the seed bank of forage seeds, and ensure rhizome death before seeding to native.

Site 2 had the most *Festuca hallii* and to a lesser extent at site 1 and 3. There is evidence that its presence in the community will increase over time. *Bromus inermis* and *Cirsium arvense* were not only in the seed bank but also found commonly in the Rumsey Parkland, along depressional sites, roadsides, and near these study sites.

Using the RoO of the 2010 reclamation criteria, these sites could have passed under non-routine application and using professional judgement. It would be appropriate to continue weed/invasive species control measures for a year or two prior to seeking a reclamation certificate.

These sites represent modified grassland. At present, many of the native species occurring on these sites are compatible with the Rumsey benchmark site (Carlson et al. 2010). With time they may progress to form a stable plant community. Although the goal of the Rumsey Parkland South Management Plan is “to preserve and protect the Rumsey Aspen Parkland ecosystem while allowing for responsible use of its resources”, there is a need to implement some weed and invasive forage control along depressional areas, ditches and access roads. Two years after seeding is a relative short time to have appreciation of *Festuca hallii* on these sites. Close monitoring, selective application of herbicide and mowing can ensure these sites trend toward the desired plant community.

2.7 References

- Carlson, J., Webb, T., and B.W. Adams. Rangeland Reference Area Monitoring Program - Prairie Area. Rangeland Management Branch, Lands Division, Alberta Sustainable Resource Development.
- Environment Canada. 2005. National climate data and information archive. Environment Canada, URL http://www.climate.weatheroffice.ec.gc.ca/Welcome_e.html. Accessed January 2012.
- Environment Canada. Invasive Plants of Natural Habitats in Canada: An Integrated Review of Wetland and Upland Species and Legislation Governing their Control. <http://www.ec.gc.ca/eee-ias/78D62AA2-55A4-4E2F-AA08-538E1051A893/invasives.pdf>
- Gramineae services ltd. 2007. Revegetation strategies for public lands: a gap analysis. Prepared for: Land Management and Rangeland Management Branches, Land Division, ASRD. Lundbreck, Alberta.
- Grilz, P., Romo, J., and J.Young. 1994. Comparative Germination of Smooth Brome and Plains Rough Fescue. *Prairie Naturalist* 26(2): 157-170.
- Johnson, D. 2011. Movement of weed seeds in Reclamation areas. *Restoration Ecology* **19**:446-449.
- Holcroft Weerstra, A. 2001. Control options for smooth brome (*Bromus inermis*) in the Rumsey Ecological Reserve: a review and summary of relevant research and experience of practitioners. Prepared for Alberta Sustainable Resource Development. Edmonton, Alberta. 42 pp.
- Mazzola, Monica B.; Chambers, Jeanne C.; Blank, Robert R.; Pyke, David A.; Schupp, Eugene W.; Allcock, Kimberly G.; Doescher, Paul S.; Nowak, Robert S. 2010. Effects of resource availability and propagule supply on native species recruitment in sagebrush ecosystems invaded by *Bromus tectorum*. *Biological*

Invasions. DOI 10.1007/s10530-010-9846-0.

http://www.fs.fed.us/rm/pubs_other/rmrs_2010_mazzola_m001.pdf

Menge, B. A. and J.P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *The American Naturalist* **39** (5).

Stevenson, M., Bullock, J., and L. Ward. 1995. Re-creating Semi-natural Communities: Effect of Sowing Rate on Establishment of Calcareous Grassland. *Restoration Ecology* **3** (4):279–289.

Strathmann, R., Terence, P., Kuris, A., Lindeman, K., Morgan, S., Pandolfi, J., and R. Warner. 2002. Evolution of local recruitment and its consequences for marine populations. *Bulletin of Marine Science* **70**: 377-396.

Tilman, D. 1997. Community invisibility, recruitment limitation, and grassland biodiversity. *Ecology* **71**: 81-92.

USDA, NRCS. 2012. The PLANTS Database (<http://plants.usda.gov>, 20 January 2012). National Plant Data Team, Greensboro, NC 27401-4901 USA.

Woosaree, J. and B. James. 2004. Revegetation of wellsites disturbances on fescue prairie in east-central Alberta. Society for Ecological Restoration, 16th International Conference. Victoria, B.C.

3.0 Foothills Fescue Grassland Restoration

3.1 Introduction

Festuca campestris (Foothills rough fescue) is the dominant native grass species of the foothills fescue grassland. It grows in association with other grasses, forbs and shrubs in response to specific ecological site conditions (IL-2010). Similar to the plains rough fescue grassland, multiple activities from various industries; Oil & gas, other industries, ranching, transportation corridors and public recreational activities, may have compromised the ecological health of the remaining fescue grassland, resulting in reduced ecosystem performance and productivity. Invasive non-native species encroachment into this remaining native fescue grassland is one of the biggest issues affecting the fescue grassland integrity. Various community groups including the ranching community, stewardship groups (Foothills Fescue Forum 2006) and government agencies acknowledge the value of retaining the ecological health and

function of the remaining fescue grassland (IL - 2010). The Energy Resources Conservation Board (ERCB) Information Letter (IL-2002) further emphasizes the importance of avoiding and conserving fescue grassland when planning developmental projects. Avoidance is not always possible, thus making reclaiming the fescue grassland to its pre-disturbed condition of paramount importance. Restoration effort is confounded by invasive non-native species such as *Poa pratensis* (Kentucky bluegrass), *Bromus inermis* (Smooth brome grass) and *Phleum pratense* (Timothy) occurring on adjacent native rangelands. Is it possible to re-establish fescue grassland in areas that were previously seeded to non-native species? Properly timed and applied herbicides, mowing and nutrient starvation techniques are some of the measures that can offer promise in controlling these undesirable species.

Woosaree and James (2007) and Desserud (2011) demonstrated some success in reclaiming plains rough fescue grassland, but reclaiming Foothills fescue grassland is a greater challenge due to climate, topography and site conditions. Currently, there is a lack of information on how to consistently and reliably restore foothills fescue grassland.

Objectives of this study were to mitigate the impact of oil & gas on sites that were previously seeded to non-native invasive species through the use of tillage, in combination with chemical treatments to reduce the spread of invasive species and seed bank in the soil. Additional objectives include:

- Determine whether the use of a cover crop could out-compete the non-native forages and enhance the site properties for seeding;
- Survey the non-native forage area to determine the extent of naturally occurring; and
- Determine nutrient availability at the site or any other factors that impedes foothills fescue seeds from getting established.
- Establish a Foothills rough fescue community once the invasive species have been eliminated.
- Monitor the rehabilitated sites for a minimum period of five years to ensure success of the rehabilitation and to prevent recurrence of the invasive species.

3.2 Methodology

Two sites along the Trans-Canada Pipeline right of way on Mac Blade and Chattaway's properties, south of Longview were targeted for the study. Mac Blade's site has approximately 80% *Bromus inermis* where as Chattaway's site consisted of approximately 80% *Phleum pratense* (Timothy) and *Cirsium arvense* (Canada thistle). The test began in late summer 2008, and consisted of four pre-seeded

treatments, replicated 3 times. Treatments included: Herbicide and no-tillage, herbicide and conventional tillage, conventional tillage with seeded barley and finally, soil fumigation with Basamid (a soil fumigant).

Each experiment is 120 m long by 4 metre width, with a 20cm buffer seeded with *Elymus trachycaulus* to prevent the occurrence of the Brome grass and Timothy into the plots.

The whole experimental area was burnt with Round-up™ herbicide at 4.46L/ha, after two weeks for full effect the treatments were applied; soil fumigation with Basamid at 397.5 kg/ha, tillage and a seeding of barley at 250 kg/hectare. *Hordeum vulgare* (Barley) was used as a treatment in some plots to deplete the nitrogen level as native species thrive better in a reduced nitrogen environment. Treatments consisted of a completely randomized block design, replicated four times at each site. Treatments were applied on October 8th, 2008 and were repeated until 2009. Soil samples were collected at the end of the growing season (2009 and 2010) and sent to Exova Laboratories for nutrient determination.

Plant surveys for naturally occurring plants within the non-native pasture along the pipeline right-of-way consisted of a floristic survey method with meander searches – one walks in a spiral pattern, starting at plot centre, in order to cover a greater area more thoroughly.

3.3 Results

3.3.1 Control of Invasive Species

Comparing the four treatments - herbicide and no-tillage, herbicide and conventional tillage, conventional tillage with seeded barley and soil fumigation with Basamid, application of Round-up proved the most efficient and practical at controlling forage grasses (Figure 16).

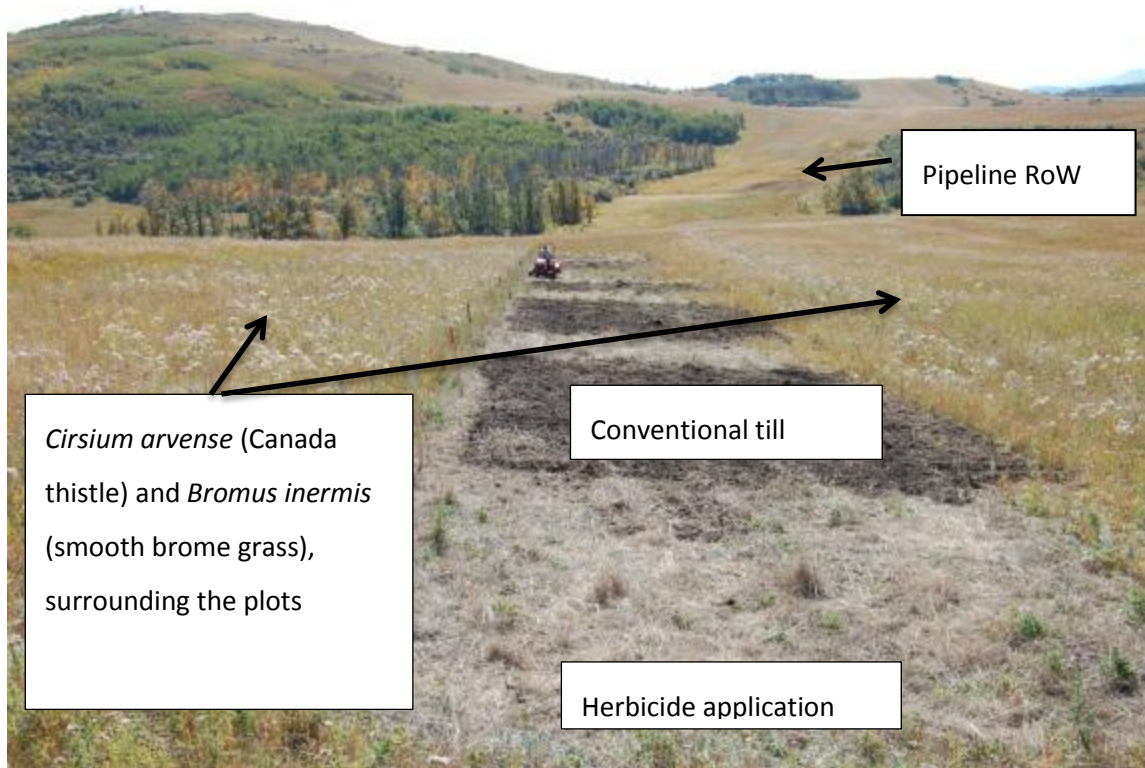


Figure 16. Effectiveness of tillage, herbicide application and Basamid on the control of non-native invasive species in fescue grassland.

Basamid had to be incorporated into the soil and is time consuming. Very few (sporadic) barley plants were observed in the till plots. After a year of weed control, the whole site was cultivated in preparation for seeding. High precipitation in June and July 2010 (81 mm and 96 mm, respectively) led to a rapid flush of weeds (Figure 17). The wetter periods also prevented access to the sites. Once the access roads were dry, making it possible to enter the sites, weed control measures had to be re- implemented. It took two years of continued herbicide application to reach a condition suitable for seeding. *Bromus inermis*, *Phleum pratense* and *Cirsium arvense* were three of the invasive species threatening this unique landscape. Annuals weeds were not a concern.



Figure 17. Re-occurrence of invasive weeds on the study plots.

In fall of 2010, both sites were seeded to a native seed mix that consisted of the major grass species of the Rough Fescue – Idaho Fescue – Parry Oat grass plant community (Adams et al. 2003), which include *Festuca campestris* (foothills rough fescue) - 57%, *Festuca saximontana* (Rocky Mountain fescue) - 15%, *Elymus innovatus* (hairy wild rye) -10%, *Festuca idahoensis* (Idaho fescue) - 5%, *Stipa richardsonii* (Richardson needle grass) - 3% and *Elymus subsecundus* (awned wheatgrass) - 5%. A seeding rate of 2000 seeds per m² (15 kg/ha) using a Fabro drill seeder. A 20 cm perimeter around the plot was seeded with *Elymus subsecundus*, in an attempt to prevent encroaching non-native grasses.

The species seeded in fall of 2010 emerged poorly. Rows of seeded grasses were sporadic. Annual weeds such as *Thlaspi arvense* (stink weed) were present in large numbers. Although annual weeds are usually not a major concern, broadleaf herbicide (Curtail M) was used to control the annual weeds to reduce competition with the grass seedlings.

3.3.2 Soil Nutrient Analysis

Soil analysis at both Mac Blade and Chattaway sites shows pH to be normal (7-7.4) and EC (electrical conductivity) to be 0.47-0.78 (dS/m). Table 2 shows the macro nutrient analysis from soil collected to 15 cm deep from each treatment at both sites.

Table 2. Nutrient status at each site, within each treatment.

Treatment	Total Nitrogen ppm	Phosphorous ppm	Potassium ppm	Sulfur ppm
Chattaway Site				
Herbicide	3	5	295	3
Tillage	8	5	316	4
Barley	3	5	279	4
Blade Site				
Herbicide	5	<5	496	7
Tillage	4	<5	477	7
Barley	8	<5	522	8

Treatments had no effects on the nutrient status. Total nitrogen, phosphorous and sulfur are fairly similar among all treatments. Potassium is the only one showing different values, but this could be due to variation at the site rather than the result of the treatments. Soils at both sites seem to be rich in organic matter (17%) when sampled to a depth of 30 cm.

3.3.3 Plant Survey within the Non-native Forage Area near the Pipeline Right of Way

A 1000 metre section of the pipeline right of way and an undisturbed area of the ROW were surveyed for occurrence of native forbs. Contrary to the view (Desserud, 2007) that native forbs cannot be found on the same section of pipeline right of way in the same region, many native forbs were found to be growing among the forage grasses on the ROW. These included:

Lupinus albifrons (silver leaf lupine), *Sisyrinchium montanum* (blue-eye grass), *Thalictrum thalictroides* (meadow rue), *Polygonatum multiflorum* (Solomon seal), *Solidago missouriensis* (Missouri goldenrod), *Galium boreale* (northern bedstraw), *Astragalus* sp (milk vetch), *Lathyrus occehorus* (pea vine), *Symphoricarpos albus* (snowberry), *Thermopsis rhombifolia* (buffalo bean), *Dodecatheon poeticum* (saline shooting star), *Artemesia ludoviciana* (prairie sage), *Rosa woodsii* (common wild rose/wood rose), *Potentilla argentea* (silver-leaf cinquefoil) and *Geum triflorum* (three-flowered avens). Occasional *Festuca campestris*, *Danthonia parryi*, and *Festuca idahoensis* plants were also recorded.

3.4 Discussion

Bromus inermis, *Phleum pratense* and *Cirsium arvense* are highly invasive, replacing native plants and changing the structure and composition of natural plant communities. The aggressive nature of these species out-competes other plants thereby preventing the coexistence of other plant species through competition for resources such as nutrients, water and light. Native forbs are part of a niche that is shade tolerant, needing little room, and many have deep taproots to access water not used by others in the community.

The Black Chernozem soils coupled with a precipitation of 177 mm in June and July provided a favourable environment to these highly invasive species. The climate in the Foothills fescue Subregion (Adams et al. 2003) is characterized by short summers with warm days and cool nights. Periods of rain followed by a dry period led to crust formation on the soil surface, thereby impeding emergence of seeded species. Thus, species like *Bromus inermis* and *Cirsium arvense* with their prolific rhizomes grew profusely and out-competed the native species. Mazzola et al. (2010) demonstrated that resource availability and propagule supply are major factors influencing establishment and persistence of native and invasive species. Increased soil nitrogen (N) availability and high propagule inputs contribute to the ability of annual invasive grasses to dominate disturbed ecosystems. Mac Blade site had approximately 80% *Bromus inermis* and the Chattaway site consisted of approximately 80% *Phleum pratense* and

Cirsium arvense, the end result is that these invasive non-native species, once seeded (Figure 18) on the landscapes imposed biological restriction and displaced the native grasses, including *Festuca campestris*.

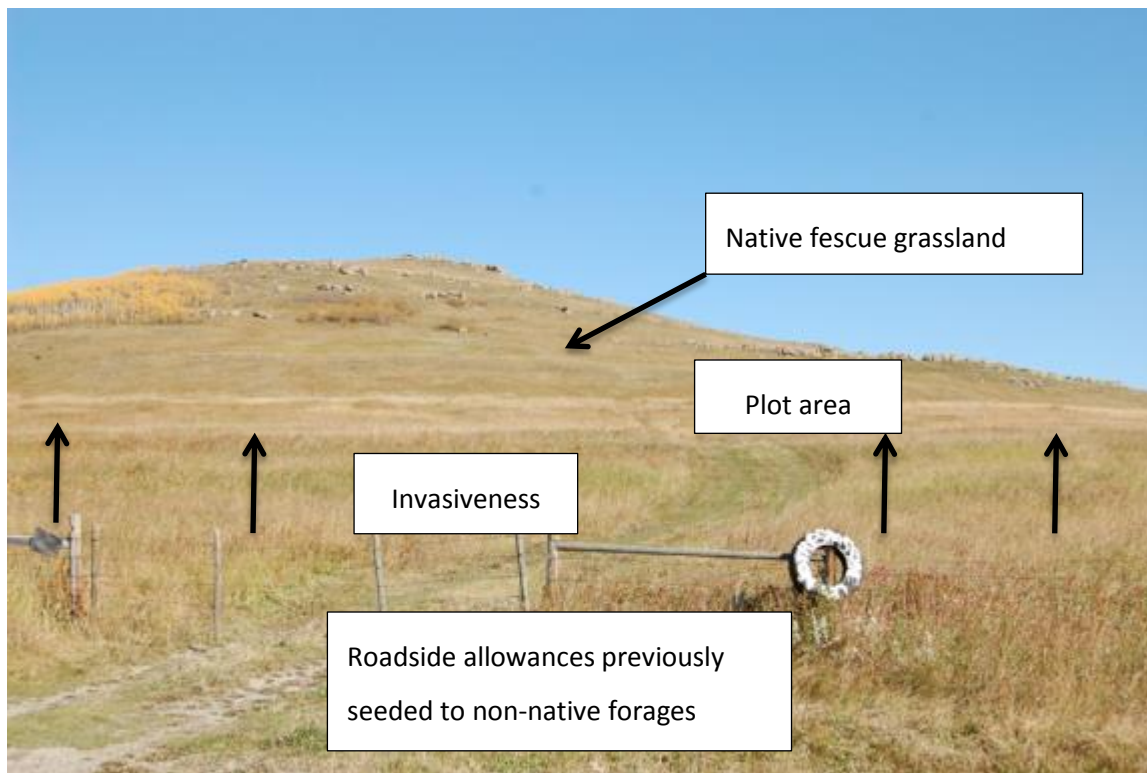


Figure 18. Non-native forage invasion into fescue grassland in the foothills fescue grassland.

Furthermore, these sites are open to grazing (wildlife and cattle), which facilitates the movement of plant propagules into the undisturbed native fescue grassland.

3.5 Conclusion

Re-establishing *Festuca campestris* proves difficult in areas that were previously seeded to *Bromus inermis* and *Phleum pratense*. None of the treatments applied seemed to be effective in the long run. There was preliminary success in controlling the non-native invasives, but periods of rain and a rich soil provide a favourable environment for regrowth. Sites closer to the roads had higher frequency and cover of non-native invasive species. Equally important in the rehabilitation of these sites is to be able to control the introduced grasses and non-native forbs (Canada thistle) on adjacent areas in order to prevent the spread of plant propagules.

Re-establishing a foothills fescue community could take up to or beyond ten years to see a modified community returned to a close approximation of native. The first seeding had poor emergence due to a lack of moisture in the weeks post seeding. The sights have been fall seeded to give the seeds natural winter conditioning and full use of spring rains.

Research and experience indicate that climate and soils play a major role in reclamation success. In native grasslands, drier environments such as those in the dry mixed grassland, with shallow soils appear to be easier to reclaim since invasive species (non-native grasses and noxious weeds) are far less competitive. It appears there are more factors controlling the success of seeded native species. At both sites even the barley cover crop was sporadic, given that barley has a high germination and is widely adaptable. In 2011, some of these factors were investigated and were reported in Woosaree and McKenzie (2011). Native fescue grasses take 2-3 years to get established and in fall of 2011, these sites were reseeded.

3.6 References

Adams, B.W., R. Ehlert, D. Moisey and R.L. McNeil. 2003. Rangeland plant communities and rangeland health assessment guidelines for the Foothills Fescue Natural Subregion of Alberta. Rangeland Management Branch, Public Lands Division, Alberta Sustainable Resource Development, Lethbridge, Pub. No. T/038 64 pp.

Desserud, P. 2006. Restoration of rough fescue grassland on pipelines in southwestern Alberta. MSc. Thesis. University of Calgary, Faculty of Environmental Design. Calgary, AB. 190 pp.

Desserud, P.A. 2011. Rough Fescue (*Festuca hallii*) Ecology and Restoration in Central Alberta. PhD Thesis. University of Alberta, Edmonton Alberta. 197 pp.

Energy Resource Conservation Board Information Letter IL 2002-1. Principles for minimizing surface disturbance in Native Prairie and Parkland Areas. <http://www.ercb.ca>.

Foothills Restoration Forum. 2006. Prospectus for a Shared Approach to Research: Conserving and Restoring Rough Fescue Grasslands.

<http://www.foothillsrestorationforum.com/storage/FRF%20Prospectus%20Draft%20%20dk.pdf>

Information Letter 2010-02. Foothills fescue grassland principles for minimizing surface disturbance. Lands Division, Rangeland Management Branch. Government of Alberta. 2 pp.

Mazzola, Monica B.; Chambers, Jeanne C.; Blank, Robert R.; Pyke, David A.; Schupp, Eugene W.; Allcock, Kimberly G.; Doescher, Paul S.; Nowak, Robert S. 2010. Effects of resource availability and propagule supply on native species recruitment in sagebrush ecosystems invaded by *Bromus tectorum*. *Biological Invasions*. DOI 10.1007/s10530-010-9846-0.

http://www.fs.fed.us/rm/pubs_other/rmrs_2010_mazzola_m001.pdf

Woosaree, J. and M. McKenzie. 2011. Evaluating the Re-vegetation Success of Foothills Fescue Grassland. Project Update, Submitted to Petroleum Technology Alliance of Canada. 19 pp.

4.0 Revegetation of Sites Affected by Salt and Hydrocarbon/Salt Contamination

4.1 Introduction

Plant species tolerance of contaminants and abilities to enhance conditions at sites is partly understood. What are the conditions that sustain vegetative productivity notwithstanding a soils problem? Is it necessary to introduce these plant growth promoting organisms (PGPR) to contaminated sites to remediate hydrocarbon affected soils? Efforts to clean up a contaminated site represent a commitment to responsible stewardship of our limited natural resources and good business practices. This study was undertaken to determine if native species have a better tolerance than non-native forage grasses to growing in disturbed environments such as those affected by pipeline breaks or produced salt water spill.

Specific objectives were to evaluate which species can effectively grow in these salts and hydrocarbons and eventually return the land to its former productive use and to evaluate if plant microbes can be attracted by living communally with the right plant species.

4.2 Methodology

4.2.1 Greenhouse Studies

A greenhouse study was designed to test the natural ability of several native species such as *Puccinellia Nuttalliana* (Nuttall's alkali grass), *Deschampsia cespitosa* (tufted hair grass), *Triglochin maritima* (seaside arrow grass), *Rumex occidentalis* (Western Dock), *Aster ericoides* (white prairie aster) *Atriplex patula* and *Agropyron repens* (quack grass) to germinate, grow and degrade salt-hydrocarbon contaminated soils. Two contaminated soils were obtained from a pipeline break near Chauvin and

Provost. The control was a normal agricultural soil. Organic liquid hume -6% (Black Earth Humates Ltd, Edmonton) and peat moss were also used as an amendment to facilitate plant growth. A 3 x 2 x 2 factorial design was used for analysis of variance in height, dry aboveground biomass, and root biomass with 3 levels of soil (3 contamination levels), 2 levels of humate, and 2 levels of peat. Three replicates were used. The ability of plants to grow successfully in these contaminated soils was ranked from 1 to 5, with 5 being excellent.

Repeated measure ANOVA with respect to time and with soil, humate, and peat as classification factors was employed in the analysis of vigor and the number of surviving plants because they were estimated twice over the growing season. GLM procedure (SAS 9.12 package) was used in the analyses.

4.2.2 Field Studies

Three field sites were used to further test growth of these native species. In 2008, Husky Energy provided access to two sites near Wainwright where a hydrocarbon spill occurred in 1999. At that time 4 tons of gypsum-green manure was applied to the site. In 2001 and 2006 calcium and nitrate-manure were applied. The sites were seeded to *Bromus inermis* as it was cultivated pasture land. The *Bromus inermis* surrounding the spill area was thick and vigorous, while the *Bromus inermis* plants growing within the spill were less vigorous and of thinner density. The site was ~80m X 15m going down a slope, pooling at the bottom. On June 12/2008 the site was seeded to native. Soil samples were taken from the high, mid and lower parts of the slope, prior to seeding.

The second Husky Energy site had poor vegetative growth and plants were sparse. There is a well head down-slope as well. The spill area is ~150mX20m. Soil samples were taken at 4 different locations heading down the slope (high, middle and lower parts of the slope).

A total of seven auger sample were collected at each location for determining baseline hydrocarbon amount.

A third site, the Zapata Energy –Leela site was identified and is located on a native sandy loam pasture near Provost (LSD 06-01-039-05-W4M). The site had a ruptured pipeline and no remediation, other than venting.

Seeding was accomplished using a drill seeder and consisted of:

Astragalus Canadensis (Canadian milk-Vetch) 5%

<i>Elymus subsecundus</i> (Awned wheatgrass)	15%
<i>Elymus trachycaulus</i> (Slender wheatgrass)	10%
<i>Bromus anomalus</i> (Nodding brome)	10%
<i>Deschampsia caespitosa</i> (Tufted hairgrass)	10%
<i>Elymus Canadensis</i> (Canada wildrye)	20%
<i>Festuca saximontana</i> (Rocky Mountain fescue)	10%
<i>Puccinellia Nuttalliana</i> (Nuttall's alkali grass)	10%
<i>Nassella viridula</i> (Green needle grass)	<u>10%</u>
	100%

At each location, seven soil samples, 1 m deep were collected from three sites of gasoline-contaminated areas in May and June 2008.

Four subsamples were obtained and mixed well. Uncontaminated soil was collected from each site and used as a control. Samples were stored in a cooler (4°C) and assessed for microbial population.

4.2.3 Microbial Growth Studies

Seven soil samples were collected from three sites of hydrocarbon contaminated areas (Husky Energy, Wainwright site 1 and 2; Zapata energy site) in May and June 2008. Four subsamples were obtained and mixed well. Uncontaminated soil was collected from each site and used as a control. Samples were stored in a cooler (4°C) and assessed for microbial population.

(1) Total bacterial population:

A 5 g sample of each soil was dissolved in 45 ml of 0.2% water agar and shaken for 0.5 hr at 200 rpm. A subsample of this initial dilution was used to prepare a dilution series (10^{-2} – 10^{-5}). The suspension (100 µl) was spread onto a nutrient agar plate (2 plates/each dilution) for total bacterial counts, cfu/g, (colony forming units) based on dry weight of soil. Plates were incubated at room temperature for 2 days. Three replications were used for each sample.

(2) Bacterial Identification:

Twenty representative bacteria colonies were transferred to potato dextrose agar (PDA) and stored. Eight of them were identified using DNA sequence analysis with three pairs of primers. Genomic DNA was extracted and processed for PCR using ITS4/5, NS1/2 and FF2/FR1 primers. PCR products were sequenced using BioEdit software and searched online for the most matched species in NCBI BLASTN database.

(1) Total bacterial population:

A 5 g sample of each soil was dissolved in 45 ml of 0.2% water agar and shaken for 0.5 hr at 200 rpm. A subsample of this initial dilution was used to prepare a dilution series (10^{-2} – 10^{-5}). The suspension of 100 μ l was spread onto a Nutrient Agar plate (2 plates/each dilution) for total bacterial counts, cfu/g (colonies forming unit, dry weight) of soil. Plates were incubated at room temperature for 2 days. Three replications were used for each sample.

(2) Bacterial Identification:

Twenty representative bacteria colonies were transferred to PDA and stored. Eight of them were identified using DNA sequence analysis with three pairs of primers. Genomic DNA was extracted and processed for PCR using ITS4/5, NS1/2 and FF2/FR1 primers. PCR products were sequenced that were edited using software BioEdit and searched online for the most matched species in NCBI BLASTN database.

4.3 Results

4.3.1 Greenhouse Studies

The greenhouse study showed that some native plant species such as Nuttall's alkali grass and tufted hair grass have the ability to grow until maturity in contaminated soils that contained F3 and F4 hydrocarbons. Several species grew successfully in these contaminated soils and were ranked from 1 to 5, with 5 being excellent vigor. Species ranking were:

1) *Puccinellia nuttalliana* - 2.8

2) *Aster ericoides* - 2.6

3) *Elymus repens* - 2.5

4) *Rumex occidentalis* - 2.4

5) *Elymus subsecundus* - 1.7

6) *Triglochin maritima* - 1.7

7) *Achillea millefolium* - 1.6

8) *Atriplex patula* - 0.8

9) *Carex* sp. - 0.7

10) *Distichlis stricta* - 0.2

Overall, soil contamination effect dominated the analyses showing consistent poor plant performance on the contaminated soil from Chauvin and particularly on the Provost contaminated soil 3. *Distichlis stricta* (salt grass), *Atriplex patula*, *Carex* spp, and *Achillea millefolium* (yarrow) although originally collected near a contaminated site at Borradaile (east of Vermillion) couldn't survive in either contaminated soil. In fact, *Distichlis stricta* (salt grass) didn't grow in clean Vegreville soil either, which could possibly indicate poor seed quality or dormancy in the seeds. *Triglochin maritima* (seaside arrow grass), *Agropyron repens* (quackgrass), western dock, and *Agropyron subsecundum* (awned wheatgrass) survived on the Chauvin soil but couldn't survive on the Provost soil. *Deschampsia caespitosa* (Tufted hair grass) and *Puccinellia nuttalliana* (Nuttall's alkali grass) (Figure 19), appeared to be the most resilient plants, which could grow on all three soils.

The peat amendment effects were both soil-specific and plant-specific. Obvious beneficial peat effect was observed on *Carex* spp., *Agropyron repens* (quack grass), western dock, *Achillea millefolium*, *Agropyron subsecundum*, which allowed these species to survive on the Provost soil and in some cases, on both soils. However, peat amendments had concurrently negative effects on clean soil such as the control soil.



Figure 19. Growth of *Puccinellia nuttalliana* (Nuttall's alkali grass) in a control soil with peat amendment (Left), the Chauvin contaminated soil plus humates (Centre), contaminated Provost soil plus peat (right).

Table 3. Concentration of hydrocarbon contaminants in soil before and after planting and with the addition of humates.

	Soil A			Soil B		
	Before Planting	After Plant Growth no humates	After Plant Growth with humates	Before Planting	After Plant Growth no humates	After Plant Growth with humates
	ppm	ppm	ppm	ppm		ppm
F2 (C10-C16)	620	220	170	710	310	470
F3 (C16-C34)	5040	2600	2160	7410	2340	4720
F4 (C34-C50)	2720	1570	1310	3560	1090	2540
F4HTGC (C34-C50+)	6380	4420	3290	8110	2340	7780
%50+	30.4	39.4	35.2	28.0	25.0	40.4

Table 3 shows Petroleum hydrocarbon parameters in two hand auger samples that exceeded the criteria for F2, F3 and F4. The addition of humate was not significant ($\alpha = 0.05$) approaching the significance level in control soil in several cases. The soil used in the study originates from a pipeline break near the Municipal District of Provost where an agricultural area of 250 m² was affected. Petroleum hydrocarbon (PHC) parameters in two hand auger samples from this site originally exceeded the CCME (Canada Wide Standard for Petroleum Hydrocarbons) (http://www.ccme.ca/ourwork/soil.html?category_id=43) criteria for F2, F3 and F4 hydrocarbons. It can be postulated that the humate amendment was not as effective in reduction of hydrocarbons because it supplied the bacteria with a more readily available source of carbon.

4.3.2 Field Studies

Husky Energy site 1 had better emergence than site 2. Site 1 had 100% of the rows covered by plants, including some annual weeds such as *Fagopyrum esculentum* (common buck wheat) (Figure 20).



Figure 20. Emergence of seeding native species on a pipeline break.

Site 1 in the second year after seeding (year 2009) showed increasing growth on the site. Other species observed included: *Bromus inermis* (smooth brome), *Thlaspi arvense* (Stinkweed), *Salsola kali* (Russian thistle), *Descurainia sophia* (Flixweed), *Amaranthus retroflexus* (Redroot Pigweed), *Taraxacum officinale* (Dandelion), *Fragaria virginiana* (Wild strawberry) and *Rosa arkansana* (Prairie rose).



Figure 21. Growth of seeded native species at site 1.

None of the *Elymus canadensis*, a native legume used in the seed mix emerged. The seeds were scarified prior to incorporating into the seed mix and could have lost viability.

Table 4 illustrates a remarkable decrease in hydrocarbon fractions recorded at site 1 during 2007 and 2010. Soil pH (7.20), EC (3.57 dS/m) and SAR (Sodium absorption ration 2.10) are favourable for plant growth. Soil pH influences availability of plant nutrients and a soil pH of 5.5-7.5 are normally desired. Any conductivity above 4 ds/m leads to the accumulation of salts, which in turn limits plant growth.

Table 4. Hydrocarbon fractions from composite 1 m core sample at site 1.

Hydrocarbon Analysis	Units	2007	2010
Benzene	mg/Kg	<0.08	<0.004
Toluene	mg/Kg	0.11	<0.005
Ethylbenzene	mg/Kg	1.40	0.01
Total Xylenes (m,p,o)	mg/Kg	15.90	0.02
F1 C6-C10	mg/Kg	603.00	7.33
F1- BTEX	mg/Kg	586.00	7.33
F2c C10-C16	mg/Kg	9230.00	208.00
F3c C16-C34	mg/Kg	21900.00	5963.00
F4c C34-C50+	mg/Kg	9600.00	3813.33
F4HTGCc C34-C50+	mg/Kg	27800.00	9226.66
% C50+	%	30.90	33.97



Figure 22. Plant cover provided by seeded native species at the Husky Energy site 2.

The Husky Energy site 2 showed seeded rows to be sparser. There is more bare-ground especially uphill towards well-site as a result of compaction by grazing livestock. The site and its surrounding areas are dominated by (Canada thistle), *Elymus repens*, *Senecio vulgaris* (Groundsel), *Hordeum jubatum* (Foxtail Barley) and *Agrostis* spp.

Seeding may have been less effective on this site as the soil is coarser and appears to be compacted (signs of heavy equipment on site by presence of deep tire tracks) and of livestock.

Some considerations about the differences in revegetation success could also be due to the level of brine spill at the site.

Table 5. Biomass production collected from 3 composite samples at the phytoremediated site 1 and 2, in 2010.

<u>Site 1</u>	<u>Dry Weight (kg/ha)</u>
Sample 1(top of plot)	1084
sample 2 (Middle of the plot)	4389
sample 3 (bottom of the plot)	6277
control	10030
<u>Site 2</u>	<u>Dry Weight (kg/hg)</u>
sample 1(top of plot)	4510
sample 2 (Middle of the plot)	2499
sample 3 (bottom of the plot)	5503
control	3059

Forage production is considerably less compared to adjacent control areas at site 1, whereas site 2 fairs well to the control. That could be due to the fact that that site 2 is influenced by compaction and growth of foxtail barley (Figure 23) in and around the site.



Figure 23. Plant cover at Husky Energy site 2.

The 2007 seeded experiment at the Zapata Energy –Leela site, located on a sandy soil native pasture near Provost had poor emergence. Seeded vegetation away from the centre of the pipeline break is doing better than those nearer to the pipeline break. Soil sampled near plants seeded in 2007 showed a 30% decrease in F3 and F4 hydrocarbon fractions. Due to the concentration of hydrocarbon present in the soil, seeded vegetation was sparse, yet thriving (Figure 24). The hydrocarbon was more severe at the centre of the plot, hardening the sandy soils making it difficult for seeds to emerge.



Figure 24. Site impacted by hydrocarbon spill from a pipeline break in a sandy soil, showing thriving *Achnatherum hymenoides* by year 2.



Figure 25. Growth of *Elymus Canadensis*, *Achnatherum hymenoides* and colonization by other native species (*Artemisia frigida* and *Carex* sp) at a petroleum hydrocarbon contaminated site in a sandy soil by year 3.

Other species found to naturally ingress into the site include *Sporobolous cryptandrus* (Sand dropseed), *Carex pensylvanica* (sun-loving sedge), *Artemisia canadensis* (Canada Wormwood), *Koleria macrantha* (June grass), *Pascopyrum smithii* (Western wheatgrass), *Aster villosa* (Hairy Golden Aster), and *Festuca saximontana* (Rocky mountain fescue)

No soil amendments were used at this site, other than improving air circulation through air turbines inserted into the soils (Figure 26).



Figure 26. Aeration of the soil to enhance degradation of hydrocarbon.

Results from soils collected near the root zone of plants naturally found growing on the site are considerably lower for F2, F3 and F4's hydrocarbon compared to soil samples collected from bare areas (Table 6).

Table 6. Analyte measured near the root zone of plants growing on site compared to bare areas on the site.

Analyte	Units	No Vegetation	Vegetation	Detection Limit
Mono-Aromatic Hydrocarbons - Soil				
Benzene Dry Weight	mg/kg	<0.004	<0.004	0.004
Toluene Dry Weight	mg/kg	<0.005	<0.005	0.005
Ethylbenzene Dry Weight	mg/kg	<0.010	<0.010	0.01
Total Xylenes (m,p,o) Dry Weight	mg/kg	<0.010	<0.010	0.01
Volatile Petroleum Hydrocarbons - Soil				
F1 C6-C10 Dry Weight	mg/kg	<12	<12	12
F1 -BTEX Dry Weight	mg/kg	<12	<12	12
Extractable Petroleum Hydrocarbons - Soxhlet				
F2 C10-C16 Dry Weight	mg/kg	1630	583	10
F3 C16-C34 Dry Weight	mg/kg	10000	6840	10
F4 C34-C50 Dry Weight	mg/kg	4510	3140	10
F4HTGC C34-C50+ Dry Weight	mg/kg	7080	6290	10
% C50+	%	13.7	23	
Moisture-Soil % Moisture	%	4.18	3.39	

4.3.3 Microbial Studies

Soils collected near plants growing on these sites revealed that bacteria were the most dominant microbes in the soils tested. Bacterial populations in most soil samples ranged from 5.7×10^8 – 4.9×10^9 cfu per gram (colony forming units). Soil samples from the Zapata contaminated site had lower bacterial population (Table 7). Fewer fungi were found in this soil. Compared to the control soil from Zapata, the bacterial composition was much different in contaminated soils. More pink, orange and yellow colored bacterial colonies were found in contaminated soils while high proportional pink-colored bacterial colonies were found in Zapata contaminated soil (Figure 27). According to the colony morphology, bacteria could be grouped into four major groups. DNA identification of eight representative isolates showed that *Bacillus* spp. was the major bacteria in the soil and possible other bacteria included *Microbacteria*, sp. and *Flavobacterium* sp. (Table 8). These bacteria may have the ability to utilize carbon and play a role in the biodegradation of crude oil in the environment. It has been reported that a mixture of *Bacillus* spp. and *Flavobacterium* sp. and other bacteria could degrade up to 78% of crude oil at suitable temperature and pH level (Rahman et al. 2002).

This experiment indicates that suitable hydrocarbon degrading bacteria already exist in the soils. Having commercial quantities of seed that can germinate and grow in these contaminated sites is more important than introducing PGPR as claimed by other researchers. However, should PGPR be an option in the strategy to remediate contaminated soils, further work is required to classify the bacterial communities in the soil and develop a biodegradation agent and method for bio-remediation of hydrocarbon contaminated lands.

Table 7. Enumeration of bacteria from soil samples collected at the hydrocarbon contaminated sites in Alberta 2008.

Sample	Source	Replication	Bacteria (CFU/g dry soil)
1	Husky Site 1	1	3.0×10^9
		2	4.5×10^9
		3	4.9×10^9
2	Husky Site 1	1	1.1×10^9
		2	1.3×10^9
		3	2.4×10^9
3	Husky Site 2	1	5.7×10^8
		2	7.3×10^8
		3	8.6×10^8
4	Husky Site 2	1	3.1×10^9
		2	2.7×10^9
		3	3.6×10^9
5	Husky Site 2 Control	1	2.2×10^9
		2	2.6×10^9
		3	2.6×10^9
6	Zapata	1	2.6×10^7
	(sandy soil)	2	1.7×10^7
		3	1.3×10^7
7	Zapata Control	1	2.5×10^9
	(sandy soil)	2	1.4×10^9

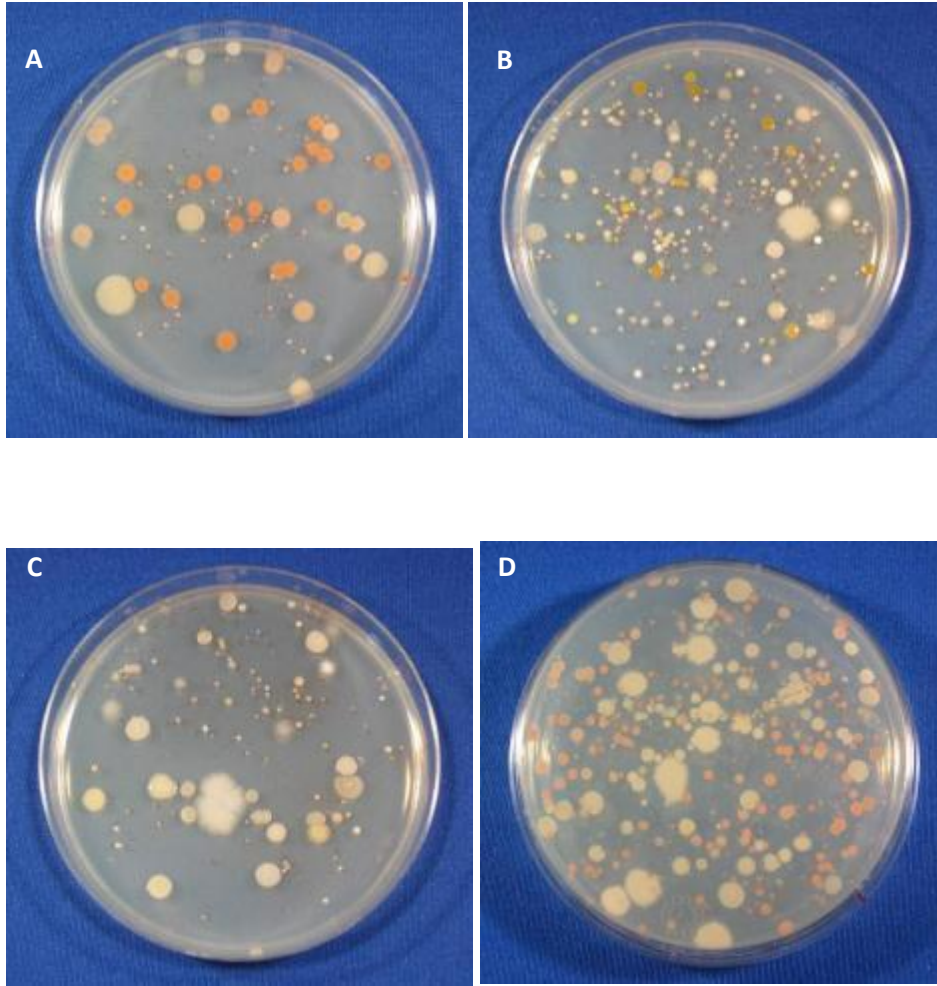


Figure 27. Morphology of bacterial colonies on nutrient agar, (A) soil sample from Zapata site, (B) soil sample from Husky site 2, (C) soil from Zapata site control and (D) back view of plate containing soil from Zapata site.

Table 8. Morphology and identification of bacteria from soil samples.

Isolate	Group	Colony Color	Identification
1	1	light pink	
2	1	pink	
3	1	pink	
4	1	pink	<i>Bacillus</i> sp.
5	1	pink	
6	1	light pink	<i>Bacillus</i> sp.
7	2	white, smooth	<i>Bacillus pumilus</i>
8	2	white flat rough	<i>Bacillus</i> sp.
9	2	white rough	
10	2	white rough	
11	2	cream white	
12	2	cream white	
13	2	cream white	
14	3	orange smooth	
15	3	orange smooth	?
16	3	orange smooth	<i>Flavobacterium</i> sp. ?
17	4	yellow	
18	4	yellow	<i>Microbacterium</i> sp.
19	4	yellow	<i>Microbacterium</i> sp.
20	4	yellow	

Note. Some colony forming units were not identifiable.

4.4 Discussion

Species used in this study show promise for remediation. Seed plots of *Deschampsia cespitosa* and *Puccinellia nuttalliana*, *Festuca saximontana* (Parkland ecotype) and *Achnatherum hymenoides* were established in Vegreville. Seeds from these species can be used in remediation, thereby reducing or perhaps eliminating the practice of land farming or “dig and dump remediation”, a 20 year old practice

in Alberta. Plant colonisation at the Zapata site has been slow as a consequence of primary succession, with seeds and propagules entering the area from surrounding vegetation. After 3 years, natural colonisation ingressed onto microsites towards the centre of the contaminated area. This trend is similar to other studies (Shu et al, 2005; Halvorson and Lang 1989, Aschenbach and Kindscher 2006). Leskiw et al. (2012) evaluated the impact of a brine release on soil and vegetation due to a gas well blowout in 1999. They concluded that average electrical conductivity was declining with time and has remained below 2.0 dS m^{-1} since 2002 and from 2002 to 2010 moss cover increased 40%, whereas shrubs decreased 30%. The most impacted plot showed higher diversity than the least impacted plots and the control (Shannon diversity index = 1.49, 1.36, 1.11 for most impacted, least impacted and control, respectively). Soil and vegetation indicated salt-affected plots were recovering naturally.

Certain arbuscular mycorrhizae (AM) and bacteria have been known to provide host plants with some tolerance of toxic conditions. Most bioremediation requires the introduction of some amendments, protein surfactants (Autry and Ellis, 1992) and microbes. Remediation of petroleum contaminated sites using plants (phytoremediation) has not always achieved the desired results within a short time, 5 years. Remediation alternatives can include on-site incineration, off-site incineration, ex-situ bioremediation, and excavation and disposal. One has to take into account the length of time it takes to treat contaminated soils to below regulatory thresholds.

Is it necessary to introduce PGPR to contaminated sites to enhance remediation on hydrocarbon affected soils or can these organisms be attracted by living communally with the appropriate plant species?

Plant Growth-Promoting Rhizobacteria (PGPR) are soil bacteria that colonize the root and increase plant growth through increasing resistance to environmental stress. Contaminated sites (pipeline breaks, accidental spills) associated with the waste products of oil and gas drilling is common on agricultural lands and prairie landscapes. Scientists have used PGPR organisms in crops to minimize expenditures in fertilizer input, maximize drought resistance, and to protect plants from pathogens. PGPR organisms in remediating contaminated soils are also being proposed for phytoremediation. Laboratory results confirmed that certain native plants are able to grow on petroleum-laden sites.

Soil samples collected at 15cm depth near the root of plants growing at Zapata confirmed the presence of naturally occurring microbes. The same conclusion cannot be made for the Husky Energy sites as cattle manure was applied to the site prior to seeding any forage crops. These bacteria may have the

ability to utilize carbon and play a role in the biodegradation of crude oil in the environment. It has been reported that a mixture of *Bacillus* spp. and *Flavobacterium* sp. and other bacteria could degrade up to 78% of crude oil at suitable temperature and pH level (Rahman et al. 2002).

4.5 Conclusion

Our objective was to see how well plants can grow and remediate soils affected by long-chain hydrocarbons. The effects of hydrocarbon and amendments on plant growth and the biological activities of the soils are critical in evaluating whether plant ecosystems and productivity can be restored. Native species used in this study showed good performance (growth, development, vigour and adequate forage production) during the study. At the Husky Energy Site, forage growth increased steadily, not yet comparable to the control but is on the right trajectory. At the Zapata site, the seeded species and naturally occurring species are doing well and showing ingression towards the most affected area on the site. The plant species that did well have also been targeted for seed multiplication and will be recommended for remediation of similarly contaminated soils. Results from this study could potentially provide an alternative to soil removal and also be useful for future remediation and reclamation practices.

4.6 References

- Autry, A. R. and Gary M. Ellis. 1992. Bioremediation: An effective remedial alternative for petroleum hydrocarbon-contaminated soil. *Environmental Progress* **124(4)**: 318–323.
- Halvorson, G. and K. Lang. 1989. Revegetation of a salt water blowout site. *Journal of Range Management* 42(1): 61-65.
- Aschenbach, T. and Kelly Kindscher. 2006. Plant Species on Salt-Affected Soil at Cheyenne Bottoms, Kansas. *Transactions of the Kansas Academy of Science (1903-)* Vol. 109, No. 3/4 (Fall, 2006), pp. 207-213.
- Leskiw, L.A., Sedor, R.B., Welsh, C.M., and T. Zeleke. 2012. Soil and vegetation recovery after a well blowout and salt water release in northeastern British Columbia. *Canadian Journal of Soil Science* **92**:(1) 179-190,
- Rahman K.S.M.; Banat I.M.; Thahira J.; Thayumanavan T.; Lakshmanaperumalsamy P. 2002. Bioremediation of gasoline-contaminated soil by a bacterial consortium amended with poultry litter, coir

pith and rhamnolipid biosurfactant [Bioresource Technology](#), Volume 81, Number 1, January 2002, pp. 25-32.

Shu, W. S., Z. H. Ye, Z. Q. Zhang, C. Y. Lan, M. H. Wong. 2005; Natural Colonization of Plants on Five Lead/Zinc Mine Tailings in Southern China. *Restoration Ecology* **13**: 49-60.

5.0 Native Species Research

5.1 Introduction

A critical part of the strategy to reclaim plant communities is to have access to appropriate plant species and knowledge on how to use them to reclaim environmentally sensitive areas to sound ecological function. Alberta Innovates Technology Futures (AITF) has been conducting research in the propagation and development of ecological varieties for re-introduction into disturbed habitats. Consultation with Alberta Sustainable Resource Development and Alberta Environment & Water (Woosaree, 2007) resulted in a list of species that are suitable for reclamation based on their ecological role in the plant community and forage value. The following describes the species that were identified to play important ecological function. These are: *Elymus innovatus* (hairy wild rye), *Oryzopsis asperifolia* (White grained mountain rice grass), *Helictotrichon hookeri* (Hooker's oat grass), *Danthonia intermedia* (timber oat grass), *Puccinellia nuttalliana* (Nuttall's alkali grass), *Festuca campestris* (foothills rough fescue), *Festuca hallii* (plains rough fescue), *Festuca saximontana* (Rocky Mountain fescue) – Parkland ecotype, *Astragalus canadensis* (Canada milk vetch), two ecotypes of (Boreal and Foothills) of *Elymus innovatus* (Hairy wild rye), *Hesperostipa comata* (needle and thread grass), *Stipa richardsonii* (Richardson's needle grass), *Deschampsia caespitosa* (tufted hair grass), *Bouteloua gracilis* (blue grama), *Vicia americana* (American vetch), *Lathyrus venosus* (pea vine), *Viburnum opulus* (low bush cranberry), *Arctostaphylos uva-ursi* (bearberry) and *Shepherdia canadensis* (buffalo berry). The objective of this study was to determine optimum propagation methodology and evaluate their potential for large scale field production.

5.2 Methodology

Germination tests were conducted on wild harvested seeds and were at three to five months of age after the dates of harvest. Seed germination consisted of placing the seeds in a petri dish on two Whatman #2 filter papers moistened with distilled water. The petri dish is incubated in the growth chamber at 22°C/15°C [day/night], 8 h day, diurnal cycles. Four replicates, each containing 25 seeds are used. Germination is recorded after 21 days as most grass species emerge in the field after 21 days. Germination of many shrub species may require acid scarification using concentrated sulphuric acid for 3 hours followed by 60 day stratification at 21°C and another 60 day stratification at 4°C prior to placing

the seeds for germination at alternating temperatures of 22°C for 8 hour light and 15°C for 16 hour dark periods.

Seeded plots were seeded using a Fabro-plot seeder (Swift Machines, Swift Current, Saskatchewan). These grasses were seeded 1-2 cm deep, with 40 cm row spacing and at a rate of 3-5 kg/ha depending on species.

The breeder seed plot represents the best plant material that was established in preparation for commercial release. These plots are established according to the guidelines set by the Canadian Seed Growers Association (CSGA circular 6, 2009). A Wintersteiger plot combine was used to harvest the seed from these plots.

5.3 Results

5.3.1 Seed Germination

Table 9 lists treatments and seed germination of shrub and grass species that can be suitable for reclamation. *Smilacina stellate*, *Thalictrum venulosum*, *Glycyrrhiza lepidota*, *Petalostemon purpureum* and *Prunus virginiana* proved difficult to germinate, despite the seeds being pre-treated to enhance germination. Other species require no pre-treatment and germinated well.

Table 9. Germination evaluation of candidate species for revegetation in forested lands.

Species		Pre-germination seed treatments	Average Percent Germination	
<i>Alnus crispa</i>	Green alder	60 day stratification at 4°C	Coll* #1	3
			Coll #2	36
			Coll #3	21
			Coll #4	0

			Coll #5	0
<i>Amelanchier alnifolia</i>	Saskatoon berry	60 day stratification at 4°C		74
<i>Arctostaphylos uva-ursi</i>	Bear berry	Acid scarification with concentrated sulphuric acid for 3 hrs. followed by 60 day stratification at 21°C followed by 60 day stratification at 4°C	Coll # 1	10
			Coll #2	10
<i>Corylus cornuta</i>	Beaked hazelnut	Prechilling		12
<i>Eleagnus commutata</i>	Silverberry	Cold stratification		92
<i>Prunus virginiana</i>	Chokecherry	Cold stratification		5
<i>Shepherdia canadensis</i>	Buffalo berry	Acid scarification with concentrated sulphuric acid for 15 minutes followed by 30 day stratification at 4°C	Coll #1	79
			Coll #2	83
<i>Symphoricarpos occidentalis</i>	Bunch berry	Acid scarification with concentrated sulphuric acid for 75 minutes followed by 20 day stratification at 21°C followed by 180 day stratification at 4°C	Coll #1	0
			Coll #2	2
			Coll #3	1
			Coll #4	2

<i>Viburnum opulus</i>	High bush cranberry	Warm and cold stratification		70
<i>Lathyrus ochroleucus</i>	Creamy peavine	None		61
<i>Oryzopsis asperifolia</i>	White-grained rice grass	None		67
<i>Oryzopsis pungens</i>	Pine rice grass	None		47
<i>Aster ciliolatus</i>	Lindley's aster	None		32
<i>Elymus innovatus</i>	Hairy wild rye	None		48
<i>Rosa acicularis</i>	Plains wild rose	Cold stratification		0
<i>Smilacina stellata</i>	Star-flowered Solomon's seal	Cold stratification		0
<i>Thalictrum venulosum</i>	Veiny meadow rue	None		0
<i>Festuca idahoensis</i>	Idaho fescue			98
<i>Glycyrrhiza lepidota</i>	Wild licorice			0
<i>Gaillardia aristata</i>	Brown-eyed Susan			80
<i>Linum lewissii</i>	Wild Blue Flax			84
<i>Agropyron smithii</i>	Western wheatgrass			99

<i>Potentilla pennsylvanica</i>	Prairie Cinquefoil			79
<i>Solidago canadensis</i>	Canada Goldenrod			73
<i>Petalostemon purpureum</i>	Purple prairie clover			5
<i>Antennaria nitida</i>	Pussytoes			39
<i>Liatris punctata</i>	Wild liatris			75
<i>Ratibida columnifera</i>	Prairie coneflower			91

*Represents different collection.

5.3.2 Field Production

At AITF's Vegreville facility, seeded plots were established (40 m x 40 m) for *Festuca hallii*, *Festuca campestris*, *Festuca saximontana* (Parkland ecotype), *Stipa richardsonii*, *Nassella viridula*, *Elymus innovatus*, *Oryzopsis asperifolia*, *Helictotrichon hookeri*, *Danthonia intermedia* (timber oat grass), *Puccinellia nuttalliana*, *Festuca campestris* (foothills rough fescue), *Festuca hallii* (plains rough fescue), *Festuca saximontana* – Parkland ecotype, *Astragalus canadensis* - two ecotypes of (Boreal and Foothills) of *Elymus innovatus*, *Hesperostipa comata*, *Stipa richardsonii*, *Deschampsia caespitosa* and *Bouteloua gracilis*.

Seed plots of native legumes (*Vicia americana* and *Lathyrus venosus*) failed as the plants were consumed by wildlife, mainly deer, ground squirrels, pocket gophers and cutworms. Methods to better safeguard these plants in a field production setting need to be developed.

Plants of *Viburnum opulus*, *Arctostaphylos uva-ursi* and *Shepherdia canadensis* were planted in a field nursery. Additionally, plants of *Viburnum opulus* were also planted within a *Bromus inermis* field to see how its growth is affected by the non-native invasive species. Figure 28 shows its growth after 5 seasons

and Figure 29 shows *Arctostaphylos uva-ursi* being transplanted in the field. All field harvested seed had good germination, which is a determinant factor in revegetation success. “ARC Grouse” green needle grass had a germination of 64 %, “ARC Badlands” blue grama 94%, “ARC Bison” plains rough fescue 84 %, Rocky Mountain fescue (Parkland ecotype) 75%, hairy wild rye 74% and “ARC Aspen” Canada milk vetch 49%. Seeds of “ARC Aspen” Canada milk vetch were not scarified prior to germination; however Tetrazolium test (TZ test) shows most seeds to be viable. *Festuca campestris*, a key species for reclamation in the foothills did not produce any seeds. This is expected for the first growing season. The plot was maintained and seed production is anticipated in the next growing season.



Figure 28. *Viburnum opulus* thriving within a *Bromus inermis* field.



Figure 29. Transplanting *Arctostaphylos uva-ursi* in the field.

Research on producing Breeder/Foundation seed for revegetation is an on-going process. Seed for “ARC Grouse” green needle grass, “ARC Badlands” blue grama (Figure 30) and “ARC Bison” plains rough fescue (Figure 31) were released in May, 2010. Commercial production was undertaken by Brett-Young Seeds Limited and seeds will be commercially available in 2012.



Figure 30. Production of a *Bouteloua gracillis* source identified variety at Vegreville.



Figure 31. *Festuca hallii* production at Vegreville in 2009-11.

5.4 Conclusion

Many of these species, especially the shrub species require special techniques such as acid scarification with concentrated sulphuric acid for 3 hrs. followed by a 60 day stratification at 21°C then followed by another 60 day stratification at 4°C (Table 9) in order to break out dormancy. For example, *Viburnum americana* (Highbush cranberry) required warm stratification (20°C for 60-90 days) for development of the radicle followed by cold stratification (1-5°C for 30-60 days) to break dormancy in the epicotyl. It then needs a day temp of 30°C and a night temp of 20°C for 60 days. (U.S. Department of Agriculture, Forest Service Handbook, 1974). Another source indicates that “the seed is best sown in a cold frame as soon as it is ripe. The germination can be slow, sometimes taking more than 18 months. If the seed is harvested 'green' (when it has fully developed but before it becomes fully ripened) and sown immediately in a cold frame, it should germinate in the spring. Stored seed will require 2 months warm followed by 3 months cold stratification and can still take 18 months to germinate (http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Viburnum+opulus)”.

Dormancy can take many forms. This includes dormancy caused by properties of the seed coat, morphological immaturity of the embryo, physiological immaturity in the seed and sometimes a combination of all the factors. Dormancy created by impermeable seed coats can generally be treated by mechanical scarification or acid scarification. Attempts to break dormancy in these species produced inconsistent results.

Table 9 lists the pre-treatment and percent germination of many of the species targeted for commercial production. Germination condition was set to 22°C for 8 hours light, 15°C for 16 hours dark alternating for all the species. *Shepherdia canadensis* (Buffalo berry) had 79-83% germination following a treatment with concentrated sulphuric acid for 15 minutes, then with 30 day stratification at 4°C.

Alnus crispa had 1 % germination. A repeat of the germination test showed a range of 0% to 36% (Table 9) among the five collections. We also found 61% germination is obtained when the seeds are planted right after harvest compared to if the seeds were stored and later germinated or planted.

Amelanchier Alnifolia, *Eleagnus commutata*, *Shepherdia canadensis* and *Viburnum opulus* had germination of 74%, 92%, 83% and 70% respectively (Table 9) and should be the focus of further plant development to meet the needs of wildlife habitat and other revegetation goals. Other non-shrubby species such as *Lathyrus ochroleucus*, *Oryzopsis asperifolia*, *Oryzopsis pungens*, and *Elymus innovatus*

also have good germination and can play an important role in returning disturbed sites to biological activity.

Arctostaphylos uva-ursi, *Rosa acicularis*, *Smilacina stellata* and *Thalictrum venulosum* proved difficult to germinate and in contemporary reclamation may be excluded from the vegetation mix due to no seed availability in commercial amounts. Alternate methods such as plant cuttings need to be investigated.

We maintained a nursery of all species germinated and found that some species transplant well while others were difficult to establish in a field conditions. For example, some species require an understory environment to thrive while others such as the *Hedysarum boreale* and *Hedysarum alpinum* became a target of grazers. Cultural practices for successful production of these candidate species need further research.