ESTABLISHMENT, FORAGE PRODUCTION AND DROUGHT RESISTANCE OF NATIVE PERENNIAL LEGUMES OF THE CANADIAN PRAIRIES

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Plant Science

University of Saskatchewan

Saskatoon

By

Shanna Marie Quilichini

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying, publication, or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Plant Sciences
University of Saskatchewan
51 Campus Drive
Saskatoon, SK, S7N 5A8 Canada

OR

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan, S7N 5A8 Canada

ABSTRACT

Forage production of perennial legume-grass mixtures and their effect on soil fertility are expected to be greatly influenced by growth environment, but many questions remain for native legume variability and performance in mixtures in semi-arid environments for forage production. Field research on four native legume species (*Astragalus flexuosus*, *Dalea purpurea*, *Hedysarum boreale* and *Vicia americana*) evaluated their establishment and early vs. late season forage production in monoculture, and in mixtures with *Bromus riparius*, in the Brown and Dark Brown soil zones of the Canadian Prairies near Swift Current and Saskatoon, SK. The experimental design was a Randomized Complete Block Design with four replicates with treatments being species mixtures/monocultures and harvest dates (July and August). The native legume-grass mixtures performed differently at the sites, with better overall establishment at Saskatoon but greater percentages of legumes present in mixtures at Swift Current. The mixtures had similar forage quality as monoculture *B. riparius*, with legumes contributing 10% or less of the forage dry matter yield at both sites. Soil nitrogen was not greater in legume-grass mixtures compared to monocultures *B. riparius*. Based on this study, native legumes would need to make up a larger proportion of forage dry matter yield to change the nutritional value and soil nutrient levels of mixture plots. In a seeding rate evaluation, the four legume species were planted in monoculture at three seeding rates (300, 200 and 100 PLS m^{-1}) and tested for forage dry matter yield near Swift Current, SK one year after seeding. While increasing seeding rates up to 300 PLS m^{-1} in the Brown soil zone corresponded to increasing seedling density and foliar cover, dry matter yield was not affected by seeding rate and there was no correlation between dry matter yield and native legume seed size (R^2=0.0895). In a greenhouse drought study, four watering regimes (100, 75, 50, 25% field water holding capacity) were applied to seedling monoculture legume and legume-grass mixtures with *B. riparius*. *A. flexuosus* was the only native legume that showed differences in above and belowground biomass by watering treatment, with greater aboveground biomass at the moderate watering levels (50% and 75%), and greater belowground biomass at the 50% watering level. Of the four legume species, *A. flexuosus* showed the greatest establishment and produced the greatest dry matter yield in monoculture at Saskatoon (2934 kg ha^{-1}, 3107 kg ha^{-1}) and Swift Current (3938 kg ha^{-1}, 3920 kg ha^{-1}) in July 2018 and August 2018, respectively.

ACKNOWLEDGEMENTS

I would like to thank the Southwest Forage Association, the Saskatchewan Forage Network, the Saskatchewan Ministry of Agriculture, Agriculture and Agri-Food Canada, and the University of Saskatchewan for funding my research project.

My supervisors for inspiring me to learn more, do more and become more; Dr. Michael Schellenberg for sharing his interest in native plants and forage research, sparking my experimental imagination, and encouraging me to test my ideas, and Dr. Bill Biligetu for his kindness, encouragement, and continued reassurance that I would eventually finish this thesis.

My graduate committee members Dr. Eric Lamb, Dr. Bruce Coulman and Dr. Timothy Sharbel and my graduate professors Dr. Chris Willenborg and Dr. Peiqiang Yu, for their guidance and teaching.

The lab assistants and technicians, fellow students and colleagues at the University of Saskatchewan Forage Breeding Laboratory and the Swift Current Research and Development Centre, particularly the Plant Ecology group, who helped me execute these projects in the greenhouse and field.

My husband, Aaron, for his love, support, and encouragement during this journey. My dad, Brian, for keeping me company on the drives to school. Friend, Douglas, for reminding me there is no limit to what I can accomplish, and many friends and family for cheering me on.

Dedicated to my late grandmothers, Annamae Dorey and Martha Gonczy, for passing on to me their love of plants.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	acid detergent fibre
AF	*Astragalus flexuosus*
BR	*Bromus riparius*
Ca^{2+}	calcium
CP	crude protein
DP	*Dalea purpurea*
DM	dry matter
gms	grams
HB	*Hedysarum boreale*
K^{+}	potassium
Mg^{2+}	magnesium
Na^{+}	sodium
NDF	neutral detergent fibre
NO_{3-}	nitrate
P	phosphorous
PLS	pure live seed
PO_{4-}	phosphate
RGR	relative growth rate
Total C	total carbon
Total N	total nitrogen
VA	*Vicia americana*

1. INTRODUCTION

Forage production in semi-arid environments, such as southwestern Saskatchewan, can be challenging due to the short growing season, limited and variable precipitation, and a high evapo-transpiration rate. Major droughts have occurred in this region relatively frequently, including historical drought in the 1930's and 1980's to recent droughts in 2001-2003 and 2017-2018 (Sauchyn and Skinner, 2001; Bonsal and Regier, 2007; AAFC, 2017). As a result, agricultural production has always been impacted by droughts in the southern Canadian prairies (Wheaton et al., 2016). The climate of this region is predicted to become more variable with respect to temperature and precipitation with a general warming trend and an overall increase in the frequency of extreme weather events such as drought (Cutforth et al., 2000; Akinremi et al., 2001; McGinn, 2010). Grazing in the southwest is restricted not only by climate, but also by low productivity and low nutritive value of forage stands in the fall and early winter (Schellenberg and Banjeree, 2012).

Planting a mixture of species can increase the productivity and quality of pastures over time through species complementarity, improving the seasonal distribution of yield and forage quality (Sleugh et al., 2000; McGraw et al., 2004; Cardinale et al., 2007). One method of increasing forage productivity is to incorporate plant species capable of producing high quality forage later in the grazing season. Native perennial legumes are of particular interest for forage production in the prairies because they have the potential to increase mineral balance (Yoshishara et al., 2013), soil nitrogen (Smoliak, 1988; Serajchi et al., 2015) and late-season forage digestibility in mixed grass stands (Mischkolz et al., 2013; Biligetu et al., 2014). In addition, diverse species mixtures with differing drought mechanisms can increase overall pasture productivity in dry environments (Tamyo-Chim and Reyes-Garcia, 2012). Incorporation of selected native species into forage stands may improve forage production in semi-arid environments during periods of drought (Mischkolz et al., 2013).

Native perennial legumes have been recognized as important companion crops when seeded in rangelands (Luscher et al., 2014), however there is a need to evaluate the productivity of a wider range of native legumes in mixtures with grasses (Scheaffer et al., 2009; Mischkolz et al., 2013; Muir et al., 2014). Most research to date has focused on single site studies using a limited number of native legume species, whereas little attention has been given to multiple species mixtures in semi-arid climates (Schellenberg et al., 2012). There is limited information available on the variability of native legumes in semi-arid environments for forage quality, productivity and drought tolerance, over time (Schellenberg et al., 2012). Biomass production of perennial mixtures and their effect on soil fertility are expected to be greatly influenced by growth environment, but many questions remain for native legume performance in mixtures (Mangan et al., 2011). Comprehensive research is not only needed for species selections and harvest management of native forage mixtures, but also optimal seeding rates and species ratios (Foster et al., 2014).

Identifying highly productive native legumes for mixed grass-forage stands in semi-arid regions will contribute to rangeland sustainability and increase the diversity of native perennial species available for forage establishment (Luscher et al., 2014). Work already conducted in southern Saskatchewan with *Dalea purpurea* (Jin et al., 2015) and *Astragalus flexuosus* (Schellenberg et al., 2014, unpublished data) have shown promise for inclusion of these species in forage production. New ecological varieties of *Hedysarum boreale* and *Vicia americana* have been developed because of increasing interest for native legumes, but they have not been evaluated in field trials in this region.

The objectives of this study were to determine the agronomic impact of including four perennial legumes in mixture with meadow bromegrass comparatively between the Brown and Dark Brown soil zones of Saskatchewan, to determine if differences exist between the selected native legume species in response to water limitation, and to determine the optimal seeding rate for direct seeding in spring. Determination of performance under drought conditions and optimal seeding rates for the selected native legumes will serve as a guide for their practical application in the Canadian prairies, where species-specific information is limited at this time.

2. LITERATURE REVIEW

2.1 Native Legume Species

2.1.1 Astragalus flexuosus

Flexile milkvetch, *Astragalus flexuosus* Douglas ex G. Don, is a native perennial legume adapted to dry sandy or clay soil and gravelly areas of the open prairies, roadsides and bluff edges (Budd, 1957; Van Bruggen, 1976; Moss, 1983; Stubbendieck and Conrad, 1989). Other common names for this species include pliant milkvetch and slender milkvetch. *A. flexuosus* flowers May to August and is mainly pollinated by native insects (Stubbendieck and Conrad, 1989; Mader et al., 2011). *A. flexuosus* is a diploid (2n=22) and can produce over 3000 seeds on a plant (Stevens, 1932; Spellenberg, 1976; Stubbendieck and Conrad, 1989). In addition to drought tolerance, this species is considered to be somewhat tolerant to saline conditions (Ajmal Khan and Weber, 2006).

Previously considered to have little, or no, forage value due to its woody stems (Williams, 1897), *A. flexuosus* has since shown fair forage value and is listed as an increaser in the plant community (Tannas, 2004; Alberta Agriculture and Forestry, 2016). Although *A. flexuosus* is rated as having low palatability, both livestock and wildlife graze this legume (Stubbendieck and Conrad, 1989). The root systems are resistant to moderately heavy trampling, but plants are unable to survive in abused or altered rangelands (Tannas, 2004). One study suggests *A. flexuosus* is favoured under moderate grazing (Patton and Nyren, 2015).

Astragalus species are known to contain nitrogen and selenium compounds which can be toxic to cattle (Williams and Parker, 1974; Williams, 1981). Three subspecies of *Astragalus flexuosus*; flexuosus, greenei and diehlii were found to contain over 25 mg NO_2/g of plant tissue (Williams and Barneby, 1977), therefore feeding trials are recommended to evaluate the potential toxicity if forage quantity and quality are shown to be promising.

2.1.2 Dalea purpurea

Purple prairie clover (*Dalea purpurea* vent. var purpurea), sometimes called violet prairie clover, violet dalea or purple parsela, is a perennial warm-season legume that inhabits dry to moderately moist habitats of the tall grass prairie, occurring most abundantly in open, upland prairies, plains, hillsides, sandy areas and woodlands (USDA, 2000; Stubbendieck et al., 2003; Reaume, 2009). *D. purpurea* is adapted to a wide range of soil types, but prefers dry and sandy soils (Stubbendieck et al., 2003; Saskatchewan Forage Council, 2007). This species flowers June to August in the Canadian prairies, and is considered to be a highly palatable decreaser in the native plant community (Stubbendieck and Conrad, 1989; USDA, 2000; Reaume, 2009). The reproduction of this plant is largely dependent on insect pollinators, although outcrossing is not a requirement for reproduction (Cane et al., 2012). *D. purpurea* is a diploid (2n=14) which can self-pollinate, however seed production doubles as a result of cross-fertilization in similar *Dalea* species, resulting in larger, plumper seeds (Stubbendieck and Conrad, 1989; Cane, 2006; Cane et al., 2012). Native bees are critical for pollination and therefore the sexual reproduction of this genus (Cane, 2006).

D. purpurea grows in small clumps with taproots up to 30cm long and produces excellent forage that is palatable and high in protein (Stubbendieck and Conrad, 1989; Raume, 2009). This legume is fairly resistant to heavy grazing, trampling and drought, but it will disappear in altered rangelands that are overgrazed (Stubbendieck and Conrad, 1989; Tannas, 2004). Studies comparing the establishment and yield of native perennial legumes found *D. purpurea* to have lower establishment, biomass yield, and seedling vigour compared to other species (Fischbach et al., 2006), but seed scarification and stratification can enhance germination (Stubbendieck and Conrad, 1989; Schellenberg and Biligetu, 2015).

Studies have shown that *D. purpurea* contains condensed tannins and can reduce the amount of *E. coli* shed by ruminants without degrading digestibility (Jin et al., 2012; Huang et al., 2015; Jin et al., 2015). This legume offers high protein (Stubbendieck and Conrad, 1989) and has higher nutritive value than alfalfa (*Medicago sativa*) (Peng et al., 2016) and sanfoin (*Onobrychis viciifolia*), therefore would be beneficial inclusion in forage pastures (Iwaasa et al., 2013). Mixtures containing *D. purpurea* have been used for re-vegetation, reclamation, and ornamental purposes, therefore seed has been commercially available since the 1980's (Stubbendieck and Conrad, 1989). Selection for specific traits such as height, timing of flowering, and lodging can be accomplished in this species without modifying other traits, making it a good candidate for forage breeding (Lindgren, 1992).

2.1.3 Hedysarum boreale

Northern sweetvetch (*Hedysarum boreale* Nutt.), is a cool season native perennial legume adapted to plains, open mountain slopes, woodlands and bluffs (Budd, 1957; Moss, 1983; Pahl and Smreciu, 1999). Although *Hedysarum* species occur in the Great Plains, they are considered uncommon due to their scattered distributions (Stubbendieck and Conrad, 1989). *Hedysarum boreale* is also called by the common names of Utah sweetvetch, boreal sweet-vetch, or plains sweet-broom. This diploid (2n=16) species is attractive to bees, similar to many native legumes, is an outcrossing species dependant on pollinators for sexual reproduction (Moss, 1983; Tepedino, 1987). Although it is able to self-pollinate, outcrossing benefits fruit development and maturation (Swoboda and Cane, 2012). *H. boreale* var. boreale has different flower morphologies and occurs in different ranges from var. alpinum (Tependino, 1987).

Hedysarum boreale can germinate under wide ranges of temperature and light, and when temperatures are high enough, can germinate in water limiting conditions (Redente, 1982). Strong taproots allow this legume to deeply penetrate the soil (Kitchen, 1992) and remain moderately drought tolerant in the early stages of growth (Redente, 1982). Due to a hard seed coat, which prevents imbibition, seeds require scarification before seeding into the field (Redente, 1982). This species grows best in the absence of competition and should be seeded in alternate rows with other species (Rosales, 2014).

Many of the *Hedysarum* species that are native to western North America are valuable for cattle forage and *Hedysarum boreale* in particular has been extensively cultivated in the United States, particularly in Utah (Mader et al., 2011; Swoboda and Cane, 2012). *H. boreale* is considered moderately nutritious throughout most of the grazing season for both livestock and wildlife, and the genus is listed as an increaser species (Pahl and Smreciu, 1999; Tannas, 2004; Swoboda and Cane, 2012). A study in Utah found this species can produce large amounts of forage in the early spring but has no value in the fall and winter (Johnson et al., 1989). This plant is able to withstand moderately heavy use and has a high reproductive capacity, therefore is a favorable species in abused and altered rangelands (Tannas, 2004).

Seed cost and availability are major limitations of utilizing this species for forage and rangeland revegetation (Redente, 1982; Bushman et al., 2007). Seed production could be increased by supplying pollinators to increase seed yield in production fields and by using a species-specific growth-staging system (Tepedino, 1987; Peel et al., 2011; Swoboda and Cane, 2012). The use of *H. boreale* for reseeding rangelands and reclaiming land has been limited because of the lack of information about the species and limited seed availability (Redente, 1982). Although *H. boreale* forms associations with native *Rhizobium* bacteria, in semi-arid environments receiving low amounts of summer precipitation, the root nodules are shed and there is no net addition of nitrogen to the soil (Carpenter and Allen, 1987; Johnson et al., 1989).

2.1.4 Vicia americana

American vetch (*Vicia americana* Muhl. ex Willd), sometimes called purple vetch, American deer vetch or stiff-leaf vetch, is a native perennial legume common in a variety of habitats including moist to dry, shady parts of upland prairies and badlands, open woods, thickets, meadows, shores and along roadsides (Budd, 1957; Van Bruggen, 1976; Moss, 1983; Stubbendieck and Conrad, 1989; Kenicer, 2008; Reaume, 2009). This species has a large native range spanning nearly all of North America and displays a huge diversity in form (Kenicer, 2008; Kennicer and Norton, 2008). The Canadian range spans from British Columbia east to New Brunswick and includes fescue grasslands and forested areas (PRFA, ND). In general, *Vicia americana* has a low, vine-like growth pattern and uses tendrils to climb onto other plants (Reaume, 2009). The variety *V. americana* var. minor Hooker however is an erect, tendril less variety that typically inhabits the drier prairies (Kennicer and Norton, 2008). Both diploid (2x) and tetraploid (4x) forms of this species exist with n=7 chromosomes (Hanelt and Mettin, 1989; Stubbendieck and Conrad, 1989; Kenicer and Norton, 2003).

Overall, *V. americana* is considered drought tolerant because of a branched taproot (Allen and Tilley, 2004; Reaume, 2009). This native legume flowers from May to August and is palatable throughout the season with high nutritional levels, averaging 20% protein content and low crude fiber (Looman, 1983; Stubbendieck and Conrad, 1989; Tannas, 2004). *V. americana* has good to excellent forage value but it disappears from rangelands under heavy use because of poor tolerance to grazing and trampling (Looman, 1983; Stubbendieck and Conrad, 1989; Tannas, 2004). However, a study of grazing intensity in Missouri suggested *V. americana* to be favoured on heavily grazed sites (Patton and Nyren, 2015). Another study in Montana found it to be unutilized by wild ungulates, but somewhat grazed by cattle (Kasworm et al., 1984). Biomass production was estimated at 49-61 kg ha^{-1} for two growing seasons in southwestern Utah (Ruyle and Bowns, 1985). Although *V. americana* is low yielding, it can provide valuable forage for wildlife and cattle and can be used as a nitrogen-fixing cover crop (PRFA, ND).

2.1.5 Bromus riparius

Meadow bromegrass (*Bromus riparius* Rhem var. Armada), is a tame grass with short rhizomes that can regrow rapidly following defoliation and is used for high intensity grazing over short periods in the cooler, moist areas of the Dark Brown and Brown soil zones of western

Canada (Knowles et al., 1993). This grass is native to Europe, Turkey and central Asia, and was introduced to North America in 1966 when a Turkish variety, Regar, was registered in the United States (Knowles et al., 1993). The same variety was later registered in Canada in 1980 and has since been widely used for pastures and hay in Alberta and Saskatchewan (Knowles et al., 1993). Armada meadow brome was registered in Canada in 2008 resulting from combined selections from the Regar, Paddock and Fleet varieties (Tremblay, 2012). *B. riparius* was chosen for this study because of its popularity in Western Canada, its compatibility with legumes such as alfalfa, cicer milkvetch (*Astragalus cicer*), bird's-foot trefoil (*Lotus corniculatus*), and sainfoin, and because it can be grazed until well into October (Knowles et al., 1993; Ogle et al., 2003; St. John et al., 2012).

Bromus riparius can grow in drylands with enough precipitation but prefers moist conditions (St. John et al., 2012). This grass has dense and fibrous roots that help prevent soil erosion (St. John et al., 2012). *B. riparius* is considered to be excellent forage for wildlife and cattle, and can produce high quality forage in late summer and fall (Knowles et al., 1993; St. John et al., 2012). This grass is selectively grazed by cattle, and remains palatable into seed set (Knowles et al., 1993). *B. riparius* can be used complementarily in mixtures, however can compete with alfalfa and other legume species, especially when moisture is abundant (Knowles et al., 1993). Alternate grass-legume seeding rows are recommended for *B. riparius* and legume mixtures (St. John et al., 2012).

2.2 Native Legumes for Forage Production

Seeding native plants in the drier region of western Canada can provide relatively stable and sustainable forage production (Schellenberg et al., 2012; Serajchi et al., 2015). Since not all native plants have characteristics that make them suitable for forage production, screening plant species potential must include an investigation of the quality in order to obtain the desired response in fed animals (Collins and Newman, 2018). Included in this assessment should be an analysis of nutritive value, potential intake and anti-quality factors, since these factors do not occur in isolation and are either positively or negatively correlated with the others (Collin and Newman, 2018). To maximize the benefits of native legumes in rangelands, both the qualitative and quantitative variations between species should be determined (Muir et al., 2019).

Introduced forage species can be competitive and may have a negative impact on the biodiversity of Canadian grassland ecosystems (Schellenberg et al., 2012). Native legumes, on the other hand, increase the plant diversity, providing benefits for wildlife and sustainable forage production (McGraw et al., 2004; Serajchi et al., 2015). The complementary effects of multiple species outweighs the importance of individual species respective productivities (Cardinale et al., 2007). High numbers of species can also help to maintain ecosystem processes in environments undergoing changes (Loreau et al, 2001). Conservation, in addition to production, and the likelihood of success once planted, are often considered by those using native seed for rangelands (Bushman et al., 2007).

Given all the benefits of including native perennial legumes in mixed forage stands, it is surprising that their utilization in the Canadian prairies remains low. The main reasons are limited access to a variety of native legume species tested for their forage value, difficulty assessing the economic cost and benefits with this lack of information, and low availability of seed. This problem is also occurring in the United States, where the use of native legume seed for forage, wildlife and grassland reclamation also remains limited due to low production and lack of available seeds (Muir et al., 2018).

2.2.1 Establishment of Native Plants

Seedling recruitment in field conditions is a combination of germination, seedling survival and seedling growth (Eriksson and Ehrlen, 2008). Under variable conditions such as moisture stress and high competition, rapid germination is the most critical factor for successful legume establishment, even though it is still not well understood (Baskin, 2003; McGraw, 2003). Although forage species with small seeds germinate well under most temperatures, they are the most difficult to establish in field conditions (Townsend and McGinnies, 1972). Where multiple species are planted together, large-seeded species have a competitive advantage over species with smaller seeds (Turnbull et al., 1999). Not including emerged seedlings, small-seeded grass species lose more seeds from the seed bank compared to larger-seeded species (Russi et al., 1992).

Despite being viable, it is common that seeds don’t imbibe water and fail to germinate in favourable conditions due to physical exogenous dormancy such as hard seed coats and other conditions such as temperature striation (Rolston, 1978). Under field conditions the breakdown of hard seeds is depth dependant, with higher germination rates occurring at the soil surface (Russi et al., 1992). Hard seeds are an ecologically significant mechanism, because they occur in species which are able to rapidly germinate after fire or allow the seeds to survive when ingested by animals (Rolston, 1978). Scarification has been found to successfully reduce physical dormancy in some native legumes and can increase the germination of hard seeded native legumes by approximately 20% (Schellenberg and Biligetu, 2015; Jones et al., 2016). Scarification can damage the seed of some species depending on the duration of abrasion (Dittus and Muir, 2010).

Determining the appropriate seeding rates for native species is challenging. Pure live seeds per metre (PLS m^{-1}) is used instead of seeds per metre to consider the purity and percent germination of various seed lots and to achieve consistent seeding rates (Houck, 2009). However, seed lot quality tests do not provide enough information to predict seeding rates and establishment for native species (Vogel, 2002). Although viable seeds are capable of germinating when conditions are suitable, dormancy must be broken to use germination as a measure of viability (Bradbeer, 1988). An objective way to measure the success of a seeding rate is to measure plant population densities 2 years post seeding (Burton et al., 2006). The quality tests of native seeds should relate the amount of seed to the density of established seedlings (Vogel, 2002).

There appears to be a wide range of suggested forage legume seeding rates with little species-specific information for native legume species. Government recommendations for forage legume seeding rates range from 40 to 150 PLS m^{-1} depending on the species (Alberta Agriculture and Food, 2018). Seeding rates for forage crops of 50-67 PLS m^{-1} in southwestern Saskatchewan row have been recommended (Leyshon et al., 1981). Comparatively, studies in the upper Midwestern US suggest 12-16 PLS m^{-1} (Fischbach et al., 2006). This variation makes economic analysis of incorporating these native species into mixtures difficult. The optimal seeding rates are likely species, soil type and climate specific, and should be evaluated for species of interest at particular locations.

Competition between species in mixtures can increase the yield of one species while proportionately decreasing the yield in another species, therefore the yields of most binary mixtures are between the yield of either component when grown in monoculture (Springer et al., 2001). There are physiological and morphological differences between legumes and grasses affecting their competitiveness which are difficult to separate because plants compete not only with other species but also with the same species (Haynes, 1980). When grown in polyculture pots, legumes have more competition from themselves than from grasses, likely because grasses take longer to recover after harvesting (Faris et al., 1986). The vigour and yield of individual species within a pasture has an effect on the overall plant composition (Haynes, 1980). Where nutrients are not limiting, highly competitive plants allocate a high percentage of biomass to the roots and can exploit large volumes of soil (Aerts et al., 1991). Competition is, however, site specific and changes with resource availability (Grimes, 1979). Suggested seedling densities are higher in the Dark Brown compared to the Brown soil zone for both monoculture legume and grass-legume mixtures in western Canada (Alberta Agriculture and Food, 2018).

2.2.2 Forage Quality

Forage quality is the potential to produce a desired response in animal performance, whereas dry matter yield is a measure of the quantity of forage produced (Ball et al., 2001; Collins et al., 2018). Quality and yield are related because highly digestible, less fibrous forages will be consumed in greater amounts (White and Wight, 1984; Robinson, 1998; Collins and Newman, 2018). Dry matter yield rather than fresh weight is used to determine a per weight basis for hay, which allows for standard testing and feed ration control (Fuez et al., 2012). Voluntary intake, digestibility and crude protein levels decrease as plants mature throughout the growing season (Collins et al., 2018; Rayburn, 2018). The concentration of fiber increases in plant stems while the ratio of leaf to stem decreases which makes digestion by ruminants more difficult (Buxton and Redfearn, 1997). Legume grass mixtures can yield greater dry matter than monoculture legumes and can have significant variation in yield, energy and protein levels of the forage produced (Cabellero et al., 1995; Simili da Silva et al., 2014).

Plant fibre consisting of cellulose, hemicellulose and lignin is negatively correlated to the amount of energy provided (Stokes and Prostko, 1998). The fibre can be separated from starch, proteins and sugars using detergent analysis and further separated into neutral detergent fibre (NDF) and acid detergent fibre (ADF) components to analyze the quality of feed (Stokes and Prostko, 1998). Crude protein is also considered when analyzing feed quality because it is a significant component of the nutrients in forage, calculated as the percent nitrogen in a sample, multiplied by a factor of 6.25 (Stokes and Prostko, 1998; Schroeder, 2012). Crude protein, similar to digestibility, is higher at early vegetative stages compared to later (Cook, 1972; Stokes and Prostko, 1998). Proteins contribute energy and amino acids to the animal and rumen microbes but are not considered to be more important than overall energy value and digestibility of forage (Robinson, 1998). Native legumes can improve the quality of a grass forage by increasing crude protein while lowering neutral detergent fibre (Posler et al., 1993; McGraw et al., 2004).

Acid detergent fibre (ADF) is composed of lignin and cellulose and is partly digestible (Robinson, 1998; Stokes and Prostko, 1998). ADF is strongly negatively correlated with the digestibility of forage, therefore low levels are desirable for digestion (Robinson, 1998; Stokes and Prostko, 1998). Comparatively, neutral detergent fibre (NDF) is all of the fibrous portion of the plant which is composed of lignin and cellulose, or the ADF, and the hemicellulose which makes up 20 - 30% of the plant cell wall (Robinson, 1998; Holtzapple, 2003; Schroder, 2012). NDF is partly digestible and negatively correlated with voluntary intake and milk production (Stokes and Prostko, 1998). As plants mature, the ratio of stems to leaves increases, increasing fiber levels in feed increase while decreasing digestibility (Stokes and Prostko, 1998; Collins and Newman, 2018). The rate of this decrease is species specific, generally with greater decreases in the digestibility of grasses compared to legumes (Stokes and Prostko, 1998; Collins and Newman, 2018).

Generally, legumes have higher crude protein levels and lower NDF compared to grasses (Government of Manitoba, 2003; Collins et al., 2018). Therefore, planting native legumes or inclusion of them in mixture can improve the quality of forage by increasing crude protein and lowering neutral detergent fibre compared to monoculture grass stands (Faris et al., 1986; Posler et al., 1993; McGraw et al., 2004; Collins et al., 2018). Plant genetics and environmental factors such as temperature and latitude also influence the NDF content of plants (Grimes, 1979; Government of Manitoba, 2003).

While assessing the overall quality of forage produced, mineral content should also be considered (Schroder, 2012). Phosphorous, calcium and magnesium are important minerals for cattle and are affected by complex relationships between soils and plants (Littledike and Goff, 1987; NRC, 2000). Deficiencies of phosphorous are common in ruminants grazing poor pasturelands and may result in poor animal growth (Goff, 2009). Milk fever, a metabolic disease which occurs predominantly in lactating cattle, can result from feed that is low in calcium, magnesium, and or, phosphorous (Boda and Cole, 1954; DeGaris and Lean, 2009). Feed that is high in sodium and potassium can also cause milk fever (Ender et al., 1971). Lactating cows in early spring have a greater demand for calcium and magnesium and can develop grass tetany and wheat pasture poisoning from grazing rapidly growing grasses which are low in these nutrients (Griffith, 1974; Grunes and Welch, 1989). One way to prevent tetany is to grow legumes in mixtures with grasses in spring grazed pastures (Griffith, 1974). Calcium, magnesium, phosphorous and potassium continue to decline from before flower budding to after plant maturity (Griffith, 1974). Maintaining diverse mixtures of wild plant species in pastures can reduce mineral deficiencies and lower the risk of toxicities since mineral concentrations are highly variable between plant species (Yoshihara et al., 2013).

2.2.3 Use in Forage Mixtures

The addition of alfalfa to grass forage stands can increase the dry matter yield and maintain the nutritional requirements of lactating cows (Kopp et al., 2003). However, there has currently been limited research conducted on the attributes and production potential of native legumes for use as livestock forage and native seeded mixtures (McGraw et al., 2004; Schellenberg et al., 2012; Mischkolz et al., 2013; Muir et al., 2014;). Studies have shown that mixtures of native species can produce higher forage yield than monocultures, yet many native legumes remain unstudied in the Canadian prairies for forage production (Serajchi et al., 2018). Determining highly productive legume grass mixtures is not as simple as just mixing as many species together as possible (Sanderson et al., 2006).

The composition and attributes of species in mixtures have been shown to be more important than the diversity of seed mixtures for nitrogen cycling and biomass production (Hammermeister et al., 2003). A study of native monoculture and polyculture establishment found mixtures with one to five species were most productive for two years after establishment compared to higher diversity mixtures (Mangan et al., 2011). There are numerous combinations of possible legume-grass seeding mixtures but there is limited information specifically on native prairie plants seeded in such mixtures (Frame, 2005; Schellenberg et al., 2012).

Studies have shown that legumes grown in pure stands are highly susceptible to weed problems, whereas mixtures with grass tend to be more competitive against weed establishment (Frame, 2005). Not only can mixtures decrease pasture weeds, studies have found the inclusion of legumes can increase forage quality compared to pure grass stands (Sanderson et al., 2006). The performance of grass-legume mixtures in terms of nutrition and yield has been attributed to the characteristics of the legume species included rather than the harvest methods (Gierus et al., 2012). The competitive nature of legume-grass mixtures is species-specific. Planting mixed grass-legume stands has been shown to reduce bloat in grazing cattle caused by the foamy gas arising from the high protein content of fast-growing forage legumes (Frame, 2005).

2.3 Drought Tolerance of Native Plants

Temperate grasslands experience both periods of drought and flood, but have become drier overall in the 20th century (Johnson et al., 2005). Some projections suggest that southwest Saskatchewan will experience drier conditions if global warming continues (Cutforth, 2000), while others refute this theory and suggest that the Canadian prairies are in fact not getting drier, but rather experiencing changes in rainfall trends (Akinremi et al., 2000). Planting more diverse species mixtures might be important for maintaining ecosystem processes and for long term carbon storage under changing environmental conditions (Loreau et al., 2001; Jungers et al., 2016).

Native legumes are suitable to a variety of climatic conditions and can maintain forage quality even under drought stress (Peterson et al., 1992; Serajchi et al., 2017). That being said, native plant populations differ genetically and phenotypically so seeds should be sourced from a similar geographic area as the intended seeding location, or chose plants with high genetic diversity which have the ability to adapt to a range of conditions (Jump et al., 2009; Bhattarai et al., 2010). Under plentiful water conditions, native legumes are able to produce more biomass and nitrogen fixation is promoted, whereas under drought stress they lose less biomass compared to non-legumes (Lauenroth and Dodd, 1979; Hofer et al., 2016). A study of sites in Sweden and Ireland showed drought-stressed legume-grass mixtures yielded similarly to monocultures grass plots under normal moisture conditions (Hofer et al., 2016).

Drought reduces plant growth and development at all life stages (Farooq et al., 2009). Although native legumes can maintain forage quality under drought stress, when seeded into mixed stands, legumes tend to decline over time due to drought, overgrazing and winter kill (Schellenberg et al., 1993). In drought conditions, legume production was found to be more affected by the soil medium compared to regional and climatic factors (Peterson et al., 1992; Daryanto et al., 2015). Like all plant species, legume biomass generally declines with drought stress but the severity appears to differ by species (Komainda et al., 2019). Phenotypic plasticity allows some plants to tolerate periods of drought by altering physical and biochemical processes (Farooq et al., 2009). Perennial legumes have a wide range of physiological and morphological drought coping strategies which can be beneficial in drought prone agricultural areas (Pang et al., 2011).

Plants can regulate hydraulic conductance in their leaves, stems and roots, particularly important for drought tolerance during critical growth stages (Vadez, 2014). Legumes react to drought by narrowing xylem cells in the roots to restrict water and slow growth during critical stages (Purushothaman et al., 2013; Vadez, 2014). The extent to which drought affects legume yield is species specific and depends on the phenological state of the plant at the time of water limitation (Daryanto et al., 2015). Shoot to root ratios for perennial forage crops can vary significantly by region and by climate in a single region (Bolinder et al., 2002). Legume species with faster relative growth rates (RGR's) have both morphological and physiological root adaptations, whereas those with slower RGR's have only morphological adaptations (Suriyagoda et al., 2012).

Drought stress can increase biomass allocation to roots and rooting depth, negatively affect nodulation, decrease shoot biomass and increase root biomass (Furlan et al., 2012; Skinner and Comas, 2018). Cool season legumes react with higher yield increases per volume of water when compared to warm season legumes (Blessing et al., 2018). The effects of drought on root biomass and depth may increase as the number of species in forage mixtures increases (Skinner and Comas, 2018). Drought resistant species typically have deep, vigourous roots, better shoot growth and greater mobilization of photosynthates compared to less resistant phenotypes (Polania et al., 2017).

2.4 Soil Fertility Improvement

Legumes can help to maintain soil nutrient levels by increasing the amount of nitrogen in pasture soils without the use of chemical applications (Serajchi et al., 2015). The soil is enriched with nitrogen through plant-bacterial association with rhizobia which infect the root hairs and fix atmospheric di-nitrogen into biologically available ammonium which can be utilized by plants (Frame, 2005). This symbiotic relationship allows legumes to increase the amount of nitrogen in the soil without addition of chemical applications to the system (Serajchi et al., 2018). *Medicago sativa* (Alfalfa) and *Lotus corniculatus* (birdsfoot trefoil) seeded at 30% with meadow bromegrass have been shown to be as profitable as nitrogen fertilized meadow bromegrass alone (Adjesiwor et al., 2017). A study in Europe showed increased biomass and nitrogen fixation with the addition of legumes to grasslands that varied by study site (Spehn et al., 2002). Since legumes have less nitrogen limitation compared to grasses, they also have a greater resistance to drought (Hofer et al., 2016). The amount of nitrogen the legume species in this study are capable of fixing in association with soil bacterium is unknown.

Nitrogen fertilization is considered economical when there is less than 25 to 30 percent legumes in mixed grass stands, increasing nitrogen at higher legume percentages increases the competitiveness of the grass and reduces the percentage of legumes (Beegle, 2014). Nitrogen is not the only soil nutrient affecting fertility, soil potassium and phosphorous are also important for the yield, quality and stand longevity of legume production stands (Griffith, 1974). Micronutrients such as manganese, copper, iron and cobalt typically are not major influences on the productivity of legume stands (Griffith, 1974).

3. EVALUATION OF FOUR NATIVE LEGUMES FOR FORAGE PRODUCTION IN THE BROWN AND DARK BROWN SOIL ZONES OF SASKATCHEWAN

3.1 Abstract

Forage production in semi-arid environments is limited by variable and low amounts of precipitation throughout the growing season. Native legumes can produce forage throughout the growing season in variable climatic conditions and can potentially increase forage quality and soil nutrients in mixed legume-grass stands. We examined the forage potential of four perennial legume species native to the prairie ecoregion in western Canada, both in monoculture and in mixture with *Bromus riparius* at Saskatoon and Swift Current, SK using four replicated Randomized Complete Block Designs at each site. Foliar cover, dry matter yield, botanical composition of mixed stands, forage quality and soil nutrients were measured in 2017 and 2018. Of the four legume species, *Astragalus flexuosus* showed the greatest potential for establishment and produced the greatest dry matter yield in monoculture at Saskatoon (2934 kg ha^{-1}, 3107 kg ha^{-1}) and Swift Current (3938 kg ha^{-1}, 3920 kg ha^{-1}) in July 2018 and August 2018, respectively. *Hedysarum boreale* and *Dalea purpurea* did not contribute significantly to the biomass in mixture plots at Swift Current where *B. riparius*/ *D. purpurea*/ *H. boreale* mixture contained 0% legumes and 100% *B. riparius*. The native legume monocultures had higher crude protein concentrations (P<.0001) compared to both the mixtures and the *B. riparius* plots throughout the growing season, and had lower levels of ADF (P<.0001) and NDF (P<.0001) in the later summer compared to the grass plots and mixtures. The results show inclusion of these native legumes in mixtures at 33% each of the seeding ratio did not increase forage yield or quality beyond *B. riparius* monocultures over a two year period.

3.2 Introduction

Proxy records suggest cycles of century scale droughts in southwestern Saskatchewan, as well as shorter scale droughts occurring in 20-25 year cycles (Vance et al., 1992; Sauchyn and Skinner, 2001). In addition, the temperature of the Canadian prairies has been getting warmer (McGinn, 2010). Although analysis of past rainfall trends suggest that the prairies are not getting drier (Akinremi et al., 2000), temperature and rainfall are negatively correlated (Cutforth, 2000). As a result, rising temperatures may change both the frequency and amount of precipitation in this region, with increases in both long- and short-term drought events predicted

(PaiMazunder et al., 2013). Breeding for perennial forage species in response to climate change is difficult and should be assessed at multiple sites over multiple years to make informed selections (Mitchell et al., 2015).

In changing environments, a larger number of species is needed to maintain steady ecosystem processes (Loreau et al., 2001). Native grassland species are more resilient to drought than introduced species since they evolved under the cyclical temperature and precipitation regimes characteristic of the environment, however they tend to yield less than introduced forages (Smoliak, 1988; Hofer et al., 2016). Although native legumes in mixed forage stands can decline because of overgrazing, winterkill and drought, they possess a wide range of drought coping strategies which can be further enhanced through genetic improvement (Schellenberg et al., 1994; Pang et al., 2011).

Using a variety of species in mixtures increases species complementary and is more productive than the effectiveness of each species in isolation (Cardinale et al., 2007). Native legumes provide environmental services to the ecosystem such as carbon sequestration, increased nitrogen contributions and improved nutrient and hydrological cycling (Sphen et al., 2002; Mahli et al., 2003; Muir et al., 2019). Increasing the diversity of plant species can also decrease the presence of weedy species and increase forage yields (Smoliak, 1988; Saskatchewan Forage Council, 2007).

In addition to increased environmental adaptation and ecosystem services, native legumes can increase not only the yield but also the quality of forage produced in mixed stands particularly later in the season when quality is typically reduced (Sleugh et al., 2000; Gierus et al., 2011; Mischkolz et al., 2013; Simili da Silva et al., 2013; Biligetu et al., 2014;). Therefore, forage mixtures containing native species help to provide relatively stable forage in semi-arid environments and produce higher yields relative to monoculture grass stands (Schellenberg et al., 2012; Serajchi et al., 2018). Inclusion of legumes in pasture mixtures can help to sequester carbon and nitrogen into the soil which is important in the face of climate change (Mahli et al., 2003).

The composition of species in mixtures is more important than the diversity of the seed mix because individual species attributes determine biomass production and nitrogen cycling

(Hammermeister et al., 2003). In fact, low diversity mixtures with one to five species have the best establishment and forage yield (Mangan et al., 2011). The forage yield of multiple species mixtures is more related to the dominant species when they are seeded in equal proportions (Sanderson et al., 2013). Depending on the species mixtures and characteristics of each component, there is significant variation in the yield and digestibility of the forage produced (Simili da Silva et al., 2014). The yield of mixtures can be between the yields of the mixture components, or may be higher or lower than component species in monocultures, due to variation in species complementary (Springer et al., 2001; Beckage and Gross, 2006). Mixtures of timothy (*Phleum pratense*), smooth bromegrass (*Bromus inermis),* red clover (*Trifolium pratense*) and white clover (*Trifolium repens*), seeded in Northern Europe and Canada, showed strong enough diversity effects to increase yield greater than monocultures over a three year period (Sturludottis, 2011).

The inclusion of native legumes therefore may not increase the quantity of forage produced, but the effect on quality should also be considered for selection in mixtures (White and Wight, 1984). Forage quality is positively correlated to voluntary intake by cattle, therefore digestibility and nutritive value should be assessed for each native legume species (Scheaffer et al., 2009; Collins and Newman, 2018). Overall, native legumes should improve the forage quality of mixed stands because they contain higher crude protein and lower NDF compared to grasses, but this depends on species and season (Posler et al., 1993; McGraw, 2004).

Native legumes can improve soil fertility by forming root associations with nitrogen fixing bacteria in the soil (Frame, 2005; Serajchi et al., 2015; Muir et al., 2019). This increase of biologically available nitrogen can increase plant yields (Sphen et al., 2002). Drought however, negatively affects root nodules and decreases the amount of nitrogen fixation that is occurring in the soil (Lauenroth and Dodd, 1979; Furlan et al., 2012). Higher diversities of plant species in arid regions can also improve the mineral balance of feed which reduces the need for mineral supplements (Goff, 2009; Yoshihara et al., 2013). Native plants can not only reduce mineral deficiencies, but also reduce toxicities by increasing the balance of nutrients in grazing animals (Yoshihara et al., 2013). Given all the potential benefits of including native perennial legumes into forage mixtures, there is a need to evaluate a wide range of potential species for use in the Canadian prairies in the future.

The establishment time of native legumes is longer than that of high yielding grasses like meadow bromegrass which creates a competitive environment for the legumes species (Cooper 1977; Hamel et al., 2008). Competition also exists between different legume species in the same mixture because of different RGR's which affect survival of each species and eventually the vegetative composition of mixed stands (Cooper, 1977). Although some consider counting individual plants to be the only reliable measure of plant establishment (Vogel and Masters, 2001), this method becomes difficult to impossible where morphological characteristics such as rhizomatous growth make determination of individual plants difficult.

We hypothesize that native legume-grass mixtures will perform differently in the Dark Brown compared to the Brown soil zone. Secondly, forage nutritive value of native legume-grass mixture will be greater than monoculture grasses due to increased crude protein contributed by native legumes, and this will be evident in the later growing season. Third, soil nitrogen levels will be greater in native legume-grass plots compared to grass only plots because of N fixation of native legumes.

The main objective of this study is to characterize the agronomic impact of native perennial legumes in mixture with *B. riparius* in the Brown and Dark Brown soil zones of Saskatchewan. This study will measure stand establishment, botanical composition, forage yield and nutritive value, soil fertility and nutrient analysis over two growing seasons at two sites Swift Current and Saskatoon, SK to determine the establishment and production potential of four native Canadian perennial legumes. This study investigates the forage potential of four native Canadian perennial legumes to assess their potential for use in mixed legume grass forage stands. Determination of species-specific seeding rates may be important for reclamation and restoration of these, and similar, native legume species.

3.3 Materials and Methods

3.3.1 Plant Material

Dalea purpurea Vent. was purchased from Pickseed (Calmar, AB), and *Vicia americana* Muhl. ex Willd was obtained from Brett Young Seed (Winnipeg, MB). *Hedysarum boreale* Nutt. was purchased from Applewood Seed Co. via Brett Young Seed. Certified seed of 'Armada' *Bromus riparius* Rehmann was obtained from the University of Saskatchewan, and

Astragalus flexuosus Douglas ex G. Don seeds were provided by the Agriculture and Agri-Food Canada Swift Current Research and Development Centre. Germination rates were 91%, 94%, 75%, 38% and 76%, for *B. riparius*, *D. purpurea*, *V. americana*, *H. boreale* and *A. flexuosus* respectively (Table 3.1). The germination factor was a correction of seed weight based on percent germination or percent viability that was used to obtain consistency in the amount of pure live seeds per metre across the species.

Table 3.1 List of species used in the experiment with respective variety, percent germination, percent viability, germination factor, and 1000 seed weight that was used for the seeding calculations.

Species	Variety	Germination (%)	Viability (%)	Germination Factor*	1000 Seed Weight (g)[††]
Bromus riparius	Armada	91	----[†]	1.09	0.05
Dalea purpurea	Subsp. Purpurea	94	----	1	0.009
Vicia americana	Subsp. americana	75	91	1.25	0.2
Astragalus flexuosus	flexuosus	76	----	1.24	0.009
Hedysarum boreale	boreale	34	89	1.62	0.09

[†] ---- Data are not available

[††] Mean 1000 seed weight where n=4.

* Germination factor calculated as (100% ÷ % germination) or (100% ÷ % viability)

The percent viability tests were provided by the seed suppliers where available for this study, whereas germination tests were conducted by the authors after the seeds were obtained. Viability, defined as the percentage of living embryos capable of germinating, was determined by certified seed testing laboratories but detailed analytical methods were not provided with the seed purchases. Tetrazolium tests are commonly used to determine whether embryos are alive by producing a red colour in living cells. Percent viability was used rather than percent germination for *V. americana* and *H. boreale* due to lower than expected rates of germination in the lab.

3.3.2 Experimental Design and Site Descriptions

The experiment was designed as a factorial, Randomized Complete Block Design (RCBD) with four replications of 22 treatment combinations of harvest date and species mixtures/monoculture stands. The experimental factors were harvest date (July and August), and species mixtures/monoculture stands (Table 3.2). Two legume species were used in each legume:grass mixture because previous studies have indicated intraspecific competition can be greater than interspecific competition and planting multiple species can have complementary over-yielding effects (Suter et al., 2007; Nyfeler et al., 2009).

The southern study site is located in the Swift Current Plateau landscape area of the Mixed Grassland ecoregion at Swift Current, SK. The Mixed Grassland ecoregion is located in the southern, semi-arid, region of the Prairie ecozone in the Brown soil zone (Acton et al., 1998). Vegetation in this region is characterized by mid and short-grasses with aspen rarely occurring in wetlands in this ecoregion (Acton et al., 1998). This plateau area is composed of Brown, loam soils and wind-blown silt which are low in organic matter and nitrogen, and underlain with limey subsoils (Acton et al., 1998; Smoliak, 1988). The more northern study site in Saskatoon, SK is located in the Saskatoon Plain landscape area of the Moist-Mixed Grassland ecoregion. The

Table 3.2 The 11 species mixtures/monoculture stands used in the experiment.

Mixture	Percent Mix	Species	Abbreviation
1	100%	*Bromus riparius*	BR
2	100%	*Astragalus flexuosus*	AF
3	100%	*Dalea purpurea*	DP
4	100%	*Hedysarum boreale*	HB
5	100%	*Vicia americana*	VA
6	33% : 33% : 33%	*B. riparius/D. purpurea/V. americana*	BR/DP/VA
7	33% : 33% : 33%	*B. riparius/D. purpurea/H. boreaele*	BR/DP/HB
8	33% : 33% : 33%	*B. riparius/D. purpurea/A. flexuosus*	BR/DP/AF
9	33% : 33% : 33%	*B. riparius/V. americana/H. boreale*	BR/VA/HB
10	33% : 33% : 33%	*B. riparius/V. americana/A. flexuosus*	BR/VA/AF
11	33% : 33% : 33%	*B. riparius/H. boreale/A. flexuosus*	BR/HB/AF

soils in this area are typical of the glacio-lucastrine landscapes of the Dark Brown soil zone with a thicker and more nutrient rich surface layer compared to the Brown soil zone (Acton et al., 1998; Smoliak, 1988).

Experimental plots were seeded on May 16, 2016 in Saskatoon and May 17, 2016 in Swift Current, Saskatchewan. Forage stands were mowed multiple times in 2016 to control weed growth during the first growing season. No fertilizer or pesticides were applied to the plots throughout the experiment. Weeds were hand rogued during the establishment year, but no control measures were taken in subsequent years.

3.3.3 Data Collection

3.3.3.1 Stand Establishment Evaluation

A visual assessment of vegetative cover by plot was completed at both sites in the fall of 2016 and again in the spring of 2018. The foliar cover of each plant species in the plot, as well as the percent of bare ground and weeds, were recorded. Measurements were taken linearly along 1 metre ruler placed randomly in plot rows 0.3m away from the edges, and visual estimation of the total coverage for each category along that metre was assessed. This procedure was repeated twice per plot to obtain a mean foliar cover for each target species in the plot. Since the target plant cover was inversely related to the combined weed and bare ground cover, only the cover by target species was statistically analyzed.

3.3.3.2 Soil Nutrient Analysis

To determine the impact of native plants on soil nutrients, an assessment of soil nutrients was conducted at both sites in springs of 2017 and 2018. Two random soil cores per plot, each at depths of 0 –30cm were taken at Saskatoon, and combined into one composite sample per plot. At Swift Current, one soil core per plot was collected at two depths of 0 –15cm and 15 –30cm. For the Swift Current samples, laboratory results from the two depths were added to give a representation of the interval 0-30cm. All collected soil samples were analyzed for nitrate (NO_{3-}), phosphate (PO_{4-}), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{2+}), total nitrogen (N) and total carbon (C), using chemical analysis at Agriculture and Agri-food Canada`s Swift Current Research and Development Centre. Technical issues with the laboratory fume hoods in Swift Current prevented analysis of the 2018 phosphorous and potassium samples as no digestions were performed during that year and up to the time of this report.

3.3.3.3 Botanical Composition

An assessment of the botanical composition of stands was conducted to compare seeded mixtures with realized species ratios and repeated for two growing seasons to observe changes over time. Two sub-samples of 0.5 m length each were clipped from each plot in early July prior to biomass harvesting. All legume species in a given plot were separated from meadow bromegrass. The separated samples were oven dried at 60 °C for 48h and weighed to give a legume to grass ratio based on dry weight. For the Swift Current site, above ground biomass from each plot was further separated into the individual legume species and weed species in the mixtures (Table 5.10).

3.3.3.4 Forage Dry Matter and Nutritive Value

Sampling for forage dry matter yield and nutritive analysis was conducted twice per growing season, with half of the plots at each site harvested in July, and the remaining half in August. At the time of harvest a 0.9m wide strip was cut from each plot, placed on a tarp and weighed. A biomass sub-sample weighing approximately 300-400 gms was obtained and dried in the forage oven for 48 hours. Dried samples were ground and analyzed for Ca^{2+}, P, Mg^{2+} and K^{+} at the Agriculture and Agri-Food Canada (AAFC) Swift Current Research and Development Centre. The samples were also analyzed for crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) at the Forage Breeding lab at the University of Saskatchewan.

Total N concentration was determined using the Leco CN 628 Element Analyser (Leco Corportation, St. Joseph, MI), then multiplying the amount of nitrogen by a factor of 6.25 to calculate the crude protein concentration. The percentage of acid detergent fibre (ADF) and neutral detergent fibre (NDF) were semi-automatically analyzed using the $ANKOM^{200}$ Fiber Analyzer (ANKOM Technology, Fairport, NY). Acid detergent and neutral detergents were used to determine the quantity of fiber residues remaining after digestion using the Filter Bag Technique (for A200 and A2001) procedures published by ANKOM (2017). The percent moisture in samples was calculated by weighing fiber residues before and after drying in the oven for 12 hours, then subtracting the percent moisture from the ADF and NDF values.

3.4 Statistical Analysis

The data was analyzed for Analysis of Variance using the mixed model (SAS statistical software, 2014) to examine the effects of species mixture and harvest date on forage yield, nutritive value and soil nutrient levels. The experimental model is Randomized Complete Block Design with 2x2 factorial treatment design with the main effects of species mixture and harvest date, and their interaction and the random effect in the model is the rep. The site and year were included as fixed effects, but there was significant site x mixture and site x year effects so data was analyzed by each site for these traits. If the treatment effect was significant at $P<0.05$, means comparisons were made using the studentized Tukey multi-treatment method. Degrees of freedom were calculated using Satterthwaite's method.

3.5 Results

3.5.1 Weather Data

The Swift Current site received greater than average precipitation in the establishment year, followed by two warmer and drier growing seasons than the long-term average in 2017 and 2018 (Table 3.3; 3.4). The Saskatoon site had high precipitation in August of 2016, followed by two drier than normal summers in 2017 and 2018.

Table 3.3 Mean monthly temperature (°C) and total monthly precipitation (mm) obtained from Environment Canada weather station in Swift Current, SK.

	Precipitation (mm)				Monthly mean air temperature °C			
	2016	2017	2018	Long-term	2016	2017	2018	Long-term
January	5.2	8.3	2.9	16.1	-8.1	-13.1	-12.9	-12.8
February	13.1	19.8	9.1	13.6	-2.1	-9.3	-18.1	-10.7
March	10.1	12.6	45.4	17.6	1.5	-5.2	-8.6	-4.5
April	25.9	22.4	9.9	22.5	6.6	4.3	-0.7	4.6
May	134.9	21.0	25.6	44.4	13.7	12.1	14.3	10.9
June	87.2	35.3	16.9	74.2	17.4	16.1	17.3	15.4
July	124.8	11.0	51.2	52.2	18.7	19.6	18.7	18.6
August	50.3	28.0	31.0	43.4	16.9	17.8	17.1	17.7
September	40.9	4.4	44.4	31.8	11.8	12.8	9.3	11.9
October	85.0	58.2	12.7	19.6	2.1	5.0	3.9	5.6
November	1.8	15.3	26.0	14.2	1.9	-9.8	-4.3	-3.4
December	9.1	10.7	12.8	15.3	-13.7	-12.3	-5.9	-9.6

Table 3.4 Mean monthly temperature (°C) and total monthly precipitation (mm) obtained from Environment Canada weather station in Saskatoon, SK.

	Precipitation (mm)				Monthly mean air temperature °C			
	2016	2017	2018	Long-term	2016	2017	2018	Long-term
January	17.3	7.4	9.4	17.3	-12.9	-13.1	-12.9	-17.5
February	7.0	9.1	6.1	13.0	-7.9	-9.3	-18.1	-14.9
March	13.9	11.3	14.7	16.0	-1.5	-5.2	-8.6	-7.5
April	3.0	18.4	9.1	21.2	5.5	4.3	-0.7	3.5
May	41.6	46.3	35.0	39.3	13.7	12.1	14.3	10.9
June	49.7	30.9	19.9	65.7	17.4	16.1	17.3	15.5
July	58.6	25.5	31.1	57.8	18.7	19.6	18.7	18.4
August	70.2	25.2	17.2	42.6	16.9	17.8	17.1	17.1
September	24.1	29.1	37.1	34.5	11.8	12.8	7.4	11.2
October	40.8	17.8	8.0	20.1	2.1	5.0	2.5	4.5
November	9.2	15.4	15.6	16.1	1.9	-9.8	-6.8	-5.8
December	9.7	6.9	4.5	15.9	-13.7	-12.3	-10.2	-13.9

3.5.2 Stand Establishment

Based on foliar cover, the success of stand establishment varied among different forage mixtures (P<.0001), between years (P<.0001) and between sites (P<.0001). There was also an interaction effect (P<.0001) of mixture x site, indicating establishment success was different for certain mixtures between Saskatoon and Swift Current (Table 3.5).

Table 3.5 The Analysis of Variance for foliar cover (%).

Factor	df†	P value % Cov
mixture (M)	10	<.0001
year (Y)	1	<.0001
site (S)	1	<.0001
M*S	10	<.0001
M*Y	10	<.0001
S*Y	1	0.086
M*S*Y	11	0.026

† Degrees of freedom

Overall, the plots had greater foliar cover at the Saskatoon site, with both sites showing increasing cover over the study period (Table 3.6). The foliar cover of the mixture plots in 2016 was similar to the monoculture of meadow brome in Saskatoon, but it was less than meadow brome in Swift Current. By 2018, there was no difference between mixtures at the Saskatoon site, and all except the mixture of *B. riparius*/ *D. purpurea*/ *H. boreale* performed similarly at the Swift Current site. *V. americana* had the greatest foliar cover of the monoculture legumes at both sites in 2016 followed by *A. flexuosus*, but by 2018 the opposite was true. *D. purpurea* had higher monoculture establishment at the Saskatoon site throughout the study and made up between 3-5% of plot cover at the Swift Current site. *H. boreale* monoculture also established better at the Saskatoon site and had disappeared from the Swift Current stands by 2018 (Table 3.6).

Table 3.6 Estimated foliar cover (%) of target species measured in September 2016 and July 2018 at Swift Current and Saskatoon sites.

	Foliar Cover (%)			
Mixture*	Swift Current		Saskatoon	
	2016	2018	2016	2018
BR	82^{a}	92^{a}	68^{a}	93^{a}
BR/DP/AF	47^{bc}	85^{ab}	52^{ab}	88^{a}
BR/VA/HB	60^{b}	87^{ab}	62^{a}	91^{a}
BR/DP/HB	55^{b}	79^{b}	66^{a}	85^{a}
BR/HB/AF	56^{b}	82^{ab}	62^{a}	85^{a}
BR/DP/VA	57^{b}	81^{ab}	65^{a}	84^{a}
BR/VA/AF	64^{b}	89^{ab}	59^{a}	88^{a}
AF	23^{d}	49^{c}	42^{bc}	65^{b}
VA	34^{cd}	9^{d}	64^{a}	40^{c}
DP	5^{e}	3^{d}	32^{c}	44^{c}
HB	4^{e}	0^{d}	26^{c}	42^{c}
P value	<.0001	<.0001	<.0001	<.0001
SEM†	3.9	3.1	3.8	3.5

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different (P > 0.05).

3.5.3 Soil Nutrient Analysis

The soil nitrate, phosphate, total nitrogen, total carbon, potassium and magnesium were different among the forage mixtures. Nitrate was the only soil nutrient tested that showed an interaction effect for mixture x site (P<.0001) and mixture x site x year (P<.0001). There was an interaction effect for mixture x year for nitrate (P=0.001), Total N (P<.0001) and Total C (P<.0001). All soil nutrients tested except sodium showed an interaction effect for site x year (P<.0001) (Table 3.7).

Table 3.7 The Analysis of Variance for soil nitrate (NO_{3-}), phosphate (PO_{4-}), total nitrogen (Total N), total carbon (Total C), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) in May 2017 and 2018 at Swift Current and Saskatoon.

Factor	df†	P value							
		NO_{3-}	PO_{4-}	Total N	Total C	K	Ca	Mg	Na
mixture (M)	20	<.0001	0.006	<.0001	0.003	<.0001	0.064	0.008	0.006
year (Y)	1	<.0001	<.0001	<.0001	<.0001	<.0001	0.001	<.0001	<.0001
site (S)	1	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.575
M*S	20	<.0001	0.006	0.023	0.625	0.017	0.064	0.008	0.006
M*Y	20	0.001	0.303	<.0001	<.0001	0.014	0.985	0.803	0.899
S*Y	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.853
M*S*Y	20	0.001	0.303	0.685	0.999	0.338	0.985	0.803	0.899

† Degrees of freedom

Soil nitrate content at the Swift Current site dropped (P<.0001) between 2017 and 2018, but it increased (P<.0001) at the Saskatoon site (Table 3.7). At the Swift Current site in 2017, mixtures which did not contain *H. boreale* had higher nitrate than other mixtures and the *B. riparius* monoculture. Interestingly the monoculture plot of *H. boreale* had the highest nitrate of all treatments in 2018 (Table 3.8). Soil phosphate levels decreased roughly three fold in Swift Current over the two growing years, and by less than half at the Saskatoon site. Nitrate increased and total nitrogen remained stable in Saskatoon site but both decreased in Swift Current from 2017 to 2018. Soil potassium was greater at the Saskatoon site, and decreased at both sites over the course of the study. Soil calcium did not change at the Saskatoon site and had no treatment differences, whereas the Swift Current site showed differences by the second growing year, with significantly greater amount found in the monoculture meadow brome and mixtures; *B. riparius*/ *H. boreale*/ *A. flexuosus*, and *B. riparius*/ *V. americana*/ *A. flexuosus*. Soil magnesium and nitrate fluctuated between treatments and between study years but no clear pattern was observed (Table 3.8; 3.9).

Table 3.8 Soil nitrate (NO_{3-}), phosphate (PO_{4-}), total nitrogen (Total N), total carbon (Total C), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) in May 2017 and 2018 at the Swift Current site sampled at a depth of 0-30cm.

Year	Mixture[*]	NO_{3-} (ppm)	PO_{4-} (ppm)	Total N (%)	Total C (%)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)
2017	BR	3	18	0.2	3.4	369	7547	1644	208
	BR/DP/AF	2	21	0.2	3.1	430	7096	1620	153
	BR/VA/HB	2	22	0.2	3.0	406	6115	1874	305
	BR/DP/HB	2	25	0.2	2.7	451	5980	1633	174
	BR/HB/AF	2	23	0.3	3.1	485	5868	2227	508
	BR/DP/VA	2	20	0.2	2.7	408	5942	1703	249
	BR/VA/AF	2	21	0.2	2.9	432	5907	1609	156
	AF	3	26	0.2	3.2	443	6787	1684	213
	VA	2	20	0.2	2.9	385	6514	1576	167
	DP	4	25	0.2	2.9	451	6129	1620	131
	HB	3	25	0.2	3.0	449	6249	1519	155
	P value	0.022	0.047	0.066	0.909	0.095	0.464	0.104	0.074
	SEM†	0.5	2.5	0.01	0.3	30.3	596.0	209.4	94.5
2018	BR	0.52^{ab}	6	0.1	1.9	125	4550^{a}	1108	205
	BR/DP/AF	0.46^{b}	6	0.1	1.7	139	3627^{ab}	1001	199
	BR/VA/HB	0.51^{ab}	6	0.1	1.8	129	3441^{ab}	1374	362
	BR/DP/HB	0.58^{ab}	7	0.1	1.6	139	3663^{ab}	1140	250
	BR/HB/AF	0.58^{ab}	6	0.1	1.5	135	3316^{b}	1151	289
	BR/DP/VA	0.45^{b}	6	0.1	1.6	134	3499^{ab}	1093	246
	BR/VA/AF	0.48^{b}	7	0.1	1.5	132	3363^{b}	1008	179
	AF	0.65^{ab}	7	0.1	1.8	138	3695^{ab}	1134	241
	VA	0.67^{ab}	5	0.1	1.8	122	3633^{ab}	983	191
	DP	0.62^{ab}	6	0.1	1.9	129	4235^{ab}	1152	236
	HB	0.82^{a}	6	0.1	1.9	138	3695^{ab}	974	133
	P value	0.011	0.127	0.220	0.434	0.665	0.016	0.150	0.481
	SEM†	0.1	0.8	0.005	0.2	12.0	322.1	142.9	78.6

*AF = *Astragalus flexuosus,* BR = *Bromus riparius,* DP = *Dalea purpurea,* HB = *Hedysarum boreale,* VA = *Vicia americana*.

† SE = Standard error of the means.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different ($P > 0.05$)

Table 3.9 Soil nitrate (NO_{3-}), phosphate (PO_{4-}), total nitrogen (Total N), total carbon (Total C), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) in May 2017 and 2018 at the Saskatoon site sampled at a depth of 0-30cm.

Year	Mixture*	NO_{3-} (ppm)	PO_{4-} (ppm)	Total N (%)	Total C (%)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)
2017									
	BR	3^{e}	7	0.2	2.1	713^{ab}	5332	993	20
	BR/DP/AF	4^{de}	8	0.2	2.1	692^{ab}	5165	996	22
	BR/VA/HB	3^{e}	7	0.2	2.1	735^{a}	5317	1012	21
	BR/DP/HB	3^{e}	6	0.2	2.1	694^{ab}	5299	975	20
	BR/HB/AF	3^{e}	6	0.2	2.1	714^{ab}	5466	1011	21
	BR/DP/VA	3^{e}	6	0.2	2.1	684^{ab}	5224	995	20
	BR/VA/AF	3^{e}	6	0.2	2.1	705^{ab}	4983	958	20
	AF	6^{bc}	6	0.2	2.0	668^{b}	5160	1014	22
	VA	5^{cd}	6	0.2	2.1	663^{b}	5348	1007	22
	DP	8^{ab}	6	0.2	2.1	652^{b}	5250	978	21
	HB	9^{a}	7	0.2	2.1	690^{ab}	5435	1004	22
	P value	<.0001	0.473	0.390	0.195	0.001	0.710	0.603	0.560
	SEM†	0.6	1.2	0.004	0.03	33.2	250.8	31.3	1.3
2018	BR	8	4	0.2	2.1	349	5693	1147	33
	BR/DP/AF	9	4	0.2	2.2	337	5636	1149	31
	BR/VA/HB	9	4	0.2	2.1	366	5170	1139	22
	BR/DP/HB	8	4	0.2	2.1	337	5686	1109	31
	BR/HB/AF	8	4	0.2	2.2	359	5601	1154	27
	BR/DP/VA	9	5	0.2	2.2	360	5350	1119	27
	BR/VA/AF	8	5	0.2	2.0	371	5397	1134	24
	AF	9	4	0.2	2.1	360	5373	1130	34
	VA	9	4	0.2	2.1	343	5636	1153	26
	DP	11	5	0.2	2.1	351	5802	1096	30
	HB	10	5	0.2	2.1	344	5601	1119	33
	P value	0.066	0.787	0.762	0.686	0.494	0.549	0.845	0.875
	SEM†	0.7	0.4	0.01	0.1	15.8	318.1	32.3	5.7

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different (P > 0.05).

3.5.4 Botanical Compositions of Legume Grass Mixtures

A high percentage of the dry matter yield for the mixtures was composed of *B. riparius* (85-100%) compared to the native legumes (0-10%) for both sites during the two study years. At the Swift Current site, the *B. riparius* mixtures containing *A. flexuosus* had the highest amount of legume forage dry matter yield contributing to the mixtures. This pattern was not observed at the Saskatoon site where mixtures with *D. purpurea* appeared promising in 2017, but by 2018, legumes contributed almost no biomass to the mixtures (Table 3.10).

Table 3.10 Botanical composition (DM%) of mixtures by functional group at the Swift Current and Saskatoon sites in 2017 and 2018.

	% of DM							
	Swift Current				Saskatoon			
Mixture*	2017		2018		2017		2018	
	Grass	Legume	Grass	Legume	Grass	Legume	Grass	Legume
BR/HB/AF	94 ± 3.4†	6 ± 3.3	85 ± 6.2	8 ± 3.8	100 ± 0.2	0 ± 0.2	100 ± 0.4	0 ± 0.3
BR/VA/HB	100 ± 0.0	0 ± 0.2	100 ± 0.4	0 ± 0.2	100 ± 0.1	0 ± 0.1	100 ± 0.0	0 ± 0.0
BR/VA/AF	94 ± 2.1	6 ± 1.8	90 ± 2.8	4 ± 1.0	100 ± 0.1	0 ± 0.0	99 ± 0.9	1 ± 0.9
BR/DP/HB	100 ± 0.0	0 ± 0.0	100 ± 0.0	0 ± 0.0	94 ± 3.1	5 ± 2.5	100 ± 0.0	0 ± 0.0
BR/DP/VA	96 ± 2.5	3 ± 1.8	99 ± 0.6	0 ± 0.3	99 ± 0.4	1 ± 0.4	100 ± 0.0	0 ± 0.0
BR/DP/AF	94 ± 1.8	6 ± 1.6	84 ± 6.5	10 ±4.5	100 ± 0.2	0 ± 0.1	99 ± 0.6	2 ± 0.8

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error.

Table 3.11 Botanical composition (DM%) of mixtures by individual species at the Swift Current site in 2017 and 2018.

	Mixture[*]	July					Aug				
		BR	DP	AF	VA	HB	BR	DP	AF	VA	HB
2017	BR/DP/AF	98 ± 1.1[†]	0 ± 0.0	2 ± 1.1	-	-	91 ± 2.3	0 ± 0.0	9 ± 2.3	-	-
	BR/DP/VA	99 ± 0.5	0 ± 0.0	-	1.0 ± 0.5	-	93 ± 4.6	7 ± 4.6	-	0 ± 0.0	-
	BR/VA/AF	96 ± 1.9	-	4 ± 1.9	0.1 ± 0.1	-	91 ± 3.6	-	9 ± 3.6	0 ± 0.0	-
	BR/HB/AF	99 ± 1.2	-	1 ± 1.2	-	0 ± 0.0	89 ± 5.9	-	11 ± 5.9	-	0 ± 0.0
	BR/VA/HB	100 ± 0.0	-	-	0 ± 0.0	0 ± 0.0	100 ± 0.4	-	-	0.4 ± 0.4	0 ± 0.0
	BR/DP/HB	100 ± 0.0	0 ± 0.0	-	-	0 ± 0.0	100 ± 0.0	0 ± 0.0	-	-	0 ± 0.0
2018	BR/DP/AF	72 ± 13.7	6 ± 5.9	9 ± 4.0	-	-	53 ± 5.0	0 ± 0.0	9 ± 5.0	-	-
	BR/DP/VA	50 ± 8.6	0 ± 0.0	-	1 ± 0.8	-	61 ± 6.0	0 ± 0.0	-	0 ± 0.0	-
	BR/VA/AF	42 ± 2.4	-	7 ± 2.6	0 ± 0.0	-	59 ± 5.3	-	4 ± 0.6	0 ± 0.0	-
	BR/HB/AF	74 ± 12.0	-	7 ± 5.5	-	0 ± 0.0	43 ± 5.1	-	14 ± 7.4	-	0 ± 0.0
	BR/VA/HB	55 ± 11.1	-	-	0 ± 0.0	0 ± 0.0	66 ± 11.9	-	-	0.7 ± 0.7	0 ± 0.0
	BR/DP/HB	54 ± 10.7	0 ± 0.0	-	-	0 ± 0.0	74 ± 10.5	0 ± 0.0	-	-	0 ± 0.0

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error

3.5.5 Forage Dry Matter Yield

The dry matter yield varied among different forage mixtures (P<.0001), by site (P=0.001), by date (P=0.048) and year (P=0.046). There was also an interaction effect (P<.0001) of mixture x year, site x year (P<.0001), harvest date x year (P=0.015), and mixture x site x year (P<.0001) indicating yield was different for certain mixtures between sites, harvest dates, sites and years (Table 3.12).

Table 3.12 The Analysis of Variance for forage dry matter yield in kg ha^{-1} in 2017 and 2018 harvested in July or August at the Swift Current and Saskatoon sites.

Factor	DF[†]	P Value
Mixture (M)	10	<.0001
Site (S)	1	0.001
Date (D)	1	0.048
Year (Y)	1	0.046
M*D	10	0.065
M*S	10	0.287
M*Y	10	<.0001
S*D	1	0.239
S*Y	1	<.0001
D*Y	1	0.015
M*S*D	10	0.094
M*S*Y	10	<.0001
M*D*Y	10	0.157
S*D*Y	1	0.965

[†] Degrees of freedom

The forage stands produced greater dry matter yields at Saskatoon in 2017, but decreased in 2018 while at Swift Current yields increased in 2018 (Table 3.13). The mixtures produced significantly more biomass compared to monoculture legumes producing similar amounts as monoculture *B. riparius*, except in August 2018 at the Saskatoon site, where *B. riparius* had the highest yield. *A. flexuosus* was the highest yielding native legume in monoculture at both sites by 2018, producing comparably to *B. riparius* monoculture and maintaining high biomass production in August (Table 3.13). *D. purpurea* did not start producing biomass until August 2017 at Swift Current. *V. americana* appeared to be productive in 2017, although its yield low in monoculture plots in 2018. *H. boreale* forage dry matter yield was generally low at Swift

Current compared to Saskatoon. Overall, the forage dry matter yields was different (P=0.048) between July and August harvests (Table 3.12), but this was not consistent between the treatments, sites or years. Mixtures of *B. riparius*/ *D. purpurea*/ *A. flexusosus*, *B.riparius*/ *H. boreale*/ *A. flexuosus* and *B. riparius*/ *V. americana*/ *A.* flexuosus had greater July yields compared to August at Swift Current in 2017. At Saskatoon in 2017, monoculture *B. riparius* and *H. boreale* had lower August yields. These treatment differences were not observed at either site in 2018, with mixtures of *B. riparius*/ *V. americana*/ *A. flexuosus* in Swift Current and *B. riparius*/ *H. boreale*/ *A. flexuosus* in Saskatoon yielding greater in August and no treatments yielding greater in July. *B. riparius* is a cool-season grass, therefore begins to senescence in the later part of the growing season and forage production was expected to be lower.

Table 3.13 Forage dry matter yield in kg ha^{-1} at the Swift Current and Saskatoon sites over two growing seasons in 2017 and 2018.

		Swift Current				Saskatoon			
Year	Mixture[*]	July	August			July	August		
				P Value	SEM			P Value	SEM
2017	BR	2748^{ab}	2285^{a}	0.349	320.0	4807^{aA}	3789^{aB}	0.019	196.8
	BR/DP/AF	3101^{aA}	1768^{abB}	0.013	268.2	4626^{a}	4378^{a}	0.521	401.4
	BR/VA/HB	2714^{ab}	2007^{ab}	0.071	227.6	5221^{a}	3960^{a}	0.194	609.6
	BR/DP/HB	2558^{ab}	2124^{a}	0.161	202.1	4663^{a}	4155^{a}	0.466	442.1
	BR/HB/AF	2983^{aA}	1942^{abB}	0.014	148.3	4858^{a}	4345^{a}	0.306	326.9
	BR/DP/VA	1956^{b}	1905^{ab}	0.908	303.5	4577^{a}	3464^{ab}	0.068	507.3
	BR/VA/AF	2471^{abA}	1870^{abB}	0.010	103.4	4406^{a}	3537^{ab}	0.183	343.0
	AF	495^{c}	1999^{ab}	0.080	506.1	1419^{b}	1829^{bc}	0.612	541.8
	VA	597^{c}	645^{bc}	0.873	284.4	56^{b}	128c	0.303	44.8
	DP	0^{c}	9^{c}	0.391	6.1	151^{b}	89^{c}	0.530	64.5
	HB	0^{c}	0^{c}	-	-	204^{bA}	40^{cB}	0.005	26.5
	P Value	<.0001	<.0001			<.0001	<.0001		
	SEM[†]	218.7	291			376.2	380.9		
2018	BR	3003^{a}	4456^{a}	0.379	1081.5	2677^{a}	2528^{a}	0.301	251.2
	BR/DP/AF	4429^{a}	3190^{a}	0.087	668.5	3125^{a}	1952^{abc}	0.937	810.8
	BR/VA/HB	2815^{a}	3378^{a}	0.532	601.0	2108^{a}	2157^{abc}	0.804	288.8
	BR/DP/HB	2756^{a}	3749^{a}	0.091	541.1	2174^{a}	2073^{abc}	0.742	211.4
	BR/HB/AF	4122^{a}	2895^{ab}	0.080	472.9	1440^{abB}	2302^{abA}	0.013	319.9
	BR/DP/VA	2577^{a}	3127^{a}	0.053	395.3	2322^{a}	1688^{abc}	0.287	383.3
	BR/VA/AF	2498^{aB}	3203^{aA}	0.048	187.2	2495^{a}	1744^{abc}	0.167	556.6
	AF	3938^{a}	3920^{a}	0.991	1117.0	2934^{a}	3107^{a}	0.784	427.3
	VA	107^{b}	0^{b}	0.326	64.3	0^{b}	0^{d}	-	-
	DP	80^{b}	34^{b}	0.237	51.8	394^{b}	761^{bcd}	0.421	300.5
	HB	95^{b}	0^{b}	0.391	67.4	0^{b}	662^{cd}	0.391	468.1
	P Value	<.0001	<.0001			<.0001	<.0001		
	SEM	470.7	662.6			453.1	374.3		

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

†† SEM = Mean standard error of the means where missing data resulted in an unbalanced statistical design.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different (P > 0.05).

A, B, C = Means within a row with the same upper case letter are not significantly different (P > 0.05).

3.5.6 Nutritive Value

The concentration of crude protein varied among different forage mixtures (P<.0001), between sites (P=0.029), between harvest dates (P<.0001) and years (P<.0001) with interaction effects of mixture x date (P=0.011), mixture x year (P<.0001), site x harvest date (P=0.024), mixture x site x year (P=0.012) and site x date x year (P<.0001). Acid detergent fiber value differed between the forage mixtures (P<.0001) and by date (P<.0001), and had interaction effect for mixture x date (P=0.013). NDF values differed between forage mixtures (P<.0001), by site (P=0.004), by harvest date (P<.0001), and by year (P<.0001) with interaction effects of mixture x year (P<.0001) and mixture x site x year (P=0.021). There were interaction effects for both ADF and NDF of site x year (P<.0001) and harvest date x year (P<.0001), and by site x date x year (P=0.016) (Table 3.14).

Table 3.14 The Analysis of Variance for concentrations of crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) in 2017 and 2018.

Factor	DF†	P Value		
		CP	ADF	NDF
Mixture (M)	10	<.0001	<.0001	<.0001
Site (S)	1	0.029	0.141	0.004
Date (D)	1	<.0001	<.0001	<.0001
Year (Y)	1	<.0001	0.237	<.0001
M*D	10	0.011	0.013	0.470
M*S	10	0.278	0.850	0.409
M*Y	10	<.0001	0.100	<.0001
S*D	1	0.024	0.383	0.053
S*Y	1	0.526	<.0001	<.0001
D*Y	1	0.928	<.0001	<.0001
M*S*D	10	0.367	0.581	0.697
M*S*Y	10	0.012	0.130	0.021
M*D*Y	10	0.419	0.318	0.613
S*D*Y	1	<.0001	0.016	0.0002

† Degrees of freedom

Crude protein concentrations of the native legumes were generally greater compared to the monoculture *B. riparius* and grass-legume mixtures. At the Saskatoon site, crude protein concentrations of the native legumes were similar between July and August in 2017 for all four legumes, but in 2018, *V. americana* did not produce adequate biomass for analyses.

At the Swift Current site, ADF concentration showed no differences among mixtures in July of 2017, but *B. riparius* and the mixtures showed greater concentrations in August compared to the monoculture legumes. The monoculture legume plots had lower concentrations of NDF during both years. The monoculture *B. riparius* and mixtures had greater ADF and NDF in August compared to July harvests.

Saskatoon had similar trends with crude protein and fibre concentrations with greater crude protein in the monoculture legume plots and high concentrations of ADF and NDF in the treatments containing *B. riparius*, especially in August. Crude protein was decreased in the August harvest across treatments, with the highest levels found in the monoculture legume plots (Table 3.15; 3.16).

Table 3.15 Concentrations of crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) at Swift Current and Saskatoon sites in 2017.

		Swift Current						Saskatoon					
		July			August			July			August		
Year	Mixture*	CP	ADF	NDF	CP	ADF	NDF	CP	ADF	NDF	CP	ADF	NDF
2017	BR	5^{b}	34	53^{a}	6^{b}	39^{ab}	56^{ab}	6^{b}	32^{ab}	54^{a}	3^{c}	39^{a}	63^{a}
	BR/DP/AF	5^{b}	38	54^{a}	4^{b}	41^{a}	63^{a}	7^{b}	31^{abc}	54^{a}	4^{c}	40^{a}	63^{a}
	BR/VA/HB	4^{b}	26	55^{a}	4^{b}	42^{a}	63^{a}	6^{b}	31^{abc}	54^{a}	3^{c}	40^{a}	63^{a}
	BR/DP/HB	5^{b}	36	55^{a}	4^{b}	42^{a}	64^{a}	6^{b}	31^{abc}	54^{a}	3^{c}	41^{a}	65^{a}
	BR/HB/AF	5^{b}	35	54^{a}	4^{b}	42^{a}	65^{a}	5^{b}	33^{a}	56^{a}	3^{c}	40^{a}	63^{a}
	BR/DP/VA	5^{b}	36	54^{a}	4^{b}	41^{a}	63^{a}	6^{b}	31^{abc}	53^{a}	3^{c}	39^{a}	63^{a}
	BR/VA/AF	5^{b}	36	54^{a}	4^{b}	43^{a}	63^{a}	5^{b}	32^{ab}	55^{a}	4^{c}	38^{a}	61^{a}
	AF	12^{a}	33	44^{ab}	9^{a}	34^{bc}	43^{c}	12^{a}	27^{cd}	33^{b}	11^{b}	31^{b}	39^{b}
	VA	10^{a}	34	34^{b}	12^{a}	30^{c}	41^{c}	12^{a}	28^{bcde}	34^{bc}	14^{a}	29^{b}	37^{b}
	DP	IS	IS	IS	12^{a}	33^{abc}	42^{bc}	13^{a}	22^{e}	29^{b}	13^{ab}	25^{b}	35^{bc}
	HB	IS	IS	IS	IS	IS	IS	13^{a}	25^{de}	24^{c}	12^{ab}	27^{b}	27^{c}
	P Value	<.0001	0.461	0.007	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	SEM†	$0.5^{\dagger\dagger}$	$3.6^{\dagger\dagger}$	$3.8^{\dagger\dagger}$	$0.6^{\dagger\dagger}$	$2.1^{\dagger\dagger}$	$2.4^{\dagger\dagger}$	$0.6^{\dagger\dagger}$	$1.0^{\dagger\dagger}$	$1.2^{\dagger\dagger}$	$0.5^{\dagger\dagger}$	$0.9^{\dagger\dagger}$	$1.4^{\dagger\dagger}$

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SEM = Standard error of the means.

†† SEM = Mean standard error of the means where missing data resulted in an unbalanced statistical design.

IS = Insufficient sample for analysis.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different (P > 0.05).

Table 3.16 Concentrations of crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) at Swift Current and Saskatoon sites in 2018.

		Swift Current						Saskatoon					
		July			August			July			August		
Year	Mixture*	CP	ADF	NDF	CP	ADF	NDF	CP	ADF	NDF	CP	ADF	NDF
2018	BR	7^{b}	34^{a}	60^{a}	5^{b}	38	65^{a}	7^{c}	35^{ab}	64^{a}	9^{abc}	36^{a}	66^{a}
	BR/DP/AF	8^{b}	33^{a}	57^{ab}	6^{b}	35	63^{a}	7^{c}	35^{ab}	65^{a}	8^{abc}	35^{a}	65^{a}
	BR/VA/HB	8^{b}	33^{a}	61^{a}	6^{b}	36	64^{a}	7^{c}	35^{ab}	64^{a}	7^{bc}	36^{a}	65^{a}
	BR/DP/HB	8^{b}	34^{a}	59^{a}	6^{b}	36	63^{a}	8^{c}	34^{ab}	64^{a}	7^{bc}	34^{a}	64^{a}
	BR/HB/AF	9^{b}	33^{a}	57^{ab}	7^{b}	35	64^{a}	7^{c}	35^{ab}	65^{a}	7^{c}	35^{a}	64^{a}
	BR/DP/VA	8^{b}	34^{a}	60^{a}	5^{b}	37	65^{a}	7^{c}	37^{a}	66^{a}	7^{bc}	35^{a}	64^{a}
	BR/VA/AF	10^{b}	30^{ab}	52^{ab}	7^{b}	35	62^{a}	7^{c}	36^{ab}	66^{a}	8^{abc}	36^{a}	66^{a}
	AF	14^{a}	27^{bc}	35^{c}	11^{a}	31	41^{b}	11^{ab}	34^{ab}	46^{b}	11^{ab}	32^{ab}	44^{b}
	VA	13^{ab}	32^{abc}	42^{bc}	IS	IS	IS	IS	IS	IS	IS	IS	IS
	DP	IS	17^{d}	27^{c}	IS	IS	IS	14^{a}	28^{b}	46^{b}	12^{a}	29^{b}	42^{b}
	HB	17^{a}	21^{cd}	27^{c}	IS	IS	IS	IS	IS	IS	9^{abc}	35^{ab}	65^{a}
	P Value	<.0001	<.0001	<.0001	<.0001	0.090	<.0001	<.0001	0.087	<.0001	0.005	0.0003	<.0001
	SEM†	1.0††	1.6††	2.9††	0.6††	1.7††	2.4††	0.8††	1.4††	1.8††	1.0††	1.4††	1.7††

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SEM = Standard error of the means.

†† SEM = Mean standard error of the means where missing data resulted in an unbalanced statistical design.

IS = Insufficient sample for analysis.

a, b, c = Means within a column with the same lower case letter are not significantly different (P > 0.05).

The amount of phosphorous, potassium, calcium and magnesium varied among the different forage mixtures. Phosphorous, calcium and magnesium varied by site (P<.0001), and phosphorous, potassium, calcium and magnesium varied by harvest date. There was also an interaction effect for phosphorous, calcium and magnesium of mixture x site (P<.0001). Interaction effects for forage magnesium were found (P<.0001) for mixture x site, mixture x year, site x date, site x year, mixture x site x date indicating high variability in magnesium between treatment effects (Table 3.17).

Table 3.17 The Analysis of Variance for forage total phosphorous (P) and total potassium (K) in 2017, and calcium (Ca) and magnesium (Mg) in 2017 and 2018 at the Swift Current and Saskatoon sites.

Factor	df†	P value			
		P	K	Ca	Mg
Mixture (M)	10	<.0001	0.020	<.0001	<.0001
Site (S)	1	<.0001	0.818	<.0001	<.0001
Date (D)	1	<.0001	<.0001	0.001	<.0001
Year (Y)	1	IS	IS	<.0001	<.0001
M*D	10	0.130	<.0001	<.0001	0.053
M*S	10	<.0001	0.161	<.0001	<.0001
M*Y	10	IS	IS	<.0001	<.0001
S*D	1	0.020	0.085	0.008	<.0001
S*Y	1	IS	IS	<.0001	<.0001
D*Y	1	IS	IS	0.859	0.972
M*S*D	10	0.173	0.149	0.0003	<.0001
M*S*Y	10	IS	IS	<.0001	0.255
M*D*Y	10	IS	IS	0.438	0.914
S*D*Y	1	IS	IS	0.002	0.085

† Degrees of freedom

IS = Insufficient sample for analysis.

Forage nutrient analysis conducted in 2017 at both sites showed an overall higher level of phosphorous, calcium and magnesium in the monoculture native legume treatments. Potassium samples in 2017 were slightly different between treatments at both sites, but no overall trend was observed. The phosphorous and potassium samples collected in 2018 are currently un-analyzed due to laboratory delays (Table 3.18; 3.19).

Table 3.18 Forage total phosphorous (P) and total potassium (K) in 2017 and calcium (Ca) and magnesium (Mg) in 2017 and 2018 at the Swift Current site.

		July				August			
Year	Mixture*	P (%)	K (%)	Ca (ppm)	Mg (ppm)	P (%)	K (%)	Ca (ppm)	Mg (ppm)
2017	BR	0.78^{b}	1.8^{ab}	2832^{bcd}	1805^{bc}	0.06^{bcd}	1.5^{abcd}	2844^{b}	2114^{bc}
	BR/DP/AF	0.78^{b}	1.9^{ab}	2608^{bcd}	1611^{c}	0.04^{d}	1.1^{cd}	2086^{b}	1849^{c}
	BR/VA/HB	0.75^{b}	1.9^{ab}	1978^{d}	1572^{c}	0.05^{cd}	1.2^{cd}	2502^{b}	1817^{c}
	BR/DP/HB	0.78^{b}	1.8^{ab}	2387^{bcd}	1590^{c}	0.04^{d}	1.0^{d}	1948^{b}	1869^{c}
	BR/HB/AF	0.78^{b}	2.1^{a}	2057^{cd}	1529^{c}	0.05^{cd}	1.2^{bcd}	1977^{b}	2168^{bc}
	BR/DP/VA	0.75^{b}	1.8^{ab}	3160^{bcd}	1781^{bc}	0.05^{cd}	1.1^{cd}	1975^{b}	1940^{bc}
	BR/VA/AF	0.75^{b}	1.9^{ab}	2451^{bcd}	1549^{c}	0.05^{cd}	1.0^{d}	2434^{b}	1977^{bc}
	AF	0.98^{ab}	1.8^{ab}	8509^{a}	2971^{ab}	0.08^{abcd}	1.6^{abc}	5817^{a}	4163^{ab}
	VA	0.11^{a}	1.7^{ab}	8896^{a}	3539^{a}	0.09^{abc}	1.7^{ab}	8026^{a}	5771^{a}
	DP	0.10^{a}	1.6^{b}	6261^{ab}	3206^{a}	0.1^{a}	1.8^{a}	7721^{a}	6311^{a}
	HB	0.095^{ab}	1.8^{ab}	5970^{abc}	2635^{abc}	0.1^{ab}	1.6^{abc}	5986^{a}	4543^{a}
	P value	<.0001	0.101	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	SEM†	0.01	0.1	833.7	275.5	0.009	0.1	629.4	484.3
2018	BR	----	----	3438^{d}	2862^{c}	----	----	4106^{b}	3036^{b}
	BR/DP/AF	----	----	3549^{d}	2776^{c}	----	----	4236^{b}	3387^{b}
	BR/VA/HB	----	----	3248^{d}	3201^{c}	----	----	4614^{b}	3593^{b}
	BR/DP/HB	----	----	3995^{d}	3225^{c}	----	----	3881^{b}	3847^{b}
	BR/HB/AF	----	----	4056^{d}	2997^{c}	----	----	4581^{b}	4802^{ab}
	BR/DP/VA	----	----	3336^{d}	2977^{c}	----	----	3845^{b}	3664^{b}
	BR/VA/AF	----	----	5235^{cd}	3785^{c}	----	----	3712^{b}	3374^{b}
	AF	----	----	7150^{bc}	4069^{bc}	----	----	8426^{a}	6218^{a}
	VA	----	----	18425^{a}	8243^{a}	----	----	IS	IS
	DP	----	----	19681^{a}	7002^{ab}	----	----	IS	IS
	HB	----	----	10636^{b}	4971^{abc}	----	----	IS	IS
	P value	----	----	<.0001	<.0001	----	----	<.0001	0.002
	SEM†	----	----	625.5††	612.3††	----	----	519.2	546.9

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

†† SEM = Mean standard error of the means where missing data resulted in an unbalanced statistical design.

IS = Insufficient sample for analysis.

--- = Analysis incomplete.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different (P > 0.05).

Table 3.19 Forage total phosphorous (P) and total potassium (K) in 2017 and calcium (Ca) and magnesium (Mg) in 2017 and 2018 at the Saskatoon site.

		July				August			
		P	K	Ca	Mg	P	K	Ca	Mg
Year	Mixture*	(%)	(%)	(ppm)	(ppm)	(%)	(%)	(ppm)	(ppm)
2017	BR	0.06^{c}	1.7^{ab}	2292^{d}	1169^{d}	0.03^{c}	1.3^{ab}	3120^{c}	1478^{c}
	BR/DP/AF	0.06^{c}	1.8^{ab}	2721^{d}	1322^{d}	0.03^{c}	1.3^{ab}	3637^{c}	1587^{bc}
	BR/VA/HB	0.05^{c}	1.8^{ab}	2386^{d}	1298^{d}	0.03^{c}	1.3^{ab}	3169^{c}	1559^{bc}
	BR/DP/HB	0.06^{c}	1.9^{ab}	2215^{d}	1171^{d}	0.02^{c}	1.2^{b}	3404^{c}	1526^{bc}
	BR/HB/AF	0.05^{c}	2.1^{a}	2023^{d}	1051^{d}	0.03^{c}	1.3^{ab}	3228^{c}	1520^{bc}
	BR/DP/VA	0.06^{c}	1.8^{ab}	3359^{d}	1366^{d}	0.03^{c}	1.3^{ab}	3155^{c}	1516^{c}
	BR/VA/AF	0.06^{c}	1.9^{ab}	2289^{d}	1164^{d}	0.03^{c}	1.4^{ab}	4406^{c}	1695^{bc}
	AF	0.1^{ab}	2.0^{a}	12093^{c}	1872^{c}	0.09^{ab}	1.7^{ab}	13563^{b}	2074^{bc}
	VA	0.1^{a}	1.3^{b}	21339^{a}	4542^{a}	0.1^{a}	1.9^{a}	18830^{a}	3520^{ab}
	DP	0.1^{a}	1.7^{ab}	16444^{b}	3278^{b}	0.1^{a}	1.4^{ab}	19177^{a}	3407^{abc}
	HB	0.09^{b}	1.4^{b}	9911^{c}	4857^{a}	0.08^{b}	1.5^{ab}	17175^{a}	5320^{a}
	P value	<.0001	0.005	<.0001	<.0001	<.0001	0.016	<.0001	<.0001
	SEM†	0.01	0.1	489.3	89.8††	0.01	0.1	764.2	409.4
2018	BR	----	----	3085^{c}	1345^{c}	----	----	2975^{c}	1323^{c}
	BR/DP/AF	----	----	2493^{c}	1222^{c}	----	----	2947^{c}	1307^{c}
	BR/VA/HB	----	----	2807^{c}	1255^{c}	----	----	2794^{c}	1361^{c}
	BR/DP/HB	----	----	2686^{c}	1265^{c}	----	----	2746^{c}	1258^{c}
	BR/HB/AF	----	----	2677^{c}	1270^{c}	----	----	2422^{c}	1190^{c}
	BR/DP/VA	----	----	2450^{c}	1213^{c}	----	----	2865^{c}	1388^{c}
	BR/VA/AF	----	----	2697^{c}	1277^{c}	----	----	2965^{c}	1835^{b}
	AF	----	----	10387^{b}	1876^{b}	----	----	10163^{b}	1835^{b}
	VA	----	----	MV	MV	----	----	MV	MV
	DP	----	----	13600^{a}	3140^{a}	----	----	14708^{a}	2879^{a}
	HB	----	----	MV	MV	----	----	1697^{c}	986c
	P value	----	----	<.0001	<.0001	----	----	<.0001	<.0001
	SEM†	----	----	514.2††	91.9††	----	----	445.5††	82.3††

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SEM = Standard error of the means.

†† SEM = Mean standard error of the means where missing data resulted in an unbalanced statistical design.

--- = Analysis incomplete.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different (P > 0.05).

3.6 Discussion

The weather at both sites was cool and wet during the establishment year compared to the long-term averages. At the Swift Current site the total yearly precipitation was among the top ten in recorded history (AAFC, 2016). This should have provided sufficient moisture to germinate and establish, regardless of drought tolerance. Despite sufficient precipitation at the Swift Current site, stand percentage was higher at the Saskatoon site. This oscillation between relatively wet and dry years matches historical climate trends in this region (Bonsal and Regier, 2007). By 2018, sustained lack of precipitation and limited spring rainfall were evident with dry spring soils, which provided plants with little moisture to start growing in the 2018 growing season. Although native plants can provide relatively stable forage in semi-arid environments (Schellenberg et al., 2012), biomass is still reduced under drought (Komainda et al., 2018). The lower dry matter yields in the second growing year are thought to be the result of the dry conditions.

Foliar cover increased over the course of the study for all plots containing *B. riparius* but did not increase for all of the native legume monoculture plots. The native legume monocultures had higher levels of foliar cover at the Saskatoon site. *A. flexuosus* and *V. americana* had the greatest foliar cover among the native legumes in monoculture at both sites. *D. purpurea* and *H. boreale* disappeared from stands by the second year at the Swift Current site. Differential survival was expected since legumes in mixtures with multiple other legumes face competitive stress from companion species and weeds (Cooper, 1977). Although the native legumes were mostly able to establish in monoculture, they contributed very little dry matter yield to mixtures, especially at the Saskatoon site. Since *B. riparius* is more competitive than the native legumes (Knowles et al., 1993), it is likely that the native legumes could not compete with this grass, especially in the Brown soil zone where nutrients are more limiting (Smoliak, 1988).

Since the sites were located in different soil zones, it was expected that the soil nutrient levels would differ between the sites. Nitrate was overall lower at the Swift Current site, reflecting the thin organic matter layer characteristic of the Brown soil zone (Smoliak, 1988). Over the course of the study, nitrate decreased in Swift Current and increased at the Saskatoon site. The native legumes may have contributed to increasing the nitrate level in Saskatoon, although the botanical composition of mixtures at that site does not suggest the legumes were a significant component of these plots. Where legumes persisted in the plots, the dry weather conditions may have resulted in nodule sloughing. Phosphorous and potassium levels decreased at both sites in 2018 which may be explained by the removal of biomass from all the plots following the August harvest in 2017. Treatment differences in soil nitrate, calcium and potassium were statistically significant but our number of experimental replication and sample size were relatively limited to detect biological differences. A long-term study may be need to precisely quantify these differences.

For botanical composition of plots it was expected that the more vigorous species would make up a greater proportion of the stand (Haynes, 1980). *B. riparius* dominated mixtures at both sites even though seeding rates were proportional between species, ie. 33%. There were greater percentages of legumes in mixtures based on forage dry matter yield at the Swift Current site, even though foliar cover was greater at the Saskatoon site. At the Swift Current site only mixtures which contained *A. flexuosus* had legumes contributing to the dry matter yield by the second growing year. The native legumes made up a small or negligible percentage of total forage dry matter yield of the mixtures by 2018 at both sites. Studies have shown that native legumes take longer to establish, and are less competitive compared to *B. riparius* (Knowles, 1993; Hamel et al., 2008).

Despite the low presence of native legumes in the dry matter yield of mixtures at both sites, the monoculture *A. flexuosus* consistently produced appreciable amounts of forage at both sites during the two study years. By 2018, *A. flexuosus* was producing as much as the mixture treatments, but did not increase the yield beyond *B. riparius* monoculture. Even though the legumes did not increase the yield of mixtures, the yields were expected to be between the yields of the individual component species (Springer et al., 2001). *D. purpurea* forage dry matter yield increased over time and was greater overall at the Saskatoon site. The forage yield of *H. boreale* monoculture plots in Saskatoon was moderate, whereas in Swift Current it was mostly absent from monoculture plots and did not contribute to the forage yield of mixtures. *V. americana* produced high forage yield at both sites in 2017, with higher yield than *A. flexuosus* at Swift Current. Low yields have previously been noted for *V. americana* (Ruyle and Browns, 1985), but it did not produce forage yield at either site in Aug 2018, possibly due to drought conditions indicating poor adaptation to such conditions. However this legume is thought to be drought resistant due to a branching root (Allen and Tilley, 2004; Reaume, 2009), therefore its lack of production in the second year may indicate that it is a short-lived species under our study environment

Legume-grass mixtures were expected to vary not only by yield, but also by digestibility (Simili da Silva et al., 2013). Our monoculture legume plot results support previous findings that native legumes can increase the quality of forage by increasing CP and reducing ADF and NDF (Faris et al., 1986; Posler et al., 1993; McGraw et al., 2004; Collins et al., 2018), particularly in the late season when digestibility and nutrient value generally decline (Schellenberg et al., 2012). This supports previous work showing the extension of forage yield and quality later in the growing season by the addition of native legumes (Sleugh et al., 2000; McGraw et al., 2004; Cardinale et al., 2007). Native legumes in monocultures had higher levels of P, Ca and Mg at both sites, supporting studies showing that their inclusion can increase mineral availability in pastures (Yoshishara et al., 2013). Forage phosphorous decreased in the late season as a result of plant maturation and senescence.

The native legume-grass mixtures performed differently in the Dark Brown and Brown soil zones, with better overall establishment at Saskatoon but greater percentages of legumes present in mixtures at Swift Current. The mixtures had similar forage quality as monoculture *B. riparius*, as legumes contributed no more than 10% total biomass of mixtures forage dry matter yield at both sites. Soil nitrogen also was not greater in legume-grass mixtures compared to monoculture *B. riparus*. Based on this study, native legumes would have to make up a larger proportion of the forage yield to significantly change the nutritional value and soil nutrient levels of mixture plots. This study focused on mixtures with *B. riparius*, but many tame and native forage grass species remain to be tested with these legumes. Given the comparative establishment of *B. riparius* to native legumes in mixture, seeding rates could be manipulated to increase the ratio of native legumes and explore changes in botanical composition, however future studies should evaluate mixtures with a less vigorous grass than *B. riparius*. Some examples of native grass species that could be tested with these legumes include northern wheatgrass (*Agropyron cristatum*), western wheatgrass (*Pascopyrum smithii*) and June grass (*Koeleria macrantha*).

4. A STUDY OF ABOVE- AND BELOWGROUND BIOMASS PRODUCTION OF NATIVE LEGUMES UNDER DIFFERENT WATERING REGIMES IN A CONTROLLED ENVIRONMENT

4.1 Abstract

Plants can modify their allocation of biomass to shoots and roots under abiotic or biotic stress such as drought, a response that differs among species. We examined the effect of different water stresses on the above- and belowground seedling growth of four selected native Canadian legumes in monoculture and in mixture with *Bromus riparius* in greenhouse conditions. The experimental design was a Randomized Complete Block Design, water regimes were 100%, 75%, 50% and 25% of the field water holding capacity, and plant materials were monocultures of *Bromus riparius*, *Astragalus flexuosus*, *Dalea purpurea*, *Hedysarum boreale*, *Vicia americana* and mixtures of *B. riparius* with combinations of these legumes. Both watering regime (P<.0001) and species monoculture/mixture (P<.0001) were found to have significant effects on shoot and root biomass production. The monoculture legume treatments showed less above and belowground biomass production compared to treatments containing *B. riparius*. For the mixtures, *B. riparius*/ *V. americana*/ *H. boreale*, *B. riparius*/ *H. boreale*/ *A. flexuosus* and *B. riparius*/ *V. americana*/ *A. flexuosus* at the 75% watering level produced comparable shoot biomass as the *B. riparius* monoculture treatments, but the inclusion of native legumes did not increase growth beyond that of the *B. riparius* monoculture. *B. riparius* produced the greatest aboveground biomass at 100% and 75% watering level, and the lowest aboveground biomass at the 25% watering level. There was no difference in the belowground biomass of *B. riparius* between the watering treatments. *A. flexuosus* was the only native legume that showed differences in above and belowground biomass by watering treatment, with greater aboveground biomass at the moderate watering levels (50% and75%), and greater belowground biomass at the 50% watering level, suggesting potential drought adaptation in this species.

4.2 Introduction

The establishment of native legumes in competition with other species is a function of their relative growth rates (RGR's) (Cooper, 1977). *B. riparius* has faster establishment rates compared to many native species (Hamel et al., 2008). Species competition is further affected by environmental stressors such as water limitation (Cooper, 1977). Once established, native legumes have lower relative losses of biomass compared to tame legume species under drought stress, but this trait is species-specific (Hofer et al., 2016; Komainda et al., 2019). Legumes have a higher competitive advantage compared to grasses when conditions are favorable for nitrogen fixation and are able to produce more biomass (Lauenroth and Dodd, 1979). The wide range of drought resistance strategies of perennial legumes should be evaluated for improved resistance through plant selections (Pang et al., 2011).

The effects of drought are evident at all plant life stages (Farooq et al., 2009). Drought resistance is generally associated with deep, vigorous root systems and faster shoot growth under water limiting conditions (Polania et al., 2017). The timing, duration and severity of drought affect the plant response and rate of growth and development (Farooq et al., 2009). Drought tolerance is a complex combination of physiological and biochemical processes, reflected by phenotypic variance of the plant (Farooq et al., 2009). The extent of yield reduction is species-specific and affected by the phenological state of the plant at the time of water stress (Daryanto et al., 2015). Overall, root systems are shallower and wider in arid climates which receive limited amounts of precipitation, this allows space to absorb the next rainfall as the root zones are quickly depleted of water (Schenk and Jackson, 2002; Guswa, 2010). The relative depth of roots however, compared to the amount of aboveground biomass, increases linearly with aridity (Schulze et al, 1996; Schenk and Jackson, 2002).

Drought not only limits growth of the legume species directly through physiological stress, but also limits the availability of nutrients for plant growth and maintenance in the environment. Drought stress can reduce the yield of native legumes by negatively affecting root nodulation, which decreases the ability to fix atmospheric nitrogen (Furlan et al., 2012; Hofer et al., 2017). Plants respond to dry conditions differently by allocating biomass either to roots or shoots (Skinner and Comas, 2010; Furlan et al., 2012). There is a need to understand the RGR’s and biomass allocation, both above- and belowground, in response to water limitation in specific native legume species, which have potential for forage production in the Canadian prairies.

The objective of this study was to determine if differences exist between the selected native legume species in response to drought stress and inter-species competition in a controlled environment by measuring the above- and belowground biomass in monocultures and in mixture with *B. riparius*. We hypothesize that the biomass reduction of native legume-grass mixtures will be less than *B. riparius* under drought stress due to greater drought resistance of the native species.

4.3 Materials and Methods

A greenhouse experiment was conducted at the Agriculture and Agri-Food Swift Current Research and Development Centre September 2017 to January 2018 and repeated September 2018 to January 2019 to compare the above- and belowground biomass accumulation of monocultures and legume:grass mixtures under varying watering treatments. Plant species were the same as the field trial (see Table 3.1). Briefly, *Bromus riparius* and four native legumes of the Canadain prairies; *Astragalus flexuosus*, *Dalea purpurea*, *Hedysarum boreale* and *Vicia americana* were used in the study. Germination rates were 91%, 94%, 75%, 38% and 76%, for *B. riparius*, *D. purpurea*, *V. americana*, *H. boreale* and *A. flexuosus* respectively.

4.3.1 Experimental Design

The greenhouse experiment was organized as a Randomized Complete Block Design with four replications. Watering levels were defined as 100%, 75%, 50% and 25% of the field water holding capacity. There were 1600 mL of 1:4 vermiculite to field soil in each pot. The field soil was collected from a field southeast of Swift Current, SK within the Brown soil zone. The water holding capacity of the soil mixture in the test pots was calculated to be 700mL (100%), 500mL (75%), 300mL (50%) and 200mL (25%) according to Gessert (1976). The experimental treatments are combinations of species monocultures/mixtures and watering levels (Table 4.1) and included 44 treatments per replication, totalling 176 test pots for each trial.

Table 4.1 Experimental treatments with group, function, number of plants per plant of each species, species and abbreviations.

Mixture	Plants Per Pot	Species	Abbreviation
1	3	*Bromus riparius*	BR
2	3	*Astragalus flexuosus*	AF
3	3	*Dalea purpurea*	DP
4	3	*Hedysarum boreale*	HB
5	3	*Vicia americana*	VA
6	1:1:1	---	BR/DP/VA
7	1:1:1	---	BR/DP/HB
8	1:1:1	---	BR/DP/AF
9	1:1:1	---	BR/VA/HB
10	1:1:1	---	BR/VA/AF
11	1:1:1	---	BR/HB/AF

Legume seeds were mechanically scarified using sand paper to weaken hard seed coats in an attempt to break exogenous physical dormancy prior to planting in vermiculite trays. The greenhouse photoperiod was 16 hours of light per day and the mean temperature was 20°C.

Seedlings were maintained in the vermiculite trays for approximately two weeks prior to transplanting and 10-52-10 fertilizer was applied weekly at 1:20 ratio with water to ensure healthy plant growth until they were transplanted to soil. The seedlings were transplanted into test pots containing the pre-measured volume of soil mixture at double the target density of three plants per pot once they reached the two true leaf stage.

After transplanting the seedlings, 150 mL of No-Damp (calcium chloride, sodium chloride and potassium chloride) at a concentration of 1:100 (v/v) applied to prevent fungal infection of newly transplanted seedlings. Test pots were watered for approximately seven days, until pots showed signs of saturation defined as pooling water on the soil surface. Once established, duplicate plants were removed from the pots in order to obtain three plants per pot for each treatment. Any weed contaminants from the soil were removed during this process.

Fertilizer was applied during the first week after transplanting using 10-52-10 at a 1:20 ratio using the lowest watering level of 200 mL, with plants at higher watering levels receiving water for the remaining volumes. Test pots were watered approximately every three days during the water application phase for both experimental runs. For treatments requiring the higher water amounts, watering was done gradually to allow enough water drainage such that pots did not overflow and reduce the volume applied. Insects (mainly aphids) in the first run of the study were controlled using biological method of placing lady bugs (*coccinellids)* in the greenhouse.

4.3.2 Data Collection

4.3.2.1 Aboveground Biomass

Three separate aboveground biomass harvests were conducted for each experiment run to obtain the aboveground dry matter for each species in test pots using the same pots throughout. Harvest 1 occurred approximately 30 days following transplanting, harvest 2 thirty days following harvest 1 and the final harvest approximately 14 days after harvest 2. The harvested material was placed in paper bags and dried in the forced air oven at 60°C for 48 hours and weighed. Comparison of the above ground biomass was made by determining dry matter yield by species for harvest 1, 2 and 3.

4.3.2.2 Belowground Biomass

After the aboveground biomass harvest, roots with soils from each test pot were placed into a Delta-T Devices Ltd. Root Washer RWC-UM-2, Cambridge, England (Delta-T, 1995) to separate roots from the surrounding soil. The root washer consists of four plastic buckets mounted over a plastic table. Garden hoses attached to each bucket swirl water pushed upward from a plastic sump pump in the root washer’s main holding tank.

Individual plants were not separated within test pots due to the intertwining nature of the roots, therefore bulk root weight was determined for each pot. After removing plants from the root washer, roots were rinsed clean. The root samples were then dried at room temperature and weighed.

4.4 Statistical Analysis

The data was analyzed for Analysis of Variance using the mixed model (SAS statistical software, 2014) to examine the effects of species monoculture/mixture and watering level on forage aboveground and belowground biomass. The experimental model was a Randomized Complete Block Design with 2 x 2 factorial arrangement. The fixed effects were species monoculture/mixture, and watering level, and their interaction, and the random effect in the model was the replication. A significance value of $P<0.05$ was used and means comparisons made using the studentized Tukey multi-treatment method at $P=0.05$. Degrees of freedom were calculated using Satterthwaite's method.

4.5 Results

4.5.1 Aboveground and Belowground Biomass by Species and Watering Level

The above-ground and belowground biomass differed among the different species monocultures/mixtures ($P<.0001$) and watering levels ($P<.0001$). There was no significant interaction effect of species x watering level on aboveground biomass ($P=0.874$) or belowground biomass ($P=0.645$) (Table 4.2).

Table 4.2 The Analysis of Variance for aboveground and belowground biomass.

Factor	df†	P value	
		Aboveground Biomass	Belowground Biomass
Mixture (M)	10	<.0001	<.0001
Water Level (WL)	3	<.0001	<.0001
M*WL	30	0.874	0.645

† Degrees of freedom

B. riparius produced the greatest aboveground biomass at 100% and 75% water holding capacities, and the lowest aboveground biomass at 25% water holding capacity. There was no difference in the belowground biomass of *B. riparius* between the watering treatments. Of the mixtures, *B. riparius*/ *D. purpurea*/ *A. flexuosus* and *B. riparius*/ *V. americana*/ *H. boreale* at the 25% watering level produced less shoot biomass compared to the other watering treatments. At 50% and 75% watering level, *B. riparius*/ *H. boreale*/ *A. flexuosus* and *B. riparius*/ *V.*

americana/ *A. flexuosus* showed the greatest belowground biomass growth compared to the high (100%) and low (25%) watering treatments (Table 4.3).

The monoculture legume treatments showed less overall above and belowground biomass compared to the mixtures containing *B. riparius*. *A. flexuosus* was the only native legume with differences in above and belowground biomass by watering treatment. The aboveground biomass was greater at the moderate watering levels (50% and75%), with the least produced at the 25% water holding capacity. *A. flexuosus* allocated more belowground biomass at the 50% watering level compared to high and low water treatments, with the least produced at the 25% water holding capacity (Table 4.3).

Table 4.3 Aboveground and belowground biomass measured for species mixtures at different watering levels in the greenhouse.

	Water Level (% field holding capacity)											
Mixture*	25	50	75	100			25	50	75	100		
	Aboveground Biomass (g pot^{-1})						Belowground Biomass (g pot^{-1})					
					P Value	SEM					P Value	SEM
BR	1.7^{aB}	2.7^{aA}	2.8^{aA}	2.2^{aAB}	0.007	0.27	2.2^{a}	2.5^{a}	3.1^{a}	2.8^{a}	0.478	0.43
BR/DP/AF	1.3^{abB}	2.4^{abA}	2.2^{abAB}	1.3^{abcAB}	0.056	0.29	1.3^{abc}	2.2^{a}	2.4^{a}	2.1^{ab}	0.076	0.31
BR/VA/HB	1.3^{abB}	2.1^{abcAB}	2.6^{aA}	1.3^{bcAB}	0.009	0.25	1.3^{bc}	1.9^{ab}	2.0^{abc}	2.1^{ab}	0.157	0.28
BR/DP/HB	1.3^{ab}	2.1^{abc}	2.0^{ab}	1.5^{ab}	0.183	0.28	1.5^{ab}	1.8^{ab}	2.1^{ab}	2.2^{ab}	0.654	0.50
BR/HB/AF	1.5^{ab}	2.5^{ab}	2.7^{a}	1.2^{bc}	0.081	0.34	1.2^{bcB}	2.1^{abA}	2.4^{aA}	1.7^{abcAB}	0.006	0.22
BR/DP/VA	1.4^{ab}	1.9^{abc}	1.9^{ab}	1.3^{abc}	0.625	0.29	1.3^{abc}	1.9^{ab}	2.2^{a}	1.9^{abc}	0.141	0.27
BR/VA/AF	1.4^{ab}	2.3^{ab}	2.8^{a}	1.1^{bc}	0.090	0.36	1.1^{bcB}	2.0^{abA}	2.3^{aA}	1.8^{abcAB}	0.005	0.22
AF	1.3^{abB}	1.7^{bcA}	1.9^{abA}	0.7^{bcdAB}	0.009	0.17	0.7^{bcdB}	1.1^{bcdA}	0.9^{bcdAB}	0.7^{cdAB}	0.024	0.15
VA	0.9^{bc}	1.3^{c}	1.3^{bc}	0.7^{bcd}	0.629	0.25	0.7^{bcd}	1.2^{bc}	0.8^{cd}	1.0^{bcd}	0.253	0.16
DP	0.2^{c}	0.2^{d}	0.2^{c}	0.2^{d}	0.710	0.06	0.2^{d}	0.1^{d}	0.1^{d}	0.1^{d}	0.267	0.024
HB	0.9^{bc}	1.2^{c}	1.1^{bc}	0.5^{cd}	0.773	0.29	0.5^{cd}	0.6^{cd}	0.6^{d}	0.4^{cd}	0.260	0.086
P value	<.0001	<.0001	<.0001	<.0001			<.0001	<.0001	<.0001	<.0001		
SEM†	0.49	0.62	0.70	0.38			0.38	0.28	0.42	0.46		

*AF = *Astragalus flexuosus*, BR = *Bromus riparius*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different (P > 0.05).

AB = Means within a row with the same upper case letter are not significantly different (P > 0.0

4.6 Discussion

Drought stress reduces biomass in all species, but the degree to which it is reduced differs by individual species (Komainda et al., 2018). Drought tolerance is physiologically complex because water deficits reduce plant growth at all developmental stages (Farooq et al., 2009). The degree of drought tolerance of a plant is a reflection of their ability to respond phenotypically (Farooq et al., 2009). Species with higher growth rates are better able to adapt to drought conditions than species with slower RGR's because they have both physiological and morphological root adaptations (Suriyagoda et al., 2012). In our experiment, *B. riparius* had a much faster RGR compared to the native legumes, producing the greatest above- and belowground biomass, regardless of watering level. The literature suggests that grass species will increase allocation of biomass to roots in water limiting conditions (Skinner and Comas, 2010), but in our study the reduction of water did not increase the allocation of biomass to roots in *B. riparius*. The roots were, however, limited by the test pot size and the results may be different in field conditions.

Even though perennial legumes are also known to have a wide range of strategies to cope with drought, water reduction in legumes was still expected to be positively correlated with decreasing yields (Pang et al., 2011; Purushothaman et al., 2013; Daryanto et al., 2015). In *Phaseolus vulgaris*, drought resistance has been correlated with deep, vigourous roots and better shoot growth (Polania et al., 2017), but deep roots do not necessarily confer drought resistance (Passioura, 1983). There is an optimal shoot:root ratio for each species which maximizes the use of water depending on the supply (Passioura, 1983). *A. flexuosus* was the only native legume that showed differences in above and belowground biomass by watering treatment, with greater aboveground biomass at the moderate watering levels (50% and75%), and greater belowground biomass at the 50% watering level, suggesting potential drought adaptation in this species.

No changes in above- and belowground biomasses were observed for *V. Americana, H. boreale* and *D. purpurea*, regardless of the water level applied. During the greenhouse study it was noted that *D. purpurea* had a slow growth rate relative to the other native legumes, and this made comparison of biomass response to watering level difficult to assess since very little biomass was produced regardless of watering treatment. The observed differences in legume biomass production are likely due to species differences, since legumes have similar water use efficiencies (Blessing et al., 2018).

Plant roots are critical to maximize hydraulic conductance of water from the soil and as the number of species in a mixture increases there is more competition for resources so roots become longer (Skinner and Comas, 2010; Valdez, 2014). During the root washing procedure it was observed the native legumes had much less root mass compared to *B. riparius*'s fibrous roots. In comparison, the native legumes were non-branched, thick taproots with small filamentous roots shooting laterally from the main root, occupying less space in the test pots. The belowground competition created in mixture pots was expected to reduce plant growth (Cahill, 2003), this theory was not supported with most mixtures yielding similar biomass compared to monoculture *B. riparius* in this study.

Severe water stress was anticipated to negatively affect the nodulation of the legumes (Furlan et al., 2012). The field soil used in test pots was collected near Swift Current, therefore the soil bacterium present were reflective of the Brown soil zone but the presence of species-specific inoculum was not confirmed. Nodules were observed on the roots of all four native legume species, but the nitrogen fixation activity was not assessed in this study. Increased levels of nitrogen from nodules were expected to increase the level of competition between legumes and grasses in mixtures (Laurenroth and Dodd, 1979). The inclusion of legumes in the greenhouse mixtures did not increase nitrogen significantly to increase growth beyond that of the *B. riparius* monoculture. For the mixtures, it is possible that nitrogen was being supplied to the test pot from the nodules, but *B. riparius* was consuming available amounts.

The study did not allow for plants to reach maturity, therefore may not have been enough time to evaluate the response of the relatively slow-growing legumes, particularly *D. purpurea*, compared to *B. riparius*. The effects on seedling growth may differ if this test had been performed on mature plants. There was above- and belowground biomass differences between the *A. flexuosus* monoculture treatments in response to water level but the effect of different relative growth rates of the legume species could not be separated from the water level response in this study.

5. EVALUATION OF THE STAND ESTABLISHMENT SUCCESS OF NATIVE LEGUMES USING DIFFERENT SEEDING RATES

5.1 Abstract

Determining the optimum seeding rate for native legumes is difficult owing to their hard seed coats, high degrees of dormancy, and staggered germination and emergence. While many generalized seeding rates are suggested in the literature, few species-specific seeding rate trials have been conducted on native legumes in Canada. We examined the effect of different seeding rates on seedling count, percent cover and dry matter yield by increasing the amount of pure live seeds per metre (PLS m^{-1}) to double and triple the generalized recommendations. A seeding rate trial was conducted in 2018 and 2019 at a site located in the Brown soil zone, near Swift Current, SK. The experimental design was a Randomized Complete Block Design, seeding rates were 100, 200 and 300 PLS m^{-1}, and species were monocultures of *Astragalus flexuosus, Dalea purpurea*, *Hedysarum boreale*, *Vicia americana*. Seedling counts showed significant differences among legume species and between the different seeding rates. Foliar cover also showed treatment differences for species and seeding rate, but the interaction of these factors on percent cover was not significant. Dry matter yield showed treatment differences between species, but it was not affected by seeding rate or the interaction of the two factors. There was no correlation between the first year forage yield and native legume seed size (r^2=0.0054, P=0.618).

5.2 Introduction

Forage stand establishment is determined by the successful germination and survival of new seedlings in plant populations (Eriksson and Ehrlen, 2008). This phase in the plant life cycle is the most important because mortality is the highest (Eriksson and Ehrlen, 2008). Generally plants with low germination have low levels of establishment (Hillhouse and Zedler, 2011). Under environmental stress and competition, rapid germination is crucial for seedling survival (McGraw, 2003). While fast germination is important, many wild species rely on life cycle strategies including staggered germination over time, which prevent seedling mortality under unfavorable conditions (Thompson, 2000). Dormancy is not synonymous with persistence of seeds in the soil, but rather functions to delay germination until certain conditions are met (Thompson, 2000).

The vigour and viability of seeds depends on both genetic and physiological factors whereas dormancy characteristics depend on the environmental conditions at seed set, development, and maturation (Kelly et al., 1992; Shaban, 2013). Exogenous physical dormancy, such as hard seed coats, are characteristics of grassland species where water availability, fire and wildlife consumption are common (Rolston, 1978; Baskin, 2003). Hard seeds are unable to imbibe water and therefore the seed coat cannot soften enough to germinate (Rolston, 1978). This physical dormancy may occur in combination with other types of dormancy such as temperature requirements (Roston, 1978). The break down of hard seed coats is greater near the soil surface, which protects the seed from germinating at unfavorable depths (Russi et al., 1992).

Different seed lots of the same species can vary in field emergence even when planted in the same environment (Shaban, 2013). Since establishment of forage legumes needs to occur in a reasonable period of time, scarification to abrade the seed coat can be used to increase the germination where hard seed coats are preventing germination (Vogel, 2002; Kimura and Islam, 2012; Jones et al., 2015; Schellenberg and Biligetu, 2015; Dittus and Muir, 2019). Survival is affected by seed size with small seeded species more likely to persist in the soil, but large seeded species more likely to survive once they have emerged from the soil (Jakobsson and Eriksson, 2000; Thompson, 2000; Hillhouse and Zedler, 2011). This differential survival of grassland species is important to understand species dynamics and abundance (Jakobsson and Eriksson, 2000).

Some legume species have comparatively greater survival under competitive stress, whether imposed by other species or by sibling plants (Cooper, 1977; Cheplick, 1992). Successful establishment of native legumes is a combination of germination strategy, seedling growth rates and competitive ability to remain in the plant population. Where native and tame species are planted in mixtures, the competitive ability of native species can be reduced (Cooper, 1977; Hamel et al., 2008). The density of native perennial legumes appears to increase linearly with seeding rate in the first year of growth (Fischbach et al., 2006). Appropriate seeding rates should be informed by research because the typical measurements of germination, purity and hard seeds are not relatable to establishment in field conditions (Vogel, 2002; Barr et al., 2017). The best way to relate seed lot characteristics to establishment is to measure seedling density following seeding for a particular seed lot (Vogel, 2002).

Due to the variation of native legume establishment success, the appropriate seeding rate is important if forage mixtures are to be successfully established (Cooper 1977; Vogel, 2002; Hamel et al., 2008; Houck, 2009). Observing how herbaceous perennial plant populations change over a range of seeding rates is useful for determining the ideal seeding rates (Burton et al., 2006). Considering the relatively high cost of native seed, it is important to determine the minimal amount required to obtain target plant densities in a given environment. For forage mixtures, the relative contribution of legume to grass seed may need to be adjusted based on this relationship.

For this study we hypothesized that the establishment of selected native legumes will increase linearly with seeding density due to lower realized germination in field compared to laboratory conditions. The objective of this study was to determine the optimal spring seeding rate for four native legume species in the Brown soil zone near Swift Current, SK. A trial was conducted to compare the establishment success and biomass yields of the selected native legumes under varying seeding rates as germination and establishment rates vary amongst the selected species.

5.3 Materials and Methods

5.3.1 Plant Material

Dalea purpurea Vent., was purchased from Pickseed (Calmar, AB), and *Vicia americana* Muhl. ex Willd was from Brett Young Seed (Winnipeg, MB). *Hedysarum boreale* Nutt. was purchased from Granite Seeds (North Lehi, UT) and *Astragalus flexuosus* Douglas ex G. Don seeds were provided by the Agriculture and Agri-Food Canada Swift Current Research and Development Centre.

5.3.2 Germination Test

A germination study was conducted to compare the cumulative germination of the four native legume species in a controlled environment. Following scarification, 50 seeds of each species were placed into an 85 mm diameter petri dish with filter paper and kept moist with distilled water at 21◦C. Seeds with radicle emergence were counted each day for 12 days. The germination rates were 94%, 75%, 66% and 76%, for *D. purpurea*, *V. americana*, *H. boreale* and *A. flexuosus* respectively.

5.3.3 Experimental Design

The field test was organized as a Randomized Complete Block Design with four replications. The two factors used in this study were legume species (*A. flexuosus* (AF)*, D. purpurea* (DP)*, H. boreale* (HB) and *V. americana* (VA)) and seeding rate (100, 200 and 300 pure live seeds per metre PLS m^{-1}). Therefore the treatments were: 1) AF100; 2) DP100; 3) HB100; 4) VA100; 5) AF200; 6) DP200; 7) HB200; 8) VA200; 9) AF300; 10) DP300; 11) HB300; 12) VA300. The test was seeded on May 18, 2018 at AAFC Swift Current Research and Development Centre using a 6-row disc seeder at a depth of 1.3 cm with three seeded rows per plot and no border rows between plots. Winter wheat pathways were seeded between replications and around the test edges. Seeds were scarified prior to seeding using a mechanical scarification machine designed for alfalfa for the small seeded species (*A. flexuosus* and *D. purpurea*) and air scarification for the relatively large seeded species (*H. boreale* and *V. america*) (Table 5.1).

Table 5.1 Seed weights for selected native legume species.

Legume Species*	1000 seed weight (gms)
AF	0.004
DP	0.01
HB	0.09
VA	0.19

*AF = *Astragalus flexuosus*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

5.3.4 Data Collection

Stand establishment was assessed by counting seedling number in July and September in 2018. The percent foliar cover was measured on 7 June 2019 using a visual assessment of vegetative cover by plot. The percent cover of each plant species was visually estimated by standing over a 1m ruler placed randomly in the middle plot rows, 0.3m from the edges and looking downwards. The percentage of the ruler covered by each plant species was recorded to give foliar cover. This procedure was repeated twice per plot to obtain a mean percent cover for the legume species in the plot. Each plot was sampled for biomass in July of 2019 by hand harvesting, using sickles, randomly selected, two 1m rows in each plot. The samples were dried in the forced air oven at 60°C for 48 hours to obtain dry weight.

5.4 Statistical Analysis

The data was analyzed for Analysis of Variance using the mixed model (SAS statistical software, 2014) to examine the effects of species mixture and seeding on foliar cover, seedling count and forage dry matter yield. The experimental model was a Randomized Complete Block Design with 2x2 factorial treatment design with the main effects of legume species and seeding rate, and their interaction and the random effect of replicate. A significance value of $P<0.05$ was used and means comparisons made using the studentized Tukey multi-treatment method at $P=0.05$. Degrees of freedom were calculated using Satterthwaite's method. Where data were not normally distributed, arcsine transformations was performed to improve normality and stabilize the sample variance (Tsai et al., 2017).

5.5 Results

The seedling counts varied among the different native legume species ($P<.0001$) and seeding rates ($P<.0001$). There was an interaction effect ($P<.0001$) between legume species x seeding rate, indicating the number of emerged seedlings was different for each species between the seeding rates. Foliar cover (%) varied among the different native legumes ($P<.0001$) and seeding rates ($P=0.012$) with no observed interaction effects. Forage dry matter yield varied among the native legume species ($P=0.005$), but not by seeding rate, and no interaction effects were observed (Table 5.2).

Table 5.2 The Analysis of Variance for seedling count (plants m-1), foliar cover (%), and forage dry matter yield (kg ha^{-1}).

Factor	df[†]	P value		
		Seedling Count	Foliar Cover	Forage Yield
Species (S)	3	<.0001	<.0001	0.005
Seeding Rate (R)	2	<.0001	0.012	0.716
S*R	6	<.0001	0.652	0.721

[†] Degrees of freedom

5.5.1 Germination Test

The native legume seeds showed species differences in patterns of germination including the number of seeds germinating per day over time, the time to maximum germination and the total percent germination. The total percent germination measured in the laboratory was used to adjust the seeding rates for the field study to ensure target pure live seeds per area was reached (Figure 5.1).

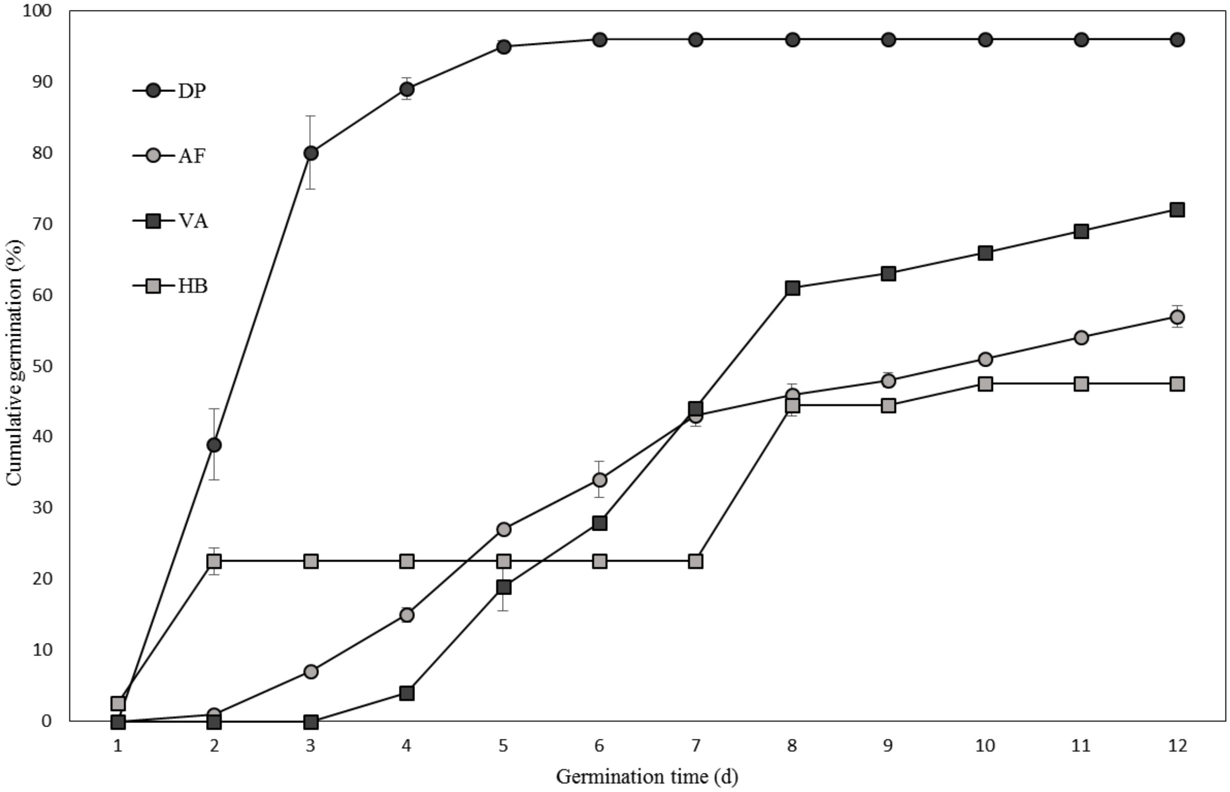

Figure 5.1 The cumulative germination of scarified native legume seeds (AF = *Astragalus flexuosus*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*) over a 12-day period.

5.5.2 Seedling count

Seedling counts were significantly different for both species and seeding rate (Table 5.3). *V. americana* had the highest number of seedlings counted in July and September 2018 at all seeding rates, comparable only to *H. boreale* at the100 PLS m^{-1} rate in September. *H. boreale* had the second highest seedling counts in July and September. The seedling count for VA and HB in both months, and DB in September, increased with increasing seeding rate. *A. flexuosus* and *D. purpurea* seedling count were comparatively lower, increasing slightly by September of the establishment year in 2018. There was a strong positive correlation (r^2=0.7797, $P<.0001$) between seed size and seedling count.

Table 5.3 Seedling count as number of seedlings per metre (count/m^2) by species and by seeding rate in pure live seeds per metre (PLS m^{-1}) for 2018.

	Seeding Rate (PLS m^{-1})									
Species*	100	200	300			100	200	300		
	July					September				
				P value	SEM†				P value	SEM
AF	8^{c}	8^{c}	5^{c}	0.111	3.4	5^{b}	12^{c}	12^{c}	0.088	4.2
DP	12^{c}	17^{c}	21^{c}	0.200	5.4	6^{bB}	17^{cAB}	35^{cA}	0.047	4.5
HB	57^{bC}	78^{bB}	119^{bA}	<.0001	5.5	93^{aB}	117^{bB}	152^{bA}	0.004	9.6
VA	80^{aC}	143^{aB}	188^{aA}	0.0004	11.6	113^{aC}	204^{aB}	274^{aA}	0.001	14.0
P value	<.0001	<.0001	<.0001			<.0001	<.0001	<.0001		
SEM	9.8	7.6	20.4			6.9	22.1	20.3		

*AF = *Astragalus flexuosus*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different ($P > 0.05$).

A, B, C = Means within a row with the same upper case letter are not significantly different ($P > 0.05$).

5.5.3 Foliar Cover

Foliar cover showed treatment differences again for species and seeding rate, but the interaction of these factors on foliar cover was not significant (Table 5.4). *V. americana* and *H. boreale* had the highest seedling counts, therefore it was not surprising that the foliar cover of these legumes was also greater than *D. purpurea* and *A. flexuosus*. Interestingly, *D. purpurea* showed rapid germination in the lab compared to the other three native legumes taking only 6 days to reach maximum germination compared to 10 days for *H. boreale* and 12 days for *V. americana* and *A. flexuosus*, yet stand establishment in the field plot was poor (Table 5.4). There was a positive correlation (r^2=0.4792, $P<.0001$) between foliar cover and seed size.

Table 5.4 Foliar cover (%) by species and for seeding rates 100, 200 and 300 pure live seeds per metre (PLS m^{-1}) for June 2018.

	Seeding Rate (PLS m^{-1})				
Species*	100	200	300		
	Foliar Cover (%)			P value	SEM†
AF	13^{bB}	43^{bA}	25^{bAB}	0.012	12.4
DP	17^{b}	35^{b}	36^{b}	0.342	10.2
HB	86^{a}	88^{a}	92^{a}	0.329	2.7
VA	65^{aB}	86^{aAB}	89^{aA}	0.027	5.6
P value	0.0001	0.0008	0.0003		
SEM†	4.3	7.9	9.2		

*AF = *Astragalus flexuosus*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

a, b, c, d, e, f = Means within a column with the same lower case letter are not significantly different ($P > 0.05$).

A, B = Means within a row with the same upper case letter are not significantly different ($P > 0.05$).

5.5.4 Forage Dry Matter Yield

Forage dry matter yield was different by species but it was not affected by seeding rate or the interaction of the two factors (Table 5.5). There was no correlation between forage yield and seed size (r^2=0.0054, P=0.618), given *A. flexuosus* and *D. purpurea* seeds are relatively small compared to *H. boreale* and *V. americana* (Table 5.5). Arcsin data transformation did not result in treatment differences between species at the 300 PLS m^{-1} seeding rate.

Table 5.5 Forage yield (kg ha^{-1}) by species and for seeding rates 100, 200 and 300 pure live seeds per metre (PLS m^{-1}) harvested July 2019 at Swift Current.

	Seeding Rate (PLS m^{-1})		
Species*	100	200	300
AF	1354^{ab}	3201^{a}	3925
DP	259^{b}	413^{c}	323
HB	2979^{a}	2543^{ab}	2406
VA	1133^{ab}	1103^{bc}	1140
P value	0.053	0.007	0.397
SEM†	660.2	475.9	1517.3

*AF = *Astragalus flexuosus*, DP = *Dalea purpurea*, HB = *Hedysarum boreale*, VA = *Vicia americana*.

† SE = Standard error of the means.

a, b, c = Means within a column with the same lower case letter are not significantly different ($P > 0.05$).

5.6 Discussion

Seed with low germination usually has poor field establishment, but success of stand establishment varies under field conditions even for species with high germinating seeds (Hillhouse and Zedler, 2011; Shaban, 2013). Since germination itself does not account for the emergence and survival of the seedling, seed lot tests do not give an accurate estimate of stand establishment (Vogel, 2002; Hillhouse and Zedler, 2011). For native plants, more accurate seeding rates can be obtained by measuring the number of emerged seedlings per gram of seed (Vogel, 2002).

There is known to be a positive correlation between seed size and seedling establishment, since larger seeds develop more vigorous seedlings which are more competitive than smaller seedlings (Turnbull et al., 1999; Jakobsson and Eriksson, 2000). These differences in seed size and seedling survival are thought to allow for different reproductive strategies in stressful environments (Marshall, 1986). Our study does support the theory that small seeded forage species are more difficult to establish (Townsend and McGinnies, 1972), since fewer seedlings emerged per area for the smaller seeded *A. flexuosus* and *D. purpurea* and they had poorer establishment compared to larger seeded *H. boreale* and *V. americana*.

Previous studies have shown linear increases in native plant establishment with higher seeding rates in the first year (Fishbach et al., 2006). In the present study, seeding rate did have a positive effect on the seedling densities of *D. purpurea*, *H. boreale* and *V. americana*, but no clear trend was observed for *A. flexuosus*. Of the four legumes, *H. boreale* and *V. americana* had the highest seedling count and the greatest foliar cover, but this did not correlate with the forage yields. This suggests that neither seedling count, nor estimates of foliar cover are accurate predictors of biomass production in native legume stands. The biomass production should be re-assessed during the second growing season since the correlation between seeding rate and biomass production begins to differ by species in years following the first production year (Fishbach et al., 2002).

The forage dry matter yield of the native legumes did not increase linearly with increased seeding rate for the four legume species. *D. purpurea* in particular is known to have low seedling vigour and low biomass production (Fishbach et al., 2006). Increasing plant densities in the plots may have created greater intra-species competition and crowded out seedlings. Research is still needed to determine to determine whether reductions from 100 PLS m^{-1} would produce comparable biomass, thereby saving cost on seed.

6. GENERAL DISCUSSION AND FUTURE RESEARCH

Research on the forage production of native legumes in multi-species stands is lacking and there is a need to study their variation in seeded mixtures (McGraw et al., 2004; Mischkolz et al., 2013; Muir et al., 2019). Research is needed not only to make species selections for mixed stands, but also to determine species-specific seeding rates and best harvest management practices (Foster et al., 2014). The assessment of native perennials should be made at multiple sites over multiple years to understand their performance (Mitchell et al., 2015).

Our experiments were conducted to better understand the agronomic characteristics of four selected native legume species to determine their forage potential in mixtures with *B. riparius* in the Brown and Dark Brown soil zones of Saskatchewan. This study offers the establishment and production potential of their inclusion in mixture with meadow bromegrass over a 3-year span.

6.1 Forage Production

There is limited information on performance of native prairie plants in seeded forage mixtures (Schellenberg et al., 2012). The establishment and production in mixtures with *B. riparius* showed the potential of combining these particular native legumes with a tame forage grass. Since each component in a mixture varies in competitiveness, we could also have assessed these legumes in mixtures with native grasses. The composition and attributes of each species in a mixture determines the overall biomass production (Hammermeister et al., 2003), but because of poor establishment in mixtures with *B. riparius*, the monoculture legume treatments gave a better indication of the legumes' agronomic potential. Future studies should include evaluation of native grass mixtures and stands seeded in alternate grass legume rows to determine the effect of competition on establishment and productivity.

Mixtures of native species have been shown to be as productive as monocultures in the long-term and as profitable as nitrogen fertilized stands (Sanderson et al., 2013; Serajchi et al., 2018). The inclusion of native legumes in this study did not significantly increase the forage production of mixtures compared to monoculture grass treatments and the percentage of legumes contributing to forage yield in mixtures was low. Competition may increase the yield of one species at the expense of another (Springer et al., 2001). The addition of the legumes did not, however, decrease forage dry matter yield compared to monoculture *B. riparius*. The monoculture legume seeding provided a better understanding of the forage yield potential of individual species at the two sites. *A. flexuosus* monoculture was able to produce as much biomass as *B. riparius* by the second growing year which was unexpected given the generally low biomass production of most native legumes compared to tame forage grasses. Grown in mixtures however, *A. flexuosus* did not increase the yield relative to the *B. riparius* monoculture. High plant density can increase plant competition within the stand but low plant density allows for more weed invasion due to slower stand establishment (Houck, 2009).

The forage nutritive value of the mixtures was generally similar to monoculture BR, but the native legume monocultures provided insight into the quality that each species would contribute if established in sufficient ratio to the grass. The native legumes had higher P, Ca and Mg, particularly in the August harvest, compared to mixture containing *B. riparius*. These higher levels of Mg and P are known to help to regulate the homeostasis of Ca in cattle, lowering the risk of milk fever (Boda and Cole, 1954; Griffith, 1974; Grunes and Welch, 1989; DeGaris and Lean, 2009). The native legumes also had higher CP, and lower ADF and NDF compared to *B. riparius*, which is consistent with previous research findings (McGraw, 2004). Native legumes maintained higher digestibility into the late growing season compared to *B. riparius* and the mixtures, supporting conclusions from previous studies (Sleugh et al., 2000; Gierus et al., 2011; Mischkolz et al., 2013; Simili da Silva et al., 2013; Biligetu et al., 2014).

These four legume species have more potential for inclusion in native rangeland restorations and long-term grazing systems rather than intensive forage production systems. Since each legume-grass mixture varies in biomass production, many complex mixtures remain to be tested (Simili da Silva et al., 2014). Detailed species compositions in Swift Current suggest that legume establishment continued to increase in the second growing season. Further studies of the long-term persistence of native legumes is recommended to determine their agronomic potential (Muir et al., 2018).

6.2 Soil Fertility

Legumes are expected to have a positive effect on nitrogen levels in the soil (Sphen et al., 2015). Nutrient additions have been shown to vary by both the legume species and by the companion species (Sphen et al., 2002). Although studies have shown grasses benefit from legumes in mixtures by receiving additional nitrogen, additional nutrients also results in greater competition from the grass (Laurenroth and Dodd, 1979; Gierus et al., 2011). The inclusion of native legumes in mixture with *B. riparius* did not significantly increase the nutrient levels in the soil at either site. While nitrogen fixation may have been occurring where legumes were established, the mixture plots were mainly composed of *B. riparius*, reducing the amount of nitrogen fixed relative to a pure legume stand , and any addition of nitrogen would have been taken up by the fast growing *B. riparius*.

Studies have shown reduced nodulation activity in legume roots during periods of drought (Furlan et al., 2012; Skinner and Comas, 2018). Nodules were observed during the greenhouse study, but did not result in increased biomass production in the mixture pots. The monoculture field plots did generally have higher nitrogen levels than plots that contained *B. riparius*, but again this may have been the result of high growth rates and production of *B. riparius* rather than nitrogen contributions from the native legumes. Since the weather during the 2017 and 2018 growing seasons was relatively hot and dry, nodulation may have been reduced in the field plots. Future studies should further explore the nitrogen fixing capabilities of these plants, particularly in dry vs. wet conditions. Microbial analysis of soil inoculum could be performed to determine if these sites had these legume species growing in them previously.

6.3 Drought Response

Some native species already possess adaptations such as drought tolerance that make them good candidates for breeding in anticipation of climate changes (Mitchell et al., 2015). Under extreme drought stress, legumes are known to be more resistant than non-legumes (Hofer et al., 2016). However during the greenhouse study it was observed that *B. riparius* seedlings maintained high above and belowground growth under low water conditions compared to the native legumes. The response of *D. purpurea* to water limitation was difficult to assess because of the low RGR of this species. *V. americana* did not appear drought tolerant, but only one seed lot was tested. For future work, the drought response of multiple varieties and seed lots should be analyzed for each legume species to increase the gene pool being tested and account for variations. A variety that is specific to the prairie environment should be selected for screening, such as *V. americana* var. Hooker which is described by Kennicer and Norton (2008).

Drought stress was expected to affect the biomass of the four legume species differently (Hofer et al., 2016; Komainda et al., 2019). Even though the semi-arid site had lower yields, *B. riparius* establishment and production was good in all treatments at both sites. The native legumes performed differently between the two sites and within each site. *A. flexuosus* was the highest yielding native legume overall, and persisted in mixtures during dry conditions in 2017 and 2018 at the Swift Current site. The other legume species did not establish well, contributed very little to the yields, or disappeared from the stands during the same period. Drought coping strategies in perennial legumes should be a goal in breeding programs (Pang et al., 2011).

Roots are important for adaptation to drought because they affect the water use of plants and the ability to draw water from the soil (Schenk and Jackson, 2002; Vandez, 2014; Polonia et al., 2017). The greenhouse study looked at the root mass produced under differential water conditions but did not assess changes in root morphology or nodulation since test pot depth was not conducive to evaluation of the whole root structures. Future studies should evaluate the change of root length, morphology and nodulation activity of different native legume species under competition and moisture stress by growing these species hydroponically in sand. Changes in root morphology under drought stress also differs between legumes and grasses (Skinner and Coma, 2010).

During the seeding rate trial, scarified *H. boreale* seed established well in the dry conditions but un-scarified seed did not establish well in the 2016 field trial, planted in one of the top ten wettest years on record in Swift Current (AAFC, 2016). Although the monoculture *H. boreale* plots did not establish, it is possible that weed pressure alone was enough competition to prevent the establishment of this species. Perhaps competition is the limiting growth factor for this native legume species in mixed stands.

6.4 Seeding Rates

The best way to determine the success of a seeding rate is to observe changes in plant populations over time (Burton et al., 2006), so we decided to measure seeding rate success by estimating plot cover and seedling number in addition to biomass yield. Legume species had a significant effect on establishment and yield of the treatments in the seeding rate trial. This is consistent with findings by Fishbach et al. (2006) that the correlation between seeding rate and biomass differs by species after the first growing year. Our study showed no significant difference in biomass with increased seeding rate in 2019. Since the data could not be normalized using data transformations, the statistical analysis was impacted by having large variance between species' forage yield, with some species yielding very little and some yielding comparatively higher. Current grassland seeding rates are inaccurate in predicting establishment success so more studies like this one are needed (Barr et al., 2017). Future work should assess whether seeding rates of less than 100 PLS m^{-1} can be used to achieve adequate stand productivity while reducing the cost of use in forage mixtures. Native forage seed is relatively expensive (Barr et al., 2017), so it is important to determine the minimum quantity that could be used to gain benefits from legume inclusion in mixtures.

Although dormant seeds are considered viable, dormancy must be broken in order to use viability as a measure of germination (Bradbeer, 1988). Hard seeds can't imbibe water and fail to germinate in otherwise favourable conditions (Rolston, 1978). The degree of legume seed hardness is influenced by the environmental conditions at maturity for each seed lot (Kelly et al., 1992). Mechanical scarification is the best method to treat large, plump seeds and is commonly used for *Melilotus officinalis* and *Astragalus cicer* L. (Acharya et al., 2006; MacKenzie and Tremblay, 2007). Mechanical scarification has only been found effective for a portion of hard seeds of *Medicago sativa*, with some requiring temperature striation in order to germinate (Hall et al., 1998). The best method of scarification is specific to each species, therefore must be tested for each (Dittus and Muir, 2009). Further studies should compare the results using scarified versus non-scarified seeds to determine whether seed dormancy is having a significant effect on stand establishment and productivity.

Germination in legumes with hard seed coats is likely also controlled by temperature fluctuations so other methods of breaking seed dormancy, such as stratification, should also be explored (Martin and Cushwa, 1966; Rolston, 1978; Baskin, 2003). Other scarification techniques such as thermal and chemical have already shown success in increasing the germination of native legume species (Jones et al., 2005). These other techniques should be explored since rapid seed germination is critical for seedling survival, especially when under drought stress (McGraw, 2003).

Native legume seeding rates are important not only for seeding forage stands, but also for native prairie restorations and land reclamations. There continues to be a need to increase the diversity of native seed available (Muir et al., 2018). Future work with native legumes should continue exploring methods such as seeding rate, row spacing, seeding depth, etc. for individual sites so that potential users can make informed decisions about the economic viability of their projects.

Of the four native legumes tested, *A. flexuosus* appears to be the most promising species for forage production in the Brown and Dark Brown soil zones of Saskatchewan. The small percentage, or absence, of the other three legumes in test plots suggests that they are challenging to establish, are out competed by *B. riparius*, or take much longer than *B. riparius* to reach mature forage stands. Continued interest in native prairies species will help to identify species with agronomic potential for future breeding and genetic improvement. These native species have the potential to not only be productive, but are also adaptable to the conditions that are characteristic of the Canadian prairies.

7. LITERATURE CITED

Acharya, S.N., Kastello, J.P., Beauchermin, K.A., Messenger, D.F. 2006. A review of research progress on cicer milkvetch (Astragalus cicer L.). Can. J. Plant Sci. 86(1):49-62.

Acton, D.F., Padbury, G.A., Stushnoff, C.T. 1998. The Ecoregions of Saskatchewan. Contributing authors: Gallagher, L., Gauthier, D., Kelly, L., Radenbaugh, T., Thorpe, J. Canadian Plains Research Centre, Saskatchewan Environment and Resource Management, University of Regina. Hignell Printing Ltd, Winnipeg, MB. 205 pp.

Adjesiwor, A.T., Anowarul Islam, M., Zheljazkov, V.D., Ritten, J.P., Garcia y Garcia, A. 2017. Grass-legume seed mass ratios and nitrogen rates affect forage accumulation, nutritive value and profitability. J. Crop Sci. 57:2852-2864.

Aerts, R., Boot, R.G.A., van der Aart, P.J.M. 1991. The relation between above- and belowground biomass allocation patterns and competitive ability. J. Oecologia. 87:551-559.

Ajmal Khan, M., Weber, D.J., eds. 2008. Ecophysiology of high salinity tolerant plants. Springer, the Netherlands.

Acton, D.F., Padbury, G.A., Stushnoff, C.T. 1998. The Ecoregions of Saskatchewan. University of Regina, Canadian Plains Research Center & Saskatchewan Environment and Resource Management. Hignell Printing Ltd., Winnipeg, MB. 205 pp.

Alberta Agriculture and Food. 2016. Alberta range plants and their classification. Government of Alberta. Agri-Facts. Agdex 134/06. 9 pp.

Alberta Agriculture and Food. 2018. Using 1,000 kernel weight for calculating seeding rates and harvest losses. Government of Alberta. Agri-Facts. Agdex 100/22-1. 5 pp.

Allen, J., Tilley, D. 2014. Plant Guide for American Vetch (Vicia americana). USDA-Natural Resources Conservation Service, Idaho Plant Materials Center. Aberdeen, Idaho 83210.

ANKOM. 2017. Acid Detergent Fiber in Feeds- Filter Bag Technique (for A200 and A2001). ANKOM Technology, NY, USA. 2pp.

ANKOM. 2017. Neutral Detergent Fiber in Feeds- Filter Bag Technique (for A200 and A2001). ANKOM Technology, NY, USA. 2pp.

Ball, D.M., Collins, M., Lacefield, G.D., Martin, N.P., Mertens, D.A., Olson, K.E., Putnam, D.H., Undersander, D.J., Wolf, M.W. 2001. *Understanding Forage Quality*. American Farm Bureau Federation Publication 1-01, Park Ridge, IL. 17 pp.

Baskin, C. 2003. Breaking physical dormancy in seeds- focussing on the lens. J. New Phytologist. 158:227-238.

Barr, S., Jonas, J.L., Paschke, M.W. 2017. Optimizing seed mixture diversity and seeding rates for grassland restoration. J. Restoration Ecol. 25(3):396-404.

Beckage, B., Gross, L.J. 2006. Overyielding and species diversity: what should we expect? J. New Phyt. 172:140-148.

Beegle, D.B. 2014. Soil fertility management for forage crops: maintenance. Penn State University, U.S. Department of Agriculture, Agronomy Facts 31-C. 6 pp.

Bhattarai, K., Bushman, S., Johnson, D.A., Carman, J.G. Phenotypic and genetic characterization of western prairie clover collections from the western United States. J. Rangeland Ecol. Manage. 63:696-706.

Biligetu, B., Jefferson, P.G., Muri, R., Schellenberg, M.P. 2014. Late summer forage yield, nutritive value, compatibility of warm- and cool-season grasses seeded with legumes in western Canada. Can. J. Plant Sci. 94: 1139 - 1148.

Blessing, C.H., Mariette, A., Kaloki, P., Bramley, H. 2018. Profligate and conservative: water use strategies in grain legumes. J. Exp. Biol. 69(3):349-369.

Bocsi, T., Allen, J.M., Bellemare, J., Kartesz, J., Nishino, J., Nishino, M., Bradley, B.A. 2016. Plants' native distributions do not reflect climatic tolerance. J. Diversity and Distributions. 22:615-624.

Boda, J.M., Cole, H.H. 1954. The influence of dietary calcium and phosphorous on the incidence of milk fever in dairy cattle. J. Dairy Sci. 37:360-372.

Bolinder, M.A., Angers, D.A., Belanger, G., Michaud, R., Laverdiere, M.R. 2002. Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. Canadian J. Plant Sci. 84(2):731-737.

Bonsal, B., Regier, M. 2007. Historical comparison of the 2001/2002 drought in the Canadian Prairies. J. Clim. Res. 33:229-242.

Bradbeer, J.W. 1988. Seed viability and vigour. Seed Dormancy and Germination. Tertiary Level Biology. Spring, Boston, MA.

Budd, A.C. 1957. Wild Plants of the Canadian Prairies. Experimental Farm Service, Canada Department of Agriculture, publication 983. Queens Printer, Ottawa, ON. 348 pp.

Burton, C., Burton, P. 2003. Growing and Using Plants in the Northern Interior of B.C.: Vicia americana Muhl. Ex. Willd. American vetch. Symbios Research & Restoration. Pp 103-106.

Burton, C.M., Burton, P.J., Hebda, R., Turner, N.J. 2006. Determining the optimal sowing density for a mixture of native plants used to revegetated degraded ecosystems. J. Restoration Ecol. 14(3):379-390.

Bushman, B.S., Larson, S.R., Peel, M.D., Pfrender, M.E. 2007. Population structure and genetic diversity in North American Hedysarum boreale Nutt. J. Crop Sci. 47:1281-1288.

Buxton, W.R., Redfearn, D.D. 1997. Plant Limitations to Fiber Digestion and Utilization. J. of Nutr. 127(5):8145-8185.

Caballero, R., Goicoechea, E.L., Hernaiz, P.J. 1995. Forage yields and quality of common vetch and oat sown at varying seeding ratios and seeding rates of vetch. J. Field Crops Research. 41:135-140.

Cahill Jr., J.F. 2003. Lack of relationship between belowground competition and allocation to roots in 10 grassland species. J. Ecol. 91:532-540.

Cane, J.H. 2006. An evaluation of pollination mechanisms for purple prairie-clover, Dalea purpurea (Fabaceae: Amorpheae). J. Amer. Midl. Nat. 156:193-197.

Cane, J.H., Weber, M., Miller, S. 2012. Breeding biologies, pollinators and seed beetles of two prairie-clovers, *Dalea ornate* and Dalea searlsiae (Fabaceae-Amorphae), from the intermountain west, USA. J. Western North Amer. Naturalist. 72(1)16-20.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M., Weis, J.J. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proc. Natl. Acad. Sci. U.S.A. 104(46): 18123-18128.

Capenter, A.T., Allen, M.F. 1987. Responses of Hedysarum boreale Nutt. to mycorrhizas and Rhizobium: plant and soil nutrient changes in a disturbed shrub-steppe. J. New. Phytol. 109:125-132.

Cheplick, G.P. 1992. Sibling competition in plants. Journal of Ecol. 80:567-575.

Collins, M., Newman, Y.C. 2018. Forages, Volume 1: An Introduction to Grassland Agriculture, 7th ed. Chapter 14: Forage Quality. Eds. Collins, M., Nelson, C.J., Moore, K.J., Barnes, R.F. John Wiley & Sons, Inc., NJ, USA. 411 pp.

Cook, C.W., Stoddart, L.A., Harris, L.E. 1956. Compartive Nutritive Value and Palatability of Some Introduced and Native Forage Plants for Spring and Summer Grazing. Utah State University, Bulletin 35, 6-1956. Pp 1-39.

Cooper, C.S. 1977. Growth of the legume seedling. J. Advances in Agronomy. 29:119-139.

Cutforth, H.W. 2000. Climate change in the semiarid prairie of southwestern Saskatchewan: temperature, precipitation, wind, and incoming solar energy. Can J. Soil Sci. 80(2): 375-385.

Daryanto, S., Wang, L., Jacinthe, P. 2015. Global synthesis of drought effects on food legume production. J. PLOS ONE 10(6): e0127401.

DeGaris, P.J., Lean, I.J. 2009. Milk fever in dairy cows: a review of pathophysiology and control principles. The Veterinary Journal 176:58-69.

Delta-T Devices Ltd., 1995. Installation Guide: Root Washer, Version 2. N. Webb ed. Cambridge, England, UK. 13 pp.

Dittus, D.A., Muir, J.P. 2010. Breaking germination dormancy of Texas native perennial herbaceous legumes. J. Native Plants. 11(1):5-10.

Ender, F., Dishington, I.W., Helgebostad, A. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemix paresis puerperalis. J. Tierphysiol Tierernahr Futtermittelkd. 28(5):233-256.

Erikkson, O., Ehrlen, J. 2008. Seedling Ecology and Evolution, Chapter 11: Seedling Recruitment and Population Ecology. Cambridge University Press, Cambridge, p. 239-254.

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

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. 2009. Plant drought stress effects, mechanisms and management. J. Agron. Sust. Dev. 29:185-212.

Fischbach, J.A., Ehlke, N.J., Peterson, P.R., Wyse, D.L., Swanson, D.R., Sheaffer, C.C. 2006. Seeding rate affects establishment of native perennial legumes in the upper Midwestern US. J. Native Plants. 7(1):61-68.

Foster, A., Vera, C.L., Malhi, S.S., Clarke, F.R. Forage yield of simple and complex grass-legume mixtures under two management strategies. Can. J. Plant Sci. 94(1): 41-50.

Frame, J. 2005. Forage Legumes for Temperate Grasslands. In association with Food and Agriculture Organization of the United Nations. Science Publishers, Inc., New Hampshire, 309 pp.

Fuez, D., Isrealsen, C., Young, A., Holmgren, L. 2012. Buying and selling corn silage or other high moisture feeds: value the feed not the water. Utah State University, Cooperative Extension, Agriculture. AG/Agribusiness/02.

Furlan, A., Llanes, A., Luna, V., Castro, S. 2012. Physiological and biochemical responses to drought stress and subsequent rehydration in the symbiotic association peanut-Bradyrhizobium sp. International Scholarly Research Network. Volume 2012, Article ID 318083, 8 pp.

Gargallo-Garriga, A., Sardans, J., Perez-Trujillo, M., Rivas-Ulbach, A., Oravec, M., Vecerova, K., Urban, O., Jentsch, A., Beierkuhnlein, C., Parella, T., Penuelas, J. 2014. Opposite metabolic responses of shoots and roots to drought. J. Sci. Reports. 4(6829):1-7.

Gessert, G. 1976. Measuring a medium's airspace and water holding capacity. Ornamentals Northwest Archives. 1(8):11-12.

Gierus, M., Kleen, J., Loges, R., Taube, F. 2012. Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. J. Animal Feed Sci. and Tech. 172:150-161.

Goff, J.P. 2009. Phosphorous Deficiency. Food Animal Practice, 5th ed. Chapter 34. pp. 135-137.

Government of Manitoba, 2003. NDF Digestibilities: A New Analysis to Evaluate Forage Quality. [Accessed 31 Jan 2020]. Available from <https://www.gov.mb.ca/agriculture/livestock/production/dairy/ndf-digestibilities-a-new-analysis.html>

Griffith, W.K. 1974. Satisfying the Nutritional Requirements of Established Legumes. In: Forage Fertilization. Eds. Mays, D.A. Madison, WI. Pp 147-169.

Grime, J.P. 1979. Plant Strategies, Vegetation Processes, and Ecosystems Properties. Chapter 1: Primary Strategies in the Established Phase. Wiley, New York, 417 pp.

Gunes, D.L., Welch, R.M. 1989. Plant contents of magnesium, calcium and potassium in relation to ruminant nutrition. 67:3485-3494.

Guswa, A.J. 2010. Effect of plant uptake strategy on the water-optimal root depth. J. Water Resources Research. 46(9):1-5.

Hall, J.W., Stout, D.G., Brooke, B.M. 1998. Alfalfa seed germination tests and stand establishment: The role of hard (water impermeable) seed. Can. J. Plant Sci. 78:295-300.

Hamel, C., Iwaasa, A., Schellenberg, M. 2008. Plant community composition in pastures seeded with native plant species in southwest Saskatchewan. Preceedings of Soil and Crop Workshop, Saskatoon, SK.

Hammermeister, A.M., Naeth, M.A., Schoenau, J.J., Biederbeck, V.O. 2003. Soil and plant response to wellsite rehabilitation on native prairie in southeastern Alberta, Canada. Can J. Soil. Sci. 83: 507-519.

Hanelt, P., Mettin, D. 1989. Biosystematics of the genus Vicia L. (Leguminosae). Annu. Rev. Ecol. Syst. 20:199-223.

Haynes, R.J. 1980. Competative aspects of the grass-legume association. Advances in Agronomy 33:227-261.

Hillhouse, H.L., Zedler, P.H. 2011. Native species establishment in tallgrass prairie plantings. 166(2):292-308.

Hofer, D., Suter, M., Haughey, E., Finn, J.A., Hoekstra, N.J., Buchmann, N., Luscher, A. 2016. Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. J. Applied Ecol. 53:1023-1034.

Holtzapple, M.T. 2003. Hemicelluloses. In The Encyclopedia of Food Sciences and Nutrition, 2nd ed. Eds. Caballero, E., Finglas, P., Toldra, F. Academic Press, Cambridge, UK. Pp 3060-3071.

Houck, M.J. 2009. Understanding seeding rates, recommended planting rates, and pure live seed (PLS). United States Department of Agriculture, Natural Resources Conservation Service, Louisiana. Technical Note No.11.

Huang, Q.Q., Jin, L., Xu, Z., Barbieri, L.R., Acharya, S., Hu, T.M., McAllister, T.A., Stanford, K., Wang, Y. 2015. Effects of purple prairie clover (Dalea purpurea Vent.) on feed intake, nutrient digestibility and faecal shedding of Escherichia coli O157:H7 in lambs. J. Animal Feed Sci. & Tech. 207:51-61.

Iwaasa, A., Wang, L.Y., Scianna, J.D., Han, G. 2013. Forage and nutritional benefits of grazing purple prairie clover and white prairie clover on western Canadian grasslands. Proceedings of the 10th Prairie Conservation and Endangered Species Conference, Patterns of Change: Learning From Our Past To Manage our Present And Conserve Our Future, Winnipeg, MB.

Jakobsson, A., Eriksson, O. 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. J. Oikos. 88:494-502.

Jin, L., Wang, Y., Iwaasa, A.D., Xu, Z., Schellenberg, M.P., Zhang, Y.G., Liu, X.L., McAllister, T.A. 2012. Effect of condensed tannins on ruminal degradability of purple prairie clover (Dalea purpurea Vent.) harvested at two growth stages. J. Animal Feed Sci. & Tech. 176:17-25.

Jin, L., Wang, Y., Iwaasa, A.D., Li, Y., Xu, Z., Schellenberg, M.P., Liu, X.L., McAllister, T.A., Stanford, K. 2015. Purple prairie clover (Dalea purpurea Vent) reduces fecal shedding of Escherichia coli in pastured cattle. J. Food Prot. 78(8):1434-41.

Johnson, D.A., Ford, T.M.J., Rumbaugh, M.D., Richardson, B.Z. 1989. Morphological and physiological variation amoung ecotypes of sweetvetch (Hedysarum boreale Nutt.). J. Range Management. 42(6): 496-501.

Jones, C.D., Stevens, M.R., Jolley, V.D., Hopkins, B.G., Jensen, S.L., Turner, D., Stettler, J.M. 2016. Evaluation of thermal, chemical, and mechanical seed scarification methods for 4 Great Basin lupine species. J. Native Plants. 17(1):5-18.

Jump, S.J., Marchant, R., Penuelas, J. 2009. Environmental change and the value of genetic diversity. J. Trends in Plant Sci. 14(1):51-58.

Jungers, J.M., Eckberg, J.O., Betts, K., Mangan, M.E., Wyse, D.L., Sheaffer, C.C. 2016. Plant roots and GHG mitigation in native perennial bioenergy cropping systems. J. GCB Bioenergy. 9:326-338.

Kasworm, W.F., Irby, L.R., Ihsle Pac, H.B. 1984. Diets of ungulates using winter ranges in northcentral Montana. J. Range Management. 37(1):67-71.

Kelly, K.M., Van Staden, J., Bell, W.E. 1992. Seed coat structure and dormancy. J. Plant Growth Regulation. 11:201-209.

Kenicer, G., Norton, S. 2008. Vicia Americana Leguminosae. The Board of Trustees of the Royal Botanic Gardens, Kew. Pp 342-349.

Kimura, E., Islam, M.A. 2012. Seed scarification methods and their use in forage legumes. Research J. Seed Sci 5(2):38-50.

Kitchen, S.G. 1992. Perennial forb life-history strategies on semiarid rangelands: implications for revegetation. Symposium on Ecology, Management and Restoration of Intermountain Annual Rangelands, Boise, ID.

Kopp, J.C., McCaughey, W.P., Wittenberg, K.M. 2003. Yield, quality and cost effectriveness of using fertilizer and/or alfalfa to improve meadow bromegrass pastures. Can. J. Animal Sci. 83:291-298.

Knowles, R.P., Baron, V.S., McCartney, D.H. 1993. Meadow bromegrass. Agriculture and Agri-food Canada: Publication 1889/E. Ottawa, ON.

Komainda, M., Kuchenmeister, K., Kuchenmeister, F., Breitsameter, L., Wrage-Monnig, N., Kayser, M., Isselstein, J. 2019. Forage legumes for future dry climates: lower relative biomass losses of minor forage legumes compared to Trifolium repens under conditions of periodic drought stress. J. Agro. Crop. Sci. 0:1-10.

Kulshreshtha, S., Wheaton, E., Wittrock, V. 2016. Vulnerability and Adaptation to Drought: The Canadian Prairies and South America. Chapter 4: The Impacts of the 2001-2002 Drought in Rural Alberta and Saskatchewan, and Canada. Eds. Diaz, H.P., Warren, J.W., Hurlbert, M.A. University of Calgary Press, Calgary, AB. 376 pp.

Lauenroth, W.K., Dodd, J.L. 1954. Response of native grassland legumes to water and nitrogen treatments. J. Range Management. 32(4):292-294.

Leyshon, A.J., Kilcher, M.R., McElgunn, J.D. 1981. Seeding rates and row spacings for three forage crops grown alone or in alternate grass-alfalfa rows in southwestern Saskatchewan. Can. J. Plant Sci. 61:711-717.

Lindgren, D.T. 1992. Variation in ornamental traits of Dalea purpurea Vent. (Purple prairie clover). J. Hort Sci. 27(8):927.

Littledike, E.T., Goff, J. 1987. Interactions of calcium, phosphorous, magnesium and vitamin D that influence their status in domestic meat animals. J. Anim. Sci. 65(6): 1727-43.

Looman, J. 1983. 111 Range and Forage Plants of the Canadian Prairies. Agriculture Canada, Ottawa, ON. 261 pp.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. J. Sci. 294:804-808.

Luscher, A., Mueller-Harvey, I., Soussana, J.F., Rees, R.M., and Peyraud, J.L. 2014. Potential of legume-based grassland-livestock systems in Europe: a review. J. Grass and Forage Sci. 69(2): 206-228.

Mackenzie, J., Tremblay, M. 2007. Successful Forage Crop Establishment. Saskatchewan Forage Council. [Viewed on 2 Feb 2020]. Available from < http://www.saskforage.ca/sfc/low/docs establishment_bulletin.pdf>.

Mader, E., Shepherd, M., Vaughan, M., Black, S.H., LeBuhn, G. 2011. Attracting Native Pollinators: Protecting North America's Bees and Butterflies. Storey Publishing, North Adams, MA. 371 pp.

Mahli, S.S., Brandt, S., Gill, K.S. 2003. Cultivation and grassland type effects on light fraction and total organic C and N in a Dark Brown Chernozemic Soil. Can. J. Soil Sci. 83(2):145-153.

Malhi, S.S., K.S. Gill, D.H. McCartney, and R. Malmgren. 2004. Fertilizer management of forage crops in Canadian Great Plains. J. Crop Sci. 1: 237–271

Mangan, M.E., Sheaffer, C., Wyse, D.L., Ehlke, N.J., Reich, P.B. 2011. Native perennial grassland species for bioenergy: Establishment and Biomass Productivity. J Agron. 103(2):509-519.

Marshall, D.L. 1986. Effect of seed size on seedling success in three species of Sesbania (Fabaceae). American J. of Botany.

Martin, R.E., Cushwa, C.T. 1966. Effects of heat and moisture on leguminous seeds. Proceedings: 5th Tall Timbers Ecology. Pp 159-175.

McGinn, S.M. 2010. Weather and Climate Patterns in Canada's Prairie Grasslands. In Arthropods of Canadian Grasslands (Volume 1): Ecology and Interactions in Grassland Habitats. Edited by J.D. Shorthouse and K.D. Floate. Biological Survey of Canada. Pp 105-119.

McGraw, R.L., Shockley, F.W., Elam, T.K. 2003. Effects of temperature on germination of 10 native legume species. Native Plants J. 4: 5-9.

McGraw, R.L., Shockley, F.W., Thompson, J.F. and Roberts, C.A. 2004. Evaluation of native legume species for forage yield, quality and seed production. J. Native Plants. 5(2):152-159.

Mitchell, M.L., Norman, H.C., Whalley, R.D.B. 2015. Use of functional traits to identify Australian forage grasses, legumes and shrubs for domestication and use in pastoral areas under climate change. J. Crop and Pasture Sci. 66:71-89.

Mischkolz, J.M., Schellenberg, M.P., Lamb, E.G. 2013. Early productivity and crude protein content of establishing forage swards composed of combinations of native grass and legume species in mixed-grassland ecoregions. Can. J. Plant Sci. 93:445-454.

Moss, E. H. 1983. Flora of Alberta: a Manual of Flowering Plants, Conifers, Ferns, and Fern Allies Found Growing Without Cultivation in the Province of Alberta, Canada, 2nd ed. Packer, J.G. eds. University of Toronto Press, Toronto, 687 pp.

Muir, J.P., Dubeux Jr., J.C.B., Dos Santos, M.V.F., Maposse, I.C., Pitman, W.D., Butler, T.J. 2014. Challenges to domesticating native forage legumes. J. Tropical Grasslands 2:94-96.

Muir, J.P., Pitman, W.D., Smith, F.S., Lloyd-Reilley, J., Shadow, R.A. 2018. Challenges to developing native legume seed supplies: the Texas experience as a case study. J. Native Plants. 19(3):225-238.

Muir, J.P., Santos, M.V.F., da Cunha, M.V., Dubeux Junior, J.C., Lira Junior, M..D., Souza, R.T.D., Souza, T.C. 2019. Value of endemic legumes for livestock production on Caatinga rangelands. J. Animal Sci. 14(2):1-12.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., Luscher, A. 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. J. Applied Ecol. 46(3):683-691.

Ogyle, D., St. John, L., Holzworth, L.K., Jensen, K.B. 2003. Meadow Brome: *Bromus biebersteinii*. United States Department of Agriculture, Natural Resources Conservation Service, Plant Guide. 44 pp.

Pang, J., Yang, J., Ward, P., Siddique, K.H.M., Lambers, H., Tibbet, M., Ryan, M. 2011. Contrasting responses to drought stress in herbaceous perennial legumes. J. Plant Soil. 348:299-314.

Pahl, M.D., Smreciu, A. 1999. Growing native plants of western Canada: common grasses and wildflowers. Alberta Agriculture, Food and Rural Development, Edmonton, AB. 118 pp.

PaiMazumder, D., Sushama, L., Laprise, R., Naveed, Khaliq, M., Sauchyn, D. 2003. Canadian RCM projected changes to short- and long-term drought characteristics over the Canadian prairies. Int. J. Climatol. 33:1409-1423.

Pang, J., Yang, J., Ward, P., Siddique, K.H.M., Lambers, H., Tibbett, M., Ryan, M. 2011. Contrasting responses to drought stress in herbaceous perennial legumes. J. Plant Soil 348:299-314.

Passioura, J.B. 1983. Roots and drought resistance. J. Ag. Water Mgmt. 7:265-280.

Paton, B., Nyren, A. 2015. Long-term grazing intensity research in the Missouri Coteau region of North Dakota: effects on plant production and composition – Final Report. Central Grasslands Research Extension Center, North Dakota State University Central Grasslands Extension Center.

Peace River Forage Association. ND. Peace Forage Seeding Tool: American Vetch. [Accessed 12 March 2020]. Available from < http://www.peaceforagetool.ca/species/american-vetch>

Peel, M.D., Waldron, B.L., Mott, I.W., Walker, S. 2011. A growth stage and heat-unit model for Hedysarum boreale. J. Native Plants. 12(1):5-12.

Peng, K., Shirley, D.C., Xu, Z., Huang, Q., McAllister, T.A., Chaves, A.V., Acharya, S., Liu, C., Wang, S., Wang, Y. 2016. Effect of purple prairie clover (Dalea purpurea Vent.) hay and its condensed tannins on growth performance, wool growth, nutrient digestibility, blood metabolites and ruminal fermentation in lambs fed total mixed rations. J. Animal Feed Sci. & Tech. 222:100-110.

Peterson, P.R., Scheaffer, C.C., Hall, M.H. 1992. Drought effects on perennial forage legume yield and quality. Agron. J. 84:774-779.

Polania, J., Rao, I.M., Cajiao, C., Grajales, M., Rivera, M., Velasquez, F., Raatz, B., Beebe, S.E. 2017. Shoot and root traits contribute to drought resistance in recombinant inbred lines of MD 23-24 x SEA 5 of common bean. Frontiers in Plant Science. 8(296):1-18.

Posler, G.L., Lenseen, A.W., Fine, F.L. 1993. Forage yield, quality, compatibility, and persistence of warm-season grass-legume mixtures. Agron. J. 85:554-560.

Purushothaman, R., Zaman-Allah, M., Mallikarjuna, N., Pannirselvam, R., Krishnamurthy, L., Gowda, C.L.L. 2013. Root anatomical traits and their possible contribution to drought tolerance in grain legumes. J. Plant Prod. Sci. 16(1):1-8.

Rayburn, E.B. 2018. Horse and Pasture Management. Eds. Sharpe, P. Chapter 13, Forages, Matching Plant Species to Your Environment, Weather, and Climate. Pp 233-243.

Redente, E.F. 1982. Sweetvetch seed germination. J. Range Management. 35(4):469-472.

Reaume, T. 2009. 620 Wild Plants of North America. University of Regina: Plains Research Centre, University of Regina. University of Toronto Press, Toronto, ON. 784 pp.

Robinson, P. 1998. Interpreting your forage test report. California Alfalfa and Forage Review, University of California. 2 pp.

Rolston, M.P. 1978. Water impermeable seed dormancy. The Botanical Review 44:365-396.

Rosales, M. 2014. Utah Sweetvetch. United States Department of Agriculture, National Resources Conservation Service, Upper Colorado Environmental Plant Center, Meeker, Colorado.

Russi, L., Cocks, P.S., Roberts, E.H. 1992. Hard-seededness and seed bank dynamics of six pasture legumes. J. Seed Sci. Research. 2:231-241.

Ruyle, G.B., Bowns, J.E. 1985. Forage use by cattle and sheep grazing separately and together on summer range in southwestern Utah. J. Range Management. 38(4):299-302.

Sanderson, M.A., Goslee, S.C., Soder, K.J., Skinner, R.H., Tracy, B.F., Deak, A. 2006. Plant species diversity, ecosystem function, and pasture management—A perspective. Can. J. Plant Sci. 87:479-487.

SAS. 2014. SAS Interface Version 3.0.5.0. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA.

Saskatchewan Forage Council. 2007. Forage Factsheet- Purple Prairie Clover. Saskatchewan Forage Council, Dryland Forage Species Adaptation CD.

Sauchyn, D.J., Skinner, W.R. 2001. A proxy record of drought severity for the Southwestern Canadian Plains. Canadian Water Resources J. 26(2):253-272.

Scheaffer, C.C., Wyse, D.L., Ehlke, N.J. 2009. Palatability and Nutritive Value of Native Legumes. NPJ. 10(3): 225-231.

Schellenberg, M.P., Banerjee, M.R. 2012. The potential of legume-shrub mixtures for optimum forage production in southwestern Saskatchewan: A greenhouse study. Can. J. Plant Sci. 82: 357-363.

Schellenberg, M.P., Biligetu, B. 2015. The effects of temperature and scarification on seed germination of three Dalea species. Can. J. Plant Sci. 95:1117-1120.

Schellenberg, M.P., Biligetu, B., Iwaasa, A.D. 2012. Species dynamic, forage yield and nutritive value of seeded native plant mixtures following grazing. Can. J. Plant Sci. 92:699-706.

Schellenberg, M.P., Waddington, J., King, J.R. 1993. Sod-seeding alfalfa in spring into established crested wheatgrass in southwest Saskatchewan. Can. J. Plant Sci. 74:293-301.

Schenk, H. Jackson, R.B. 2002. Rooting depths, lateral root spreads and belowground/above-ground allometries of plants in water-limited ecosystems. J. Ecol. 90:480-494.

Schroeder, J.W. 2012. Interpreting forage analysis. North Dakota State University Extension Services, AS-1080. 2 pp.

Schulze, E.D., Mooney, H.A., Sala, O.E., Jobbagy, E., Buchmann, N., Bauer, G., Canadell, J., Jackson, R.B., Loreti, J., Oesterheld, M., Ehleringer, J.R. 1996. Rooting depth, water availability, and vegetation cover along an aridity gradient in Patagonia. J. Oecologia. 108:503-511.

Serajchi, M., Lamb, E.G., and Schellenberg, M.P. 2015. Forage Production and Crude Protein of Seven Native Species in a 4-Year Stand., Soil and Crops Workshop, Saskatoon, SK, Canada, March 23-24, 2015.

Serajchi, M., Schellenberg, M.P., Mischkolz, J.M., Lamb, E.G. 2018. Mixtures of native perennial forage species produce higher yield than monocultures in a long-term study. Can. J. Plant Sci. 98(3): 633-647.

Shaban, M. 2013. Study on some aspects of seed viability and vigor. Int. J. Advanced Biological and Biomedical Research. 1(12):1692-1697.

Simili da Silva, M., Tremblay, G.F., Belander, G., Lajeunesse, J., Papadopoulos, Y.A., Fillmore, S.A.E., Cabreira Jobim, C. 2014. Forage energy to protein ratio of several legume-grass complex mixtures. J. Animal Feed Sci. and Tech. 188:17-24.

Skinner, R.H., Comas, L.H. 2010. Root distribution of temperate forage species subjected to water and nitrogen stress. J. Crop Sci. 50:2178-2185.

Sleugh, B., Moore, K.J., George, R., Brummer, E.C. 2000. Binary legume-grass mixtures improves forage yield, quality, and seasonal distribution. Agron. J. 92(1):24-29.

Smoliak, S. 1988. The Northern Great Plains—Canadian connection. J. Rangelands. 10(5):210-212.

Sorensen, J.T., Holden, D.J. 1974. Germination of native prairie forb seed. J. of Range Management. 27(2):123-126.

Spehn, E.M., Scherer-Lorenzen, M., Schmid, B., Hector, A., Caldeira, M.C., Dimitrakopoulos, P.G., Finn, J.A., Jumpponen, A., O'Donnovan, G., Pererira, J.S., Schulze, E.-D., Troumbis, A.Y., Korner, C. 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. J. Oikos. 98(2):205-218.

Spellenberg, R. 1976. Chromosome numbers and their cytotaxonomic significance for North American Astragalus (Fabaceae). J. Taxon. 25(4):463-476.

Springer, T.L., Aiken, G.E., McNew, R.W. 2001. Combining ability of binary mixtures of native, warm-season grasses and legumes. J. Crop Sci. 41:818-823.

Stevens, O.A. 1932. The number and weights of seed produced by weeds. American J. of Botany. 19(9):784-794.

St. John, L., Tilley, D., Jensen, K. 2012. Plant Guide for Meadow Brome (Bromus biebersteinii). UDSA-Natural Resources Conservation Services, Aberdeen Plant Materials Center. Aberdeen, Idaho 83210.

Stokes, S.R., Prostko, E.P. 1998. Understanding forage quality analysis. AgriLife Extension, Texas A&M System. L-5198, 3-98.

Stubbendieck, J., Conrad, E.C. 1989. Common Legumes of the Great Plains: An Illustrated Guide, 1st ed. University of Nebraska Press, Lincoln, NE 685588, USA. pp. 329.

Stubbendieck, J., Hatch, S.L., Landholt, L.M. 2003. North American Wildland Plants, 6th ed. University of Nebraska Press, 501 pp.

Sturludottis, E. 2011. Forage Quality and Yield in Grass-Legume Mixtures in Northern Europe and Canada, Master's thesis, Faculty of Physical Sciences, University of Iceland, pp 62.

Suriyagoda, L.D.B., Ryan, M.H., Renton, M., Lambers, H. 2012. Adaptive shoot and root responses collectively enhance growth at optimum temperature and limited phosphorus supply of three herbaceous legume species. J. Annals of Botany 110:959-968.

Suter, M., Ramseier, D., Guesewell, S., Connolly, J. 2007. Convergence patterns and multiple species interactions in a designed plant mixture of five species. J. Oecologica. 151:499-511.

Swoboda, K.A., Cane, J.H. 2012. Breeding biology and incremental benefits of outcrossing for the restoration wildflower, Hedysarum boreale Nutt. (Fabaceae). J. Plant Species Biol. 27:138-146.

Tamayo-Chim, M., Reyes-Garcia, C. 2012. A combination of forage species with different responses to drought can increase year-round productivity in seasonally dry silvo-pastoral systems. J. Agroforest Syst. 84:287-297.

Tannas, K. 2004. Common Plants of the Western Rangelands. Volume 3. Alberta Agriculture, Food and Rural Development, Edmonton, AB. 505.

Tepedino, V.J. 1987. Bee visitors of sweetvetch, Hedysarum boreale boreale (Leguminosae), and their pollen-collecting activities. Great Basin Naturalist. 47(2):314-318.

Thompson, K. 2000. Seeds: The Ecology of Regeneration in Plant Communities, 2nd ed. Chapter 9: The Functional Ecology of Soil Seed Banks. Eds. M. Fenner. p. 215-235.

Townsend, C.E., McGinnies, W.J. 1972. Temperature requirements for seed germination of several forage legumes. Agron. J. 64:809-812.

Tremblay, M. 2012. New Crested Wheatgrass and Meadow Bromegrass Varieties Provide western Canadian Forage Producers with Enhanced Production. Eds. Dokken-Bouchard, F. Crop Production News 34(1):5-6.

Tsai, A.C., Liou, M., Simak, M., Cheng, P.E. 2017. On hyperbolic transformations to normality. J. Computational Stat. & Data Analysis. 115:250-266.

Turnbull, L.A., Rees, M., Crawley, M.J. 1999. Seed mass and the competition/colonization trade-off: a sowing experiment. J. Ecol. 87:899-912.

Vadez, 2014. Root hydraulics: the forgotten side of roots in drought adaptation. J. Field Crops Research. 165:15-24.

Van Bruggen, T. 1976. The Vascular Plants of South Dakota, 1st ed. The Iowa State University Press, 538 pp.

Vogel, K. 2002. The challenge: High quality seed of native plants to ensure successful establishment. J. Seed Tech. 24:9-15.

Weigelt, A., Steinlein, T., Beyschlag, W. 2005. Competition amoung three dune species: the impact of water availability on belowground processes. J. Plant Ecol. 176(1):57-68.

White, L.M., Wight, J.R. 1984. Forage yield and quality of dryland grasses and legumes. J. Range Mangement. 37(3):233-236.

Williams, M.C. 1981. Nitro compounds in foreign species of Astragalus. J. Weed Sci. 29(3):261-269.

Williams, M.C., Barneby, R.C. 1977. The occurrence of nitro-toxins in North American Astragalus (Fabaceae). J. Brittonia. 29:310-326.

Williams, M.C., Parker, R. 1974. Distribution of organic nitrates in Astragalus. J. Weed Sci. 22(3):259-262.

Williams, T.A. 1897. Grasses and Forage Plants of the Dakotas. U.S. Department of Agriculture, Division of Agrostology, Bulletin No.6. Government Printing Office, Washington.

Yoshihara, Y., Mizuno, H., Ogura, S., Sasaki, T., Sato, S. 2013. Increasing the number of plant species in a pasture improves the mineral balance of grazing beef cattle. J. Animal Feed Sci. and Tech. 17(2013):1.