CHARACTERIZATION OF NEW SAINFOIN POPULATIONS FOR MIXED ALFALFA PASTURES IN WESTERN CANADA

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Dedication

I dedicate this thesis to my wife, Cynthia Akorfa Sottie and our two daughters, Deanne

and Edlyn for the love and care they showed me throughout this work, and also to my

parents, Solomon Sottie and Agnes Kabutey for their support.

Abstract

A series of experiments were conducted to compare nine new sainfoin (*Onobrychis viciifolia* Scop.) populations developed to persist in mixtures with alfalfa (*Medicago sativa* L.) under a multiple-cut management to mixtures with the standard check variety of sainfoin, Nova. In trials at Lethbridge under rain-fed and irrigated conditions, new sainfoin populations produced higher ($P<0.05$) dry matter (DM) yields as compared to Nova. New cultivars persisted for three production years in mixed stands with alfalfa accounting for >20% DM at each harvest. Condensed tannin (CT) concentrations in new sainfoin populations were similar to Nova and were higher during the bud as compared to the vegetative and flowering stages. One of the new sainfoin populations, LRC-3519 seeded in alternate rows with alfalfa caused a reduction ($p<0.001$) in bloat incidence as compared to mixed Nova-alfalfa stands. Bloat incidence was reduced by 98% in LRC-3519-alfalfa stands as compared to Nova-alfalfa stands. Average daily gains (>1.0 kg d^{-1}) of steers on alfalfa-sainfoin mixed pastures were similar ($p>0.05$). Seeding of these new sainfoin populations in mixed stands with alfalfa could prove to be a means of taking advantage of the productivity of mixed forage legume pastures while preventing bloat in grazing ruminants.

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List of Abbreviations

AB: Alberta

ADF: Acid detergent fibre

ADG: Average daily gain

CP: Crude protein

CT: Condensed tannins

DM: Dry matter

DMD: Dry matter degradability

LAI: Leaf area index

LRC: Lethbridge Research Centre

OM: Organic matter

PEG: Polyethylene glycol

NDF: Neutral detergent fibre

SK: Saskatchewan

VFA: Volatile fatty acids

Chapter 1

1.0 Introduction

Forage legumes are widely used in many grassland farming areas around the world because of their nutritional value for livestock and also their ability to fix atmospheric nitrogen into the soil. There has been increased scientific knowledge of legume agronomy and utilization under both grazing conditions and in forage conservation (Rochon et al., 2004). Improved legume varieties have been developed through advances in plant breeding and the economic potential of legumes is also recognized.

When forage legumes contain moderate levels of secondary compounds such as condensed tannins and flavonoids they offer some advantages to livestock nutrition by increasing efficiency of N utilization within the digestive tract, reducing pasture bloat, providing resilience to parasites and reducing methane emissions from enteric fermentation into the environment (McMahon et al., 1999; Theodoridou et al., 2011). The superior nutritive value and voluntary feed intake characteristics of forage legumes results in improved growth performance of ruminants provided with legume-rich as compared grass-dominated pastures (Frame, 2005).

Beef production has been enhanced through the widespread use of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pretense* L.) in North America. The growth performance of beef cattle on alfalfa pastures is similar to that obtained in feedlots (Popp et al., 2000). However, the high risk of cattle bloating is one of the major reasons why farmers are reluctant to graze livestock on alfalfa pastures and

other bloat-causing legumes such as clover. However, there are other legume forages, notably sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus* L.) that do not cause bloat in ruminant livestock because they contain moderate concentrations of condensed tannins.

Studies have shown that incorporation of as little as 10% DM sainfoin in alfalfa pastures reduced the risk of pasture bloat (McMahon et al., 2000; Wang et al., 2006). However, the sainfoin cultivars available in western Canada are not able to persist in mixtures with alfalfa over multiple grazing seasons and thus their ability to reduce the risk of bloat in cattle grazed on these mixed legume pastures gradually declines (Acharya, 2007). Several new sainfoin populations have been developed at the Lethbridge Research Centre (LRC) using new and old germplasm selected from mixed alfalfa-sainfoin stands grown under multiple-cut management.

The hypothesis is that selected sainfoin populations would persist in mixed alfalfa-sainfoin pastures for multiple grazing seasons and therefore be effective in preventing bloat in cattle grazing mixed alfalfa-sainfoin pasture.

The main objectives of this thesis are to determine in pure stands of alfalfa, sainfoin and in alfalfa-sainfoin mixed pastures: 1. biomass production, re-growth pattern and persistence of sainfoin populations; 2. forage quality, palatability and condensed tannin content of alfalfa and sainfoin populations and 3. bloat incidence and growth performance of stocker cattle on alfalfa-sainfoin mixed pastures.

<div align="center">**Chapter 2**</div>

2.0. Literature Review

2.1 Forage

Forage is vegetative food of any kind for animals especially domestic animals, maintained on pasture, browse, and mast (refers to nuts collectively) (Barnes, 1980). In the broad sense, forage refers to plant or plant parts fed to livestock or game animals used for grazing or harvested for feed by animals. Wheeler (1980) referred to forage as all vegetation eaten by grazing livestock and went on to classify pastures as follows:

1. Native pastures that are almost exclusively indigenous species.

2. Natural pastures that consist largely of indigenous species but also contain a significant proportion of volunteer introduced species.

3. Improved pastures that contain communities entirely or principally of sown species, to which in most cases fertilizer has been applied.

4. Sown pasture generally restricted to areas where original plant communities have been destroyed by ploughing and replaced by introduced grasses and legumes almost invariably with the addition of fertilizers.

Ruminant livestock production in Canada, US, Australia, Europe and other parts of the world relies heavily on forages. Forage production is the foundation of Canada's beef and dairy industries. The beef and dairy industries are the second and third ranking primary agriculture sectors after the grain sector (Statistics Canada, 1996). It is estimated that two-thirds of the feed protein in Canada comes from hay, grazing of

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forages and fodder corn production (McQueen and Buchanan-Smith, 1993). Cultivated

forages have been widely adapted to various regions of Canada with significant

production coming from lands not suited to annual crops. The majority of the forage-

based livestock industry is situated in western Canada where management of

cultivated forages is integrated with that of rangeland resources. The four western

provinces have 96% of the 26 million ha of Canadian rangeland used for livestock

production. Forages are frequently grown in rotation with cereal and oilseed crops.

Canada's cold-temperate climate dictates winter feeding of livestock with

preserved forages often being used for periods extending from October to May

depending on location and annual weather.

2.1.1. Importance of forage legumes

Forages play an important role in the diets of ruminants by providing protein,

energy, minerals and vitamins. Although the popularity of intensive feedlot production

of beef has increased over the last 20 years, it is estimated that at any time, three-

fourths of the cattle in North America can be found on pasture (Wheeler and Mochrie,

1980). In recent times, the bio-fuel industry has dramatically increased the cost of

grain-based cattle production. The increasing demand for grain for human food and

the rising cost of fuel indicate that greater reliance will soon have to be placed on

forages as a source of nutrition for livestock.

The nitrogen content of legumes varies among species, and with cultivation and

harvest management practices. The crowns of forage legumes are the main areas of

nitrogen deposition in the plant and the nitrogen in the roots contribute significantly to plant regrowth after harvest.

2.1.1.1 Feeding value of forage legumes

Forage legumes in general have higher protein, pectin, carotene and vitamin content than grasses but have lower levels of water soluble carbohydrate, cellulose and hemicellulose (Frame, 2005). Their composition can be classified into cell content (protein, water soluble carbohydrate, lipids, minerals, organic acids and pectin) and cell wall constituents (cellulose, hemicellulose and lignin). The ratio of cell wall constituents to cell contents gives an indication of the nutritive value of the forage. The ratio influences the availability and digestibility of nutrients; which has implications for voluntary feed intake in ruminants. If harvested at the vegetative stage, the higher digestibility of legumes as compared to grasses and cereals, results in higher voluntary feed intake in ruminants consuming legumes (Minson, 1982).

The nutrient composition of forage legumes are generally influenced by the stage of growth; particularly in upright-growing species such as alfalfa and sainfoin, where the leaf: stem ratio decreases greatly with advancing maturity. However, in species like white clover, there is continued generation of new leaves and petioles from the stolon network, concurrent with advancing maturity of previously generated foliage and this reduces the decline in nutritional quality with advancing maturity (Frame, 2005).

There has been improvement in live weight gains of cattle grazed on legume monocultures or legume-rich forage as compared to grass (Frame et al., 1998; Popp et

al., 2000). This is due to the higher nutritive value and voluntary feed intake of forage legumes as compared to grass.

2.1.1.2 Nitrogen fixation

Most leguminous forages fix nitrogen from the atmosphere into the soil thereby improving soil fertility. Atmospheric nitrogen is fixed by bacteria of the genera *Rhizobium* and *Bradyrhizobium*, which infect the root hairs of the legume families and thus induce the formation of benign plant galls (nodules) and live symbiotically with the host plant (Frame, 2005). The host plant supplies energy via carbohydrate from photosynthesis to the bacterial rhizobia, while the rhizobia supply the host plant with nitrogenous compounds through fixation.

Stoa and Zubriski (1969) reported that wheat yields were nearly 50% higher from land cropped to alfalfa for 3 years as compared to those cropped for the same period to a non-legume (corn, wheat, and flax crop sequence) without nitrogen fertilization. Even after non-legume fields were fertilized with 67 kg N ha^{-1}, yields of wheat were 10 to 15% higher in fields that had been previously used to grow alfalfa. Meyer (1987a) reported that barley grain yields following four to six plantings of legumes were increased by 7 to 68% compared with barley following wheat without fertilization. After fertilization (75 kg N ha^{-1}) barley yields were 12 to 15% greater when they followed a legume crop as compared to wheat. Consequently, inclusion of a legume within crop rotations can reduce the amount of chemical N fertilizer needed to optimize crop yields.

2.1.2. Land use and revenue from forages

The importance of forages in Canada can be seen in the area of land used and revenue generated from pasture (Tables 2.1). The advantages of forage legumes stems not only from their ability to reduce production costs and environmental impact of livestock production systems but also from the fact that beef produced forage is perceived by consumers to be more 'natural' than beef produced in intensively managed feedlots (Frame et al., 1998).

Table 2.1. Area of pasture land (ha) and share of area (percentage) of pasture land on beef and dairy farms with grazing cattle,[1] by revenue class, Canada, 2001

Gross farm receipts (dollars)	Tame or seeded pasture		Native pasture		Total pasture (ha)
	ha	%	ha	%	
Less than 25,000	488,410	26.8	1,336,240	73.2	1,824,650
25,000 to 49,999	812,420	29.4	1,947,700	70.6	2,760,130
50,000 to 99,999	1,444,740	28.4	3,641,170	71.6	5,085,900
100,000 to 249,999	2,120,870	24.8	6,422,190	75.2	8,543,060
250,000 to 499,999	684,290	20.4	2,671,930	79.6	3,356,210
500,000 or more	X	X	F	F	3,040,700
Other[2]	X	X	X	X	2,169,380
All revenue classes	6,610,590	24.7	20,175,740	75.3	26,786,330

Notes: Due to rounding figures may not add up to totals.
1. Includes only farms that report having grazing cattle and that also report obtaining 51% of more of their gross farm receipts from either beef or dairy.
2. "Other" comprises farms that did not specify their annual gross receipts.
X: Suppressed to meet the confidentiality requirements of the *Statistics Act.*
F: Too unreliable to be published.
Data source: Statistics Canada, 2001, Farm Environmental Management Survey.
Table source: Statistics Canada, 2004, *Farm Environmental Management in Canada*, "Grazing Management in Canada," Catalogue number 21-021-MWE.

2.2. Species seeded for mixed pastures

2.2.1. Alfalfa (*Medicago sativa* L.)

There are many types of forage used in the cattle production systems in Canada but alfalfa is by far the most important forage because of its high yield, wide adaptation and persistence in fertile, well drained soils and excellent feeding value for ruminants. Alfalfa is the most widely used forage in western Canada where it is grown on approximately 2.6 million ha (Statistics Canada, 1996). According to Berg et al. (2000) alfalfa has been bred since the turn of the 20th century to improve its quality, productivity and adaptation to the agronomic conditions in North America. However, owing to its propensity to cause bloat, alfalfa was rarely grazed in Canada and with the exception of a few varieties was not bred based on its properties and responses to grazing. The demand for grazing alfalfa however rose with the demand for improved productivity and reduced cost of pastured forage (Berg et al., 2000).

Alfalfa is sometimes referred to as the "Queen of cultivated forages" because it has high feeding value compared to most commonly grown forages. It is one of the few forage species with the potential, under proper grazing management to sustain gains in beef cattle of 1 to 1.5 kg d^{-1} a level comparable to gains in feedlots (Popp, et al, 1997; Joyce and Brunswick, 1977; Popp et al, 2000).

Alfalfa is a widely grown herbaceous perennial legume. It is well adapted to a wide range of climatic and soil conditions. It has survives winter conditions where temperatures decline to below -27°C in Alaska and Western Canada. However, it does not grow very well on acid soils (Hanson and Barnes, 1974) or poorly drained soils. It produces more protein per hectare than any other crop for livestock and is an

important source of vitamin A. It helps minimise pollution by reducing the need for chemical nitrogen fertilizer and it deep-rooted properties reduces both with and water soil erosion.

Popp et al. (2000) indicated that alfalfa can be used to maximize beef production on pastures as a monoculture or as a dominant species in a forage mixture. However, pasture bloat and rapid ruminal protein degradation can result in losses of high level of nitrogen in the form of urea when ruminants are grazed on alfalfa-based pastures (Dahlberg et al, 1988), even though protein deposition in growing ruminants is increased as compared to most other pasture systems. This can result in increased N concentrations in the excreta with potential consequences for the environment. The feeding value of alfalfa's protein is often not fully realized because it is subject to extensive proteolysis, both during ensiling and in the rumen (McDonald et al., 1991). The utilization of the protein in alfalfa forage by livestock can be inefficient (~70% digested N lost in the urine) due to rapid degradation of alfalfa protein in the rumen. This phenomenon can result in an imbalance between the supply of nitrogen and carbohydrate in the rumen, reducing the efficiency of microbial protein synthesis. Alfalfa also does not persist in infertile or poorly drained soils and is susceptible to infestation by potato leafhopper (*Empoasca fubae*) and alfalfa weevil [*Hypera postica* (Gyllenhal)]. However bloat is the most serious problem that limits the grazing of alfalfa pastures by cattle.

2.2.2. Sainfoin (*Onobrychis viciifolia* Scop.)

Sainfoin is also known as holy grass, St. Foin and cock's head. The name sainfoin was derived from two French words, 'sain' and 'foin' meaning 'healthy hay'.

2.2.2.1. Description

Sainfoin is a potentially long-lived perennial legume with many erect or sub-erect, hollow stems and grows to heights of 100 cm (Frame, 2005) (Fig. 2.1). The root system develops from a single seedling tap root, which may branch in older plants. Many fine lateral roots develop from the deep tap root and these provide sites for nodule development (Fig. 2.2). Nodules are orange-white in colour and range in size from a 1 mm spheroid-shaped structure to a large 3 x 6 mm wedge-shaped structure. Leaves are pinnate with 5 to 14 pairs of leaflets and a terminal leaflet. The inflorescence is an erect raceme bearing many pinkish flowers on long axillary stalks. The pods contain a single kidney-shaped seed. Seeds are large and are brown or black in colour. Sainfoin begins to grow in the spring before other perennial forage legumes and flowers about two weeks before alfalfa (Goplen et al., 1991). The blooming and ripening period of sainfoin is also shorter than alfalfa. During the fall season, it develops a low, rosette growth that remains green for most of the winter.

Fig. 2.1. Sainfoin

Fig. 2.2. Roots of sainfoin with nodules

2.2.2.2. Breeding

The purpose of most breeding programs is to improve biomass yield, persistence and grazing tolerance. Sainfoin is self-incompatible and is cross-pollinated mainly by honey bees. Diploid plants with a chromosome number of $2n=2x=14$ and tetraploid plants, $2n=4x=28$ have been described.

Sainfoin is divided into two main types, 'Common' and 'Giant'. Giant and common sainfoins are taxonomically indistinguishable based on growth behaviour (Fortune, 1985) but giant types generally flower at least twice in a growing season while the common types flower once and then adopt a rosette habit. Most common type cultivars grow slowly in the establishment year but if managed well will persist better than the giant types. The giant type is more vigorous in the first year of establishment but persists only for a relatively short period of time.

2.2.2.3. Cultivars

Examples of sainfoin cultivars and countries where they were developed include Melrose and Nova (Canada), Fakir (France), Eski, Remont and Renumex (United States of America), Emry (Hungary), Zeus and Vala (Italy) and Othello (Australia). Melrose was the first cultivar to be developed in Canada in 1963 followed by Nova, which was released in 1980. Nova has more spring vigor and winter-hardiness than Melrose and yields 7% more biomass (Goplen et al. 1991).

2.2.2.4. History and Distribution

Borreania et al (2003) indicated that sainfoin has been used as a forage crop in Europe and Asia for several centuries. It was introduced to the United States of America (USA) in 1786 but cultivation was limited until the 1960's when more widely adapted varieties were grown in Montana and parts of western Canada (McCartney and Horton, 1997). Since the release of the cultivar 'Eski' in 1964, sainfoin has been used in the USA for hay and pasture crop under both dryland irrigated conditions. In Europe, sainfoin has declined over the last 40 years owing to its low persistence and poor regrowth after spring harvest.

2.2.2.5. Characteristics

Attributes that have contributed to the cultivation of sainfoin include: its bloat-safe properties, high nutritive value, winter hardiness, drought tolerance, resistance to alfalfa weevil (Bolger and Matches, 1990; Ditterline and Cooper, 1975), and higher yield than alfalfa in many areas of the north-western United States. It is well adapted to dry and calcareous soils and grows best on deep, well-drained soils at pH levels of 6.0 and above (Frame, 2005). Sainfoin is easy to establish as the seeds germinate readily and produce vigorous seedlings that grow rapidly. It grows well on a range of soil types including Brown, Dark Brown and Black, unless there is a lack of moisture.

The interest in sainfoin began with the need for alternatives to alfalfa due to the spread of alfalfa weevil and pasture bloat (Bolger and Matches, 1990). Sainfoin is comparable to alfalfa in forage quality and its impact on the average daily gain of cattle (Parker and Moss, 1981). Moreover, the low to moderate levels of condensed tannins in sainfoin prevents bloat and reduces protein degradation during ensiling and

in the rumen. As a result, the absorption of protein in the small intestine is increased in comparison with some legumes. Sainfoin's non-bloat characteristic gives it an advantage over alfalfa for grazing. Furthermore, feeding sainfoin results in a higher daily weight gain, in cattle and young lambs, than other legumes (e.g. *Lotus corniculatus* L.) utilised at a comparable stage of growth. As with all legumes, sainfoin has the ability to fix nitrogen enabling it to produce substantial yields without the use of chemical nitrogen fertiliser, making it attractive candidate for use in crop rotations. Sainfoin can be conserved as silage or hay so as to provide high quality forage for ruminant livestock.

2.2.2.6. Biomass yield

Dry matter yields of sainfoin are quite variable depending on growing conditions, ranging from 7 to 15 t DM ha^{-1} (Frame, 2005; Goplen et al., 1991). Yields are about 20% less than alfalfa, likely as a result of lower leaf area index (LAI), a less erect canopy structure and less efficient nitrogen fixation (Frame, 2005). Sainfoin is not as efficient at fixing nitrogen as alfalfa, or white or red clover.

2.2.2.7. Use and management of sainfoin as forage and fodder

Sainfoin is suited for harvesting as hay as it grows upright and can be easily cut. It is best harvested at the half- to full-bloom stage since it has poor regrowth (Goplen et al., 1991). It can be seeded as a monoculture or in mixtures with grasses. Highest yields have been reported for pure stands (Goplen et al., 1991), but sainfoin does grow well in mixtures of grasses such as Russian wildrye and crested wheatgrass

under dryland conditions. However, under irrigation sainfoin/grass mixtures have yielded less than sainfoin alone (Goplen et al., 1991).

Sainfoin retains its leaves longer than alfalfa and can be harvested at a more mature stage without as much loss in quality with the highest biomass yields obtained when it is harvested between the 75 and 100% bloom stage. Sainfoin may tolerate light grazing at the bud stage and still yield a suitable crop of hay, but once if it is harvested after full bloom, regrowth is poor.

2.2.2.8. Use of Sainfoin and Alfalfa in mixtures

The effects of sainfoin on digestion of alfalfa were investigated *in vitro* and *in vivo* by Wang et al (2007). Fresh alfalfa and sainfoin were incubated in an artificial rumen (Rusitec) in ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 (as fed) and also as hay. Disappearances of dry matter and nitrogen from sainfoin were 77 and 65%, respectively of those from alfalfa. Protease and endoglucanase activities, NH_3-N and methane production declined as level of sainfoin in the diet increased. Inclusion of sainfoin with alfalfa improved fermentation in laboratory scale silos and digestion of the silage in in vitro batch cultures. It was proposed that sainfoin tannins reduced the degradation of forage protein without affecting the digestibility of the non-protein cell wall fraction and that the nutritive value of alfalfa/sainfoin mixtures preserved as hay or silage was improved relative to alfalfa alone.

McMahon et al. (1999) fed alfalfa alone or with early to full-bloom sainfoin (at 10 or 20% of ad libitum alfalfa dry matter intake) or with sainfoin hay or pellets, to eight Jersey steers in crossover trials conducted over 4 years. They observed that

including sainfoin in the diet reduced the incidence of bloat by 45 to 93%, irrespective of the form that it was fed. Co-feeding sainfoin with alfalfa markedly reduced the incidence of bloat in cattle. When consumed with alfalfa, tannins within sainfoin reduce proteolysis in the rumen and increase the absorption and efficiency of utilization of alfalfa proteins by ruminants (McMahon et al. 1999).

Research conducted at the Lethbridge Research Centre demonstrated that mixing sainfoin with alfalfa at concentrations of 10-12% (DM basis) reduced bloat (McMahon et al., 1999; Wang et al, 2006) due to presence of condensed tannin in sainfoin.

Sainfoin is considered a promising forage for dry rangeland areas of western USA and Canada (McGraw and Marten, 1986). Introducing a highly productive perennial legume into Canada could result in substantial increases in dry matter production on pasture and increases in root biomass and soil carbon. Unfortunately, currently available sainfoin cultivars in Canada (Melrose and Nova) fail to persist in mixtures with alfalfa and their poor re-growth limits their value as a pasture forage for grazing ruminants.

2.2.2.9. Palatability and animal gains on sainfoin

Feed intake by grazing ruminants is controlled by sensory mechanisms including taste, smell, texture, viscosity and temperature (Provenza, 1995). Goatcher and Church (1970) indicated that short and long-term feed intake regulation is controlled by taste while olfactory responses are less important. There is evidence that food selection involves interactions between the senses of taste and smell (Provenza, 1995). If given

a selection, ruminants generally select forages that are high in nutrients and low in toxins (Provenza and Balph, 1990). Acharya (1998) observed that the palatability of sainfoin was comparable to alfalfa while other studies have suggested that alfalfa is more palatable than sainfoin (Gesshe and Walton, 1981; Marten et al., 1987). In contrast, Parker and Moss (1981) found that 6-month old heifers preferred sainfoin hay to alfalfa hay.

Average daily gains of 0.80 and 0.86 kg d^{-1} have been measured for steers grazing pure sainfoin pastures (Mowrey et al., 1992; Marten et al., 1987), which are comparable to gains in cattle grazing alfalfa pastures. Average daily gain and feed consumption did not differ for 319 kg heifers fed either sainfoin or alfalfa hay for 60 days (Parker and Moss, 1981).

2.2.2.10. Limitations

The most serious limitation of sainfoin as reported by Auld et al. (1977) is its susceptibility to root and crown rot caused by *Fusarium solani* (Mart.). Within 3 months of planting, over 50% of sainfoin seedlings were infected with *F. solani* but symptoms did not usually develop until after two years. In between the third and fifth years, many plants died and stands became so depleted that forage yield was severely reduced (Auld et al. 1977).

2.3. Tannins

Tannins are a very diverse family of polyphenolic compounds that can be subdivided into condensed (CT) or hydrolysable (HT) tannins. Both types of tannins

form complexes with proteins, polysaccharides and other macromolecules (Morrison et al, 1995) and reduce the degradation of protein in the rumen (Wang et al 2006). The HT occur mainly in fruit pods and plant galls and their degradation products are absorbed across the small intestine and can be toxic to ruminants (McLeod, 1974; Dollahite et al. 1962). HT are often found in leaves of tropical trees and browse shrubs and not in forage legumes.

2.3.1. Condensed tannins

CT are high molecular weight phenolics which occur in a range of herbaceous legumes and tree leaves. They are present mainly in the leaves and stems of plants but can also be present in the flowers of some plants. CT bind forage proteins as the plant is masticated ruminants and protect them from microbial digestion in the rumen often without significantly decreasing intestinal digestion or amino acid absorption (Wang et al., 2007, Dahlberg et al., 1988). CT-protein interactions are mainly based on hydrophobic and hydrogen bonding. CT bind with protein at near-neutral pH (pH 5.5-7.5) to form CT-protein complexes. These complexes dissociate and release the protein within the abomasum where pH is often less than 3.5.

Min et al (2003) reported that the nutritional effects of CT in forages could be assessed by administering polyethylene glycol (PEG) into the rumen as it selectively binds with CT thus preventing the CT from binding proteins in the rumen. A study by Min et al. (2000; 2002) showed that CT reduced protein solubilisation, reduced rumen protein degradation and rumen proteolytic activity and proteolytic bacterial populations. The decline in proteolysis reduced deamination and the release of

ammonia into the rumen (Min et al., 2003; McMahon eta l., 1999). Increasing CT concentration increased the amount of undegraded feed protein flowing out of the rumen without reducing the amount of microbial protein synthesized (Min et al., 2003).

2.3.2. CT in Ruminant Nutrition

CT in forages are considered anti-nutritive factors for ruminants, owing to their capacity to bind macromolecules such as fiber and proteins (McAllister et al., 2005). From studies carried out by Leinmuller et al. (1991), they observed that at concentrations exceeding 6% of dietary dry matter (DM), CT depressed feed intake, the digestibility of fiber and protein, and the growth rate of ruminant livestock. According to Barry et al. (1986), although formation of CT-plant protein complexes occurs at CT concentrations of less than 4% of diet dry matter, feed intake is seldom affected. More recently, forage CT have been studied as potentially beneficial agents in ruminant nutrition (McAllister et al., 2005). The reversible and pH dependent nature of tannin-protein interactions enhances the resistance of CT-bound protein to microbial degradation in the rumen, yet it enables dissociation of the complexes in the abomasum. This relationship can increase the proportion of plant protein that reaches the small intestine. As a result, CT containing forages have been reported to improve wool growth (Reis, 1979), milk production (Wang et al., 1996) and lambing percentage in sheep (Min et al., 1999; Luque et al., 2000).

2.3.3. CT and animal health

CT in the rumen become bound to cell coat polymers of bacterial cells after forages containing CT are masticated by ruminants. Dietary CT induce changes in the morphology of several species of rumen bacteria (Jones et al., 1994) and the proteolytic activity of rumen bacteria are inhibited (Bae et al., 1993; Jones et al., 1994). When forages containing CT are fed to ruminants, the CT prevent the formation of the stable froth in the rumen which contributes to the development of pasture bloat. The risk of bloat in cattle grazing alfalfa can be reduced by providing CT at 1.7% of dietary DM (Waghorn and Jones, 1989). Li et al. (1996) suggested CT levels as low as 1.0 mg g^{-1} DM in alfalfa pasture could help reduce the risk of bloat in cattle.

Molan et al. (1999; 2000) showed that CT-containing forages can act against parasitic nematodes by breaking their life cycle and thereby reducing the contamination of pasture with infective larvae.

CT may also reduce the amount of methane (CH_4) produced in cattle consuming forage-based diets (McMahon et al., 1999), a factor that could improve energetic efficiency in cattle and reduce their contribution to greenhouse gases.

Waghorn (1990) and Morris and Robbins (1997) have proposed that due to the positive effects of condensed tannins on digestion in ruminants, pathways for their synthesis be genetically engineered into legume species prone to provoking bloat.

2.4. Bloat in cattle on pastures

The incidence of pasture bloat in cattle increases if legume forages such as alfalfa and clover (Howarth et al., 1991) are introduced into cultivated pastures. Bloat

has also been observed with vegetative wheat pastures, but the incidence of bloat with this forage is less than with alfalfa and clover (Horn, 1997). Bloat is a digestive disorder that causes fatality and reduces production. It is a serious problem to livestock producers, especially regions of western North America, eastern Australia, New Zealand and South America (Majak et al, 1995). Bloat manifests itself in the accumulation of gas in the rumen and reticulum resulting in distension of the left flank (Fig. 2.3) and impairment of digestive and respiratory function to the point that it may cause death.

There are generally two types of bloat, frothy and free-gas in cattle, depending if they are raised either on pasture or in a feedlot. Free-gas bloat is usually associated with high starch feeds and arises as a result of acidotic rumen stasis. It may also occur as a result of a physical obstruction or damage to the cardia or esophagus and is frequently associated with hardware disease. Free-gas bloat is not common in animals on pasture as it is in the feedlot. As frothy bloat (Cheng et al., 1998) is the primary form of bloat associated with grazing ruminants, it is the focus of this review.

Fig. 2.3. A bloated steer.

2.4.1. Causes of pasture bloat

Bloat is a complex condition that is difficult to predict under field conditions and this has led to varied and conflicting theories of its cause. There are three factors that have generally been accepted as prerequisites for the development of frothy bloat in cattle on pasture. These factors were initially proposed by Howarth et al. (1986) and are:

1. A readily digestible feedstuff or highly digestible high-protein forage such as alfalfa, clover or wheat, that results in rapid gas production and proliferation of ruminal microbial populations.

2. Presence of fine feed particles that promote the coalescence of gas bubbles in rumen contents and thereby restrict the release of fermentation gases. These fine particles arise from the fragmentation and rupture of chloroplasts.

3. Conditions favourable for ruminal bacteria to produce an excessive amount of exopolysaccharides or bacterial slime that further contributes to the establishment of stable foam that traps fermentation gases.

Ruminants harbour active populations of bacteria, protozoa and fungi within the rumen. These rumen microorganisms enable the ruminant to digest fibrous feeds, such as grasses and legumes. During digestion, rumen microorganisms produce large quantities of CO_2 and CH_4. These gases separate from liquid and solid contents and rise to the top of the rumen, where they coalesce as free gas bubbles. Gas is pushed towards the front of the rumen to the esophageal opening by rumen contractions (Majak et al., 2003b) and is released from the rumen through eructation. Eructation usually occurs about once per minute over a duration of about 10 seconds. Rate of gas production in the rumen increases after feeding and therefore the rate of eructation also increases to expel the produced gas from the rumen.

In bloated animals, the eructation mechanism is inhibited (Reid and James, 1985, Cheng et al., 1998) and therefore the rate of gas production in the rumen exceeds the rate at which it is expelled. With frothy bloat, the gas becomes trapped in rumen fluid leading to formation of small bubbles which fill the rumen cavity and inhibit the nerve endings that control the release of gas into the esophagus during eructation (Galyean and Rivera, 2003). The rumen becomes tympanitic when the rate

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of gaseous discharge from the rumen is less than the rate of gas produced by microbial fermentation. Pressure is placed on the diaphragm and lungs and this affects the animal's ability to breath, potentially resulting in death.

In frothy pasture bloat (Fig. 2.4), high soluble protein concentrations in the forage, along with its rapid fermentation and possibly other unidentified characteristic of the animal contribute to the formation of foam (Gaylean and Rivera, 2003). Additionally, the rapid lysis and release of proteins from plant cells upon their entry into the rumen increases the viscosity of the rumen fluid and is a contributing factor to pasture bloat (Howarth et al., 1977).

Fig. 2.4. Foaming rumen content from steer grazing pure alfalfa pasture.

Saponins, are secondary metabolites found in a range of natural plant sources that are known to have foaming properties. They are abundantly present in alfalfa and

clover and are thought to also contribute to the frothy bloat observed with these plant species (Sen et al., 1998). However, Majak et al. (1980) examined the role of saponins in bloat using high- and low-saponin cultivars of alfalfa. The high-saponin cultivar had twice the concentration of saponin as the low-saponin cultivar but there was no significant difference in the occurrence of bloat between the two cultivars.

An animal's physiology can also play a role in its susceptibility to bloat. Cattle are more prone to bloat than other species such as sheep or deer and the vulnerability varies widely among individuals (Ayre-Smith, 1971; Colvin and Backus, 1988). Ruminants with a high propensity to bloat maintain comparatively high volumes of fluid in the rumen (Cockrem et al., 1987b) and may retain more digesta in the rumen for a longer time owing to a slower rate of passage of digesta (Okine et al., 1989). Howarth et al., (1986) proposed that some of these traits are heritable, raising the possibility that one could select for cattle with a reduced propensity to bloat.

The higher foam production in bloat-prone cattle could be attributed to slower rates of passage of the liquid phase of ruminal contents. The ruminal passage rate is an important factor in the aetiology of legume bloat. Slower clearance enhances microbial activity and promotes gas production. Rapid clearance decreases microbial gas production, enhances protein bypass and reduces the chances of bloat (Majak et al., 1995). However, there were no differences in the rumen volume of steers that were more prone to bloat when grazing alfalfa as compared to those that were less prone (Majak et al., 1986). A specific salivary protein (bSP30) was found to be more prevalent in cattle that were less bloat-prone (Rajan et al., 1996) but the volume of salivary proteins did not differ between the two groups of cattle (Carruthers and

Morris, 1993). However, it is not known if this protein has a direct role in bloat prevention. The role of microbial communities in the development of frothy bloat also is not fully known. It has been suggested that *Streptococcus bovis* plays a role in its aetiology of feedlot bloat (Gutierrez et al., 1959). Min et al. (2006) used 16S rDNA to show that there were differences in the rumen microbial populations of bloated and non-bloated steers. The occurrence of bloat or susceptibility to bloat has not been definitely related to qualitative or quantitative changes in populations of ruminal microbes (Dawson et al., 1997) but it is hoped that metagenomic analysis will help in understanding the role(s) of rumen microorganisms in the aetiology of frothy bloat.

2.4.2. Classification of forages based on bloat-causing abilities

Wang, et al. (2012) classified the bloat risk of forages into three categories, "bloat-causing", "moderate-risk" or "low-risk" (Table 2.3). The type of forage in a pasture has a major influence on the risk of bloat in grazing cattle. Forages that are digested rapidly in the rumen are classified as bloat-causing and those that are digested slowly are usually low-risk. Forages that lie between these two categories are considered to be of moderate-risk of causing bloat.

Table 2.2. Classification of forages based on bloat-causing tendency.

Bloat-causing	Moderate-risk	Low-risk
Alfalfa	Arrowleaf clover	Sainfoin
Sweetclover	Berseen clover	Birdsfoot trefoil
Red clover	Persian clover	Cicer milkvetch
White clover	Spring wheat	Crownvetch
Alsike clover	Oats	Lespedeza
Kura clover	Rape (canola)	Fall rye
Winter wheat	Perennial ryegrass	Most perennial grasses

(Adapted from Wang et al., 2012)

2.4.3 Bloat control

2.4.3.1 Development of alfalfa populations

The rapid initial rate of digestion (IRD) of alfalfa in the rumen was what prompted research into breeding cultivars with slower rate of ruminal digestion. Coulman et al. (2000) developed the cultivar, AC Grazeland, which exhibited a 15% reduction in the IRD when compared to Beaver, the unselected parent. On average, there was a 56% reduction in the incidence of bloat in cattle grazing AC Grazeland as compared to Beaver in multi-locational trials in western Canada (Berg et al., 2000). When AC Grazeland was compared with Alfagraze, another grazing-tolerant alfalfa cultivar, there was no significant difference found in their bloat-reduction abilities

(Hall et al., 2001). Even though there was reduction in the incidence of bloat with AC Grazeland, bloat still occurred and thereby the risk of this condition in grazing cattle was not eliminated.

2.4.3.2. Plant and animal management strategies

A number of plant and animal management strategies can be employed to control the occurrence and severity of frothy bloat.

2.4.3.2.1. Forage maturity

The most important factor in managing pasture bloat is the degree of maturity of the alfalfa at the time of grazing. The risk of bloat is highest at the vegetative (pre-bud) stage and declines as the plant matures (Majak et al., 2003b). In an experiment carried out at Kamloops over a period of two years, three groups of cattle were fed alfalfa at the vegetative, bud and bloom stages. A total of 129 cases of bloat were recorded with vegetative alfalfa as compared to 20 cases at the bud stage and no bloat in cattle at the bloom stage. The leaf-to-stem ratio of the plant declined as the plant matured resulting in a decrease in the chloroplast particles in the rumen. Bloating did not occur in cattle that were fed alfalfa at the bloom stage, possibly because there was a decrease in chloroplast particles that contribute to the formation of froth in the rumen. Bloat can be controlled at the vegetative stage if the forage is swathed and allowed to wilt prior to feeding (Majak et al., 2001). Wilting a swath for 24 to 48 hours has been found to decrease and in some incidences eliminate pasture bloat. When dew is allowed to dry off before animals are allowed into alfalfa pasture it

reduces the risk of bloat. Mayland et al. (2007) believe that it is the dew that increases the risk of pasture bloat but this assertion has not been fully explained. As plant height affects the leaf-to-stem ratio, alfalfa with heights of 20 to 25 cm causes more bloat than those with heights of 51 to 75 cm (Majak et al., 2003b).

2.4.3.2.2. Type of cultivated forage

Many studies have shown that grazing cattle on grass-legume mixed pastures reduces the incidence of bloat, especially in continuous grazing systems. It has been suggested that the alfalfa should constitute no more than 50% of the mixture to minimize the incidence of bloat (Majak et al., 2003). If cattle graze both grass and legumes at the same time, the initial rate of digestion is reduced and the risk of frothy bloat is reduced. The nutritional value of these pastures has been demonstrated in various studies using cattle grazing mixtures of grass and alfalfa (Popp et al., 2000), perennial ryegrass and white clover (Papadopoulos et al., 2001) and alfalfa and orchardgrass (Viera et al., 2010). It is important that the growth stage of alfalfa in the mixed stand be monitored and if possible grazing avoided when alfalfa is at the vegetative stage as cattle may selectively consume only alfalfa. Bloat has been reported with grass-legume mixtures where the legume accounted for less than 15% of the mixture (Majak et al., 2003b). The period after cutting or intensive grazing of alfalfa-grass mixed stands is also of higher bloat risk as alfalfa usually recovers faster than most grass species after harvest. The grass that is used in the mixture must ideally have about the same or similar seasonal growth and re-growth patterns as alfalfa. Two meadow bromegrass cultivars, Paddock and Fleet, released by the Saskatoon Forage

Breeding Program in 1987 have regrowth characteristics that are adequate for use in a 50:50 percent alfalfa-grass seeding mixture.

Pasture bloat is a major deterrent to the grazing of alfalfa-based pastures despite the potential profitability that this system may offer. Some cattle producers in western Canada would rather use solely grass or bloat-safe legumes pastures, rather than risk pasture bloat. The annual cost of bloat in terms of reduced pasture productivity has been estimated to be about $30 to $50 million in Canada (Acharya, 2007), US$ 310 million in the USA, AU$ 180 million in Australia and NZ$ 25 million in New Zealand (Tanner et al., 1997).

Statistics on annual bloat losses of cattle that graze legume pastures is limited. It is however believed that the losses are around 1.5 percent in western Canada (Majak et al., 2003b). If one selects to use only grass pastures it should be done with an appreciation that legumes can increase pasture productivity from 10 to 70% depending on the legume used (Majak et al., 2003b).

2.4.3.2.3. Cereal/grass silage-legume mixtures

In studies by Bretschneider et al. (2001) and Bretschneider et al. (2007), it was demonstrated that feeding corn silage prior to grazing alfalfa reduced the incidence and severity of bloat. Corn silage reduced the initial rate of digestion of alfalfa and also the number of bacteria in the rumen. Santos et al. (2010) observed that bloat incidence in sheep fed spineless cactus was reduced when the feed was supplemented with Bermuda grass hay. In this study, Bermuda grass hay was more effective in controlling bloat than soybean hulls.

2.4.3.2.4. Soils and farming practices

More bloat has been observed in some parts of western Canada as compared to others. More cases have been reported in the Gray Wooded soil zones as compared to the Dark Brown and Brown soil zones (Majak et al., 2003b).

The mineral composition of alfalfa has not been found to be an accurate predictor of bloat risk. Bloat has been associated with high levels of potassium and low levels of sodium in the rumen (Hall et al., 1988) and with the level of nitrogen fertilizer applied to alfalfa (Stewart et al., 1981). It is believed that high levels of nitrogen fertilizer increase the soluble proteins in the plant which contribute to the formation of stable froth in the rumen (Stewart et al., 1981). The role of plant mineral composition in the occurrence of bloat is however not conclusive.

Incidence of bloat is increased in cattle on irrigated alfalfa pastures as compared to dry-land pastures. Irrigation may contribute to the lushness of the pasture and protein content of alfalfa (Majak et al., 2003b). Immediately after a killing frost, the risk of alfalfa causing bloat is increased and it is suggested that the forage be allowed to stand for at least a week after frost to reduce the risk of bloat.

2.4.3.2.5. Grazing management

Continuous grazing on alfalfa based pastures has been found to be a management strategy to reduce the incidence of bloat. Bloat increases when cattle are grazed intermittently as compared to continuously. It has been observed that more pasture bloat occurs when grazing is interrupted because of bad weather, or when cattle are not grazing continuously because of attacks from biting flies. Grazing

continuously helps increase the consistency of meal size and thereby moderate the rate of gas production over the course of the day, reducing the risk of pasture bloat (Dawson et al., 1997).

The incidence and frequency of bloat in cattle grazed on alfalfa was less than with cattle fed alfalfa green-chop in confinement. Bloat incidence was also higher when cattle were fed alfalfa green-chop in the morning (07.00 – 08.00 h) as compared to later in the day (11.00 – 12.00 h) (Majak and Hall, 1993). Rumen chlorophyll content was higher with morning feedings as compared to mid-day (Majak and Hall, 1993), suggesting that differences in the integrity of the chloroplasts may play a role in this difference.

2.4.3.2.6. Anti-foaming Agents/Feed Additives

Many oils and detergents can be used to prevent and treat pasture bloat. They are usually administered into the rumen via stomach tube. Oils assist in emulsifying the froth in the rumen. Many vegetable oils are known to show efficacy in the prevention and treatment of bloat and some oils such as coconut and palm kernel oils are known to have bactericidal activity against Gram-positive bacteria (Petschow et al., 1996). Yabuuchi et al. (2007) reported that the activity of *Streptococcus bovis* was inhibited by lauric acid but that the bacterium could adapt after repetitive culturing with lauric acid.

Many feed additives have been assessed for their ability to prevent or treat bloat but many are not effective. Some of these include: household detergent (Tide®), chromium (glucose tolerance factor), disodium phosphate (14% in mineral salt), a

commercial flocculant (Betz 1190®) and Silent Herder® mineral mix (Majak et al., 2003b).

Antibiotics have also been administered to ruminants with the aim of reducing the activity and gas production from rumen microbial populations to control bloat. Ionophores such as rumensin and lasalocid and other pluronic detergents have been assessed as bloat preventatives. Rumensin in a slow-release bolus form was able to reduce bloat by about 80% in confined experiments with alfalfa green-chop (Majak et al., 2003b) and by about 50% under grazing conditions(Hall et al., 2001). This was attributed mainly to reduction in protozoal activity in the rumen. The ionophore, lasalocid has been found to be effective in controlling feedlot but not pasture bloat (Majak et al., 2003b).

Bloat Guard® has been used in pure irrigated alfalfa stands to prevent the occurrence of pasture bloat. It has been administered in salt-molasses block and in liquid molasses through a lick feeder. The blocks worked best when they were placed throughout the pasture at a density of about one block for every 10 head of cattle (Majak et al., 2003b).

There are feed additives that can be included in the drinking water of grazing cattle to prevent bloat. Blocare 4511® was the first industrial water-soluble de-foaming agent to be looked at in North America. It was used widely in Australia and New Zealand for the control and prevention of pasture bloat. Multi-locational trials conducted in western Canada by Stanford et al. (2001) using Blocare 4511® for the prevention of bloat proved the effectiveness of the product. Two additional trials were carried out in western Canada to verify the efficacy of Blocare 4511® in preventing

bloat (Majak et al., 2001; Stanford et al., 2001). Currently, this product is not registered for use in Canada.

However, there is a water-soluble product similar to Blocare 4511® called Alfasure™, which was manufactured in Alberta and is available in Canada for bloat control. Inclusion of Alfasure™ in drinking water or spraying it on alfalfa prior to grazing effectively prevents bloat in cattle (Majak et al., 2005). Alfasure™ was shown to reduce the viscosity and stability of foam in rumen fluid, changes that could account for its ability to supress bloat (Wang et al., 2006c).

2.4.3.2.7. Use of low-risk bloat forages

The establishment of pastures using non-bloating legumes such as sainfoin (*Onobrychis viciifolia* Scop.), cicer milkvetch (*Astragalus cicer*), birdsfoot trefoil (*Lotus corniculatus*) or crownsvetch (*Coronilla varia*) prevents pasture bloat. However, growth performance of cattle on these legumes is often lower than with alfalfa.

The potential of bloat may be reduced by establishing pastures containing mixtures of alfalfa and sainfoin or other bloat-free legumes. Sainfoin is one of the most common condensed tannin-containing forage legumes grown in North America.

Research conducted at the Lethbridge Research Centre demonstrated that incorporation of sainfoin into alfalfa forage at concentrations of 10-12% (DM basis) reduced bloat (McMahon et al, 2000; Wang et al, 2006a). In another experiment McMahon et al. (1999) fed alfalfa herbage alone or with early to full-bloom sainfoin herbage (at 10 or 20% of ad libitum alfalfa dry matter intake) or with sainfoin hay or

pellets. Including sainfoin in the diet reduced the incidence of bloat by 45 to 93% irrespective of the form in which the sainfoin was supplied. It was concluded that co-feeding sainfoin with alfalfa can markedly reduce the incidence of bloat in cattle.

2.5. Conclusion

Alfalfa-sainfoin mixed pastures could be one of the most logical approaches to bloat prevention in cattle on alfalfa pastures. It is easier to implement than swathing and wilting alfalfa. The ease of administration to cattle as compared to the use of Alfasure, and other bloat prevention methods makes it a cost effective way of preventing bloat. The alfalfa-sainfoin mixed pastures can maintain high animal productivity as compared to grass pastures.

Chapter 3

New sainfoin populations for bloat-free alfalfa pasture mixtures in western Canada[1]

3.1. Introduction

Beef production on pastures can be maximized by the use of alfalfa (*Medicago sativa*

L.) as a monoculture or dominant species in the forage mixture (Popp et al., 2000).

However, pasture bloat is a major deterrent to the grazing of alfalfa-based pastures

despite the high growths that are obtainable (Popp et al., 2000). The fear of pasture

bloat prevents cattle producers from using alfalfa-based pastures and this, some

suggest, costs Canadian cattle producers Can$30 to Can$50 million per year in lost

potential pasture productivity, a figure that has grown with the increasing cost of

grain-based diets. To avoid this loss, numerous bloat control strategies have been

developed (Majak et al., 1995), including the breeding of AC Grazeland, a bloat-

reduced alfalfa cultivar (Coulman et al., 2000), and the use of 'Alfasure,' an alcohol

ethoxylate/pluronic detergent administered through water (Berg et al., 2000).

However, AC Grazeland is not completely bloat-safe and has only been used in

conjunction with other strategies to manage alfalfa bloat. Alfasure, although highly

effective for controlling bloat is problematic under conditions where water supply for

the cattle cannot be completely controlled.

Unlike alfalfa pasture, bloat does not occur in pastures with high-tannin containing

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[1] Acharya, S., E. Sottie, B. Coleman, A. Iwaasa, T. McAllister, Y. Wang and J. Liu. 2013. New sainfoin populations for bloat-free alfalfa pasture mixtures in western Canada. Crop Sci. 53:1-11.

forage legumes such as sainfoin (*Onobrychis viciifolia* Scop.) (Berg et al. 2000).

McMahon et al. (1999) demonstrated that as little as 10% DM sainfoin can provide

sufficient condensed tannins to make mixed alfalfa pastures bloat-safe. In addition,

when consumed with alfalfa, the presence of tannins from sainfoin reduces the

proteolysis of alfalfa proteins in the rumen and increases the efficiency of protein

utilization by ruminants (McMahon et al. 2000). Earlier work concluded that even

though crude protein (CP) concentration of sainfoin is lower than the CP concentration

of alfalfa at the same morphological stage, the presence of moderate level of

condensed tannins can protect protein fractions from degradation during ensiling and

in the rumen (Borreani et al. 2003). This and the greater intake potential of sainfoin

over alfalfa has the potential to make it a preferred forage legume for cattle and dairy

producers (Albrecht and Beauchemin, 2003).

Sainfoin is resistant to the alfalfa weevil [*Hypera postica* (Gyllenhal)] (Ditterline and

Cooper 1975) and provides earlier spring grazing or hay production than alfalfa

(Bolger and Matches, 1990). This crop is also more drought- and cold-tolerant than

alfalfa, is widely adapted, and grows on alkaline soils. In general sainfoin forage

quality (Carleton et al. 1968; Frame et al., 1998) and average daily gain of cattle

(Parker and Moss 1981) grazing this crop compares favourably with alfalfa. In

addition, condensed tannins may also reduce methane (CH_4) production in cattle

consuming forage-based diets (McMahon et al. 1999), a factor that could improve

energy efficiency and reduce their contribution to greenhouse gases. In spite of these

advantages this crop is not utilized to its potential in western Canada due to low forage

yields. Although some reports indicate that sainfoin yield can be equal to or greater

than alfalfa depending on environment, in western Canada yields have been historically less than alfalfa (Hanna and Smoliak, 1968). Producers in this area would utilize sainfoin more if they could grow mixed alfalfa and sainfoin pastures. However, information on alfalfa-sainfoin mixed stand performance is scant at best and nonexistent for western Canada.

Earlier studies in western Canada indicated that presently available sainfoin cultivars do not persist well in mixtures with alfalfa or exhibit regrowth comparable to alfalfa after grazing (Jefferson et al., 1994). These authors concluded that alfalfa-sainfoin mixtures are not stable or sustainable in semiarid environments. Several new sainfoin populations were developed at the Lethbridge Research Centre (LRC) using new and old germplasm and selections from mixed alfalfa-sainfoin stands under multiple-cut management. The main objectives of this study were to determine if new sainfoin germplasm developed for their ability to survive with alfalfa perform better than old cultivars in mixed alfalfa pastures and in pure hay stands. To achieve this objective, biomass production, persistence, and regrowth after cutting of these new populations under pure and mixed stands with alfalfa were compared in different eco-climatic zones of western Canada.

3.2 Materials and Methods

3.2.1. Treatment structure

Several new sainfoin introductions, newly developed LRC populations, and two registered sainfoin cultivars for western Canada (Nova and Melrose) were used to determine their forage yield potential under irrigated and rain-fed conditions at Lethbridge, AB. Sainfoin population designations, their origin, parentage and the method of population development are presented in Table 3.1. All populations and cultivars included in the study except 3509

belong to *Onobrychis viciifoila* subsp. *viciifolia.* LRC 3509 waso a different species i.e. *Onobrychis arenaria* (Kit.) DC. Subsp. *arenaria*, popularly known as Siberian sainfoin. AC Longview alfalfa (Acharya and Huang, 2000) was used to compare forage yield of the sainfoin populations in pure stands under hay-cutting management. Four of these populations (LRC-3900, LRC-3901 and LRC3902 and Nova) were also used in trials under irrigated conditions in Lethbridge, AB (49°41'57.33"N, 112°45'49.61"W, Dark Brown Soil Zone); Saskatoon, SK (52° 7'59.79"N, 106°38'3.02"W; Dark Brown Soil Zone) and Swift Current, SK (50°16'53.89"N, 107°45'26.77"W; Brown Soil Zone) in pure and mixed stands with AC Grazeland alfalfa.

For both hay-cutting management and frequent harvest tests, the plots were arranged in a randomized complete block design with four replications and were harvested to a 10-cm canopy height. For hay-cutting management trials, plots were harvested when the alfalfa check was at 5 to 10% bloom and for frequent harvest trials the plots were harvested when the crops attained a canopy height of ~40 cm. In the two Saskatchewan locations (Saskatoon and Swift Current) the individual plots were 2 m by 6 m, with 6 rows per plot seeded 30 cm apart in monoculture, or with 3+3 rows of sainfoin and alfalfa seeded in alternate rows at the same row width. At Lethbridge (irrigation), for both hay-cutting management and frequent harvest tests the plot sizes were ~ same but had 10 rows per plot seeded 18 cm apart in the monoculture plots and 5 + 5 rows of sainfoin and alfalfa seeded in alternate rows using 18 cm between rows in the mixed plots. Plots were irrigated twice with ~ 25 mm water before first cut and between cuts or as needed. For the rain-fed hay-cutting management trial at Lethbridge only five rows (36 cm apart) were planted in the

same plot area. Seeding rates for alfalfa and sainfoin were adjusted to obtain a 50/50 mix
(on the basis of viable seed numbers) of the two crops in mixed plots.

3.2.2. Establishment protocol

Levelled and uniform plots (with 6.5 – 7.5 soil pH) where legumes had not been grown for
at least 2 yr followed by at least one fallow yr were chosen for the trials. A small amount of
N (16.6 kg ha^{-1}) and an adequate amount of P (83.3 kg ha^{-1}) were applied at seeding
through application of an 11-15-0 fertilizer mix. After this point no fertilizer was applied
for the duration of the experiment. A preseeding application of glyphosate [N-
(phosphonomethyl)glycine] at a rate of 1 L ha^{-1} was used to control perennial and grassy
weeds. Edge [n-ethyl-α,α,α-trifluoro-N-(2-methylallyl)-2,6-dinitro-p-toluidine] (Mosanto,
Canada) was worked into the soil just before seeding (at 8.9 kg ha^{-1}). Clipping at the
establishment phase was necessary in some plots to control weeds. The plots were seeded
in early June 2008 at all locations.

3.2.3 Data collection

Forage was harvested using a small-plot forage harvester with a sickle mower (Hege 212,
manufactured in D-7112 Waldenburg, Germany) for forage and frequent harvest trials at
Lethbridge and Saskatoon. Fresh weight was recorded for each plot and a 500-g random
sample was collected from each plot for determining moisture content of the fresh material.
The plot yield was expressed as dry matter (DM) yield in kg ha^{-1}. For mixed plots in
frequent harvest trials a 50 cm by 50 cm area was hand-harvested at random just before the
plots were harvested for forage yield to determine species composition in the mixtures.

Care was taken to include equal number of sainfoin and alfalfa rows during sampling. At Swift Current, species composition was determined from a 0.3 m^2 quadrat and the biomass yield was determined for each plot by hand-harvesting 1 m^2 area when the canopy height was ~ 40 cm.

3.2.4. Statistics

The experimental design used at each location was a randomized complete block design with four replications. Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 2005) for each location separately using year as a random effect. Means were then compared using the least squares mean linear hypothesis test (LSMEANS/PDIFF). Since year was a repeated measure, various variance-covariance structures were fitted and the best model was selected for the final analyses. A separate analysis was done to test for the proportion of sainfoin that persisted in mixed stands over years. Fisher's protected LSD test (Steel and Torrie, 1980) was used to evaluate differences among means for significance. Unless otherwise specified, treatment effects were declared significant at $P<0.05$.

Table 3.1. Designation, parentage, country of origin, selection method used and clonal composition of sainfoin populations used in the study.

Population designation	Parent population	Origin	Selection method used and proportion of selected clones
Melrose	Cultivar	Western Canada	Original population
Nova	Cultivar	Western Canada	Original population
Shoshone	Cultivar	Wyoming, USA	Original population
Delaney	Cultivar	Montana/Wyoming, USA	Original population
LRC 3401	Emyr	England	300 individual plants selected after 3 spring applications of 0.5 l ac^{-1} of glysphosate
LRC 3402	Perly	England	Original population
LRC 3422	Kazakhstan	Kazakhstan	Original population
LRC 3432	Remont	Montana, USA	300 individual plants selected after 3 spring applications of 0.5 l ac^{-1} of glysphosate
LRC 3506	CN 45635	PGRC (China)	Original population
LRC 3507	CN 31800	PGRC (China)	Original population
LRC 3509	CN36246	PGRC (China)	Original population, Siberian sainfoin (*Onobrychis arenaria* subsp. a*renaria)*
LRC 3511	Eski	Montana, USA	Original population
LRC 3519	Splendid	Romania	300 individual plants selected after 3 spring applications of 0.5 l ac^{-1} of glysphosate
LRC 3900*	230 clone synthetic	Alberta, Canada	LRC 3432 35%; LRC 3519 19%; Melrose 13%; Perly 13%; LRC 3401 8%; Eski 5%; Chinese accessions 5%; and Kazakhstan 2%
LRC 3901	176 clone synthetic	Alberta, Canada	LRC 3401 26%; LRC 3519 24%; Chinese accessions 12%; Nova 9; Remont 8%; Eski 7%; Melrose 6%; Perly 5%; and Kazakhstan 3%
LRC 3902	200 clone synthetic	Alberta, Canada	LRC 3519 45%; LRC 3432 14%; Eski 10%; Emyr 7%; Chinese accessions 7%; Nova 5%; Melrose 5%; Perly 5%; and Kazakhstan 2%

Note: All populations and cultivars except 3509 are *Onobrychis viciifoila* subsp. *viciifolia.*

The components of LRC 3401, 3432 and 3519 were selected from 30-m x 30-m plots planted in solid-seeded rows with 1-m spacing. Clones from 300 selected plants from each population were planted in three separate isolated breeding nurseries from which seed was harvested in bulk.

*The components of the 3900 series were seeded in alternate rows with AC Longview alfalfa in 2001 spring in plots 6 m x 18 m in area. These plots were cut in the fall of 2001 and three times each year in 2002 to 2004. In spring 2005 the best plants that survived most were selected and their clones were transplanted to three separate breeding nurseries for seed production in the proportion noted above and harvested in 2006.

3.3. Results and Discussion

3.3.1. Weather data

In 2009, the total monthly precipitations were below long-term average for the respective locations (Fig. 3.1). This was particularly noticeable for the months of May and June in Lethbridge and Swift Current, and May in Saskatoon. In 2010, 2011, and 2012 total precipitation during the growing season were higher than the long-term average at all locations. The monthly total precipitation for July and August in 2012 at Swift Current was also lower than long-term averages for these months. The average monthly temperatures varied among years but followed the same pattern as the long-term averages recorded at the three locations.

3.3.2. Forage yield trials

Forage yield trials at Lethbridge under rain-fed (established in 2007) and irrigated (established in 2008) conditions indicated that the year, population and year x population interaction effects were significant. Since the year x population was significant, means for each year for each population are presented separately in Tables 3.2 and 3.3.

Lethbridge
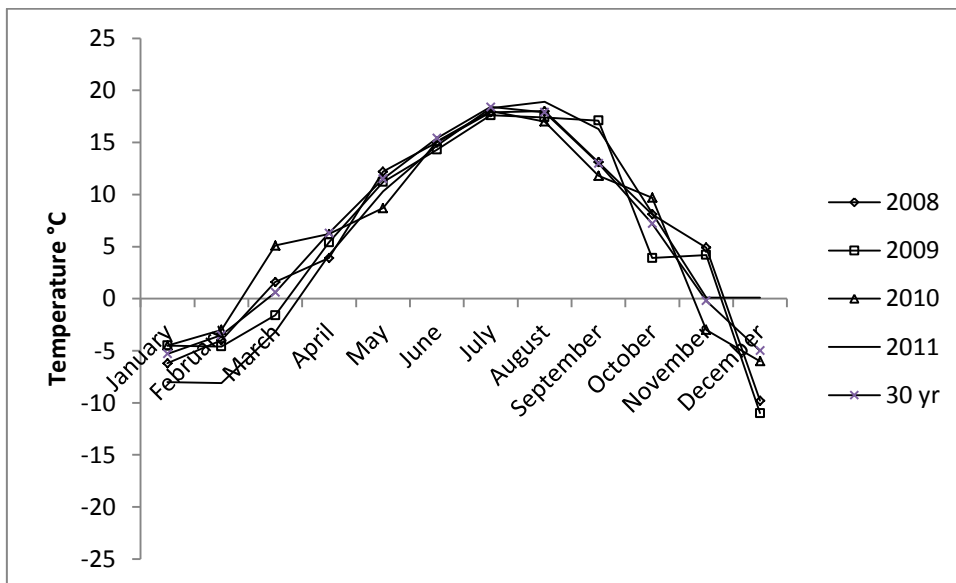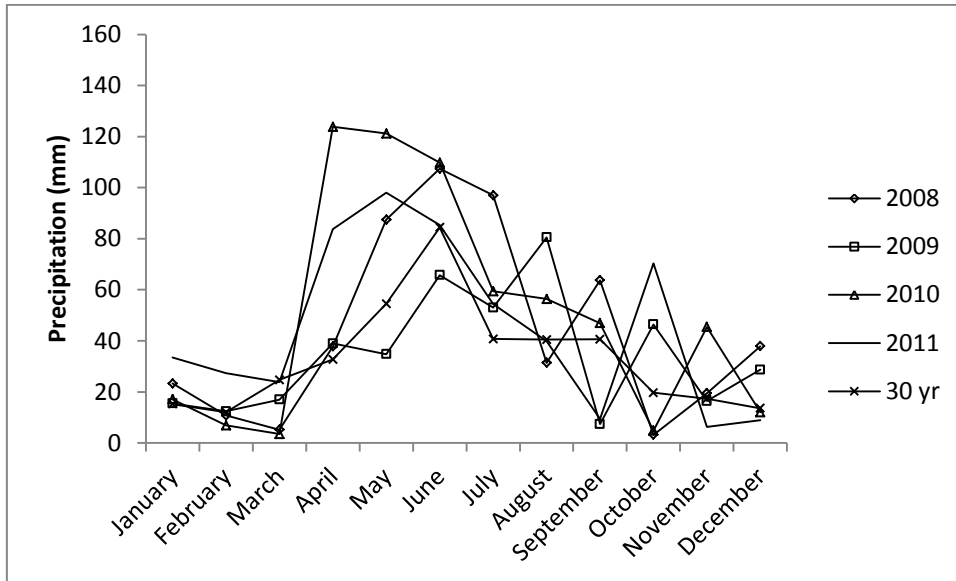
Saskatoon
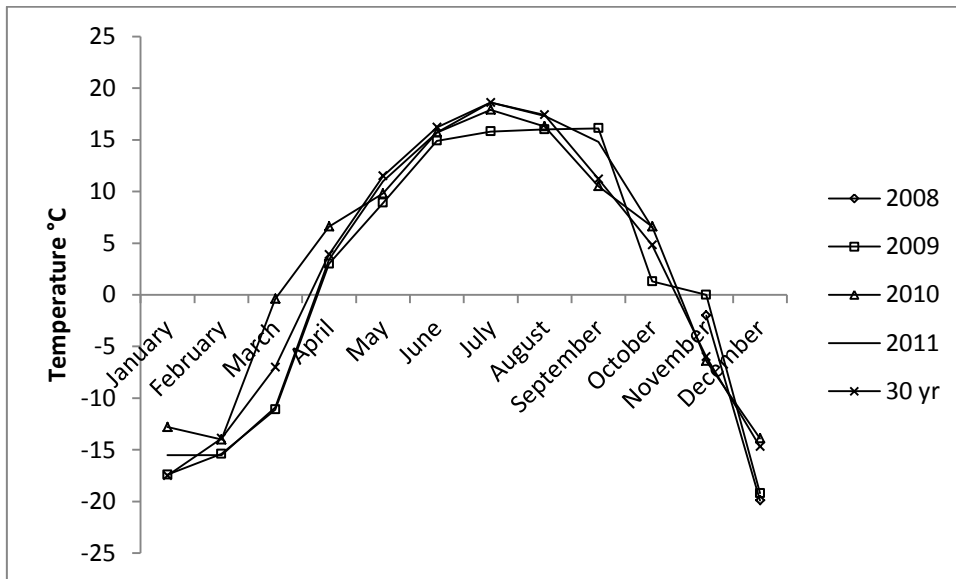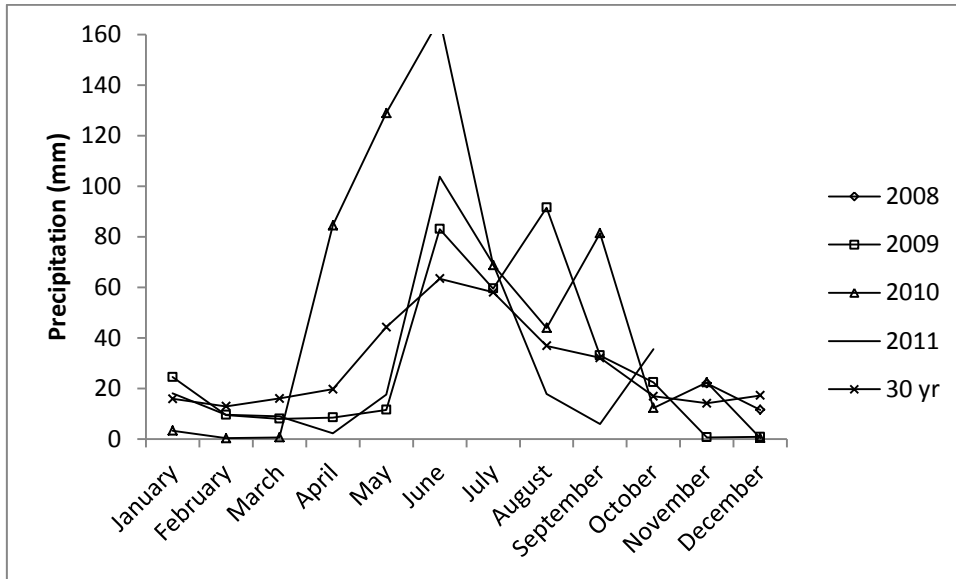
Swift Current
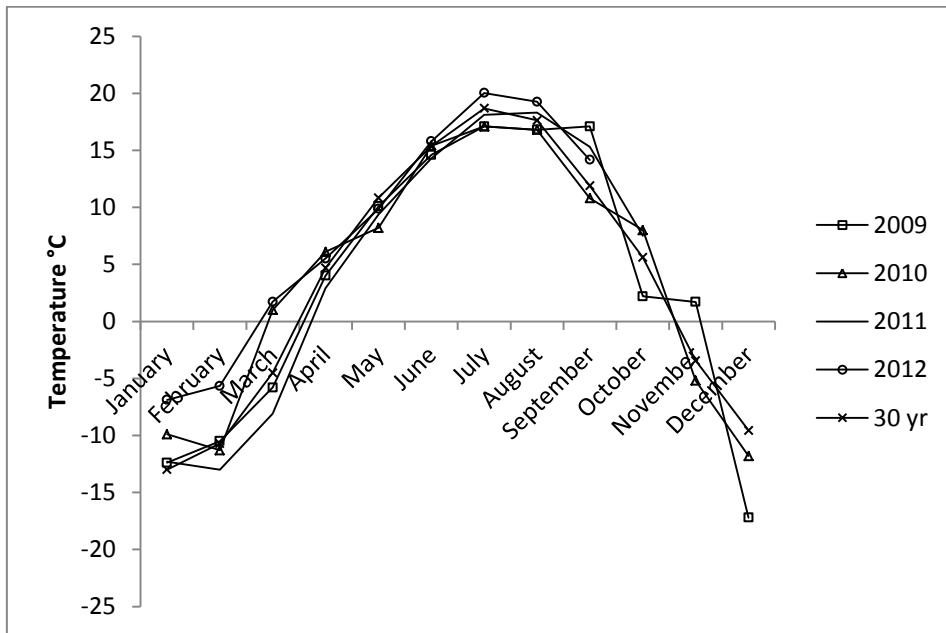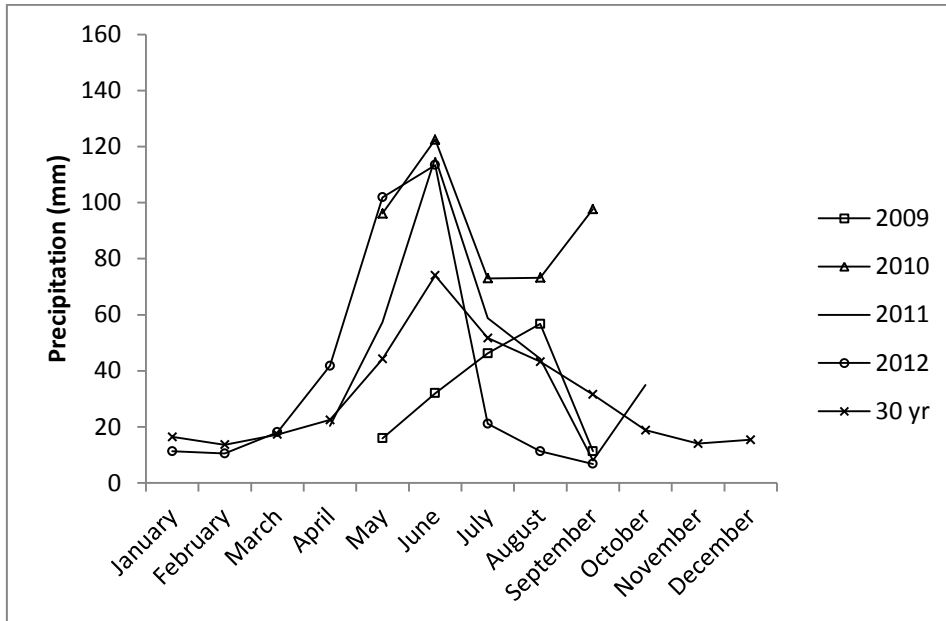
Fig. 3.1. Total monthly precipitation and average monthly temperature for 2008 to 2011 and 30-yr average for Lethbridge, AB; Saskatoon, SK; and 2009 to 2012 for Swift Current, SK.

Table 3.2. Mean total yearly dry matter (DM) yield of 10 sainfoin populations and a high-yielding alfalfa cultivar established in 2007 at Lethbridge, AB under rain-fed conditions.

| | Total DM yield from two cuts | | | | | | | |
| | 2008 | | 2009 | | 2010 | | | |
Populations	Yield	% Nova[‡]	Yield	% Nova	Yield	% Nova	3-yr mean	% Nova
	kg ha^{-1}							
Nova	9602	100	3381	100	4759	100	5914	100
Melrose	9913	103	4232	125	5737	121	6627	112
LRC 3401	10406	108	4195	124	4824	101	6465	109
LRC 3432	10096	105	5229	155	7593	160	7639	129
LRC 3509[§]	9605	100	3329	98	4435	93	5788	98
LRC 3511	10365	108	3697	109	4757	100	6273	106
LRC 3519	11062	115	4452	132	8566	180	8026	136
LRC 3900	10673	111	4239	125	4900	103	6604	112
LRC 3901	11326	118	5607	166	6078	128	7671	130
LRC 3902	11179	116	5767	171	8188	172	8378	142
AC Longview[¶]	9772	102	5316	157	8060	169	7716	130
Year means	10363a		4495c		6373b		7009	
LSD (p=0.05)[˙]	1068		1398		1410		913	

[‡] % Nova = (mean DM yield of a population/mean DM yield of Nova for the year) x 100

[§] *Onobrychis arenaria* subsp. A*renaria*

[¶] AC Longview alfalfa used was developed for high yield and disease resistance in western Canada.

Year mean yields followed by different letters are significantly different (restricted LSD at p = 0.05).

[˙]Mean treatment differences in a year greater than the LSD value are significantly different at (p = 0.05).

Table 3.3. Mean total yearly forage yield of 12 sainfoin populations and a high yielding alfalfa cultivar established in 2008 at Lethbridge, AB under irrigation.

	Total DM yield							
	2009		2010		2011			
Population	Yield	% Nova[‡]	Yield	% Nova	Yield	% Nova	3-yr mean	% Nova
	kg ha^{-1}							
Nova	11149	100	10452	100	7734	100	9778	100
Melrose	12433	112	12875	123	9480	123	11596	119
Delaney	12151	109	12546	120	9128	118	11275	115
Shoshone	12438	112	11106	106	9186	119	10910	112
LRC 3401	13069	117	11915	114	7709	100	10898	111
LRC 3432	11535	103	11074	106	7065	91	9891	101
LRC 3509[§]	11257	101	11155	107	8725	113	10379	106
LRC 3511	10804	97	12201	117	9248	120	10751	110
LRC 3519	14670	132	12447	119	9625	124	12247	125
LRC 3900	12192	109	10535	101	7734	100	10154	104
LRC 3901	13128	118	11294	108	8633	112	11018	113
LRC 3902	15352	138	11632	111	8868	115	11951	122
AC Longview[¶]	12752	114	12186	117	10809	140	11916	122
Year means	12533a		11648b		8765c		10982	
LSD (p=0.05)[˙]	1882		1973		1230		1234	

Total DM yield consisted of three cuts in 2009 and two cuts each in 2010 and 2011.

[‡] % Nova = (mean DM yield of a population/mean DM yield of Nova for the year) x 100

[§] *Onobrychis arenaria* subsp. A*renaria*

[¶] AC Longview alfalfa used was developed for high yield and disease resistance in western Canada.

Year means followed by different letters are significantly different (restricted LSD at p = 0.05).

[˙] Mean treatment differences in a year greater than the LSD value are significantly different at (p = 0.05).

For the rain-fed test established in 2007, the 2008 mean yield was higher (p < 0.05) than the other two years (Table 3.2). The mean yield for 2009 (4495 kg ha^{-1}) was lower (p < 0.05) than the 2008 (10,363 kg ha^{-1}) and 2010 mean yields (6373 kg ha^{-1}). Like most

perennial forage crops, sainfoin DM yields were normally highest in the first production year and thereafter progressively declined in subsequent years, a response observed by others in western Canada (Hanna and Smoliak, 1968). Significantly lower ($p < 0.05$) yields at Lethbridge in the second as compared to the third production year is not normal and may have been due to dry conditions in 2009 (Fig. 3.1). In this test 3-yr average biomass yield was higher ($p < 0.05$) for four newly developed sainfoin populations (3432, 3519, 3901, and 3902) than Nova (Table 3.2). This is a positive attribute of the new populations as they were developed for their ability to survive with alfalfa under a frequent harvest regime and DM yield in pure stands was not a primary selection criteria.

Four (3519, 3900, 3901 and 3902) of the eight newly developed sainfoin populations included in this rain-fed trial produced higher ($p < 0.05$) DM than Nova in the first production year. In the second and third production years, three newly developed sainfoin populations (3432, 3901, 3902; and 3432, 3519, 3902; respectively) yielded higher ($p < 0.05$) DM than Nova (Table 3.2). AC Longview alfalfa was not the highest yielding entry during the three production years. The increased yield performances of newly developed populations indicate that these populations could be used in pure stands without sacrificing biomass yield. The Siberian sainfoin (*O. arenaria*) produced similar or slightly less DM yield than Nova during the three years, including over the dry year in 2009.

In the 2008-established irrigated test, mean yield over years (Table 3.3) gradually decreased, which is the normal yield pattern in perennial forage trials. Three-year mean DM yield of 3519, 3901 and 3902 (newly developed populations) and two cultivars Melrose and Delaney, included in this test were higher ($p < 0.05$) than Nova. Four (3401, 3519, 3901 and 3902) out of eight newly developed sainfoin populations had higher ($p <$

0.05) DM yield than Nova in the first production year. But, in the second (2010) and third (2011) production years only (3519 and 3511 and 3519 populations produced higher (p < 0.05) yields than Nova. AC Longview alfalfa was not the highest yielding entry in this irrigated test over the first two production years. In the third production year, AC Longview alfalfa was the highest yielding entry and its DM yield was higher (p < 0.05) than Nova. The 40% increase in AC Longview alfalfa yield in the third production year was probably responsible for making its 3-yr mean DM yield higher (p < 0.05) than Nova. The fact that some of these newly developed sainfoin populations yielded close to a high-yielding alfalfa cultivar is worth noting as they were developed for their ability to survive in frequently harvested mixed stands and not for their DM yield in pure stands. The Siberian sainfoin (*O. arenaria*) produced slightly higher DM yield than Nova in all three years, but its 3-yr mean did not differ from Nova.

3.3.3. Frequent Harvest Trials

3.3.3.1. Lethbridge Test

The results of trials at Lethbridge, AB established in 2008 under irrigation identified sainfoin population x year interaction (p < 0.05). In this trial, DM yield in 2009 was lower (p < 0.05) than in2010 and 2011 (Table 3.4). Usually DM yields in perennial forage stands are higher in the first year of production than in subsequent years. Extremely dry conditions in March to May in 2009, a period when irrigation water is unavailable, likely reduced DM yield of the first cut.

Two of three new populations (3900 and 3902) developed at LRC produced higher (p < 0.05) DM yields than Nova over the three production years in pure stands (Table 3.4). New

populations 3900 and 3902 yielded 26 and 23% more ($p < 0.05$) DM, respectively than

Nova. The mean yield of 3901 did not differ from Nova as it was only 7% higher. The

higher DM yields of the new sainfoin populations suggest that these populations could be

used for hay or pasture production in this area.

Mean yields for all mixed stands and AC Grazeland alfalfa were greater ($p < 0.05$) than

Nova (Table 3.4). Superior yield for mixed stands of Nova/Grazeland (16%) over Nova

was expected as in pure stands Grazeland DM yields were 19% higher than Nova. Mixed

stands of the three new sainfoin populations had mean yields that were 11 to 15% higher

than mixed stands of Nova/Grazeland. This indicates that the new sainfoin populations

could be used in mixed stands under irrigation to take advantage of their higher biomass

yield. Total biomass production, however, is not the most important consideration from the

perspective of bloat prevention as alfalfa in a mixed stand can produce compensatory

growth when the proportion of sainfoin is reduced, thus maintaining DM yield of the stand

(Jefferson et al., 1994).

Table 3.4. Mean total yearly dry matter (DM) yield of four sainfoin populations in pure and mixed stands with AC Grazeland alfalfa established in 2008 at Lethbridge, AB under irrigation and with frequent harvest.

Population and Mixture	Total DM yield kg ha^{-1}							
	2009	% Nova[‡]	2010	% Nova	2011	% Nova	3-yr mean	% Nova
LRC 3900	14310	127	14956	118	13927	136	14398	126
LRC 3901	12136	108	12855	101	11764	115	12252	107
LRC 3902	14903	132	14137	111	12932	126	13990	123
Nova	11280	100	12708	100	10236	100	11408	100
3900/Grazeland	14432	128	15295	120	15091	147	14924	131
3901/Grazeland	13324	118	14733	116	15244	149	14433	127
3902/Grazeland	14358	127	14525	114	15619	153	14834	130
Nova/Grazeland	12187	108	13211	104	14404	141	13267	116
AC Grazeland[¶]	10869	96	13691	108	16328	160	13629	119
Year means	13089b		14012a		13949a		13682	
LSD (p = 0.05) [˙]	1942		2503		2772		1824	

Total DM yield consisted of three cuts in all three years.

[‡] % Nova = (mean DM yield of a population/mean DM yield of Nova for the year) x 100

[¶] AC Grazeland alfalfa used was developed for low initial rate of digestibility at Saskatoon, SK.

Year mean yields followed by different letters are significantly different (restricted LSD at p = 0.05).

[˙] Mean treatment differences in a year greater than the LSD value are significantly different at (p = 0.05).
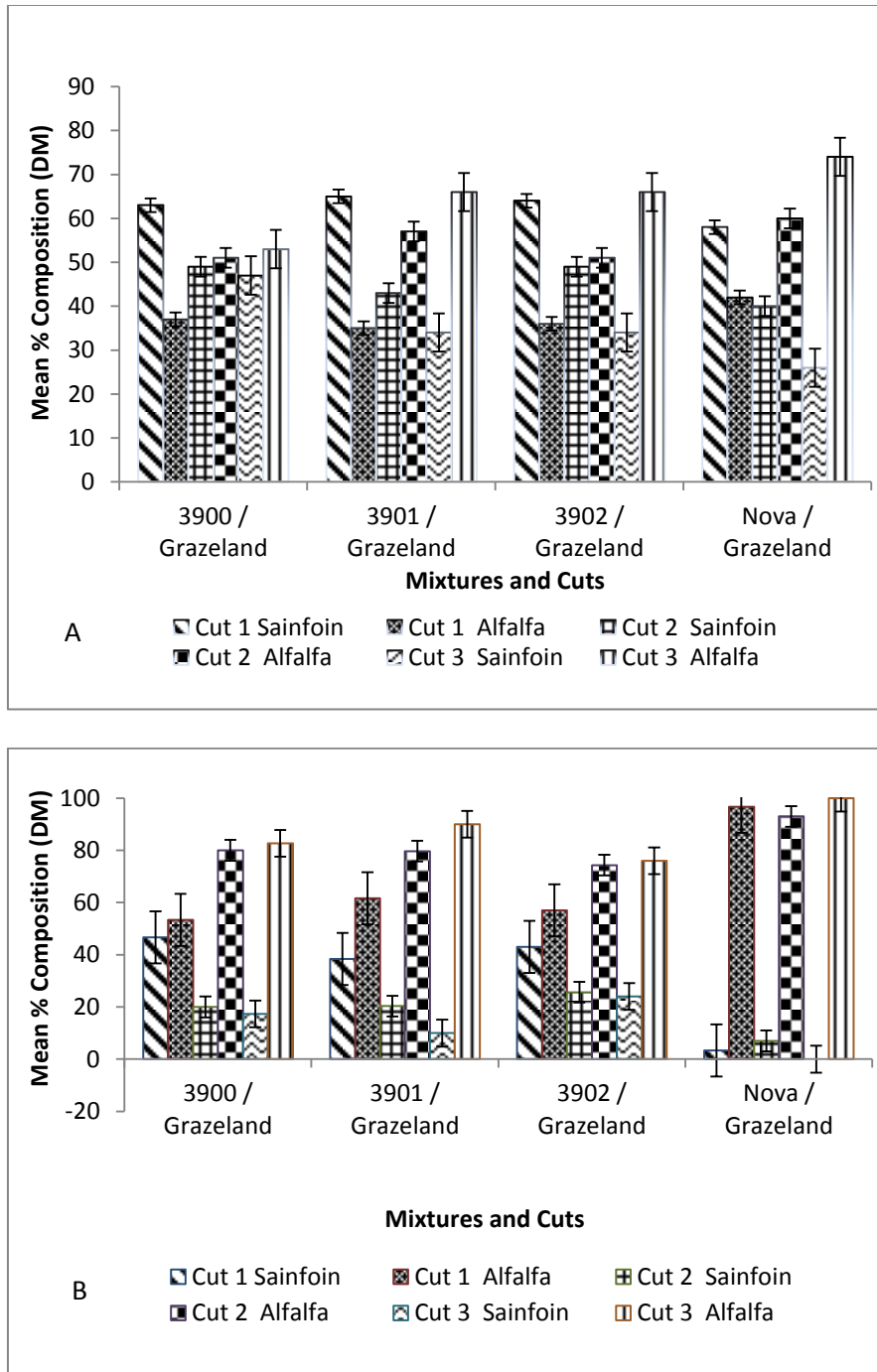
Fig. 3.2. Proportion of sainfoin and alfalfa in frequently cut plots observed in three cuts in (A) 2009 and (B) 2011 mixed stands established in 2008 at Lethbridge, AB.

For bloat-prevention in grazing cattle, the proportion of sainfoin in the mixed alfalfa pasture is of paramount importance. At first cut in the first production year, the proportion of sainfoin (DM basis) was higher (~ 20%) than alfalfa in all four combinations (Fig 3.2 A), even though seeding rate was targeted for a 50:50 plant stand. This was expected as sainfoin plants grow more rapidly in spring and mature earlier than alfalfa, resulting in a higher proportion of the DM at first cut. At second and third cuts, the proportion of alfalfa increased resulting in a decline in sainfoin in all mixed stands. In the first production year, the proportion of Nova declined from 58% in the first cut to 40% at second and 25% at third cut. These proportions are well above the minimum level of 10% to prevent bloat in cattle grazing mixed stands. It is important to note that this minimum proportion (10%) of sainfoin to prevent bloat was determined under confined feeding conditions where the amount of alfalfa and sainfoin in the diet was controlled (McMahon et al., 1999). For bloat-free grazing on a commercial scale where proportional intake of the two forages would be uncertain, it would likely be desirable to have higher proportion of sainfoin in mixed stands. In a grazing trial where separate strips of sainfoin and alfalfa were planted, 35% sainfoin reduced bloat by 77% in grazing cattle as compared to pure alfalfa (Wang et al., 2006). In the third production year, the proportion of Nova was less than 10% at first and second cut (Fig. 3.2 B) and was undetectable at third cut. In fact, at Lethbridge, Nova comprised <10% of the forage in the second production year. Therefore, it can be said that the newly developed populations have the potential to persist in mixed stands with alfalfa for at least three production years, with populations 3900 and 3902 contributing >20% of the biomass in third production year across all three cuts. Consequently, these populations have the potential to reduce the risk of bloat in cattle rotationally grazing mixed pasture.

3.3.3.2. Saskatoon Test

A year x population interaction was also observed for the plots established in 2008 in Saskatoon, SK. Again, due to spring drought in 2009, the mean DM yield for less ($p <$ 0.05) than the other two years (Table 3.5). The three newly developed sainfoin populations in pure stand yielded 5 to 13 % more biomass than Nova over the three years. Mean DM yields in pure stands of sainfoin were lower ($p < 0.05$) than in mixed stands and AC Grazeland. At this location, DM yield of AC Grazeland was higher ($p < 0.05$) than the sainfoin populations either in pure or mixed stands.

Table 3.5. Mean total yearly dry matter yield of four sainfoin populations in pure and mixed stands with AC Grazeland alfalfa established in 2008 at Saskatoon, SK under rain-fed conditions.

Population and Mixture	Total DM yield kg ha^{-1}							
	2009	% Nova[‡]	2010	% Nova	2011	% Nova	3-yr mean	% Nova
LRC 3900	3926	123	9602	102	8640	124	7389	113
LRC 3901	3102	97	9595	102	7901	113	6866	105
LRC 3902	3556	111	9103	97	8404	120	7021	108
Nova	3201	100	9415	100	6975	100	6530	100
3900/Grazeland	4651	145	11445	122	12334	177	9477	145
3901/Grazeland	4543	142	11036	117	12197	175	9259	142
3902/Grazeland	4569	143	11492	122	11130	160	9064	139
Nova/Grazeland	4432	138	10812	115	11151	160	8798	135
AC Grazeland[¶]	5386	168	12015	128	13368	192	10256	157
Year mean	4152b		10502a		10233a		8296	
LSD (p = 0.05)[˙]	889		1387		1944		1219	

Total DM yield consisted of three cuts in all three years.

‡ % Nova = (mean DM yield of a population/mean DM yield of Nova for the year) x 100

¶ AC Grazeland alfalfa used was developed for low initial rate of digestibility at Saskatoon, SK.

 Year mean yields followed by different letters are significantly different (restricted LSD at p = 0.05).

˙ Mean treatment differences in a year greater than the LSD value are significantly different at (p = 0.05).
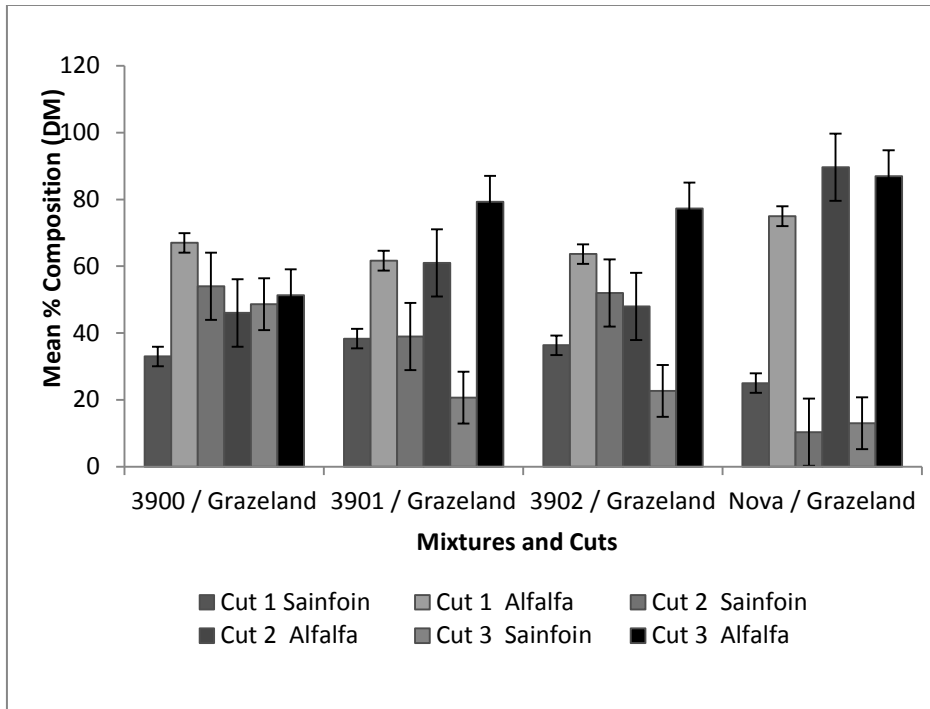
Fig. 3.3. Proportion of sainfoin and alfalfa in frequent harvest plots observed in three cuts in 2011 mixed stands established in 2008 at Saskatoon, SK.

At Saskatoon all mixed stands were seeded to have 50:50 sainfoin:alfalfa plant populations. However, from the first production year (2009) the proportions of sainfoin were lower than alfalfa. In the first cut, the proportion of sainfoin varied between 25 and 45% in mixed stands and further declined with subsequent cuts. In (2010, moisture conditions were more favourable and the proportions of sainfoin varied between 48 and 60% at the first cut. At second cut, the proportion of Nova declined to 17% whereas selected populations continued to account for >45% of DM in mixed stands (data not included). In 2011, the proportion of new sainfoin populations in mixed stands did not drop below 20%, even at the third cut. In contrast, Nova declined from 25% in the first cut to 10 and 11%, in the second and third cuts, respectively (Fig. 3.3 The proportion of sainfoin in mixed stands did not drop below 20% for selected populations even after the third cut. These data imply that

any one of the three new populations could be planted in mixed alfalfa pasture to reduce the risk of bloat over at least a 3 yr period.

3.3.3.3. Swift Current Test

Production conditions in Swift Current, SK differed substantially from the other two locations. Due to a severe drought in 2009 at Swift Current, only one cut was taken in the first production year. Mean DM yields for all populations and mixtures were extremely low (< 1000 kg ha^{-1}) and variable, making the data unfit for statistical analysis. Consequently, data were recorded in 2010, 2011 and 2012 with two cuts being obtained in 2010 and 2011, but as in 2009 only a single cut was possible in 2012.

As a population X year interaction was observed total DM yield was reported by year (Table 3.6). DM yield in 2011 was higher ($p < 0.05$) than in 2010 and 2012. DM yields in 2011 in all plots were higher than 2010 and 2012. Typically DM yields are the highest in the first production year, but moisture deficiencies in 2009 to 2012 may have altered this yield pattern. Pure stands of Nova yielded higher ($p < 0.05$) mean DM compared to the new sainfoin populations from 2010 to 2012 (Table 3.6). Yields from pure stands of Nova did not differ from the DM yield of mixed stands in 2010. In 2011 and 2012, all mixed stands produced higher mean yield than pure Nova. In 2011 mean DM yields for 3900 and Nova mixed stands were higher ($p < 0.05$) than pure stands of Nova, whereas in 2012 DM yields for the mixed stands with 3901, 3902 and Nova were higher ($p < 0.05$) than pure Nova. Mean yields for 2010 and 2011 at Swift Current (when two cuts could be taken) were lower than the yields at the other two locations. Dry and variable environmental

conditions may have favored the older cultivar Nova, when it was managed in a single-cut

production system.

Table 3.6. Mean total yearly dry matter (DM) yield of four sainfoin populations in pure and mixed stands with AC Grazeland alfalfa cultivar established in 2008 at Swift Current, SK under rain-fed conditions.

Population and Mixture	Total DM yield kg ha^{-1}							
	2010	% Nova[‡]	2011	% Nova	2012	% Nova	3-yr mean	% Nova
LRC 3900	4741	66	6151	87	2845	76	4579	76
LRC 3901	4959	69	5865	83	2982	80	4602	77
LRC 3902	5031	70	5425	77	3172	85	4542	76
Nova	7219	100	7069	100	3724	100	6004	100
3900/Grazeland	6643	92	8477	120	4117	111	6412	107
3901/Grazeland	6492	90	7549	107	4463	120	6168	103
3902/Grazeland	6771	94	7927	112	4426	119	6374	106
Nova/Grazeland	7101	98	8251	117	4684	126	6679	111
AC Grazeland[¶]	6790	94	7772	110	4273	115	6278	105
Year Mean	6194b		7165a		3854c		5738	
•LSD (p = 0.05)	640		912		535		407	

Note: 2009 data for DM yield was not included in the analysis as the DM yield was too small (<900 kg ha^{-1}) and the single-cut data were variable. Instead, single-cut data for 2012 was included to determine the 3-yr means for this trial.
‡ % Nova = (mean DM yield of a population/mean DM yield of Nova for the year) x 100
¶ AC Grazeland alfalfa used was developed for low initial rate of digestibility at Saskatoon, SK.
 Year mean yields followed by different letters are significantly different (restricted LSD at p = 0.05).
˙ Mean treatment differences in a year greater than the LSD value are significantly different at (p = 0.05).
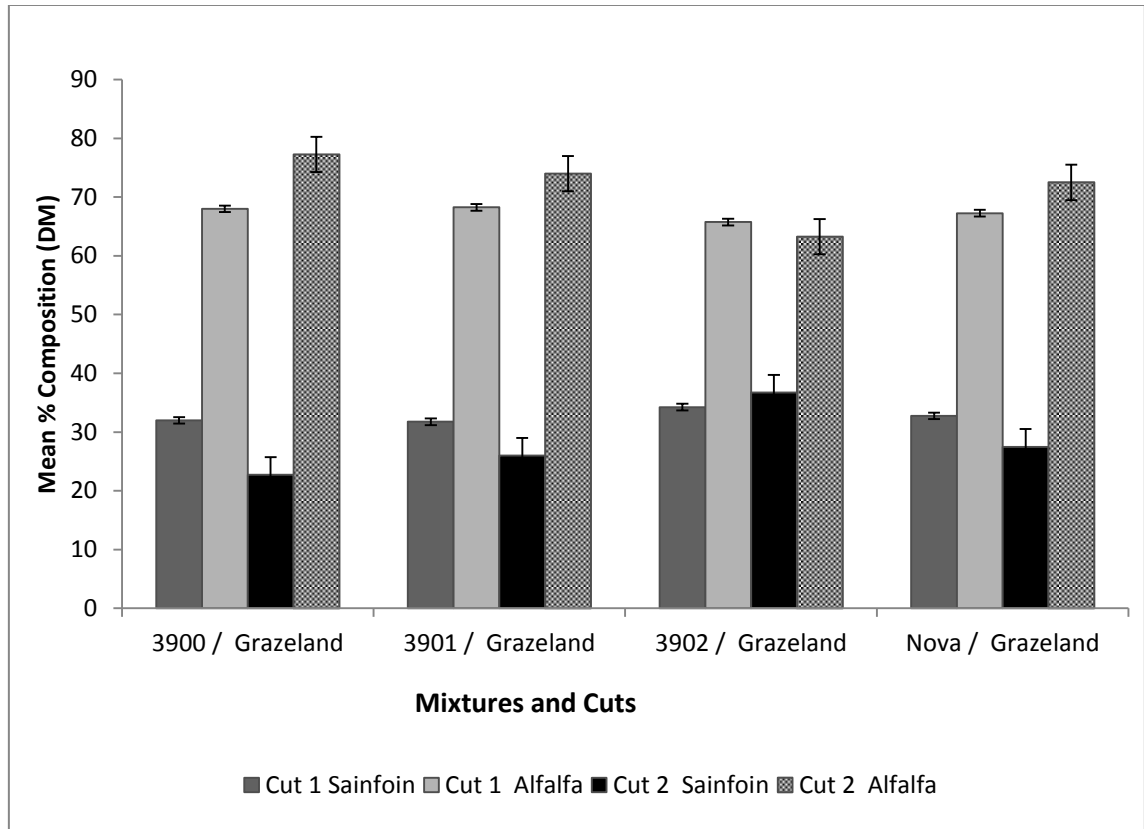
Fig. 3.4. Proportion of sainfoin and alfalfa in frequent harvested plots observed in three cuts in 2011 mixed stands established in 2008 at Swift Current, SK.

In Swift Current, where only one or two harvests could be taken per year, sainfoin populations behaved differently in mixed stands as compared to other locations. In 2011, the proportion of alfalfa was >60% in all mixed stands (Fig. 3.4). Nova accounted for >25% of the DM in mixed stands after the second cut, a level similar to newly developed populations. The only population that was numerically higher than Nova after the second cut in 2011 was 3902. Relatively poor performance of the new populations in pure stands at this location indicates that new sainfoin populations need to be developed which are adapted to the extremely variable and dry conditions in regions such as Swift Current to be viable in mixed stands with alfalfa.

3.4. Conclusions

This study was initiated to determine if the sainfoin populations selected at Lethbridge for their ability to survive under a multiple-cut system with or without alfalfa could be used in alfalfa pastures as a possible means to prevent or reduce bloat in western Canada. In this multi-location and multi-year study, no severe winter kill was noticed in pure or mixed stands of any of the sainfoin populations included, although the persistence of sainfoin was reduced over time. At Lethbridge (under rain-fed and irrigated conditions) and Saskatoon (rain-fed), new sainfoin populations produced higher DM yields than Nova in pure stands over three production years. However, at Swift Current, an established sainfoin cultivar Nova, yielded higher than selected sainfoin populations. This means that pure stands of the new sainfoin populations could be used to improve hay or pasture production in most, but not all regions of western Canada. For improved performance under extremely dry and variable conditions such as Swift Current, further populations would have to be developed. From the present study it seems possible that for locations with high-stress environments multiple-cut sainfoin may not have an advantage over single-cut sainfoin.

At Lethbridge and Saskatoon it was clear that the new sainfoin populations have potential in high performance grazing systems (alfalfa pasture) when the mixed stands would be harvested multiple times during the growing season. The new populations not only survived in mixed alfalfa stands for 4 yr under a harvest schedule to simulate grazing conditions, they accounted for sufficient DM (20%) in the stand to meet the criteria for reduced risk of bloat. This study suggested that under rotational grazing conditions Nova would be unlikely to significantly reduce the risk of pasture bloat after first production year. Our observation at Swift Current did not find that mixed stands of the selected

sainfoin populations were superior to Nova. In 2012 a decline in the proportion in Nova/Grazeland mixed plots was noticed. It is possible that the new populations may show their superiority over a longer period of time or under actual grazing as the stands were not cut very frequently at this location.

This study also indicated that that in most cases, mixed alfalfa-sainfoin stands are more productive than pure sainfoin stands, and therefore would be likely preferred by producers as a means of maximizing DM production while preventing bloat and maximizing the yield of beef from pasture.

Chapter 4

Condensed tannin concentration, botanical and in vitro fermentation characteristics of sainfoin populations

4.1. Introduction

Forage legumes that contain condensed tannins (CT) such as sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus* L.) do not cause bloat in ruminants (Berg et al., 2000; Wang et al., 2006; McMahon et al., 1999). CT bind to proteins through hydrophobic and hydrogen bonding in a pH-dependent/reversible manner (Min et al., 2003), reducing the formation of the stable foam that causes bloat in ruminants (Li et al., 1996; McMahon et al., 1999). CT can also improve the nutritional value of forage protein and have been reported to improve milk production in grazing ewes and dairy cows fed fresh forage (Wang et al., 1996; Woodward et al., 2000). However, high levels of CT may lower forage intake and digestibility (Barry and McNabb, 1999).

Li et al. (1996) found that as little as 1.0 mg CT g^{-1} DM in forage legumes prevented pasture bloat. McMahon et al. (1999) reported that 10% of sainfoin DM fed as hay, pellets or fresh herbage along with green chop alfalfa was sufficient to reduce the incidence of bloat by 45 to 93% in steers. When consumed with alfalfa, the presence of CT from sainfoin reduces the proteolysis of alfalfa and increases protein absorption and utilization by ruminants (McMahon et al., 1999). Wang et al. (2006) recorded a 77% reduction in the incidence of bloat in cattle grazing alfalfa/sainfoin mixed pasture containing 35% sainfoin.

The objectives of this study were to describe the botanical characteristics and condensed tannin concentrations in old and newly developed sainfoin populations and to ascertain the effect of sainfoin CT on in vitro digestion. Polyethylene glycol (PEG), which is known to deactivate CT (Waghorn and Shelton, 1997) was added to in vitro digestions to assess the effect of sainfoin CT on rumen fermentation.

4.2. Materials and methods

4.2.1. Forage establishment

Pure stands of four sainfoin populations comprising Nova, LRC-3401, LRC-3432 and LRC-3519 were established in the spring of 2009 with four replicate plots arranged in a randomized complete block design (RCBD). Plots were 2 m x 6 m with 10 rows seeded 20 cm apart in each plot. Sainfoin seeds were inoculated using Royal Peat legume inoculant (Becker Underwood, Saskatoon, Canada) prior to seeding. At the time of seeding, N and P fertilizer were applied at the rate of 10 and 60 kg ha^{-1} respectively, to aid in pasture establishment. Perennial and grassy weeds were controlled using glyphosate (N-(phosphonomethyl) glycine) prior to seeding and annual weeds were controlled by mowing the crop twice during the establishment year on June 11 and July 19 using a small plot forage harvester. Residue was baled manually removed from the plots.

4.2.2. Botanical characteristics

Plant height and stem thickness was measured for 12 plants when plots were at 50% flower. Height was measured as the distance from base of crown to the tip of longest stem and stem thickness was measured using a caliper. The number of days for the plots to reach 50% flower was recorded.

4.2.3. Sample preparation and analysis

In 2010, forages were collected by clipping plants 5 cm above ground from three different locations in each plot during the vegetative (<10% bud), bud (>10% bud but no flowers), early flower (<30% of stems flowered) and late flower (>80% of stems flowered with >30% of flowers on raceme open) stage on May 18, June 1, June 15 and July 2, respectively. A subsample of forage harvested at the early flower stage was separated into leaves, stems and inflorescence. A second set of forage samples were collected by clipping plants at 5 cm above ground at three different locations in each plot after primary growth on 1 June, 2010 and after regrowth on 6 August, 2010. These samples were used to measure CT levels in primary growth and regrowth samples during the bud stage.

Plant samples from each plot were composited into single sample resulting in four samples per population. Forage samples were dried at 55°C in a forced-air oven for 72 h. Dried samples were ground to pass a 1-mm screen in a Wiley mill and analyzed for N by combustion - mass spectrometry (NA 1500, Carlo Erba Instruments, Milan, Italy), acid detergent fibre (ADF) and amylase-treated neutral detergent fibre (aNDF) using an Ankom fiber Analyzer 200 (ANKOM Technology Corp., Macedon, NY,

USA) according to the procedure of McGinn et al. (2004), and extractable condensed tannins (ECT) as described by Terill et al. (1992). Purified ECT from whole plant sainfoin in the vegetative stage of growth was used as a reference standard in the determination of ECT. Determination of ECT for each forage sample was carried out in duplicate using a 500 mg of oven-dried, ground forage. Samples were extracted three times with a 20 mL solution of 7:3 (vol/vol) acetone/water solution containing 0.1% ascorbic acid and 10 mL diethyl ether [4.7:2.0:3.3 acetone:water:diethyl ether (vol/vol)]. Solutions were vortexed, centrifuged and a mixture of butanol/HCl [95% butan-1-ol: 5% HCl (36%) (vol/vol); 6 mL] was added. The mixture was incubated in a boiling water bath for 75 min. Solutions were cooled on ice and immediately read at 550 nm using a spectrophotometer (UltraSpec Plus 4054, Pharmacia, Baie d'Urfe', QC).

4.2.4. In vitro assessment of rumen fermentation characteristics of sainfoin

Whole plant material at the bud stage of growth was used to assess the effect of CT on in vitro ruminal degradability. Comparison was made on the effect of sainfoin CT on ruminal fermentation characteristics in the presence or absence of PEG (MW, 3,350; Sigma) in the incubation fluid. Inoculum was prepared using fresh rumen fluid collected 2 h after morning feeding from two rumen cannulated Holstein cows fed a 300:700 (DM hasis) barley grain:barley silage diet. Rumen fluid was strained through 4 layers of cheesecloth and transported in a thermos under anaerobic conditions prior to combining with mineral buffer as described below. Cows were cared for according to the standards of the Canadian Council of Animal Care (1993).

Ground samples (600 mg) of whole plant sainfoin from the four sainfoin populations were weighed in triplicate into 120 ml serum bottles (Wheaton, Millville, USA). Samples were incubated with and without PEG at 2.3 g L^{-1} of phosphate:carbonate buffer solution (McSweeney et al., 1999). Rumen fluid was diluted 1:2 (v/v) in the anaerobic phosphate:carbonate buffer solution as described by Goering and Van Soest (1970) and as modified by Thoedoridou et al. (2011). No NH_4HCO_3 was used in preparation of the bicarbonate buffer which consisted entirely of $NaHCO_3$ so as to increase the likelihood that N would be a limiting substrate for rumen microbes. Aliquots (40 ml) of the buffered rumen fluid were dispensed into previously warmed serum bottles at 39 °C and flushed with N_2. Serum bottles were sealed with butyl rubber stoppers and incubated for 24 h in a shaking water bath at 39 °C. Three blanks with and without PEG of buffer rumen fluids without sainfoin samples were also included as controls.

Concentrations of ammonia in rumen fluid were determined by the phenol-hypochlorite method (Weatherburn, 1967). Volatile fatty acids were analyzed by gas chromatography using a Hewlett Packard Model 5890 series Plus II gas-liquid chromatograph equipped with a 15 m x 0.53 mm i.d. NUKOL fused silica capillary column (Supelco Canada, Mississuauga, ON).

Dry matter degradability (DMD) was estimated as the final weight expressed as a percent of the initial weight.

4.2.5. Statistical analyses

Data was analyzed using analysis of variance with the MIXED Procedure of SAS

(2009). Arithmetic means were calculated for plant populations using PROC means.

Fixed effects were the stage of maturity and plant part, and CT concentration was

considered as a variable effect. Treatment (sainfoin populations) and PEG treatment

were main effects and replication was considered as a random effect. Tukey-Kramer's

multiple comparison test was used to identify differences (p<0.05) in CT

concentrations between plant stage of maturity, and plant parts.

4.3. Results

4.3.1. Botanical characteristics

Plant heights during primary growth for Nova, 3401, 3432 and 3519 were 78, 87, 92

and 88 cm respectively, and 38, 39, 40 and 40 cm, respectively after regrowth. Stem

thicknesses were 6.0, 6.5, 7.0 and 7.0 mm and days to 50% flower were 40, 40, 34 and

35 for Nova, 3401, 3432 and 3519, respectively (Table 4.1).

4.3.2. Chemical Characteristics

There were no differences (p>0.05) in the CP, NDF or ADF concentration among

populations at the vegetative stage. Similarly, there were no differences at the bud,

early flower or late flower stages (Table 4.2).

Table 4.1. Plant heights (cm), stem thickness (cm) and days to 50% flower of Nova
and three selected sainfoin populations at primary growth and regrowth stages

	Nova	3401	3432	3519	P value
Plant height (cm)					
Primary growth	78.0[b]	87.0[a]	92.0[a]	88.0[a]	0.03
Regrowth	38.0	39.0	40.0	40.0	0.54
Stem thickness (mm)	6.0	6.5	7.0	7.0	0.36
Days to 50% flower	40	40	34	35	

Plant heights and stem thickness of the primary growth and regrowth taken when
plants were at 50% flowering.
Means followed by different letters are significantly different.

Table 4.2. Mean crude protein, neutral detergent fibre and acid detergent fibre (g kg^{-1}
DM) of four sainfoin populations at different stages of maturity at Lethbridge, AB.

	Nova	3401	3432	3519	SE	P value
	g kg^{-1} DM					
Vegetative						
CP	225	232	230	229	3.5	0.62
ADF	252	255	244	255	3.2	0.72
NDF	275	278	268	276	3.0	0.55
Bud						
CP	217	211	210	208	3.0	0.78
ADF	263	256	266	258	2.4	0.56
NDF	289	287	290	283	3.5	0.63
Early flower						
CP	195	192	190	194	2.2	0.64
ADF	366	357	352	351	2.4	0.48
NDF	400	394	387	390	3.2	0.58
Late flower						
CP	178	177	170	172	2.4	0.72
ADF	381	380	386	375	3.3	0.57
NDF	420	426	431	419	2.6	0.65

Vegetative stage: < 10% bud
Bud stage: > 10% bud and no flower
Early flower: > 50% stems flowered with < 30% of flowers on raceme opened
Late flower: > 80% stems flowered with > 30% of flowers on raceme opened

Table 4.3. Extractable condensed tannin concentration of plant parts, after primary growth and regrowth, and at different maturities in four selected sainfoin populations at Lethbridge, AB.

	Nova	3401	3432	3519	SE	P value
	g kg^{-1} DM					
Plant part						
Leaf	35.7^a	35.3^a	31.3^a	32.7^a	0.4	0.56
Inflorescence	17.4^b	18.6^b	17.2^b	18.0^b	0.3	0.52
Stem	8.9^c	9.4^c	10.5^c	9.1^c	0.3	0.61
Cut						
Primary growth	33.2^b	33.6^b	32.9^b	33.1^b	0.4	0.62
Regrowth	34.6^a	34.8^a	34.0^a	34.2^a	0.3	0.78
Maturity stage						
Vegetative	30.2^c	31.2^c	29.2^c	30.0^c	0.4	0.38
Bud	33.7^a	33.9^a	32.8^a	33.3^a	0.4	0.42
Early flower	31.6^b	32.1^b	30.1^b	32.1^b	0.2	0.12
Late flower	28.9^d	29.1^d	28.6^c	28.5^d	0.3	0.54

Primary growth contained 37% leaves and 63% stem
Regrowth contained 46% leaves and 54% stem
Vegetative stage: < 10% bud
Bud stage: > 10% bud and no flower
Early flower: > 50% stems flowered with less than 30% of flowers on raceme opened
Late flower: > 80% of stems flowered with greater than 30% of flowers on raceme opened
Mean values with different superscripts within the same column differ at $p < 0.05$

4.3.3. CT concentrations

Plant parts

There was no difference (p>0.05) in CT concentrations in the leaves of the four sainfoin populations (Table 4.3). Similarly, there was no difference in CT concentrations in the stems or inflorescence among populations. Leaves had higher (P < 0.001) CT concentrations than inflorescences, which in turn were higher in CT than stems (Table 4.3).

Plant maturity

All populations had similar (p>0.05) CT concentrations at the vegetative stage of maturity. Similarly, there was no difference in the concentration of CT at the bud stage among sainfoin populations. Both the early and late flower stages of all populations also had similar CT concentrations (Table 4.3). All populations had the highest concentration of CT concentration at the bud stage, followed by the early flower, vegetative and late flower stages (Table 4.3).

4.3.4. Rumen fermentation characteristics

There were no differences in the total VFA, NH_3-N or DMD among the different sainfoin populations after 24 h of incubation (Table 4.4). Including PEG in the incubation media increased (p<0.05) ammonia accumulation, but did not alter the concentration of total VFA or DMD among sainfoin populations (Table 4.4).

Table 4.4. *In vitro* fermentation characteristics of four sainfoin populations after 24 h of incubation.

	Nova		3401		3432		3519		SE	P value		
	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG		P	T	PxT
pH	6.5	6.6	6.4	6.5	6.4	6.5	6.5	6.6	0.1	NS	NS	NS
DMD (%)	69.4	70.6	69.2	70.6	68.3	70.4	70.0	70.5	2.2	NS	NS	NS
End products												
NH_3-N (mM)	28.8	32.6	28.9	32.6	29.6	33.1	28.3	32.6	0.5	NS	*	*
Total VFA (mM)	129.7	131.2	129.4	130.9	130.1	131.1	129.1	131.1	7.6	NS	NS	NS
Molar proportion												
(% of total VFA)												
Acetic acid	57.9	57.4	58.2	57.1	58.4	57.5	57.8	57.2	0.9	NS	NS	NS
Propionic acid	17.9	18.4	18.2	18.5	18.1	18.5	18.0	18.3	0.3	NS	NS	NS
Butyric acid	8.7	8.4	8.6	8.4	8.5	8.3	8.7	8.2	0.2	NS	NS	NS
Acetic:Propionic	3.23	3.12	3.20	3.09	3.20	3.11	3.21	3.13	0.1	NS	NS	NS

NS : Not significant
* Significant at p<0.05
P : sainfoin population
T : PEG treatment.

4.4. Discussion

LRC-3432 and LRC-3519 matured earlier than Nova and LRC-3401. The two attained 50% flower 6 and 5 days before Nova and LRC-3401. This implies LRC-3432 and LRC-3519 could be grazed or cut for hay earlier than Nova and LRC-3401.

As plants mature, the cell wall content increases leading to reduction in CP and increases in ADF and NDF concentrations (Frame, 2005), a response observed in this study. The late flower stage of sainfoin had higher ADF and NDF, and lower CP concentrations as compared to the other growth stages. Plant maturation leads to reduction in the proportion of leaves and increase in the proportion of stems and as the leaves contain more CP than stems there is reduction in CP concentration of the whole plant (Sheaffer et al., 2009).

Sainfoin leaves had the highest concentration of CT followed by the inflorescence and stem, respectively. Sainfoin is different from most CT-containing forage legumes grown in western Canada such as *Dalea purpurea* (purple prairie clover), *Lotus corniculatus* (birdsfoot trefoil) and *Trifolium hybridum* (alsike clover), which have the highest CT concentrations in their inflorescence (Berard et al., 2011; Jin et al., 2012). The CT concentrations for these species increase with plant maturity, but with sainfoin CT concentration decreases with plant maturity (Berard et al., 2011). Although sainfoin has CT in the inflorescence and stem, most CT are concentrated in the leaves. As the plant matures, the leaf:stem ratio declines resulting in a reduction in the concentration of CT in the whole plant. Barry (1989) speculated that the inflorescence

of some plants contains CT to protect reproductive organs from attack by invasive organisms.

The CT concentrations in the sainfoin populations in this study were within the range reported by McMahon et al. (2000) and Berard et al. (2011). Berard et al. (2011) reported a wide range in CT concentration from 16.3 to 94.4 g kg^{-1} for six different sainfoin cultivars grown in different locations in western Canada. It has been reported that CT concentrations may be affected by climate (Anuraga et al., 1993; Lees et al., 1994), soil fertility (Barry and Forss, 1983) and plant maturity (McMahon et al., 2000) attributing to wide variation in CT concentrations. Even though the germplasm used in this study differed, there were no differences in their CT concentrations, possibly because they were grown under similar environmental conditions and harvested at the same stage of maturity.

Inclusion of PEG in the medium to inactivate the biological activity of CT increased (p<0.05) NH_3-N concentrations in the incubation medium as compared to incubations without PEG. This observation was consistent with previous studies by McMahon et al. (2000) and Theodoridou et al. (2011) where biologically active sainfoin CT decreased NH_3-N concentration in the rumen fluid. This suggests that sainfoin protein was not as rapidly degraded in rumen fluid and hence less NH_3-N was released as a result of the deamination of amino acids. It is also possible the sainfoin CT directly reduced proteolytic activity in the rumen (Jones et al., 1994; Waghorn et al., 1998) through inhibition of proteolytic bacteria (McMahon et al., 2000). Barry et al. (1986) observed an increased efficiency in N utilization in the rumen of sheep fed high tannin

L. pedunculatus compared to sheep fed Lotus treated with PEG, which led to reduction in urinary N excretion.

It has been reported that when dietary CT exceed 50 g kg^{-1} DM, there is reduction in ruminal digestion of DM (Terrill et al., 1992). The CT concentration of the sainfoin populations in this study were lower than 50 g kg^{-1}, a factor that may have resulted in DMD not differing between sainfoin incubated with or without PEG. Reduction in rumen ammonia concentration was the most common rumen fermentation parameter affected by presence of CT in the diet (Waghorn, 2008). The fact that production of total VFA from sainfoin was not affected by addition of PEG during in vitro digestion is also in agreement with the lack of a difference in DMD.

4.5. Conclusion

This study indicates that condensed tannins were present in the leaves, stems and inflorescence of sainfoin with the highest concentration in leaves. The bud growth stage of sainfoin had the highest condensed tannin concentration, but the concentration of CT decreased with advancing maturity of the plant. Sainfoin CT reduced N degradation in in vitro ruminal batch cultures without affecting DMD suggesting that it may offer the advantage of improving the efficiency of N utilization in ruminants without negatively impacting feed conversion efficiency.

Chapter 5

Alfalfa pasture bloat can be eliminated by intermixing with newly developed sainfoin population

5.1. Introduction

Alfalfa is one of the most important forage legumes grown in western Canada. Beef cattle (*Bos taurus* L.) weight gains on alfalfa pastures under proper management are comparable to those achieved in confined feedlot systems (Popp et al., 2000). Live weight gains of $1.0 - 1.5$ kg d^{-1} (Popp et al., 1997) and gains of 440 to 820 kg ha^{-1} (Burris et al., 1993) have been reported for steers grazing alfalfa. However, the risk of frothy bloat is a major deterrent to the grazing of pure alfalfa pastures by cattle (Dalberg et al., 1988). The protein in alfalfa is rapidly fermented in the rumen and contributes to the formation of stable foam that contributes to pasture bloat (Wang et al., 2012). Bloat is often responsible for mortalities in cattle grazing alfalfa (Wang et al., 2012) as well as those in feedlots (Nagaraja et al., 1998) and is recognized as an important digestive disorder. The risk of bloat has prompted most producers to limit the level of alfalfa and other bloat causing legumes in pastures to less than 30% (Veira et al., 2010).

Legumes that contain condensed tannins (CT) such as sainfoin and birdsfoot trefoil (*Lotus corniculatus* L.) do not cause bloat in ruminants (Berg et al., 2000). CT bind to proteins through hydrophobic and hydrogen bonding in a pH-dependent/reversible

manner (Min et al., 2003), reducing the formation of stable foam and the incidence of bloat (Li et al., 1996; McMahon et al., 1999). Condensed tannins can also improve the nutritional value of forage protein and have been reported to improve milk production in grazing ewes and dairy cows fed fresh forage (Wang et al., 1996, Woodward et al., 2000). However, high levels of CT may lower forage intake and digestibility (Barry and McNabb, 1999).

Li et al. (1996) found that as little as 1.0 mg CT g^{-1} DM is needed in forage legumes to prevent pasture bloat. McMahon et al. (1999) reported that about 10% of sainfoin DM fed as hay, pellets or fresh herbage in a fresh alfalfa diet was sufficient to reduce bloat incidence by 45 to 93% in confined steers fed in pens. When consumed with alfalfa, CT in sainfoin reduce the proteolysis of alfalfa and increases protein absorption and utilization by ruminants (McMahon et al., 1999). Wang et al. (2006) recorded a 77% reduction in the incidence of bloat in cattle grazing alfalfa/sainfoin mixed pasture that contained 35% sainfoin. Acharya (1998) observed that the palatability of sainfoin was comparable to alfalfa while other studies have suggested that alfalfa is more palatable than sainfoin (Gesshe and Walton, 1981; Marten et al., 1987). Average daily gains of 0.80 and 0.86 kg d^{-1} have been measured for steers grazing pure sainfoin pastures (Mowrey et al., 1992; Marten et al., 1987), gains which are comparable to those in cattle grazing alfalfa. The limitation of sainfoin is its low persistence under grazing and in mixtures with alfalfa as well as its poor regrowth after grazing (Mowrey and Matches, 1991). A new sainfoin population LRC-3519, has been selected at the Lethbridge Research Centre (LRC), AB, Canada for its regrowth properties after cutting and has been shown to persist in mixed alfalfa stands in western Canada

(Acharya et al., 2013). However, the bloat-reduction/prevention property of this new population needs to be evaluated. Our working hypothesis was that this new population would be more persistent in mixed pastures with alfalfa than an older cultivar Nova, and thereby reduce the occurrence of pasture bloat in cattle grazing alfalfa/sainfoin mixed pastures. Nova was used for comparison as this cultivar is one of the two registered for commercial production in Canada and it is presently the standard check cultivar in western Canada.

The objectives of this study were: 1) to evaluate the performance of a new sainfoin population (LRC-3519) in mixed alfalfa stands compared to check cultivar Nova; 2) to assess anti-bloat efficacy of the new population compared to Nova due to improved persistence when mixed with alfalfa; and 3) to determine the feed preference of cattle for alfalfa and sainfoin when both are available for grazing in the pasture at the same time.

5.2. Materials and methods

The study was conducted in 2010, 2011, and 2012 grazing seasons at the LRC, Lethbridge, Alberta, Canada, at 49° 42' N latitude 112° 47' W longitude with an altitude of 899 m above sea level. Alfalfa-sainfoin mixed pastures were grown on a slightly alkaline clay loam dark brown Chernozem soil (Larney and Janzen, 2012). Lethbridge has a semi-arid climate with an average maximum temperature of 12.3 °C and an average minimum temperature of −1.1 °C with an average precipitation of 365 mm yr^{-1} (Environment Canada, 2013).

5.2.1. Determination of bloat occurrence and ruminal fermentation parameters of cattle grazing alfalfa/sainfoin mixed pastures

Pasture establishment

The pastures were established on June 19, 2008 and seasonally grazed in the three study years. The two mixed pasture treatments consisted of the cultivar Nova sainfoin (Hanna, 1980), presently used as check cultivar in western Canada, and one of the newly developed populations LRC-3519 grown in mixtures with a high alfalfa variety, AC Blue J (ABJ) (Acharya et al., 1995). LRC-3519 was developed at LRC based on selection for enhanced regrowth in a multiple cut system and persistence in mixed alfalfa stands under irrigation. The two pasture mixes (Nova/ABJ and 3519/ABJ) were organized in a randomized complete block design (RCBD) with four replicate pastures per treatment. Mature (3-4 year old) ruminally-fistulated Angus steers were grazed on these mixed pastures and observed for the occurrence of bloat.

The alfalfa/sainfoin mixed pastures were 4050 m^2 with all pastures seeded with alfalfa and sainfoin in alternate rows with 18 cm row spacing (except in the preference study) using a 5 m wide no-till press-wheel type drill seeder (John Deere, Canada). Seeding rate were at 5 and 15 kg ha^{-1} pure live seed for alfalfa and sainfoin respectively, to achieve a 50:50 herbage density. Alfalfa and sainfoin seeds were inoculated using Dormal® (Becker Underwood, USA) and Royal Peat (Becker Underwood, Canada) inoculants respectively prior to seeding. At the time of seeding, N and P fertilizer was applied at the rate of 10 and 60 kg ha^{-1} respectively, to aid in pasture establishment.

Perennial and grassy weeds were controlled using glyphosate (N-(phosphonomethyl) glycine) prior to seeding and annual weeds were controlled by mowing the crop twice during the establishment year. Residue was manually removed from the fields. In 2009, pastures were cut twice on June 11 and July 19 using a forage harvester, Hege 180 (Wintersteiger, Austria) equipped with a sickle bar mower and the forage baled and removed from the paddocks.

Prior to the bloat studies, the pastures were rotationally grazed twice in each of 2010 (June 28 to August 31) and 2011 (June 19 to August 29) using steers at a stocking rate of 4.0 AUM ha^{-1} year^{-1}, resulting in a season-long stocking rate of 8.6 steers ha^{-1}. To maximize bloat occurrence this study was carried out from September 29 to October 21 in 2010 and September 26 to October 19 in 2011 on second harvest regrowth when both alfalfa and sainfoin were at the vegetative stage. At the time of study, the mean proportions of sainfoin (on a dry matter basis) in the pastures were 7 and 29% in 2010; and 5 and 28% in 2011 for Nova and LRC-3519, respectively.

Forage samples were collected in both study years by clipping plants with shears within a 0.36-m^2 quadrat at four different locations in each plot at a height of 5 cm above ground at both the primary and regrowth stages before steers were turned into the pastures. These samples were manually separated into alfalfa and sainfoin and dried in a forced-air oven at 55˚C for 72 h to determine dry matter (DM) yield and the proportion of sainfoin and alfalfa in pasture on a DM basis.

Steers were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Assessment of pasture bloat

Pasture bloat was assessed using the procedure described by Majak et al. (1995), and Veira et al. (2010). The study was conducted using 10 Angus steers (615 ± 36 kg) fitted with ruminal cannulae. The steers were adapted in a separate alfalfa/sainfoin mixed pasture for 7 days prior to the start of the experiment. Steers were randomly divided into two groups of five and assigned to two treatments in a crossover design with each group being exposed to both treatments. To ensure daily exposure to fresh pasture, pastures were divided into 14 paddocks using electric fence and each paddock was grazed for 1 d only. The steers were allowed to graze the pastures for 6 h d^{-1} from 0800 to 1400, and were held in a fenced area with access to water, but no feed each night. This management approach maximizes the risk of bloat in cattle grazing alfalfa (Majak et al., 1995).

The steers were observed and scored for bloat using visual scoring of 0 to 3; employing the system of Paisley and Horn (1998). Scores '1' and '2' were classified as sub-acute bloat and '3' as acute bloat. The length of each crossover test continued until the sum of incidences of bloat from both groups reached a minimum of 24. Any steer with a bloat score of 3 was haltered and its cannula opened to relieve pressure. At the end of the daily grazing period, the cannula of each steer was opened and rumen condition was scored to document the degree of frothiness of rumen contents. Multiple bloats on the same day by the same animal were considered as one case of bloat although the number of distensions per animal-day was recorded. Immediately after completion of the 6-h grazing period and when bloat occurred, rumen fluid was collected from four locations within the rumino-reticulum of each steer, and was

prepared by straining the rumen contents through four layers of cheesecloth. The pH of rumen fluid was measured using a portable laboratory pH meter (Hach Company, USA), subsampled and processed for determination of ammonia nitrogen, and volatile fatty acids as described below.

Samples analysis

Dried forage samples were ground to pass a 1-mm screen in a Wiley mill and were analyzed for N by combustion - mass spectrometry (NA 1500, Carlo Erba Instruments, Milan, Italy), acid detergent fiber (ADF) and amylase-treated neutral detergent fiber (aNDF) using a Ankom fiber Analyzer 200 (ANKOM Technology Corp., Macedon, NY, USA) as described by McGinn et al. (2004), organic matter (OM) by ashing samples at 550˚C for 6 h (AOAC, 1999; #943.01), and extractable condensed tannins (ECT) by the procedure described by Terill et al. (1992). Purified ECT from whole plant sainfoin in the vegetative stage of growth was used as a reference standard in the determination of ECT. Determination of ECT for each forage sample was carried out in duplicate using a 500 mg oven-dried ground sample. Samples were extracted three times with a 20 mL solution of 7:3 (vol/vol) acetone/water containing 0.1% ascorbic acid and 10 mL diethyl ether [4.7:2.0:3.3 acetone:water:diethyl ether (vol/vol)]. Solutions were vortexed and centrifuged. A mixture of butanol/HCl [95% butan-1-ol: 5% HCl (36%) (vol/vol); 6 mL] was added to the solution and the mixture was incubated in a boiling water bath for 75 min. Solutions were cooled on ice and absorbance was immediately read at 550 nm using a spectrophotometer (UltraSpec Plus 4054, Pharmacia, Baie d'Urfe', QC, Canada).

Concentrations of ammonia in rumen fluid were determined by the phenol-hypochlorite method (Weatherburn, 1967). Volatile fatty acids were analyzed by gas chromatography using a Hewlett Packard Model 5890 series Plus II gas-liquid chromatograph equipped with a 15 m x 0.53 mm i.d. NUKOL fused silica capillary column (Supelco Canada, Mississuauga, ON, Canada).

5.2.2. Determination of forage preference by cattle grazing alfalfa and sainfoin pastures

Alfalfa and LRC-3519 sainfoin monoculture strips were seeded in spring 2010 leaving a 36 cm gap between strips. Each strip consisted of 10 rows of alfalfa next to 10 rows of sainfoin and were 48 m long and 3.6 m wide using 36 cm row spacing. Crop strips were systematically alternated within the pasture. The pasture was divided by electric fencing into four paddocks of 48 m x 7.6 m with each paddock containing a single strip of alfalfa and sainfoin. Forage DM yields were determined before and after each grazing as described below. Each paddock e was further divided into five using electric fencing with each fenced section being grazed for a single day. In the summers of 2011 and 2012, eight Angus steers fitted with permanent ruminal cannulae were randomly assigned in pairs to each paddock and allowed to graze 0800 to 1030 h. Pastures were grazed in the vegetative stage for 5 days to estimate forage preference. Four trained observers were assigned to observe steers grazing the two forages within each paddock with the location of each steer (i.e. alfalfa or sainfoin) recorded every 10

min throughout the 2.5 h grazing period. Duration that each steer spent on alfalfa or sainfoin were summed and used to estimate forage preference.

After each grazing day, three 0.36-m^2 herbage samples were clipped from each plot from both the grazed and adjacent enclosed areas to a height of 5 cm above ground. Forage production was calculated as the total dry weight of each clipped species in the enclosed area and the amount of forage utilized was estimated as the difference between the total forage clipped from the ungrazed area as compared to the corresponding grazed area. Forage samples were dried at 55˚C in a forced-air oven for 72 h to determine DM yield and the amount of forage consumed by steers. Sub-samples were also used for the determination of nitrogen, ADF, aNDF and OM as described above. Degree of preference was based on the level of consumption (Chavez, 2011) and time spent grazing (Veira et al, 2010) for each of the two forages. Preference rating was calculated as ratio of alfalfa DM consumed to sainfoin DM consumed by cattle (Gesshe and Walton, 1981).

5.2.3. Statistical Analysis

Data for DM yield and sainfoin proportion were analyzed using PROC MIXED procedure of SAS using replication as random effect to evaluate the main effects and interactions of year, pasture (Nova/ABJ or 3519/ABJ) and growth period (primary, 1st regrowth or 2nd regrowth) (SAS Institute Inc., 1999). Since the year x pasture x growth period interaction was significant, means for each year and growth period were presented and discussed. Comparisons of feed quality among the three forages were

made using analysis of variance followed by least square significant difference test for mean separation (LSMEANS with the PDIFF procedure of SAS) where a treatment effect was identified as significant. Data for rumen fermentation characteristics were analyzed separately for each year using SAS PROC MIXED procedure. Bloat occurrence were tested using a chi-square test and treatment effects were considered significant at $p < 0.05$.

5.4. Results

Mean total pre-grazing DM yield of LRC-3519/AC Blue J was higher ($p < 0.05$) than that of Nova/AC Blue J before the two grazing periods in 2011, but not in 2009 and 2010 (Table 5.1). Proportions of LRC-3519 sainfoin was higher than that of Nova in the mixed stands at first and second regrowth in 2009 and at all three harvests grazing cycles (primary and regrowths) in 2010 and 2011 (Table 5.2). Proportions of Nova sainfoin in pastures dropped rapidly and by the second year at the first regrowth stage it accounted for only 8% pasture DM as compared to LRC-3519 which accounted for 30%.

Table 5.1. Mean pre-grazing herbage dry matter (DM) yield (kg ha^{-1}) of two types of sainfoin in mixed pastures with AC Blue J alfalfa (ABJ) over three production years.

Year and harvest	Nova/ABJ	3519/ABJ	SE	P value
DM yield (kg ha^{-1})				
2009				
Primary growth (11 June)	6289	6456	361	0.88
1st regrowth (19 July)	5803	6604	348	0.46
Year total	12092	12760	466	0.63
2010				
Primary growth (28 June)	8638	9730	311	<0.01
1st regrowth (06 August)	1302	1575	82	0.72
Year total	9939	11305	368	<0.01
2011				
Primary growth (19 June)	6573	7903	418	<0.01
1st regrowth (02 August)	1512	2005	159	0.07
Year total	8085	9908	536	0.03

DM yields were means of samples collected immediately before mowing in 2009 and the initiation of grazing in 2010 and 2011 on rotationally stocked paddocks. Samples were collected on the days steers were put on pastures. DM yields are means of samples collected from four different locations in each pasture. Pastures were not grazed in 2009, just mowed and baled.

Table 5.2. Mean percentage of Nova and LRC-3519 (3519) sainfoin DM in pre-grazed sainfoin/AC Blue J (ABJ) mixed pastures rotationally grazed by steers over three production years.

Year, harvest and (date)	Nova	3519	SE	P value
	Mean percent			
2009				
Primary growth (11 June)	55	55	2	0.97
1st regrowth (19 July)	44	52	3	0.01
2nd regrowth (15 September)	25	45	2	<0.01
2010				
Primary growth (28 June)	43	53	4	0.01
1st regrowth (06 August)	8	30	3	<0.01
2st regrowth (29 September)	7	29	3	<0.01
2011				
Primary growth (19 June)	30	49	3	<0.01
1st regrowth (02 August)	5	30	2	<0.01
2nd regrowth (26 September)	5	28	2	<0.01

Pastures were not grazed in 2009 but were harvested and baled. Data presented are means of forage samples collected from four different locations in each pasture immediately before first, second and third harvests in 2009 and before first, second and third grazing cycles in 2010 and 2011.

AC Blue J alfalfa had higher (p<0.05) CP concentration than both Nova and LRC-3519 sainfoin in 2010 and 2011, but lower OM (p<0.05) in samples collected during the bloat study (Table 5.3). The ADF and aNDF concentrations were similar between the two forage species. The mean extractable condensed tannin (ECT) content of Nova and LRC-3519 sainfoin did not differ (p>0.05) (Table 5.3). Extractable condensed tannins were not detected in alfalfa. In the preference study samples, AC Blue J had higher (p<0.05) CP in 2011 and 2012; and higher aNDF in 2011 but lower (p<0.05) OM and similar ADF in both years (Table 5.3).

Table 5.3. Mean crude protein (CP), neutral detergent fiber (aNDF), acid detergent fiber (ADF), organic matter (OM) and extractable condensed tannin (ECT) concentration of AC Blue J alfalfa (ABJ) and Nova and LRC-3519 (3519) sainfoin collected from pastures used for in the bloat study in 2010 and 2011 and the preference study in 2011 and 2012.

	ABJ	Nova	3519	P[♣]	ABJ	Nova	3519	P
Bloat Study			2010				2011	
CP (g kg^{-1})	264[a]	220[b]	217[b]	0.02	262[a]	218[b]	221[b]	0.01
aNDF (g kg^{-1})	326	310	326	0.06	293	283	269	0.04
ADF (g kg^{-1})	265	269	272	0.52	255	251	249	0.62
OM (g kg^{-1})	884[b]	909[a]	917[a]	0.03	892[b]	910[a]	915[a]	0.04
ECT (g kg^{-1})	0	30.4	29.0	0.07	0	30.6	29.2	0.06
Preference study			2011				2012	
CP (g kg^{-1})	247[a]		221[b]	0.03	239[a]		224[b]	0.04
aNDF (g kg^{-1})	359[a]		303[b]	0.02	332		326	0.61
ADF (g kg^{-1})	298		294	0.86	287		278	0.23
OM (g kg^{-1})	904		910	0.43	895[b]		912[a]	0.04

Values are means of three 0.36-m^2 samples taken every 3 days during the bloat study and every day during the preference study. Values within a row followed by different superscripts differ significantly at p<0.05.
[♣] Probability of F values (ANOVA)

Table 5.4. Total bloat incidence, number of multiple distensions and highest bloat score in steers grazing two types of sainfoin at ~ 6 and 30% in mixed pastures with AC Blue J alfalfa in 2010 and 2011.

Year	2010			2011		
	% sainfoin			% sainfoin		
Parameter	Nova	3519	P	Nova	3519	P
	(7)	(29)	value	(5)	(28)	value
Total No. of bloat incidences	43	5	<0.01	48	1	<0.01
No. of multiple distensions (steer-day)$^{-1}$	5	0	<0.01	8	0	<0.01
Total no. of steers with bloat score						
1	28	4	<0.01	32	1	<0.01
2	10	1	<0.01	8	0	<0.01
3	5	0	<0.01	8	0	<0.01
Bloat incidences h^{-1} over a 6-h grazing period						
0 - 1	4	2	<0.01	7	0	<0.01
1 - 2	15	3	<0.01	20	1	<0.01
2 – 3	16	0	<0.01	12	0	<0.01
3 – 4	5	0	<0.01	6	0	<0.01
4 – 5	2	0	<0.01	3	0	<0.01
5 – 6	1	0	<0.01	0	0	

Values in parenthesis under Nova and 3519 represent percent composition (DM basis) of the two sainfoin populations in the mixtures with alfalfa immediately prior to the bloat study. Significance of bloat incidence and number of multiple distensions were determined using Chi-square tests.

In 2010, the total number of steers experiencing bloat (bloat incidence) was about 90% (43 out of 48 bloat incidences) in Nova/alfalfa mixed pastures a level that was higher (p<0.001) than in LRC-3519/alfalfa pastures, which had only 10% of the incidences (5 out of 48 bloat incidences) (Table 5.4). The highest bloat score (severity) recorded on Nova/alfalfa plots was 3, which was an acute form of frothy bloat, while the highest score on LRC-3519/alfalfa plots was 2. In 2011, 98% of the recorded incidences of bloat occurred in steers grazing Nova/alfalfa plots with the highest severity being 3. This is compared to a bloat incidence of 2% in steers grazing LRC-3519/alfalfa with the severity of bloat never exceeding a score of 1. There were 12 and 17% multiple bloats per steer d^{-1} out of the total incidences of bloat that occurred in steers on Nova/alfalfa plots in 2010 and 2011, respectively (Table 5.4). About 80% of bloat incidences were recorded within 3 h after the steers were released onto pasture (Table 5.4) and most bloat incidences were observed within the first 6 d of the trial in both years (Figs. 5.1 and 5.2). Incidences of bloat became less frequent as the trial continued.
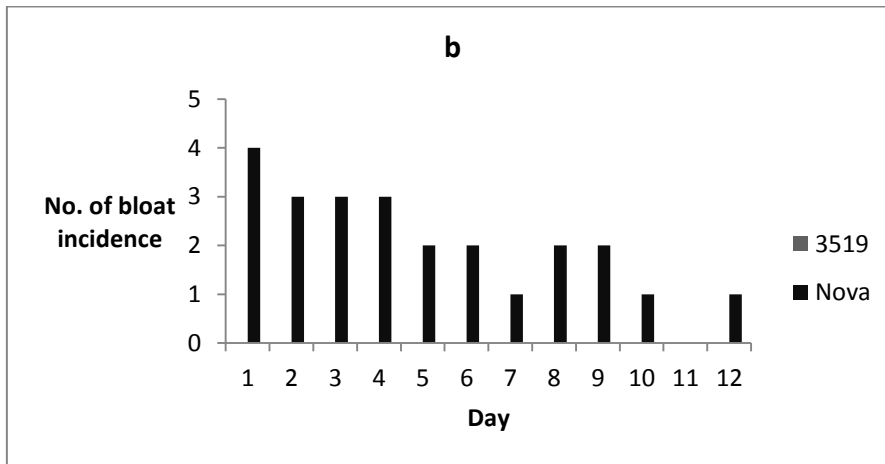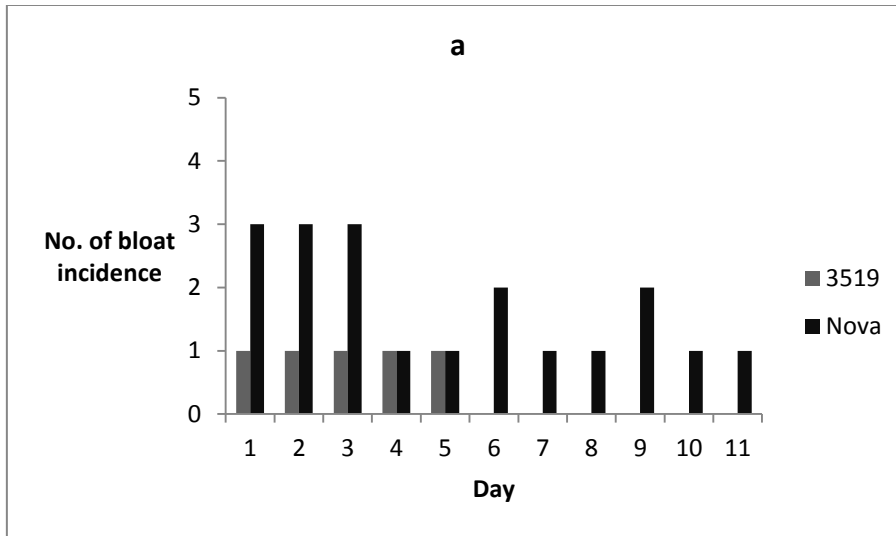
Fig. 5.1. Number of bloat incidences observed daily in steers grazing Nova and LRC-3519 sainfoin in mixtures with AC Blue J alfalfa in 2010 during grazing periods (a) 1 from 29 September – 9 October and (b) 2 from 10-21 October.
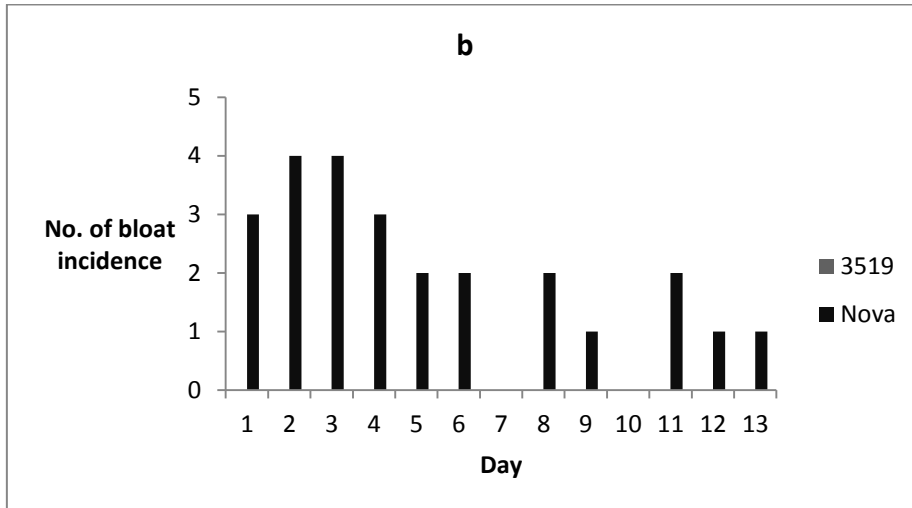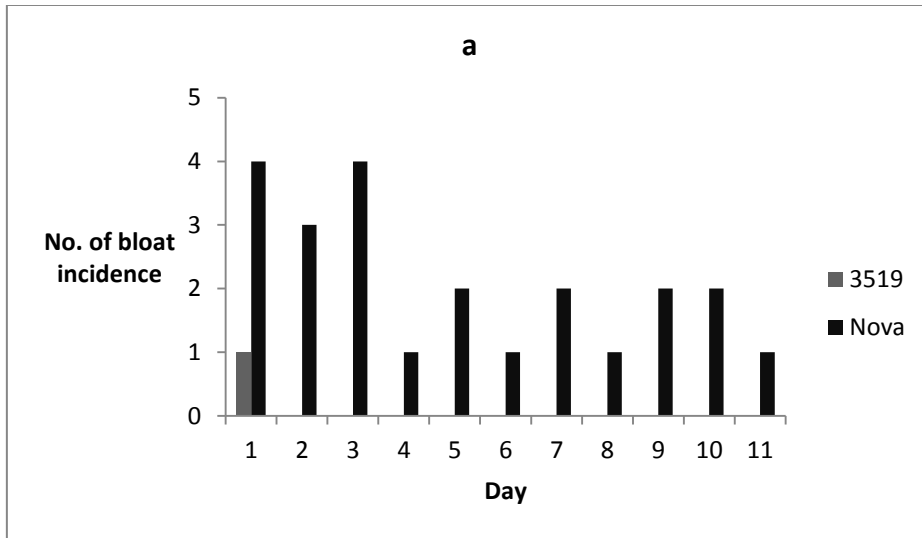
Fig. 5.2. Number of bloat incidences observed daily in ten steers grazing Nova and LRC-3519 (3519) sainfoin in mixtures with AC Blue J alfalfa in 2011 during grazing periods (a) 1 from 26 September – 6 October and (b) 2 from 7-19 October.

Rumen fluid obtained from cattle grazing Nova/alfalfa pastures had higher (P< 0.05) ammonia but similar VFA concentration and molar proportions of individual VFA as compared to that obtained from cattle grazing LRC-3519/alfalfa pasture in both years (Table 5.5).

The mean rumen pH of bloated steers was slightly lower than that of non-bloated steers (Table 5.6). Similarly, the pH of rumen fluid from steers grazing Nova/alfalfa steers was only numerically lower than that from steers grazing LRC-3519/alfalfa.

Table 5.5. Fermentation characteristics of rumen fluid collected from steers grazing Nova/AC Blue J (ABJ) or 3519/ABJ mixed sainfoin/alfalfa pastures in 2010 and 2011

	2010				2011			
	Nova/ ABJ	3519/ ABJ	SE	P value	Nova/ ABJ	3519/ ABJ	SE	P value
pH	6.0	6.1	0.1	0.08	5.9	6.1	0.2	0.06
NH_3-N (mM)	24.6	20.7	1.3	0.04	24.7	20.6	1.1	0.04
Total VFA (mM)	110.9	106.3	1.4	0.39	112.0	108.3	0.9	0.41
molar proportion (% of total VFA)								
Acetic acid	64.3	64.3	0.3	0.38	64.6	64.4	0.4	0.30
Propionic acid	20.7	21.4	0.4	0.73	21.1	21.4	0.3	0.72
Butyric acid	10.7	10.5	0.4	0.52	10.5	10.6	0.4	0.86
Isobutyric acid	1.0	0.8	0.2	0.43	0.9	0.9	0.3	0.79
Acetate:Propionate	3.2	3.2	0.2	0.87	3.1	3.1	0.3	0.87

VFA = Volatile Fatty Acids
Values for each year are means (n=24) of samples collected for the two periods during the bloat study.

Table 5.6. Mean rumen fluid pH of bloated and non-bloated steers grazing Nova/AC Blue J (ABJ) and LRC-3519/ABJ sainfoin/alfalfa mixed pastures in 2010 and 2011.

				Rumen pH				
Year	Nova/ ABJ	3519/ ABJ	SE	P value	Bloated	Non- bloated	SE	P value
2010	6.0	6.2	0.2	0.06	6.0	6.1	0.2	0.09
2011	5.9	6.1	0.2	0.07	5.8	6.0	0.3	0.08

pH values were recorded immediately after steers were taken off pasture (after 6-h grazing) and whenever any steer was relieved after a bloat score of 3.
Rumen fluid pH were recorded from all steers on the two treatments during the last four days of each grazing period and in 30 bloated and 30 non-bloated steers.
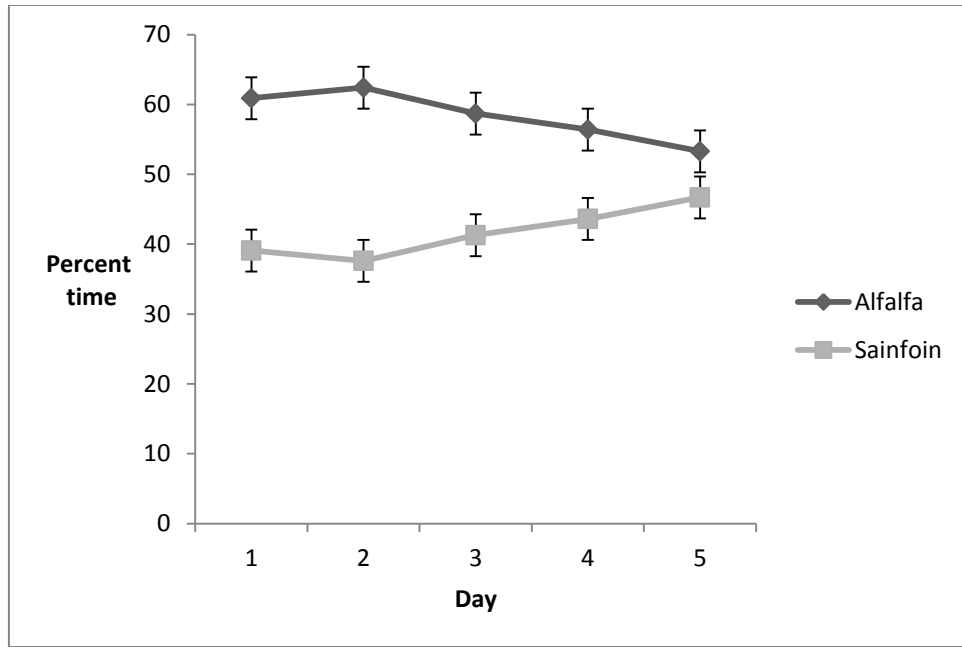
Fig. 5.3. Mean percent time spent grazing AC Blue J alfalfa and LRC-3519 sainfoin ($\pm$ SE) by steers in 2011. The time steers spent grazing sainfoin or alfalfa was estimated over a 2.5 h grazing period.
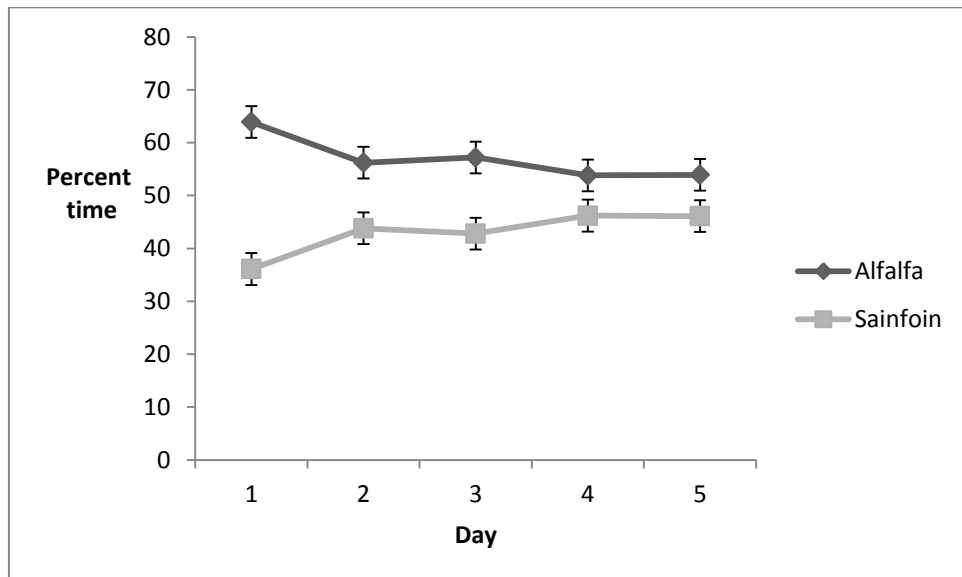
Fig. 5.4. Mean percent time spent grazing AC Blue J alfalfa and LRC-3519 sainfoin ($\pm$ SE) by steers in 2012. The time steers spent grazing sainfoin or alfalfa was estimated over a 2.5 h grazing period.

Table 5.7. Mean forage DM yield (kg ha^{-1}), DM consumed (kg ha^{-1}) by steers and percent time spent by these steers grazing alfalfa and sainfoin pasture strips in 2011 and 2012.

	2011				2012			
	Alfalfa	Sainfoin	SE	P value	Alfalfa	Sainfoin	SE	P value
DM yield (kg ha^{-1})	7554	9577	306	0.06	4994	3297	128	0.04
DM consumed (kg ha^{-1})	2492 (57)	1915 (43)	79	0.04	2048 (63)	1164 (37)	84	0.03
% time spent grazing	58	42	3	0.04	55	45	4	0.04
Preference rating	1.0	0.8	0.3	<0.01	1.0	0.6	0.3	<0.01

Values in parenthesis under forage DM consumed represent the percentage of alfalfa and sainfoin consumed of total DM intake.
Preference rating is the ratio of DM utilized by steers for alfalfa and sainfoin.

Grazing steers consumed more ($p<0.05$) alfalfa than sainfoin DM (Table 5.7) as result

of spending more time grazing alfalfa (57%) than sainfoin (43%) (Figs. 5.3 and 5.4).

Steers spent from 55 to 80% of the total time grazing alfalfa during the first two days

of each grazing period but this duration dropped to about 50 to 60% of the time from

the third day onward. In both 2011 and 2012, the amount of alfalfa DM consumed

(2492 and 2048 kg ha^{-1} respectively) was higher ($p<0.05$) than sainfoin (1915 and

1164 kg ha^{-1}). Steers consumed between 14 and 26% more alfalfa than sainfoin in

2011 and 2012, respectively (Table 5.7). Preference (ratio of DM forage utilized) of

alfalfa to sainfoin was 1.0:0.8 and 1.0:0.6 in 2011 and 2012, respectively (Table 5.7).

Pre-grazing DM yield of sainfoin did not differ t ($p=0.062$) from alfalfa in 2011 but

was lower ($p=0.043$) in 2012 (Table 5.7).

5.4. Discussion

Higher DM production and proportion of the new sainfoin population in the mixed

stand (Tables 5.1 & 5.2) was expected as the new population (LRC-3519) was

developed using germplasm selected for survival and growth in mixed alfalfa stands

under a multiple cut system. This observation was previously recorded for LRC-3519

as well as other sainfoin populations that were selected at LRC using similar criteria

(Acharya et al. 2013). In this study DM yields of LRC-3519 in pure stands were 11

and 35% higher than Nova under rain-fed and irrigated conditions, respectively.

Even though bloat could occur in all seasons, to maximize the risk of bloat these trials

were carried out in the fall when cool overnight temperatures (0 to 10 °C), in

combination with moderate daytime temperatures (15 to 20˚C) are known to increase the risk of bloat (Majak et al., 1995; Berg et al. 2000). At this time, cool temperatures also delayed maturation and extended the vegetative growth phase of the forage crops to build up the organic reserves in the roots for over wintering and the initiation of spring growth (Frame, 2005). All steers bloated when grazing Nova/alfalfa in both years except one steer in 2010 that never bloated on either pasture, an observation that suggests that physiological differences among animals is also a factor that determines the likelihood of bloat. Variability in bloat has been observed earlier among individual animals and among different forage species (Ayre-Smith, 1971; Clark and Reid, 1974). Out of five bloat cases (10%) recorded on LRC-3519/alfalfa plots in 2010, all were observed in one steer during the first grazing period. The same steer had 3 out of the 5 multiple distensions on Nova/alfalfa plots during the second grazing period. This steer had the characteristics of a chronic bloater (Cockrem et al., 1987), but the physiological cause of this trait could not be readily identified.

About 30% (DM basis) sainfoin in the mixed sainfoin/alfalfa stand reduced the incidence of bloat by up to 98%. This contrasts with observations of Wang et al. (2006) who recorded 77% bloat reduction in steers grazing an alfalfa/sainfoin mixed pasture with 35% sainfoin in the mixture. The reduced control of bloat in Wang et al. (2006) may have been due to differences in the level of sainfoin in the pasture as well as preference of steers for either alfalfa or sainfoin as the two crops were seeded in monoculture strips. In the present study, forages were seeded in alternate rows so that steers had access to both forages in close proximity which may have contributed to the lower bloat incidence. Higher preference for alfalfa (55 to 80% time spent) observed

in this study may also explain why a higher bloat incidence was observed by Wang et al. (2006).The reduction in bloat incidence in LRC-3519/alfalfa vs. Nova/alfalfa plots (Table 5.6) indicates that the higher proportion of sainfoin and consequently higher amount of condensed tannin in the diet was likely able to bind the soluble protein in alfalfa and thereby reduce the formation of the stable foam associated with bloat (Waghorn and Jones, 1989; McMahon et al., 1999).

In this study, the earliest incident of acute bloat occurred in Nova/alfalfa plots 38 min after steers were released into the pasture. The early onset of bloat may be due to the rapid release of plant cell contents in the rumen (McArthur and Miltimore, 1969). Reid et al. (1962) indicated that about 65% of the soluble protein in the feed can be released during mastication hence raising the possibility that formation of stable foam could occur shortly after consumption. About 80% of bloat cases were recorded within 3 h after the steers were released into pastures in the morning (Table 4). After 3 h, both the incidence and severity of bloat decreased, a pattern similar to that observed by Hall and Majak (2000). All steers that experienced acute bloat (bloat score of 3) and were relieved, bloated again (multiple distension) the same day. About 90% of the steers that experienced acute bloat received a bloat score of 1or 2 (sub-acute) while 10% were assigned an acute bloat score of 3 by end of the 6-h grazing period.

All the bloat incidences that occurred in cattle on LRC-3519/alfalfa plots were sub-acute, whereas 16% of the bloat incidences recorded in cattle that grazing Nova/alfalfa plots were acute. This shows that as compared to Nova, LRC-3519 persisted longer in mixed alfalfa mixtures and produced more DM while reducing the incidence of bloat in grazing steers by 90 and 98% in 2010 and 2011, respectively.

The pH of rumen fluid in this study (Table 5.6) was similar to that recorded by McArthur and Miltimore (1969) in cattle grazing alfalfa and ladino clover. These researchers recorded pH before grazing and at a 6-h interval, and observed a decline in rumen pH over time with those cattle that bloated exhibiting a slightly lower pH as compared to those that did not. These values fall within the range of normal rumen pH values measured in cattle consuming forage, indicating that acidosis does not play a role in pasture bloat. A reduction in the ammonia nitrogen concentration of rumen fluid from steers grazing LRC-3519/alfalfa as compared to those grazing Nova/alfalfa may be due to higher amounts of condensed tannins in the LRC-3519/alfalfa pastures. CT may have inhibited proteolytic bacteria or resulted in an increase in the formation of CT-protein complexes. It may also suggest that amino acids from sainfoin protein were less available for deamination or for direct incorporation into microbial cells (McMahon et al, 1999). This could lead to more plant protein escaping degradation by rumen microorganisms, possibly increasing the quantity of amino acids available for absorption in the small intestine.

Plant preference varies among ruminant species and even among individual of the same species (Ivins, 1955). Some forage species are consumed if they are the sole choice provided to cattle, but are discriminated against or even rejected if offered in combination with other forages (Vallentine, 2001). Preference of ruminants for forages has been defined as a function of their degree of consumption (Chavez, 2011) and the time spent grazing (Veira et al, 2010). An increase in consumption and more time spent grazing is indicative of an increased preference for that type of forage. The time spent grazing and amount of forage consumed in this study indicate that alfalfa

was preferred to sainfoin. The preference rating of 1.0:0.8 or 1.0:0.6 for alfalfa and sainfoin in this study is similar to results reported by Gesshe and Walton (1981). In their study they compared the preferences of six grasses and three legumes and found that alfalfa was preferred over sainfoin across a range of growth stages. At the vegetative, flowering, and seed set stages, relative preferences were 1.5:0.5, 1.5:0.7 and 1.3:0.8 for alfalfa and sainfoin, respectively. Although the relative palatability of these two species was different, both forages would have been readily consumed by cattle if they were offered as the sole feed source (Marten et al., 1987).

The steers observed in this study preferred to graze alfalfa when first released into the pasture, followed by sainfoin. The time spent grazing sainfoin increased with time from an average of 20 to 45 percent. It is possible that steers that bloated while grazing LRC-3519/alfalfa pasture did not consume sufficient sainfoin during the first two days of grazing so as to prevent bloat.

The diurnal effect (Fisher et al., 2002) of cattle preference for these forages was not considered in this study. In the current study, cattle were only grazed during the morning so as to maximize the risk of bloat. There is anecdotal evidence that risk of bloat is lower if cattle are released onto pastures in the afternoon. Therefore, it is possible that the preference for the crops may have been different if cattle had been released into pastures at a different time of the day. In the future, a study should be undertaken with the time of day as a variable to examine its impact on forage preference. Cattle could first be adapted to pastures that have higher levels of sainfoin and that they could likely be subsequently moved to pastures that contain lower levels of sainfoin without causing bloat.

5.5. Conclusion

This multi-year study was initiated to determine if a new sainfoin population developed for its ability to survive under a multi-cut system with alfalfa could be used to prevent bloat in cattle rotationally grazing alfalfa pasture. Yield from pastures decreased over time for mixed alfalfa/sainfoin pastures for both new and old sainfoin populations. But, yield performance of the mixed alfalfa stand with the new sainfoin population was significantly higher than Nova in second and third year stands. The proportion of new sainfoin in the mixed stand remained more than 20% higher than Nova, even though both sainfoin types were present in the pastures at similar levels at the beginning of the experiment. Bloat severity and incidence in alfalfa mixed stands with new sainfoin (LRC-3519) was much less (~95%) than with Nova. Neither the nutritional quality or levels of CT differed between the two sainfoin populations, the higher proportion of DM from LRC-3519 in the mixed stand during grazing (two cycles per year) was likely instrumental in the significant reduction in bloat. This study suggests that the new sainfoin populations, capable of providing 25-30% biomass in mixed alfalfa pastures over three years, can be used to reduce the risk of bloat even when steers appeared to prefer alfalfa over sainfoin.

Chapter 6

Biomass production and cattle performance on alfalfa/sainfoin mixed pastures in western Canada

6.1. Introduction

Alfalfa is by far the most productive forage legume crop in western Canada (McCartney and Horton, 1997; Popp, 1995). This, coupled with its high nutritional quality (Mir et al., 1994; Dahlberg et al., 1988), and its rapid digestibility (Dahlberg et al., 1988), make it one of the few forage crops capable of sustaining beef cattle growth of 1 to 1.5 kg d^{-1} (Popp et al., 2000) and gains of 535 to 923 kg ha^{-1} (Burris et al., 1993; Joyce and Brunswick, 1977; Meyer et al., 1956). However, cattle grazing alfalfa can bloat, reducing the rate of gain and in extreme cases causing death (Majak et al., 1995). In addition, the rapid degradation of alfalfa protein in the rumen causes a large portion of nitrogen to be converted to urea in the liver and excreted in urine, reducing the efficiency of N utilization (Dahlberg et al., 1988).

Sainfoin is a high quality perennial forage legume (Spedding and Diekmahns, 1972; Sottie et al., 2012) containing condensed tannins (McMahon et al., 1999; Wang et al., 2006), with the capacity to produce high dry matter (DM) yields (Fortune, 1985; Goplen et al., 1991; Acharya et al., 2013). Interestingly when sainfoin is consumed with alfalfa, the presence of tannins in sainfoin reduces the ruminal proteolysis of alfalfa protein in the rumen and reduces the risk of bloat (McMahon et al., 1999). The

tannin/protein complex formed in the rumen dissociates in the abomasum and enables

plant protein to be digested and amino acids to be absorbed in the small intestines

thereby increasing the efficiency of protein utilization (McMahon et al., 1999). Bloat

reductions of up to 77 - 98% have been observed in cattle grazing alfalfa/sainfoin

mixed pastures with 25 - 35% sainfoin DM in the mixture (Wang et al., 2006; Sottie et

al., 2012). In addition Li et al. (1996) and McMahon et al. (1999) indicated that a

minimum of 10% sainfoin DM in mixtures with alfalfa will control pasture bloat, but

the incidence of bloat reached 47% in the study by McMahon et al. (1999) when the

sainfoin was fed as hay or fresh herbage along with green chop alfalfa to confined

Jersey steers. It has been reported that steers grazing pure sainfoin pastures gained

between 0.86 and 0.96 kg d^{-1} (Mowrey et al, 1992; Parker and Moss, 1981); a rate of

gain comparable to that achieved in cattle grazing alfalfa pastures. However, sainfoin

has poor re-growth after haying and grazing (Bolger and Matches, 1990) and seldom

produces as much biomass as alfalfa in pure stands. In mixed stands with alfalfa the

reduction in biomass associated with sainfoin can be reduced while the risk of bloat is

reduced as compared to cattle grazing pure alfalfa pastures (Acharya et al., 2013;

Sottie et al., 2012).

A major weakness of sainfoin cultivars (e.g. Melrose and Nova) registered for western

Canada is that they fail to persist in alfalfa pastures and grow back at a much slower

rate than alfalfa after grazing. New sainfoin populations were selected for their ability

to persist in mixed alfalfa stands at the Lethbridge Research Centre (LRC), AB,

Canada (Acharya et al., 2013). The new sainfoin populations were targeted to have a

similar growth pattern as alfalfa under multiple-cut management. These populations

were developed with the objective of maintaining a minimum of 25 percent of the DM in mixed pastures as sainfon over the grazing season under rotational grazing (Acharya et al., 2013). Some of the populations developed at LRC were found to perform better in mixed stands with alfalfa in multiple cut systems as compared to older cultivars registered for commercial production in western Canada (Acharya et al., 2013). One of the new populations was found to reduce the incidence of bloat in cattle grazing mixed alfalfa stands by 98%, a reduction far greater than that achieved with the older sainfoin variety, Nova (Sottie et al., 2012).

Forage production and animal live weight gains are good indicators of pasture productivity (Large et al., 1984) with different grazing systems having an impact on the productivity and the persistence of plant species in pastures. Greater changes in the botanical composition of pastures have been reported with continuous vs rotational grazing systems (Walton et al., 1981).

In the present study it was the hypothesized that the alfalfa/sainfoin mixed pastures with newly developed sainfoin populations would have higher DM yield, resulting in improved growth performance of steers as compared to pastures that contained an older sainfoin cultivar. It was also proposed that sainfoin would be more persistent in rotational as compared to continuous grazed pastures.

The objectives of this study were to estimate pasture productivity, the botanical composition and nutritional quality of alfalfa - sainfoin mixed pastures grown at two different eco-climatic zones in western Canada. Pasture productivity was also assessed by measuring the growth performance of steers grazed at both locations.

6.2. Materials and methods

6.2.1. Experimental locations

Three experiments were conducted, with a rotational and a small plot continuous grazing at LRC, Alberta and a rotational grazing experiment at the Semiarid Prairie Agricultural Research Centre, Swift Current, Saskatchewan (SPARC).

The LRC pastures were located at 49° 42' N latitude 112° 47 W longitude at 899 m above sea level. In the summer of 2010, 2011 and 2012 mixed alfalfa–sainfoin stands established on June 19, 2008 were grazed continuously. The mixed pastures were grown on a slightly alkaline clay loam dark brown Chernozem soil (Larney and Janzen, 2012). The climate at Lethbridge is semi-arid with an average maximum annual temperature of 12.3 °C and an average minimum annual temperature of −1.1 °C and receives on average 365 mm of precipitation each year (Environment Canada, 2013).

SPARC is located at 50° 16 'N latitude 107° 44' W longitude at 825 m above sea level in Swift Current. Pastures were established on a brown Swinton silt loam soil (Ayres et. al., 1985). Swift Current has an average maximum annual temperature of 9.7 ℃ and an average minimum annual temperature of -2.5 ℃ and receives on average 330 mm of precipitation per year (Environment Canada, 2013).

6.2.2. Pastures and Experimental Design

Pasture treatments consisted of AC Blue J alfalfa (BJ) in mixtures with an old sainfoin cultivar, Nova and three new sainfoin populations (LRC-3401, LRC-3432 and LRC-3519). Sixteen mixed pastures of 4050 m^2 size were seeded in 2008 in a replicated Randomized Complete Block Design (RCBD). All four alfalfa-sainfoin pasture treatments were seeded at 5 and 15 kg ha^{-1} alfalfa and sainfoin respectively, to achieve a 50:50 ratio of the two populations on biomass basis. Alfalfa and sainfoin were seeded in alternate rows with row spacing of 30 cm. All 16 pastures were divided by electric fences and each plot was further divided into paddocks for rotational grazing. The LRC pastures were irrigated when soil moisture was low. In 2009, the LRC pastures were cut on June 11 and July 19 using a forage harvester and the material was removed after harvest.

A similar experiment with same experimental design, row spacing, seeding rate and pasture size was established in 2009 and grazed in 2010 and 2011 at SPARC, Swift Current, SK. At this location Beaver was used as the alfalfa cultivar in the mixed pastures. Pastures at Swift Current were not irrigated (rain-fed).

In 2009, four more pastures were seeded at LRC with AC Grazeland alfalfa in mixtures with Nova, LRC-3401, LRC-3432 or LRC-3519 at the same seeding rate as for previous sites and with plot sizes of 3.6 m x 24 m. Each pasture type was replicated four times in a RCBD.

6.2.3. Animals and Grazing Management

All steers used in this study were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

i. LRC rotational grazing experiment

Eighty Hereford steers with mean live weight of 365 ± 3.9 kg were used in 2010 in this experiment. The steers were blocked by live weight and randomly assigned to treatment. Initial weights were determined as the average of weights from two consecutive weighings (d-1 and 0). Stocking rates were set at 4.0 AUM ha^{-1} (8.6 steers ha^{-1} season^{-1}). Prior to the start of grazing, steers were adapted to alfalfa hay for 14 days prior to introduction into experimental pastures when the sainfoin was in full bloom and alfalfa was in 80% bloom on June 28, 2010. In 2010, grazing was delayed as a result of abundant precipitation in May and June and the risk of damaging pastures through trampling. Water and a mineral block (Windsor TM, Canada with composition NaCl: 98%, I: 100 mg kg^{-1}, Co: 50 mg kg^{-1}, Zn: 7500 mg kg^{-1}, Mn: 5000 mg kg^{-1}, Cu: 2500 mg kg^{-1}, Fe: 2500 mg kg^{-1} and Se: 120 mg kg^{-1}) were freely available to all steers in each pasture. Steers were weighed every four weeks during grazing period and on two consecutive days at the end of the grazing period.

The experiment was repeated in 2011 and 2012 with 64 (329 ± 3.6 kg) and 48 (320 ± 4.2 kg) Hereford steers. Sainfoin was in 40% bloom and alfalfa was in 15% bloom when steers were released to graze pastures in 2011 and 2012. Alfasure® (Rafter 8 products, Calgary) containing polyoxypropylene polyoxyethylene glycol (non-ionic block polymer) was dispensed into the drinking water to prevent pasture bloat in steers at a rate of 50 ml Alfasure® to 100 l water.

ii. SPARC rotational grazing experiment

Eighty Red Angus steers with mean live weights of 385 ± 29.5 kg and 330 ± 17.9 kg were used at SPARC in 2010 and 2011, respectively. Steers were blocked by live weight and assigned to pastures as at LRC. The pastures were grazed by steers to utilize 50-60% of the forage during the grazing season. Similar to the experiment at LRC in 2010, steers were placed on pasture when alfalfa was in 80-90% bloom and sainfoin was in full bloom. In 2011, alfalfa was in 15-20% bloom and sainfoin, 50-60% when steers were released onto the pasture. All steers had free access to water and a mineral salt block (Magnum TM 120 with composition NaCl: 98%, I: 100 mg kg^{-1}, Co: 50 mg kg^{-1}, Zn: 7500 mg kg^{-1}, Mn: 5000 mg kg^{-1}, Cu: 2500 mg kg^{-1}, Fe: 2500 mg kg^{-1} and Se: 120 mg kg^{-1}) but Alfasure® was not administered in the drinking water.

iii. LRC small plot continuous grazing

Ten Hereford steers with average live weights of 365 ± 3.9 kg, 330 ± 3.6 kg and 320 ± 4.2 kg in 2010, 2011 and 2012 respectively were allowed to graze all the alfalfa/sainfoin mixed pastures to the end of the grazing season. Alfalfa hay was provided to steers as supplemental feed when pasture was grazed to about 80%. Water with Alfasure® (50 ml Alfasure® to 100 l water) and mineral salt block were freely available to all steers.

6.2.4. Sample collection and analysis

The DM biomass yield of each rotationally grazed paddock was determined by clipping four quadrats (0.36-m^2) with hand shears at a height of 5.0 cm above ground prior to grazing. The same procedure was used to estimate the amount of forage consumed after steers were taken off each paddock by collecting residual herbage (Large et al., 1984; Popp et al., 1997). Before each grazing cycle, forage samples were collected from all paddocks to determine the botanical composition of alfalfa and sainfoin in all pastures. Biomass production on continuous stocked pastures was estimated from forage samples collected before grazing. The collected samples were manually separated into alfalfa and sainfoin and dried in a forced air oven at 55°C to estimate total dry matter (DM) (forage) production, forage utilization and the percent of sainfoin and alfalfa in the mixed pasture.

Forage samples in each paddock within a pasture were composited into four samples for analyses. Dried forage samples were ground to pass a 1-mm screen and were analyzed for N by combustion - mass spectrometry (NA 1500, Carlo Erba Instruments, Milan, Italy), acid detergent fiber (ADF), amylase-treated neutral detergent fiber (NDF) as described in section 5.2.1. Organic matter (OM) was estimated by ashing samples at 550°C for 6 h (AOAC, 2004-2005).

6.2.5. Statistical Analysis

Data were analyzed statistically using the MIXED model procedure of SAS (SAS Institute Inc. 1999). Forage composition was analyzed as a repeated measures analysis to test period (grazing cycle) and year effects. Year effects in treatment combinations

were determined by multiple mean comparisons using PDIFF (SAS Institute, Inc. 1999). Pasture production and utilization and live weight gain were analyzed using the GLM procedure with pasture plot treated as the experimental unit. The model to compare DM forage utilized by year used a repeated measures design. Data was pooled by paddock and grazing cycle with treatment as the fixed effect in the model. Year and replicates were considered to be random. Differences in climatic conditions over years and variations in productivity of pastures resulted in a year x treatment interaction, therefore results for each year were reported separately. Differences among treatment means were tested using Tukey test at a significance level of 0.05.

6.3. Results

In 2009, the total monthly precipitations were below the long-term average at each of the respective locations (Figs. 6.1 and 6.2). This was particularly noticeable for the months of May and June in Lethbridge and Swift Current. In 2010, 2011, and 2012 the total precipitations during the growing seasons were higher than the long-term averages at all locations. The monthly total precipitation for April, May and June for both locations were higher than 30-year averages. The monthly total precipitation for July and August in 2012 at Lethbridge was lower than long-term averages. The average monthly temperatures for the years were variable but followed the same pattern as the long-term average for the two locations.
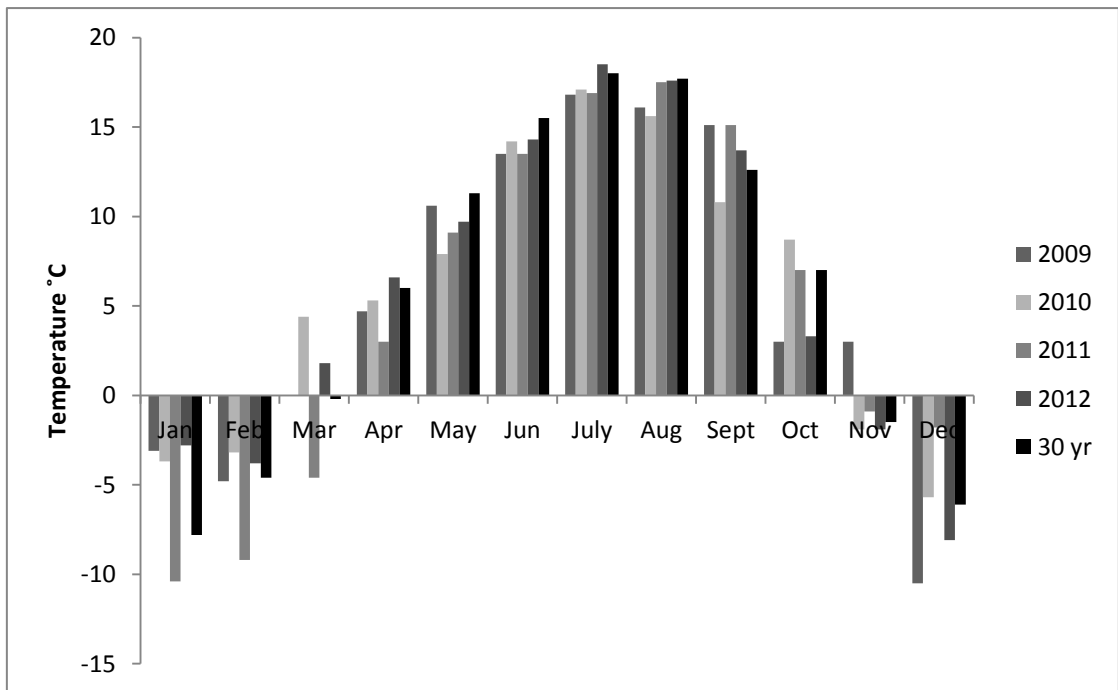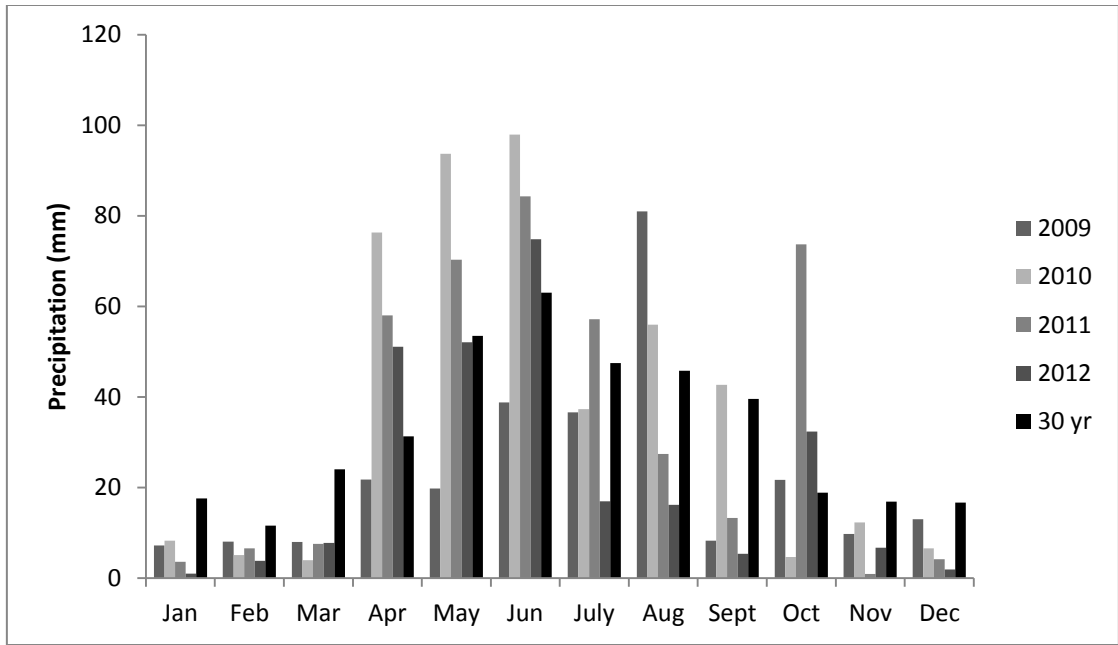
Fig 6.1. Total monthly precipitation and mean monthly temperature for 2009 to 2012 and 30 year average for Lethbridge, AB.
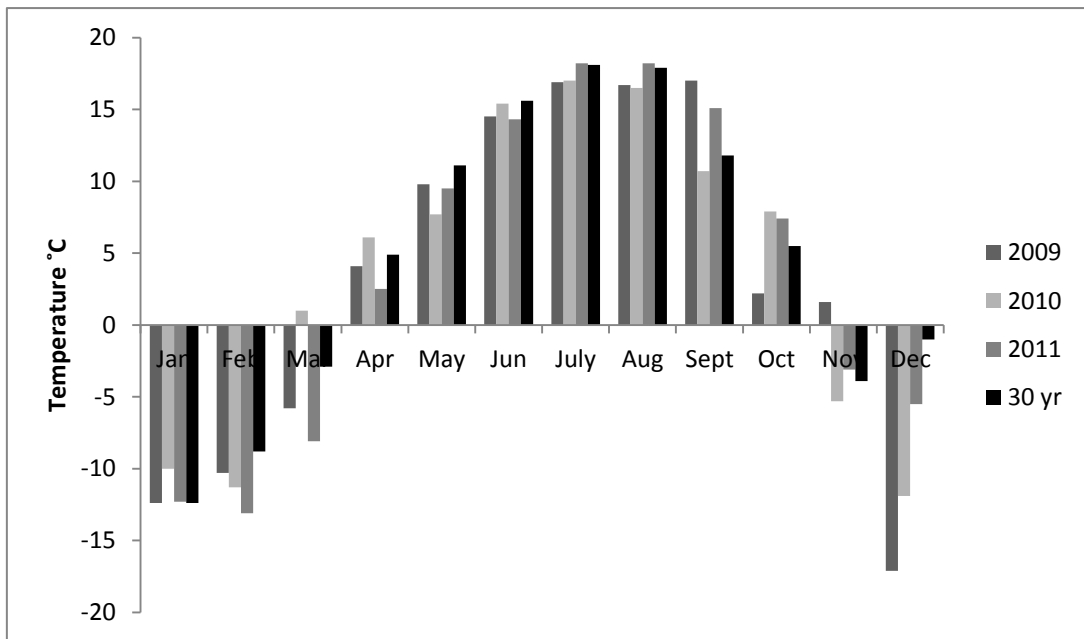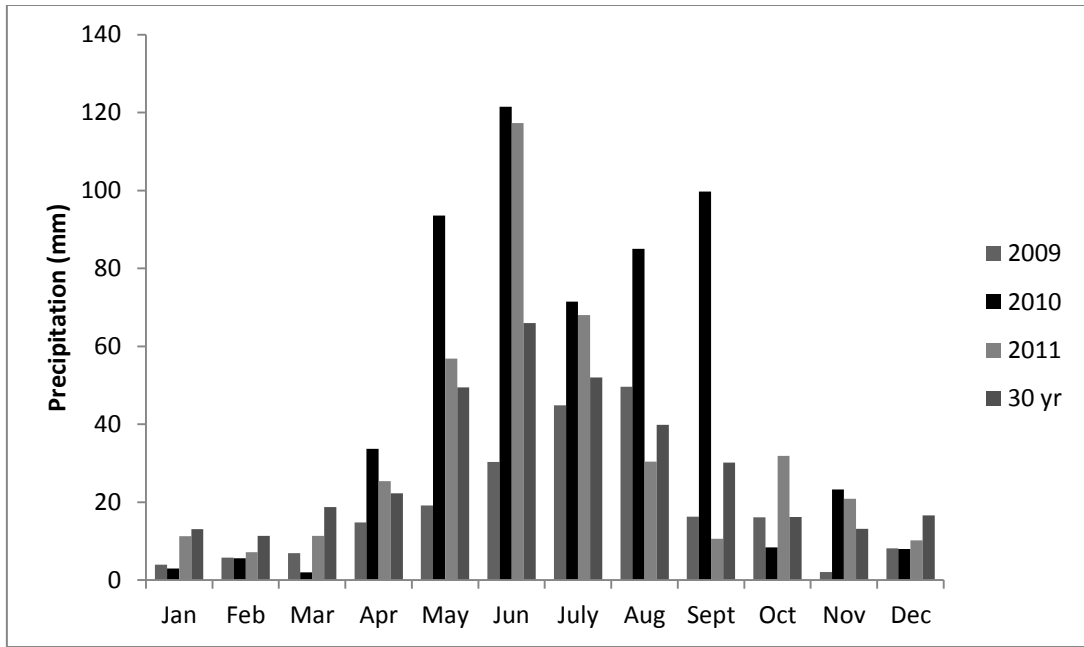
Fig. 6.2. Total monthly precipitation and mean monthly temperature for 2009 to 2011 and 30 year average for Swift Current, SK.

6.3.1. Forage production and utilization

There were no difference ($p>0.05$) in mean forage DM production (kg ha^{-1}) among treatments in 2009 in Lethbridge (Table 6.1). In 2010, mean forage DM production, as estimated before two cycles of grazing, for 3432/BJ was higher ($p<0.05$) than Nova/BJ but did not differ ($p>0.05$) from 3519/BJ and 3401/BJ. In 2011, the mean forage DM yield followed a similar trend as in 2010. In 2012, there was no difference ($p>0.05$) among the three new sainfoin population mixtures (3401/BJ, 3432/BJ and 3519/BJ) in DM yield, but all were higher ($p<0.05$) than Nova/BJ. The 3432/BJ mixture had the highest forage DM yield over the four years, which was similar ($p>0.05$) to that of 3519/BJ and 3401/BJ but higher ($p<0.05$) than Nova/BJ. It is also important to note that the mean forage DM yields of all the mixtures declined ($p<0.05$) over time, but at different rates (Table 6.1). The reduction in DM yield over four years for Nova/BJ, 3401/BJ, 3432/BJ and 3519/BJ were 47, 33, 32 and 36 %, respectively.The mean forage DM yields for all four forage mixtures were similar ($p>0.05$) in both 2010 and 2011 at Swift Current (Table 6.1).

Table 6.1. Forage production and utilization of alfalfa/sainfoin mixed pastures under rotational and continuous grazing in Lethbridge, AB. and rotational grazing in Swift Current, SK.

Lethbridge rotational grazing

	Nova/BJ*	3401/BJ	3432/BJ	3519/BJ	P-value
Forage DM production (kg ha^{-1})					
2009	12092	11778	12602	12760	0.876
2010	9939^a	10209^{ab}	11978^b	11305^{ab}	0.001
2011	8085^a	8850^{ab}	9684^b	9908^b	0.001
2012	6419^a	7833^b	8586^b	8150^b	0.018
4 year mean	9133^a	9668^{ab}	10713^b	10530^b	0.001
Forage DM utilized (kg ha^{-1})					
2010	5268	5615	5749	5539	0.788
2011	4851	5133	5036	5152	0.645
2012	3916	4073	4293	4238	0.676

Swift Current rotational grazing

	Nova/Br*	3401/Br	3432/Br	3519/Br	
Forage DM production (kg ha^{-1})					
2010	5374	4653	4592	5255	0.662
2011	2987	3079	3286	2666	0.245
Forage DM utilized (kg ha^{-1})					
2010	2687	2559	2617	2680	0.854
2011	1762	1724	1742	1706	0.782

Lethbridge continuous grazing

	Nova/Gr*	3401/Gr	3432/Gr	3519/Gr	
Forage DM production (kg ha^{-1})					
2010	6760^a	8553^{ab}	10097^{bc}	10257^c	0.014
2011	6107^a	7789ab	9127^b	9177^b	0.013
2012	4078	3601	4440	3743	0.642
3 year mean	5648^a	6647ab	7880^b	7725^b	0.005

* BJ: AC Blue J alfalfa; Br: Beaver alfalfa; Gr: AC Grazeland alfalfa.
Lethbridge rotational grazing was under irrigation. Swift Current rotational grazing and Lethbridge continuous grazing were under rain-fed conditions.
Forage DM production in the Lethbridge rotational grazing system were the mean yields of three cuts in 2009 and mean yields of two cuts in 2010, 2011 and 2012.
Forage DM production in Swift Current and Lethbridge continuous grazing were mean yields of a single cut.
Mean values in the same row with different superscripts differ significantly (p<0.05)

Mean DM yields of 3432/Gr and 3519/Gr mixtures at LRC under continuous grazing

and rain-fed conditions were higher ($p<0.05$) than Nova/Gr in 2010 and 2011, but not

in 2012 (Table 6.1). In 2012, there were no differences ($p>0.05$) in mean DM yields

among any mixtures. The 3 year mean DM yields for 3432/Gr and 3519/Gr were

higher ($p<0.05$) than Nova/Gr but DM yields of this mixture were similar ($p>0.05$) to

that of 3401/Gr (Table 6.1).

Mean DM yields of all the mixed populations at SPARC under rotational grazing and

rain-fed conditions were markedly lower than yields recorded at LRC under rotational

grazing and irrigation or continuous grazing and rain-fed conditions.

The amount of forage consumed by grazing steers in all four treatments were similar

($p>0.05$) at both LRC and SPARC (Table 6.1). It is worth noting that the steers at both

locations were only allowed to utilize 50-60% of the available forage in the paddock.

6.3.2. Botanical composition

In 2009, the proportion of sainfoin in all mixtures at Lethbridge were 25% or higher

over all three cuts even though there were reductions ($p<0.05$) from cut to cut in the

amount of Nova and 3401 in pastures (Table 6.2). In 2010, all mixtures had over 40%

sainfoin before grazing but, after grazing, DM proportions of Nova and 3401 dropped

to 8 and 13%, respectively. For mixtures with 3432 and 3519, the proportion of

sainfoin was 30 and 28%, respectively. In 2011 and 2012 all the mixtures had either

close to or above 30% sainfoin before the first grazing. However, only mixtures with

3432 and 3519 retained more than 25% sainfoin after regrowth.

At Swift Current pasture regrowth was poor and pastures were not grazed for a second pass. Proportions of Nova and 3401 sainfoin DM in the regrowth were 11 and 17% respectively, whilst 3423 and 3519 accounted for a higher ($p<0.05$) proportion of DM proportions at 42 and 30%, respectively (Table 6.2). The proportion of 3432 (39%) DM in the primary growth in 2011 was higher ($p<0.05$) than Nova (25%), 3401 (26%) and 3519 (26%).

Under continuous grazing and rain-fed conditions in Lethbridge, the DM proportion of Nova was 9% higher than 3432 in 2010, but mean differences did not differ among the four populations (Table 6.2). The mean proportion of sainfoin DM varied between 50 and 54% in pastures in 2011 and in 2012, mixtures with 3401 had the highest (68%) proportion of sainfoin ($p<0.05$) in the stand (Table 6.2).

Table 6.2. Botanical composition (% DM basis) of sainfoin in alfalfa/sainfoin mixed pastures under rotational and continuous grazing in Lethbridge, AB. and rotational grazing in Swift Current, SK.

Sainfoin (% DM of total herbage)					
Lethbridge rotational grazing					
	Nova/BJ*	3401/BJ	3432/BJ	3519/BJ	P-value
2009					
1st cut (June 11)	55	52	54	55	0.724
2nd cut (July 19)	44	45	52	52	0.214
3rd cut (Sept. 15)	25a	30a	48b	45b	0.025
2010					
Primary growth (June 28)	43a	40a	50b	53b	0.041
Regrowth (Aug. 6)	8a	13a	30b	28b	0.001
2011					
Primary growth (June 19)	30a	40ab	52c	49bc	0.038
Regrowth (Aug. 2)	5a	13a	43c	30b	0.001
2012					
Primary growth (June 19)	28a	30a	47b	44b	0.032
Regrowth (July 27)	5a	11a	40c	29b	0.001
Swift Current rotational grazing					
	Nova/Br*	3401/Br	3432/Br	3519/Br	
2010					
Primary growth (July 14)	33ab	27a	39b	30a	0.042
Regrowth (Sept 21)	11a	17a	42c	30b	0.001
2011					
Primary growth (July 4)	25a	26a	39b	26a	0.001
Lethbridge continuous grazing					
	Nova/Gr*	3401/Gr	3432/Gr	3519/Gr	
2010 (June 28)	61	57	52	57	0.054
2011 (June 24)	53	51	50	54	0.615
2012 (June 20)	52a	68c	60b	64bc	0.032

* BJ: AC Blue J alfalfa; Br: Beaver alfalfa; Gr: AC Grazeland alfalfa.
Forage regrowth in 2010 but no regrowth in 2011 in Swift Current due to poor precipitation. No regrowth in continuous grazing pastures in Lethbridge as steers were retained on pasture throughout the grazing season.
In 2009, there was no grazing in Lethbridge but the paddocks were cut and baled three times.
Means in the same row with different letters differ significantly (p<0.05).

6.3.3. Chemical composition

Alfalfa had higher CP content (p<0.05) than all sainfoin populations (Table 6.3) at both LRC and SPARC in all years. In 2010, the CP content of all the forages was lower than subsequent year(s) at both locations. There were no differences (p>0.05) in NDF, ADF and OM content among populations within each year. At both locations, the NDF and ADF content in 2010 were higher (p<0.05) than in 2011 and 2012 at LRC. Similarly, NDF and ADF in forages collected in 2010 at SPARC were higher (p<0.05) than in 2011.

Table 6.3. Mean crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and organic matter (OM) (g kg^{-1} DM) of AC Blue J alfalfa, Beaver alfalfa and Nova, LRC-3401 (3401), LRC-3432 (3432) and LRC-3519 (3519) sainfoin populations at Lethbridge, AB. and Swift Current, SK.

Forage						
Lethbridge, AB						
	Alfalfa[¶]	Nova	3401	3432	3519	P-value
2010						
CP	189a	171b	164b	168b	171b	0.042
NDF	423	429	393	404	409	0.174
ADF	344	369	366	345	363	0.263
OM	912	897	902	905	908	0.482
2011						
CP	248a	224b	223b	219b	227b	0.034
NDF	383	369	351	337	356	0.232
ADF	301	289	316	304	307	0.375
OM	921	896	894	901	903	0.322
2012						
CP	258a	222b	210b	220b	214b	0.032
NDF	372	386	395	392	385	0.345
ADF	314	331	324	328	315	0.386
OM	897	885	889	892	889	0.614
Swift Current, SK						
2010						
CP	184a	166b	160b	157b	156b	0.032
NDF	442	445	457	457	456	0.424
ADF	369	378	386	391	385	0.365
OM	931	929	923	929	928	0.654
2011						
CP	236a	217b	221b	214b	219b	0.042
NDF	384	392	386	394	390	0.655
ADF	322	331	335	327	336	0.546
OM	910	894	902	888	892	0.434

[¶] AC Blue J alfalfa in Lethbridge, Beaver alfalfa in Swift Current

Means in the same row with different letters differ significantly (p<0.05).

6.3.4. Animal Performance

The length of grazing season at LRC was 43 d in 2010, 41 d in 2011 and 61 d in 2012. At SPARC, it was 21 d in 2010 and 29 d in 2011. The (ADG) in kg d^{-1} for steers on all pasture treatments in each year were similar ($p > 0.05$) at both LRC and SPARC (Table 6.4). In 2010, the ADG of steers in all treatments was lower than in subsequent year(s) at both LRC and SPARC. The live weight gains of steers (kg ha^{-1}) were highest for all treatments in 2012 at LRC followed by 2011 and the lowest in 2010. At Swift Current, the live weight gains (kg ha^{-1}) in 2011 for all mixed stands were higher than those of 2010.

Table 6.4. Growth performance of steer on four alfalfa/sainfoin mixed pastures under rotational grazing in Lethbridge, AB and Swift Current, SK.

	Grazing treatments				
Lethbridge, AB.					
	Nova/BJ[¶]	3401/BJ	3432/BJ	3519/BJ	P-value
	Length of grazing season (d)				
2010	43	43	43	43	
2011	41	41	41	41	
2012	61	61	61	61	
	Average daily gains (kg d^{-1})				
2010	0.84	0.83	0.88	0.89	0.82
2011	1.19	1.20	1.19	1.21	0.87
2012	1.13	1.11	1.16	1.15	0.71
	Live weight gains (kg ha^{-1})				
2010	311	307	325	329	0.32
2011	420	423	420	427	0.80
2012	593	582	608	598	0.56
Swift Current, SK.					
	Nova/Br[¶]	3401/Br	3432/Br	3519/Br	P-value
	Length of grazing season (d)				
2010	21	21	21	21	
2011	29	29	29	29	
	Average daily gains (kg d^{-1})				
2010	0.80	0.72	0.70	0.79	0.65
2011	1.10	1.20	1.15	1.11	0.51
	Live weight gains (kg ha^{-1})				
2010	144	130	126	143	0.62
2011	274	299	287	277	0.55

[¶] BJ: AC Blue J alfalfa; Br: Beaver alfalfa
Stocking rate = 8.6 steers ha^{-1}
Live weight gain ha^{-1} = Stocking rate (SR) x ADG x grazing season length

6.4. Discussion and Conclusion

Lower DM yield at SPARC as compared to LRC was expected as Swift Current weather conditions (Figs. 6.1 and 6.2) are less suitable for plant growth (Acharya et al., 2013). During the growing season, Swift Current normally experiences lower rainfall and higher temperatures than Lethbridge and this may have contributed to the lower forage DM yields observed in this study. Furthermore, the new sainfoin populations were selected at Lethbridge, so it is possible that they may been better adapted to Lethbridge than to Swift Current (Acharya et al. 2013). The two alfalfa cultivars used at the two locations also differed and this could also account for the differences in forage DM yields as the two alfalfa cultivars may have had inherent differences in DM productivity. The DM yields of 3432 and 3519 in mixtures with alfalfa were higher than that of Nova/alfalfa under both rotational and continuous grazing systems at LRC. Over time, there were reductions in the DM proportions of sainfoin in all mixtures particularly after the first grazing in 2010 at LRC under rotational grazing. but 3432 and 3519 accounted for over 25% of pasture DM throughout the grazing periods whereas Nova and 3401 fell below 15% after the first grazing cycle in all years. As proportions of sainfoin DM below 15% in the mixed alfalfa pasture dramatically increases the risk of bloat, these pastures may not be suitable for unsupervised grazing (Wang et al., 2006, Sottie et al. unpublished). All the sainfoin populations grew back the following year before the first grazing cycle. This was similar to the percentages recorded under continuous grazing where they all had levels of sainfoin above 30% in all three production years (Table 6.2).

The relative proportions of sainfoin (on a DM basis) in the primary growth were higher (p<0.05) than that measured after regrowth. During the winter months both sainfoin and alfalfa enter dormancy (Rohde and Bhalerao, 2007) hence there is not much competition for soil nutrients or other growth factors during this time. After this rest period both forages grow vigorously in spring. Alfalfa recovers quickly after the first grazing or haying and competes with sainfoin for soil nutrients, reducing the proportion of sainfoin in the stand. However, two of the three new sainfoin populations, 3519 and 3432 yielded over 25% DM even after grazing or haying in all years at Lethbridge under rotational grazing. These two populations should be appropriate for use in mixed alfalfa pasture where a reduction in bloat risk during rotational grazing is a priority.

The results showed all treatments had similar nutritional quality and DM intake, which may explain the similar ADG of steers among treatments at LRC and SPARC. The ADG observed were similar to that reported for pure alfalfa pastures (Popp et al., 2000; Schlegel et al., 2000) and comparable to that observed in feedlots. This was expected as the mixed stands had very high proportion (>70%) alfalfa and alfalfa pastures are known to promote high ADG in cattle. The lower ADG recorded at LRC and SPARC in 2010 resulted from the fact that rain delayed the introduction of steers to pasture until after both legumes were fully mature. The higher ADF and NDF and lower CP concentration in these mature forages were indicative of their lower nutritional quality (Table 6.3).

The yield of beef per hectare in the current study of 126 – 299 kg ha^{-1} at SPARC and 307 – 608 kg ha^{-1} at LRC were within ranges reported by Popp et al. (1997) and

Schlegel et al. (2000) even though the length of the grazing season was far shorter in the present study. Popp et al. (1997) reported yields of 107 to 370 kg ha^{-1} in steers grazing alfalfa/grass mixed pastures for 110 – 142-d with stocking rates of 1.1 and 2.2 steers ha^{-1} whereas Schlegel et al. (2000) reported 159 to 689 kg ha^{-1} on pure alfalfa stands grazed for 85 – 118-d with stocking rates of 3.7 to 7.7 steers ha^{-1}. The higher stocking rate of 8.6 steers ha^{-1} used in the present study did not adversely influence live weight gains as compared to the two studies cited above. This may have been due to the fact that the steers in the current study were allowed to only graze 50 to 60% of the forage material available in each pasture.

Although direct statistical comparison between the two locations is not appropriate there was a clear difference in productivity between the two locations. Length of grazing season and live weight gains per hectare at LRC were almost double of those observed at SPARC (Table 6.4). This was expected as available moisture at Lethbridge could be controlled through irrigation. As at SPARC, grazing seasons under rain-fed conditions in semi-arid regions are usually shorter due to the low mean annual precipitation. According to Heady (1975) in some temperate climates, the forage production year is distinctly cyclic and plant growth is usually concentrated in a limited growing season. This results in one distinct forage supply cycle (or sometimes more) during the year consisting of different phases and thus requires appropriate forage and animal management to match forage yields (production cycle) (Vallentine, 2001).

The ADG were similar in the different eco-climatic zones and the ADG were similar to gains reported for steers on alfalfa pastures (Popp et al., 2000). The high reduction

in pasture bloat reported when grazing cattle on alfalfa/sainfoin mixed pastures (Sottie et al., 2012) and the ADG comparable to pure alfalfa pastures give the mixed pasture an advantage over grazing pure alfalfa pastures which expose grazing cattle to high risk of bloat.

No bloat occurred in grazing cattle at LRC as 'Alfasure' was used to ensure bloat-free grazing in stands with lower proportions of sainfoin. At Swift Current no bloat control agent was used and still no incidences of bloat were observed in grazing cattle. This may have been due to high proportion (>25%) of sainfoin in the mixed pastures at this site as was observed by Sottie et al. (2012).

From the observed biomass production it is clear that the populations developed at Lethbridge were probably not the best for the arid environment of Swift Current. Further selections in arid environment for biomass improvement of mixed alfalfa/sainfoin stand will be necessary for closing the gap in biomass productivity in these locations where continuous grazing for a short growing season would be preferred. However, at LRC, the two new populations, 3432 and 3519 had higher persistence (>25%) inmixed stands and higher biomass production with alfalfa than Nova. These two new populations could be used in mixtures with alfalfa when high animal gains and reduction in the risk of bloat in cattle on alfalfa pastures is the target.

7.0 General Discussion

In hay trials at Lethbridge, four of the new populations (LRC-3432, LRC-3519, LRC-3900 and LRC-3902) under rain-fed conditions and three (LRC-3519, LRC-3901 and LRC-3902) under irrigated conditions produced higher ($P < 0.05$) DM yields than Nova. In another set of tests where multiple harvests were taken at Lethbridge (irrigation) and Saskatoon (rain-fed), the new sainfoin populations produced higher ($P < 0.05$) DM yields than Nova in pure stands and persisted for three production years in mixed alfalfa stands where they accounted for >20% of forage DM. Proportions of Nova in mixed alfalfa stands decreased ($p > 0.05$) after the first production year. At Swift Current, however, new sainfoin populations produced lower ($P < 0.05$) DM yield than Nova in pure stands and similar DM yields and proportions in mixed alfalfa stands. The new populations tested may help improve hay or pasture production in most parts of western Canada.

CT concentration in Nova and three new sainfoin populations (LRC-3401, LRC-3432 and LRC-3519) whole plants were similar ($P > 0.05$). CT concentrations were higher ($P < 0.05$) in the leaves, followed by the inflorescence, which were higher ($P < 0.05$) than concentrations in the stems.

Effect of sainfoin CT on rumen fermentation characteristics after 24 h *in vitro* incubation resulted in higher NH_3-N concentration ($P < 0.05$) when PEG was added to the incubation media. Addition of PEG to the media did not affect ($P > 0.05$) DMD and VFA after 24 h of incubation.

LRC-3432 and LRC-3519 matured earlier than Nova. The two attained 50% flowering 5 days earlier Nova.

LRC-3519 persisted better in alfalfa mixed stands (29% of total DM) compared to Nova (5%) after two cycles of rotational grazing. Bloat incidence and severity in steers were reduced ($P < 0.001$) by 98% in LRC-3519 mixed alfalfa stands compared to Nova mixtures.

ADG of steers on four different alfalfa-sainfoin mixed pastures was 1.2 kg d^{-1} at Lethbridge and Swift Current. Percent DM of LRC-3432 (28 – 30%) and LRC-3519 (30 -43%) in alfalfa mixed stands were higher ($P < 0.05$) than Nova (5 – 8%) after first grazing cycle at Lethbridge. New sainfoin populations, LRC-3432 and LRC-3519 can be used in alfalfa pastures for high ADG and bloat prevention as they produced higher DM yields and proportions than Nova.

Further studies could be carried out on:

1. Seeding of new sainfoin populations in established pure alfalfa pastures to determine the establishment and persistence of sainfoin in alfalfa pastures.

2. Rejuvenate alfalfa-sainfoin mixed stands with new sainfoin populations after three production years.

3. Establish pure sainfoin stands using new populations to determine grazing tolerance, pasture longevity and cattle performance.

4. Further characterization with regard to what it is in alfalfa that causes bloat and whether 'Alfasure' could be used to try to determine that since whatever Alfasure interacts with negates the factor(s) that lead to the bloat condition.

8.0. References

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9.0. Appendix

Appendix 1. Extractable condensed tannin concentration in whole plant of Nova and three new sainfoin populations harvested at Lethbridge, AB. and Saskatoon, SK.

	Lethbridge	Saskatoon	P value
	g kg^{-1} DM		
LRC-3900	33.0	43.8	<0.01
LRC-3901	33.1	43.6	<0.01
LRC-3902	33.7	43.0	<0.01
Nova	33.4	43.4	<0.01