Evaluation of a Non-genetically Modified Reduced-Lignin Alfalfa Cultivar in Mixtures with Grasses

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Table of contents

List of Tables

List of Figures

Abstract

Several reduced-lignin alfalfa (*Medicago sativa* L.) cultivars [GM (genetically-modified) and non-GM] are now available locally and are thought to be a tool that could be used in the dairy industry to increase forage digestibility and intake . Non-GM reduced-lignin cultivars may have more potential locally due to the limited acceptability of GM cultivars in eastern Canada and as they can be grown in mixtures with cool-season grasses [i.e., timothy (*Phleum pratense* L.) or tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.)]. We evaluated the performance of a non-GM reduced-lignin alfalfa cultivar compared to a standard (non-GM) alfalfa cultivar when mixed with perennial grass species (timothy and tall-fescue) to determine if a reduced-lignin alfalfa cultivar can provide advantages in terms of forage quality and yield in eastern Canada. Dry matter yield, nutritive attributes, and the yield contribution of each species were determined. Irrespective of the alfalfa cultivar, alfalfa-timothy mixtures produced lower grass yields due to drought conditions experienced during the study, but forage quality was higher when compared to alfalfa-tall fescue mixtures. The value of lower quality alfalfa-tall fescue mixtures was compensated by their greater yields. The contribution to yield of grasses was low, alfalfa in all treatments contributing the most (70-80%) to forage yields. Significantly higher yields and lower forage quality were observed when plants were harvested at the early flower stage of alfalfa when compared to early bud stage. Irrespective of the alfalfa stage at harvest or the grass it was associated with, the reduced-lignin non-GM alfalfa cultivar failed to significantly improve the nutritive value of alfalfa-grass mixtures compared to the standard alfalfa cultivar, an average 4% reduction (or 23 g kg^{-1} of NDF) in neutral detergent fiber concentration being observed across both production years, with no differences in acid

detergent fiber, lignin concentrations, or yields. Therefore, the reduced-lignin non-GM alfalfa cultivar evaluated thus appears to have limited potential locally when used in mixtures with grasses.

Résumé

Plusieurs cultivars de luzerne (*Medicago sativa* L.) à faible teneur en lignine [GM (génétiquement modifié et non-GM] sont maintenant disponibles et sont considérés comme un outil qui pourrait être utilisé dans l'industrie laitière pour augmenter la digestibilité et la consommation des fourrages. Les cultivars à faible teneur en lignine non-GM peuvent avoir plus de potentiel dans l'est du Canada car ils peuvent être cultivés en mélange avec des graminées et que les cultivars GM sont peu acceptés localement. Nous avons comparé la performance d'un cultivar de luzerne à faible teneur en lignine non-GM à celle d'un cultivar standard de luzerne en mélange avec des graminées [i.e., fléole des près (*Phleum pratense* L.) or fétuque élevée (*Schedonorus arundinaceus* (Schreb.) Dumort.)] afin de déterminer si la luzerne faible en lignine peut améliorer la qualité et les rendements de fourrages dans l'est du Canada. Les rendements de fourrages, la valeur nutritive et la contribution au rendement de chaque espèce ont été déterminés. Quel que soit le cultivar de luzerne, les mélanges de luzerne-fléole des près ont produit moins de graminées en raison des conditions de sécheresse rencontrées au cours de l'étude, mais la qualité des fourrages était meilleure comparativement aux mélanges de fétuque élevée-luzerne. La qualité inférieure des mélanges de fétuque élevée-luzerne a été compensée par leurs rendements plus élevés. Des rendements de fourrages plus élevés et une qualité inférieure ont été observés lorsque les plantes ont été récoltées au stade début floraison comparativement au stade bouton. Dans tous les traitements, la luzerne a contribué environs 70 à 80% du rendement total des fourrages. Indépendamment du stade de la luzerne à la récolte ou de l'espèce de graminée dans le mélange la luzerne non-GM à faible teneur en lignine n'a pas amélioré de façon

significative la valeur nutritive des mélanges luzerne-herbe comparativement au cultivar standard de luzerne, une réduction moyenne de 4 % (ou 23 g kg^{-1} de NDF) de la concentration de fibres au détergent neutre étant observée en moyenne pendant les deux années de production, aucun différence dans la concentration de fibres au détergent acide, de lignine ou les rendements étant observé. Le cultivar de luzerne non-GM à faible teneur en lignine évalué semble donc localement avoir un potentiel limité lorsqu'il est utilisé dans des mélanges avec des graminées.

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Contribution of Authors

This thesis is written in the form of a traditional thesis according to the “Guidelines Concerning Thesis Preparation” of McGill University. My role was to conduct most of the experimental procedures and develop the text of the thesis. Dr. Philippe Seguin provided help in the design of experiments, supervision and technical assistance throughout this study. He contributed significantly to the edition and correction of various drafts of the thesis. Dr. Shyam Bushansingh Baurhoo designed the experiments and provided help on experimental strategies, supervision, and funding throughout this project.

List of Abbreviations

NDF	Neutral Detergent Fiber
ADF	Acid Detergent Fiber
ADL	Acid detergent Lignin
CP	Crude Protein
NDFD	Neutral Detergent Fiber Digestibility
TDN	Total Digestible Nutrients
GM	Genetically Modified
N	Nitrogen

Chapter 1 GENERAL INTRODUCTION

1.1 Background

The dairy sector is a longstanding and staple part of Canada's history acting as a sustainable and critical economic driver for the country with approximately 80% of the dairy farms being in the provinces of Ontario and Quebec (Canadian dairy Information Centre, AIMIS, 2018). For such an important sector to be thriving, especially at the individual farmer level, cost of production is a critical factor, mainly regarding feed costs. Given careful attention to ingredients that meet the high nutrition requirements of the dairy cattle, we can understand that forage nutritive value is a crucial component. The forage fiber concentration and fiber digestibility are critical, as high-quality forages can reduce requirements for high-cost concentrated feeds. Data from experts of Lactanet (unpublished data, 2016) demonstrated that the cost of concentrated feeds (i.e., corn grain, soybean meal, etc.…) per hectoliter of milk produced is reduced from $12.51 to $9.81/hL when the neutral detergent fiber (NDF) concentration of forages is reduced from 53% (the Provincial average) to 48%. At the farm scale, this represents a reduction in input costs of $16,000/year and if applied to the entire Province of Quebec to $100 million. Such values underline the importance of high-quality forage in dairy production. One approach to address the need for high-quality forages has been the development of low-lignin (or reduced-lignin) alfalfa (*Medicago sativa L.*) cultivars (Fisher, 2017). There are two main types of low-lignin cultivars: the genetically modified (GM) and non-GM cultivars. The GM low-lignin cultivars are also often resistant or tolerant to glyphosate, while non-GM low-lignin cultivars are developed using conventional breeding and selection methods. Non-GM low-lignin cultivars may be more suited to eastern Canada as forages are most commonly grown in mixtures with cool-season grass species, none of which are currently available with the glyphosate-resistance trait.

The general aim of this study was to compare the performance of a non-GM low-lignin alfalfa cultivar to a standard (non-GM) alfalfa cultivar when mixed with perennial cool-season grass species. Specifically, we aimed to determine if low-lignin alfalfa cultivars can provide advantages in terms of forage yield and forage quality when used in mixtures with cool-season grasses, given that alfalfa is usually grown in mixtures with grasses on the vast majority of acreage in eastern Canada. Two types of alfalfa (low-lignin and standard/regular cultivars) were evaluated in mixtures with different grass species (tall fescue - *Schedonorus arundinaceus* [Schreb.] Dumort. or timothy - *Phleum pratense* L.) and were also harvested at two stages of alfalfa development (bud and early-flower stages). The evaluation of the alfalfa-grass treatments harvested at different stages of development is essential given that one of the advantages of low-lignin alfalfa cultivars is that they can theoretically be harvested at a more advanced stage of development than regular cultivars resulting in comparable nutritive values but greater yields and greater stand persistence. While this has been demonstrated for solo-seeded alfalfa (Grev et al., 2017; McCaslin et al., 2014), it remains to be demonstrated if this advantage will be observed when used in alfalfa-grass mixtures.

1.2 Objectives

The specific objectives of this study were thus to contrast the effects of the various treatments (alfalfa type, grass species in the mixture, and alfalfa stage of development at harvest) on:

1. Forage yields,
2. Botanical composition (i.e. proportions of alfalfa, grass, and weeds),
3. Nutritive value of the alfalfa-grass mixtures,
4. Alfalfa persistence,
5. Nutritive value of experimental silages from different alfalfa-grass mixtures.

1.3 Hypotheses:

The hypotheses of this study were,

- Delaying harvest to obtain higher yields may affect the quality of the grasses.
- The benefits of the low-lignin varieties (as observed in pure stands) on forage quality will be less when grown in mixtures with grasses.

Chapter 2 LITERATURE REVIEW

2.1 Alfalfa

Alfalfa (*Medicago sativa* L.), the single most grown forage legume in Canada is often referred to as “Queen of Forages” (Hanson et al., 1988). Although its geographical origin is said to be in the middle east, alfalfa was cultivated long before the recorded history, and now it is widely grown in almost every part of the world (Hanson and Kehr, 1972). The importance of alfalfa has increased in the early 20th century resulting in a substantial increase in the acreage of alfalfa under cultivation (Stewart, 1926). Several merits of alfalfa over other forage crops can explain in part its important expansion; these include its wide range of adaptability, high yields, high protein content and desirable mineral content, and also its ability to improve soil quality (Hanson et al., 1988). Alfalfa was first introduced in eastern Canada in 1871 and gradually spread throughout the provinces of Quebec, Ontario and rest of the Atlantic provinces (Hanson et al., 1988). The more severe winters of western Canada made it initially hard for alfalfa to grow and survive well until the introduction of extremely winter hardy types. Less than 0.4 million ha of alfalfa was cut for hay in between 1941-1951, which expanded rapidly to 1.8 million ha and increased by six-fold by 1981. Most recent data report that alfalfa and alfalfa-based mixtures in total are grown on an estimated 3.8 million ha in Canada (Statistics Canada, 2016 estimate).

2.1.1 Morphology and anatomy

Alfalfa belongs to the Fabaceae family commonly called "legumes" (Hanson et al., 1988). Unsurprisingly, alfalfa not only is the species of greatest economic value in the *Medicago* genus but also it is the world's most important forage (Small, 2010). Alfalfa being a deep-rooted crop, the taproot grows up to 6 meters long and gradually decreases in thickness from top to bottom. The roots bear nodules which host symbiotic nitrogen fixing bacteria (Cosgrove et al., 2003). A 10-15 cm enlarged, or highly branched crown is found closely attached to the taproot from which the new buds are commonly borne (Stewart, 1926). The young stems arising from the shoot apex grow rapidly until the reproductive stage measuring 50-70 cm in length and 6mm in thickness (Hanson et al., 1988). The stems are often marked with reddish brown or brownish purple which are hollow but partly filled with white pith. The trifoliate, compound, pinnate leaves arise alternatively from the stem showing alternate phyllotaxy. The flowers are borne in clusters on each branch and are seen in different colours, although being predominantly purple (Hanson and Kehr, 1972).

2.1.2 Adaptation: climate, water and soil

Although alfalfa is widely grown under a variety of conditions, it is highly adapted to certain climatic and soil conditions where it display a better performance (Hanson et al., 1988). Temperature, rainfall and humidity are the three important things to be considered while discussing the climatic requirements of alfalfa. Based on the season and soil in which alfalfa is grown, the water requirements vary. Anyhow, it can be grown under conditions where the rainfall is around 100 cm (Hanson and Kehr, 1972). The optimum temperature required for the growth of alfalfa is specific and different for each growth stage (vegetative, bud and flower) and for each plant parameter (such as dry weight, dry matter (DM) accumulation, number and size of leaves, tiller height) under consideration. The number of days to flowering decreases with increase in temperature up to a certain point but as the temperatures go up flowering is delayed which may be because of slowing down of internal processes responsible for flowering due to high temperatures (Pearson and Hunt, 1972). The damp hot climates may favour the growth of aggressive weeds which may smother the crop. Alfalfa is favoured by low humid, warm and dry air, and moist soil which is not waterlogged (CFIA, 2012). Alfalfa can tolerate slight or moderate unfavorable soil conditions provided the climatic conditions are favourable. Although it can be grown on wide range of soils, a well-drained soil is an absolute requirement for its successful establishment, long stand-life and optimum production. Soil pH is one of the key limiting factors and a pH of 6.5 – 7 (neutral) is desired for the successful growth of alfalfa (Peters et al., 2005). Lower pH may limit the extent of symbiotic nitrogen fixation by the crop (Min, 2011) and may require liming to grow alfalfa in acidic soils.

2.1.3 Soil improvements and nitrogen relations

There are undoubtedly many factors responsible for the success and expansion of alfalfa but one of the most significant traits is its ability to enrich the soil. Its ability to produce higher yields of rich, palatable, nutritious forage under a wide range of soil and climatic conditions has given the merit to the crop over other forages (Fishbeck et al., 1987). The finger shaped, less than half-inch sized nodules inhabiting the symbiotic rhizobium bacteria fixes the atmospheric nitrogen. Alfalfa have the potential to fix an average of 450 kg N ha^{-1} $year^{-1}$ through symbiotic N_2 fixation (Fishbeck et al., 1987). Therefore, N fertilizers are not generally recommended and even when applied not only they do not improve the yield or nutritional quality but interfere with nodule formation and nitrogenase activity (Oliveira et al., 2004).

2.2 Forage quality

Forage quality, a combination of forage nutritive value and forage intake, in a broader sense can be defined as the potential of a forage to produce a desired response in the animal to be fed or simply as the performance of the animal when fed with forages (Ball et al., 2001). Forage nutritive value which is defined as the total amount of digestible energy (total digestible nutrients or TDN) is a very important factor determining forage quality (Ball et al., 2001). Understanding the cell wall structure, cell contents and the mechanics of forage digestion in the rumen of dairy animals is very important in order to fully understand and determine forage quality. The cell wall development of plants occurs in two phases where the cell elongation occurs with deposition of pectins, xylans and cellulose in the primary growth phase and subsequent thickening of cell wall with lignin deposition during secondary growth phase (Jung and Allen, 1995). The amount of lignified and non-lignified tissues present in the cell wall influences the forage digestibility as the non-lignified tissues gets digested rapidly and the lignified tissues remain undigested by the rumen microbes (Wilson, 1994). The cellulolytic bacteria attaching to the surface digests the cellulose and hemicellulose rapidly. The close packing of the very thick middle lamella and primary cell wall and arrangement of lignified cells in multicellular strands in the secondary cell wall makes it inaccessible to the rumen microbes and hence resulting in the poor digestion of those areas. Although the optimal forage quality differs depending on the class of animals being fed (dairy cattle/lactating cows/sheep/horses), the main attributes of a good quality forage includes forage digestibility, intake potential, protein content, fiber and mineral content. Digestible energy is one of the most important attributes of forage quality as energy is a driving factor for the development, maintenance and milk production of the animal. The energy supplied in the forage feed depends upon the digestion in the animal and absorption of energy sources in the plant. Acid detergent fiber (i.e., ADF) represents the indigestible fraction (cellulose, lignin) of the forage (Figure 2.2) which is insoluble in acid detergent solution and is inversely proportional to the digestibility (Newman et al., 2009). Higher the ADF concentration, lower will be the digestibility of the forage meaning lower forage quality. The forage samples are boiled for 60 minutes in acid detergent solution to determine ADF in which hemicellulose becomes soluble and leaves out cellulose and lignin (Ball et al., 2001; Mertens, 2002). Acid detergent lignin (i.e., ADL) measures the accurate amount of lignin present in the feed, which is a major influencer of digestibility, feed intake potential and is performed on the ADF residue (Hindrichsen et al., 2006). The maximum amount of forage that can be consumed by the animal, forage intake, is another important attribute of the forage quality. The intake potential depends up on the rate of digestion and the palatability of the forage (Buxton, 1996). Neutral detergent fiber (i.e., NDF) represents the total fiber fraction of the forage (hemicellulose, cellulose, and lignin) estimating the residue that is insoluble in neutral detergent solution

(Figure 2.2) and is inversely proportional to the intake potential (Newman et al., 2009). The higher the NDF concentration is the lower will be the forage feed intake by the animal. A forage sample is boiled for 75 minutes in neutral detergent solution in which most of the cell wall contents (cellulose, lignin, hemicellulose) dissolves leaving out few insoluble fibres (Mertens, 2002). Even though the fiber content of the cell is well expressed by ADF and NDF, they do not measure how digestible the fiber is. Neutral detergent fiber digestibility (i.e., NDFD) measures the digestibility of fiber fraction in the rumen at a specified level of feed intake or in a specific time and estimates TDN and intake potential (Ball et al., 2001). The protein requirements of the animals which is usually expressed as crude protein (i.e., CP) is met by the nitrogen content in the forages which is divided into true protein and nonprotein N (Figure 2.2) and is affected by several factors like maturity and part of the plant the animal consumes. The CP content is inversely proportional to the plant maturity and is high in the early cut alfalfa (Ball et al., 2001; Hancock et al., 2014). The two most important attributes of forage quality, digestibility energy and intake, are inversely proportional to the fiber content of the forage (Putnam et al., 2008). Hence the increase in percentage of ADF and NDF results in decline of energy and intake potential. However, the very low concentrations of effective fiber content can cause physical (rumen) problems as the fiber stimulates chewing, rumination and saliva production in the animal (Putnam et al., 2008). The balance of mineral content in the forage fed is nutritionally important not only to meet the requirements of the animals but the excess amounts of some minerals like K, Se may cause harm to the animal. Generally, ash content (an indication of total minerals available) is inversely proportional to the digestible energy (Putnam et al., 2008).

2.2.1 Factors affecting the nutritive value of forage

Important characteristics like digestible energy, intake potential and fiber content are influenced by several factors which in turn affect the nutritive value of the forage. One of the major and critical agronomic factors that affects forage quality is the plant maturity at harvest. More mature plants are high in fiber, which affect the digestion rates thus reducing the intake potential. The ADF and NDF concentrations increase as the plant matures reducing the CP and NDF digestibility (Putnam and Orloff, 2016). The leaf: stem ratio has been an important factor in improving the forage quality as the leaves have a higher CP content and, also doesn't form lignified secondary cell walls like the stems. So, a higher leaf stem ratio is associated with a higher forage quality (Nelson and Moser, 1994). Yield/quality trade-off is another important factor affecting the quality of forages which puts forage producers in a dilemma whether to favor forage quality or yield. The morphological and physiological changes occurring in the maturing plant results in higher yields but lower forage quality (Figure 2.1). The outcome of early harvesting at immature growth stages would be higher quality forage but lower yields. Agricultural producers can influence the forage quality by managing the harvest schedule. Intensive cutting schedules could cause more harm to the crop rather than aiding in producing higher quality forage by making the stands thin and allowing the weeds to invade, as a more intensive management may reduce plant populations and jeopardize winter survival and stand persistence (Putnam and Orloff, 2016). Temperature, an important environmental factor, affects the functioning and presence of the metabolic and anabolic products and cell wall content – synthesis of lignin is increased during high temperatures, which in turn affect the forage quality (Nelson and Moser, 1994). The forage quality of alfalfa is affected by several other factors like soil type where the quality is higher when grown on certain soils like heavy clay loams and soil fertility where the presence of some minerals like N , P, and K can increase or decrease the forage quality (Mueller and Orloff, 1994). The varietal/species differences can also have profound impact on the quality. For example, the multifoliate varieties in alfalfa can have a higher leaf: stem and exhibit superior forage quality than the trifoliate varieties (Ball et al., 2001). Weed management is also a factor which affect the quality of the forage. The time of day of harvest, insect and diseases incidence, and irrigation also influence the forage quality in a more minor ways and often doesn't have a strong effect like plant maturity or the leaf/stem ratio (Putnam and Orloff, 2016).

2.3 Reduced-lignin alfalfa

Digestibility and intake potential, the two main attributes of forage quality are influenced by several factors and the cell wall composition is one of the main factors that influence them. Lignin is a complex, phenolic polymer deposited into the cells walls as the plant matures and is responsible for the rigidity, water and mineral transport in the plant vascular system (Getachew et al., 2011). The deposition of lignin into the stems as the plant matures becomes a limiting factor forcing the growers to harvest early, thus compromising yield. The lignin present in the cell walls makes it challenging for the rumen bacteria to access the digestible material present in the interior layers of the cell walls. This situation leaves a lot of undigested material in the rumen of the animal and most of it exits the rumen undigested. Not only the extent of digestion is reduced cutting down the energy supplied to the animal but also the slow digestion process fills the rumen quickly which decreases the intake potential of the animal (Jung et al., 2012). In contrast, the rate of digestion and rate of passage is high when the animals are fed with non-lignified tissues/low-lignin containing tissues like leaves (Jung et al., 2012). Thus, reducing the lignin content in the fiber can increase the digestibility and intake potential of forages (Undersander et al., 2009).

Realizing that the regulation of lignin content would be a key step in the process of successfully improving the forage quality, researchers and companies developing alfalfa cultivars have devoted considerable efforts in the past decade to the development of reduced-lignin varieties. Scientists from Forage Genetics International (FGI) in a partnership with The Samuel Roberts Noble Foundation (Noble Foundation) and the U.S. Dairy Forage research center developed a reduced-lignin variety, HarvXtra, through genetic engineering (GM alfalfa) (FGI, 2014). The synthesis of the lignin is catalyzed by a group of enzymes including caffeoyl-CoA-3-O-methyltransferase (CCoAOMT) and caffeic acid-3-O-methyltransferase (COMT) (Jung et al., 2012). The down regulation of these enzymes through gene-silencing and antisense approaches by making use of phenylalanine ammonia-lyase (PAL)2, a pathway gene promoter from bean for tissue specific expression resulted in reduced lignin concentrations in plants from 13 – 24 % and increased digestibility and intake compared to traditional alfalfa plants (Getachew et al., 2018; Guo et al., 2001). The HarvXtra trait in almost all the GM reduced-lignin varieties also include the glyphosate (Round-Up) resistance trait. The Alforex company has developed a reduced-lignin variety, Hi-Gest360, through conventional breeding techniques (non-GM alfalfa) with the goal of improving the overall forage quality (Alforex, 2015). The conventional breeding approach had two main components – selection for altered cell wall composition and direct selection for in vitro rumen digestion of cell walls. The lignin concentration is here reduced by selection for low ADL (Jung et al., 2012). A concurrent increase in the concentrations of CP and a 7-12% decrease in the ADL and NDF is observed when more frequent cuts are taken with shorter intervals in the varieties (HarvXtra) developed through genetic modification (Grev et al., 2017). The variety Hi-Gest 360, developed through conventional breeding efforts has shown to have 7-10% less lignin compared to regular varieties (Sheaffer, 2015). One of the advantages of low-lignin varieties is that they theoretically can be harvested with a wider harvest window as they maintain their nutritive value even up to 10 days longer (Figure 2.3) than the regular varieties (Grev et al., 2017). The producers can harvest less frequently resulting in lesser number of harvests, with higher yields and a similar forage nutritive value than with stands of regular alfalfa managed more intensively (Min et al., 2016). The concerns of many forage producers in eastern Canada is associated with the potential unwanted gene flow from GM to non-GM alfalfa and the practice of growing alfalfa locally often in mixture with grasses, which are not yet glyphosate resistant, makes the reduced-lignin non-GM cultivars a preferable option (Min et al., 2016).

2.4 Alfalfa in mixtures with grasses

Growing alfalfa in combination with grasses in comparison to alfalfa grown alone may increase the stand persistence and the absence of cross linkages in lignin in grasses results in higher digestible fiber, which improves the overall forage nutritive value of the alfalfa-grass mixture (Aponte et al., 2019). The neutral detergent fiber digestibility (NDFD) is also higher in binary mixtures compared to alfalfa in monocultures (Aponte et al., 2019). The other main reason to grow alfalfa in mixtures with grasses is due to the increased forage yield and stand persistence which is because of the protection offered by the grass component to alfalfa from winterkill thus improving the winter survival of alfalfa (Aponte et al., 2019; Bélanger et al., 2014; Malhi et al., 2002). The improved stand persistence helps in increasing the overall yield of the legume-mixture component due to niche complementarity where the two components use resources in a different way or different resources and the species diversity reduces the weed incidence in the stand (Nyfeler et al., 2009; Sanderson et al., 2012). There is a considerable increase in CP concentration and a lower fiber concentration is seen in grass-legume mixtures compared to pure grass stands (Ball et al., 2001). The legume components enhance the soil N content by fixing atmospheric nitrogen which is rapidly depleted by the grass forcing the legume to fix more. As they differ in their root systems (tap root vs fibrous roots) they also absorb nutrients from different depths (Aponte et al., 2019; Nyfeler et al., 2009). This not only reduces the input costs as 80% of N required for forage production is met by N_2 fixation but also helps in weed control and prevents soil erosion (Rasmussen et al., 2012; Sleugh et al., 2000).

2.5 Perennial grasses most commonly cultivated locally

2.5.1 Timothy

One of the main forage grasses grown in Canada in mixtures with alfalfa is timothy, due to its winter hardiness and the ability to grow in cool and moist places (Pomerleau-Lacasse et al., 2019; Gilles, T & Lebas F, 2015). It has a shallow fibrous root system. The bulbs formed through the enlarged internodes serves as storage for reserves enabling it to survive the winter (Lacefield et al., 1980). Timothy has pale green coloured, smooth and hairless leaves growing up to 30-45 cm. The relative ease of seeding, establishing and fast-growing nature of timothy makes it a popular cool season grass (Gilles,T & Lebas F, 2015). It can be grown on moist, clayey and fine textured soils. Timothy is not drought tolerant and cannot withstand sandy soils (Cosgrove, 2009). A pH of 5.5 to 7.0 is best suitable for growing timothy and it cannot grow well in saline soils. It is a highly palatable grass and is higher in quality than many other cool season grasses but declines rapidly with maturity giving a narrow window for harvest (Gilles,T & Lebas F, 2015). The yields are up to 9 t/ha depending on the time (higher in spring than in summer) and maturity of the crop during harvest (Bélanger and McQueen, 1996).

2.5.2 Tall fescue

Another important cool season grass forage used for growing with alfalfa in binary mixtures is tall fescue. The wide adaptive nature, ease of establishment and ability to survive harsh conditions makes it preferable to other grass species (Henson, 2001). Tall fescue is a deep rooted, robust plant growing up to 1m high. The leaves are shiny with distinctive ribs on the upper side. Like timothy it also grows well on soils with a pH of 5.5 – 7.0 and is better adapted to soils with high fertility. It is drought tolerant and has a high regrowth potential (Henson, 2001). The rough nature of the leaves, and the possible presence of toxic compounds that may decrease the animal performance makes it less palatable and less desirable compared to other species (Cosgrove, 2009).

Tables and Figures

Figure 2.1 Effects of the developmental stage of plants on their yield and nutritive value taken from (Blaser et al., 1986).

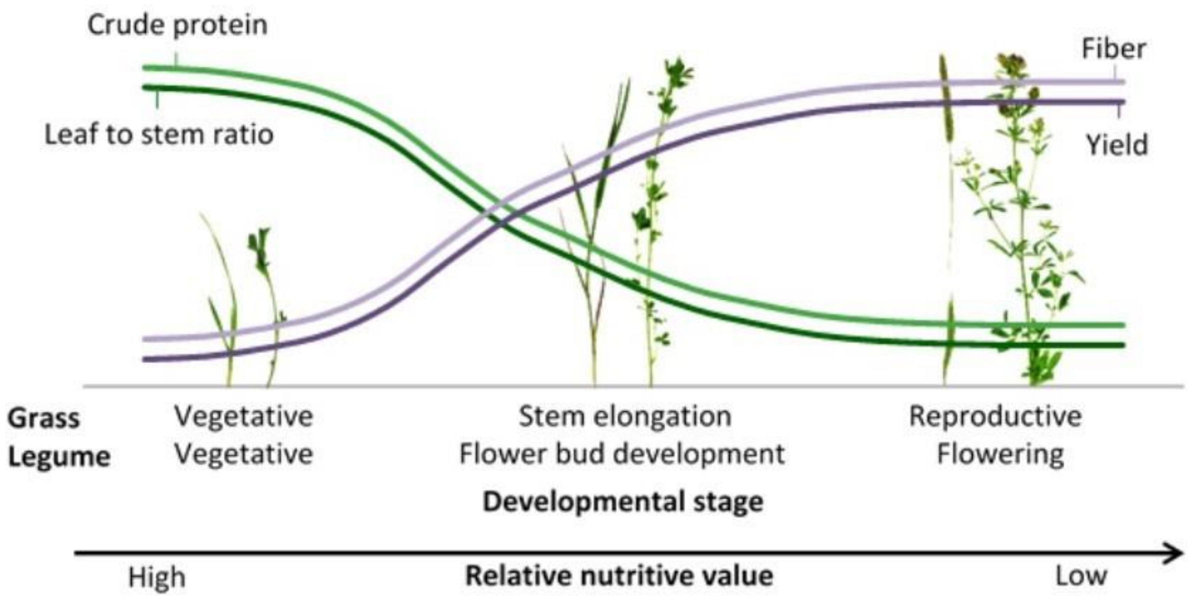
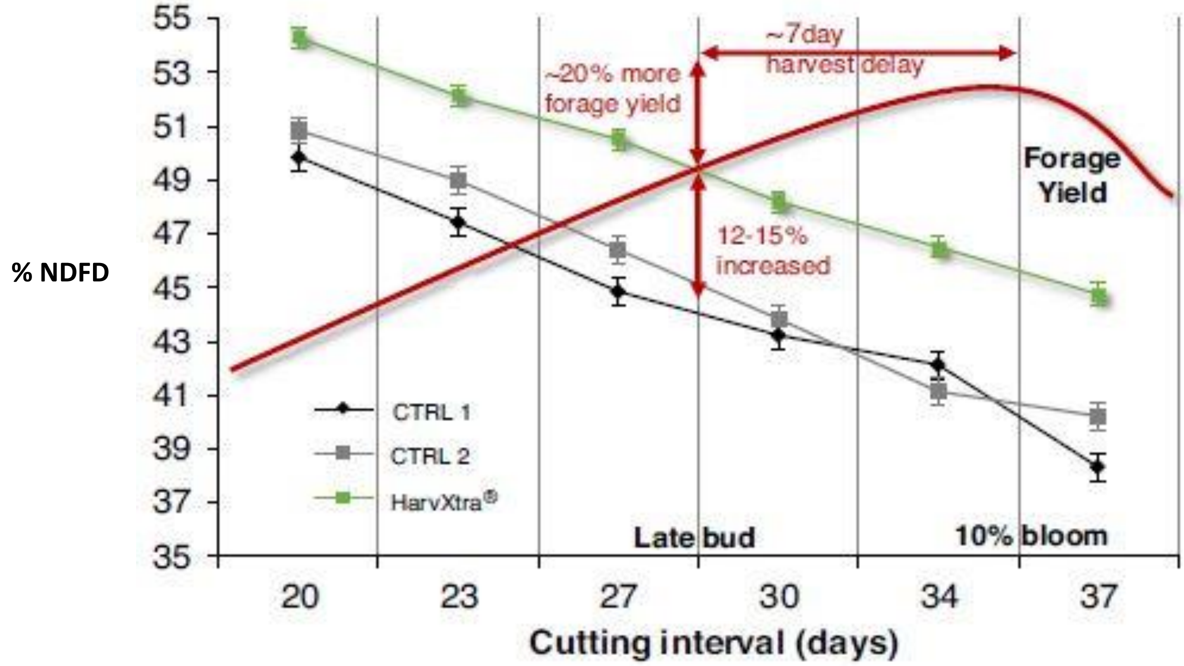


Figure 2.2 Forage analytic fractions and chemical constituents. Taken from Ball et al. (2001).

Portion of forages

<table>
<tr><th colspan="4">Analytic fractions</th><th colspan="2">Chemical constituents</th></tr>
<tr><td colspan="4">Moisture</td><td colspan="2">Water</td></tr>
<tr><td rowspan="13">Dry matter</td><td colspan="3">Ash</td><td colspan="2">Minerals and sand</td></tr>
<tr><td rowspan="12">Organic matter</td><td rowspan="4">Neutral detergent fiber (NDF)</td><td rowspan="3">Acid detergent fiber (ADF)</td><td colspan="2">Cellulose</td></tr>
<tr><td colspan="2">Lignin</td></tr>
<tr><td colspan="2">Fiber-bound and heat-damaged nitrogen</td></tr>
<tr><td colspan="3">Hemicellulose</td></tr>
<tr><td rowspan="8">Neutral detergent solubles
(NDS)</td><td rowspan="4">Neutral detergent soluble carbohydrates (NDSC)</td><td colspan="2">Fructans
Glucans
Pectic acids</td></tr>
<tr><td rowspan="3">NSC</td><td>Sugars</td></tr>
<tr><td>Starches</td></tr>
<tr><td>Organic acids</td></tr>
<tr><td rowspan="3">Crude protein (CP)</td><td colspan="2">Non-protein nitrogen</td></tr>
<tr><td colspan="2">True proteins</td></tr>
<tr><td colspan="2">Fiber-bound and heat-damaged nitrogen</td></tr>
<tr><td>Ether extract</td><td colspan="2">Esterified fatty acids
Pigments
Waxes</td></tr>
</table>

Figure 2.3 Neutral Detergent Fiber Digestibility (NDFD, reflecting forage quality) data obtained from cutting management trials in two reference alfalfa cultivars compared to HarvXtra alfalfa taken from (Barros et al., 2018).



Chapter 3 MATERIALS AND METHODS

3.1 Site and treatments description

Experimental plots were sown in Sainte-Anne-de-Bellevue, QC, Canada (45°25'33.5" N 73°55'52.9" W) in May 2017. A total of eight treatments were evaluated which included four alfalfa-grass treatments and two stages of development of the alfalfa at harvest. The four alfalfa-grass treatments included: i) standard alfalfa (cv. Standfast) seeded in mixture with tall fescue; ii) standard alfalfa seeded with timothy; iii) low-lignin conventional alfalfa (cv. High-gest) seeded with tall fescue; and iv) low-lignin conventional alfalfa seeded with timothy. Alfalfa was seeded at a rate of 13 kg/ha on a PLS (pure-live seed) basis, tall fescue at 5 kg/ha PLS and timothy at 8 kg/ha PLS. Seeding was done in plots of 1.3 by 5 m with an 18-cm row spacing using a Fabro seeder (Swift Current, SK, Canada) at a target depth of 1.5 cm. All treatments were harvested at two stages of development according to the alfalfa component: early-bud stage and early flowering stage. Treatments were assigned to a randomized complete block design with split-plot restriction and each treatment being replicated four times. The stage of development at harvest and treatments were assigned to main-plot and the alfalfa type and grass species combinations were assigned to sub-plots.

Plots were fertilized prior to seeding based on soil test results following local recommendations (CRAAQ, 2010). The insecticide Matador 120 (Lambda – Cyhalothrine 120g/L) was applied to the plots at the rate of 83 mL/ha twice in the seeding year, on July 2nd and again on July 20th to control a severe outbreak of potato leafhopper (*Empoasca fabae*).

3.2 Field data collection and sampling

In the seeding year, all plots were harvested twice to control weeds, no data or sampling was done. In post-seeding years, plots were harvested three to five times depending on the stage of development of alfalfa treatment. A 4 × 0.6 m area was harvested at a 7 cm height from the ground using an experimental flail mower and yield was recorded. For each plot, a 500 g subsample was collected and dried in a forced-air oven at 55°C for 48 h to determine yields on a dry matter basis.

At each harvest, forage from a 0.35 m^2 permanent quadrat in an area representative of the plot's composition was also cut using clippers to determine the botanical composition in each plot. Plants were then separated by species (i.e., alfalfa, tall fescue or timothy, and weeds) with each component being dried in a forced-air oven at 55°C for 48 h to determine their yield contribution on a dry matter basis. Samples from the 500 g sub-samples were subsequently grinded through a 1 mm screen using a Wiley mill (Standard model 4, Arthur H. Thomas Co., Philadelphia, PA) for determination of the nutritive value. Finally, to assess alfalfa establishment and persistence, stem density was also determined in 70 by 36 cm quadrat per plot in May and October of each year.

At the second harvest in the second post-seeding year, a 1 kg sub-sample of fresh biomass from each plot was also ensiled manually in experimental mini-silos made of PVC tubing for 45 days at room temperature. After the period of ensiling, the silos were opened and a sample of 25 g from the thoroughly mixed silage was homogenized in 250ml of distilled water for 1 min. The pH of the water was tested immediately, the rest of the ensiled forage samples were frozen for further analysis. The material was then sub-sampled, and 500 g sub-samples were dried in a forced-air oven at 55°C for 48 h and were subsequently grinded through a 1 mm screen using a Wiley mill for determination of the nutritive value.

3.3 Laboratory analyses

Ground subsamples of 0.5-1.0 g from each plot were scanned by visible and near infrared reflectance spectroscopy (NIRS) using a NIRS system DS 2500 monochromator (Foss, Silver spring, MD). Laboratory analyses were performed on a set of selected samples from each production year to measure the nutritive value of the forage. All the samples selected in NIRS were analysed for neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), dry matter, and crude protein (CP) content.

Neutral detergent fiber analysis was done by using heat-stable α-amylase which is insensitive to EDTA and without the use of sodium sulphite as it attacks lignin and dissolves cross linked proteins as described in Van Soest et al. (1991). Acid detergent fiber analysis was done by following the standard procedures mentioned in 973.18 section of Official Methods of Analysis (AOAC, 1990) using the acid detergent solution. Both the NDF and ADF procedures were performed with an Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY) using F57 filter bags. Each sample was analyzed for crude protein (N × 6.25) using a Leco Nitrogen Analyzer (TruSpec Nitrogen Determinator System; Leco Corp., St. Joseph, MI). Dry matter and ADL analyses were conducted using standard procedures (AOAC, 1990).

3.4 Statistical analyses

Data were analyzed using a 2-way ANOVA using the GLM procedure of SAS (SAS, 2014) where mixtures and stage of development at harvest of treatments were fixed factors and the blocks were a radom factor. Differences among treatment means were tested using Least Square Differences, LSD (SAS, 2014) and statistical significance was be declared at $P < 0.05$, differences at $P < 0.1$ being also mentioned.

Chapter 4 RESULTS AND DISCUSSION

4.1 Environmental conditions

During the establishment year in 2017, precipitation and average daily temperature were both above the 30-year averages (580 vs. 516 mm and 16 vs. 14°C), with 277 mm of precipitation just in the months of April and May (Tables 4.1 and 4.2).

In the first and second production years (i.e., 2018 and 2019), there were prolonged dry spells coupled with higher temperatures. In 2018, precipitation was only 418 mm from April to September or 143 mm in the months of April and May with a mean temperature of 17°C. In 2019, total precipitation was 480 mm with 204 mm just in months of April and May, and with much less rainfall in the later months. The mean temperature from April to September averaged 15°C.

In the winter of 2017 to 2018, there were several days with mean temperature above 0°C and 0 cm ground snow cover recorded (Figure 4.1). Such winter conditions could have affected alfalfa winter survival and affected regrowth of grasses in the following year. The initial winter days of 2018-2019 also had several days with almost 0 ground snow cover and mean temperature above 0°C which might have caused severe winterkill in few experimental plots (Figure 4.2)

4.2 Yields

4.2.1 Total forage dry matter yields

No interactions were observed at harvest between the alfalfa stages of development and the mixtures in both production years. Total forage yields in the first production year were comparable with minimal differences observed among mixture treatments (Table 4.3). The reduced-lignin alfalfa–tall fescue mixture had slightly lower yield (i.e., 6.68 Mg ha^{-1}, although not significant) compared to other treatments, probably due to the fact that this specific treatment had lower alfalfa yield. Although there were significant differences in the grass yields between timothy and tall fescue treatments, those differences were compensated for by differences in alfalfa yields, albeit not different statistically, leading to similar total yields in the first production year.

Total forage yields in the second production year were overall 18% greater in tall fescue mixtures with both alfalfa cultivars (i.e., standard and reduced-lignin) compared to timothy-based mixtures (Table 4.3), mainly due to significantly higher yields of the grass component in the mixture, but also, albeit not significant, of the alfalfa component. In both production years, total forage yields were highest at the first harvest and were greatly reduced in subsequent harvests, which may be due to poor regrowth of grass components after the first harvest (Tables 4.4 and 4.5).

In both production years, the alfalfa stage of development at harvest had significant effect ($P<0.05$) on total annual forage yields. Overall, forage yields were 20 to 30% lower

(Table 4.3) when harvested at the early bud stage of alfalfa compared to early flower stage. Our later finding occurred despite one or two additional cuts at the early bud stage.

4.2.2 Alfalfa dry matter yields

Minimal differences in alfalfa yield among the mixture treatments were only observed (*P*= 0.09) in the first production year, whereas no differences were observed in the second production year. As for total forage yields, significant differences (*P*<0.05) in alfalfa yields were observed between the two-alfalfa stage of development at harvest in both years. Alfalfa yields were 40% higher when plants were harvested at early flower stage compared to early bud stage. There were no interactions between stages and mixtures treatments during both production years.

In the first production year, alfalfa-timothy mixtures, regardless of the alfalfa cultivar, produced higher alfalfa yields than the tall fescue-based mixtures (average of 6.22 and 5.14 Mg ha^{-1}, respectively). These difference in alfalfa yields among mixture treatments were compensated for by opposite differences in grass yield contributions, leading to similar total yields between mixture treatments. Alfalfa in the various mixture treatments represented 70 to 90% of the annual total yield in the first production year (Table 4.6). Alfalfa yields in the second production year were overall lower compared to yields in the first production year in all mixture treatments except for the reduced lignin alfalfa-tall fescue treatment, for which alfalfa yields increased compared to the first production year.

In contrast to our results, other studies conducted in southwestern Quebec reported consistently higher alfalfa yields when mixed with timothy compared to when mixed with tall fescue over three production years (Pomerleau-Lacasse et al., 2019). Although alfalfa yields decreased from the first harvest to subsequent harvests, the contribution of the alfalfa component to the total yield increased which is again due to an even poorer regrowth of grass components (Tables 4.4 and 4.5). The decreasing alfalfa yields in subsequent harvests may be due to lower DM accumulation in late summer and early fall than spring which was also reported by others (Brink et al., 2010). A study by Bélanger et al. (2014) suggest that higher alfalfa yields in first production year compared to the second observed in mixtures with grasses could not only be due to reduced alfalfa population associated with winterkill, but also due to competition from the associated grass counterparts.

The lower alfalfa component in the mixtures when harvested at early bud favored the growth of weeds leading to higher contribution of weeds at early bud harvests than at early flower. As the alfalfa matures, the energy reserves are replenished and minimize the potential for winterkill ultimately increasing the alfalfa contribution to the total yields. This better persistence of alfalfa-grass mixtures and suppressed weed growth with harvests at the early flower stage has been reported by others who also reported similar findings indicating the better persistence and weed control in alfalfa-grass mixtures harvested at the early flower stage than at early bud stage (Bélanger et al., 2020; Pomerleau-Lacasse et al., 2019).

4.2.3 Grass dry matter yields

Grass yields among mixture treatments differed significantly ($P<0.001$) in both production years (Table 4.3). The effect of stage of development of the alfalfa component at harvest was observed ($P<0.05$) only during second production year and, no interactions between stage and mixture were observed ($P>0.05$) during both production years. The grass yields were higher by 15% when harvested at the early flower stage compared to the early bud stage (Table 4.3). In both production years, the two tall fescue mixture treatments produced significantly higher grass yields on average than timothy treatments regardless of the alfalfa cultivar with which they were mixed with. Yield differences between the grass treatments averaged 0.82 and 0.78 Mg ha^{-1} across alfalfa cultivars in the first and second production year, respectively (Table 4.3).

The grass species contribution to total forage yields was less than ideal in most mixtures ranging between 6 and 22% depending on the mixture and production year (Table 4.6). Such overall low yield contributions and yields differences observed between tall fescue and timothy treatments may be explained in part by lower than average precipitation in the first production year (up to 20% less than the 30-year average rainfall recorded locally) coupled with higher than average daily temperatures (Tables 4.1 and 4.2). These drought conditions may have contributed to the overall poor performance of timothy. Indeed, timothy has been consistently reported to have poor drought tolerance leading to limited regrowth potential and persistence in such conditions (Cosgrove, 2009).

The contribution of both grass species to the total annual DM yields was reduced significantly from first harvest to subsequent harvests, where in some treatments timothy yield contribution was reduced to <7 % (Table 4.6). Such low grass contributions to yield in years with important drought events were also reported previously by others in the region including Pomerleau-Lacasse et al. (2019). However, in contrast to our observations, they reported that both tall fescue and timothy had similar regrowth patterns in face of drought. In the present study, significant differences (*P*<0.01) between these two grasses were observed with timothy overall performing very poorly. Indeed, timothy accounted only for 10 % on average of the total yields in alfalfa – timothy grass mixtures, this contribution being reduced from 17 to 2% from first cut to subsequent cuts (Tables 4.4 and 4.5). Tall fescue overall performed better than timothy accounting for 19% of the total annual yield in alfalfa-tall fescue mixtures, while maintaining this contribution across harvests.

Our results indicate that, although timothy is reported to have greater winter hardiness, tall fescue performed better in the current climatic conditions showing higher regrowth potential and better persistence in mixtures with alfalfa. The grass yields were similar at both early bud and early flower except that timothy showed increased yields in 2019.

4.2.4 Weed dry matter yields

The weed dry matter yields were comparable among the treatments and over the two production years. Although there were no significant differences (*P*>0.05) or effect of weed yields on total DM yields, a slight increase in weed yields from 2018 to 2019 was observed. Small differences (*P*<0.1) were observed among the treatments when the botanical composition of the mixtures was estimated during both production years. The weed component in the treatments ranged only from 3 to 6% (Table 4.6), being slightly higher in second production year and inconsistently increased or decreased in the subsequent cuts after the first harvest with most of the treatments having almost no weeds (Tables 4.4 and 4.5). In second production year, the higher weed growth was maybe due to decreased alfalfa yields as a result of winterkill favoring weed growth (Table 4.3). In both production years, significant effects (P<0.05) of stage at harvest and interactions between stage at harvest and mixtures were observed. Weed growth was higher when plots were harvested at the early bud stage compared to the early flower stage. Both types of grasses (i.e., timothy and tall fescue) and alfalfa varieties (standard and reduced-lignin) had similar effects on weed incidence. The most common weeds in our experimental plots were lamb's-quarters (*Chenopodium album* L.), shepherd's-purse (*Capsella bursa pastoris* L.), and barnyard grass (*Echinochloa crusgalli* L.).

4.3 Forage nutritive value

In both production years, significant main effects were observed for NDF and CP concentrations. There were no significant interactions for any parameters except for a Stage x Mixture interaction (P<0.01) for ADL in the first production year, reflecting that in most cases mixture treatments performance did not differ according to the alfalfa stage of maturity at harvest.

In both production years, the NDF concentration was significantly higher with tall fescue treatments compared to timothy (P<0.01; by 40 and 21 g kg^{-1}, in 2018 and 2019 respectively; Table 4.7). Although few studies (e.g., Pelletier et al., 2010) reported that tall fescue grown in pure stands had lower NDF concentration than timothy, results similar to ours were reported locally by Pomerleau-Lacasse et al. (2019) and Bélanger et al. (2017) in studies comparing alfalfa-tall fescue mixtures to other alfalfa-grass mixtures. This higher NDF concentration of alfalfa-tall fescue mixtures compared to alfalfa-timothy mixtures could be due in part to differences in the grass component contribution to the total forage yield as grasses tend to have higher NDF concentrations compared to legumes (Ball et al., 2001). Indeed, the percentage of tall fescue was significantly greater than that of timothy (24 vs 13%, respectively) when averaged over both production years (Table 4.6). For ADF concentration, small differences in the first production year were observed (P=0.056) among mixture treatments, the ADF concentration of tall fescue treatments being 12 g kg^{-1} greater compared to timothy treatments. Surprisingly, in both production years, the ADL percentage was similar (P>0.05) among all four treatments. Crude protein differed significantly (P<0.01) among the mixture treatments, decreasing over the two production years and being higher with alfalfa-timothy treatments compared to tall fescue in both production years. This difference may be mainly due to slightly higher (although not significant) alfalfa percentage in the alfalfa-timothy mixtures compared to alfalfa-tall fescue.

The effect of reduced-lignin alfalfa cultivar was observed only in terms of NDF, with an average 4% or 23 g kg^{-1} reduction in NDF concentration across both production years. The reduced-lignin alfalfa – grass mixtures did not differ from standard alfalfa mixtures in terms of ADF concentration, lignin concentration, and forage yields. Locally, similar results were reported for pure alfalfa stands by Boucher et al. (2020) with non-GM alfalfa cultivars selected for improved digestibility or reduced fiber content not performing differently from standard cultivars.

The stage of development of alfalfa at harvest had significant effects (*P*<0.05) on forage quality. The plants harvested at early bud stage had higher forage quality compared to the plants harvested at the early flower stage, having lower NDF, ADF and ADL concentrations and higher CP concentration.

4.4 Nutritive value of ensiled forage

The nutritive value of ensiled forage was determined at the second harvest in the second production year. Most of the results of silage analysis were similar to the fresh forage. There were differences between mixture treatments only for NDF and CP concentrations (Table 4.8). As for fresh forage, tall fescue treatments add higher NDF concentrations and lower CP concentrations. Again, this could be due to the higher percentage of tall fescue component in alfalfa grass mixtures compared to timothy in this harvest (16 vs 8%) and over the two production years (24 vs 13%). A significant effect of the alfalfa stage of development at harvest was also observed for the silage nutritive value, with forage from plots harvested at the early bud stage of alfalfa having a greater nutritive value (i.e., lower NDF, ADF and ADL concentrations and higher CP concentration) compared to those harvested at the early flower stage. There were no significant interactions between alfalfa stage at harvest and mixture treatments.

4.5 Alfalfa persistence

Alfalfa stems were counted in the spring and fall of the two production years to assess the alfalfa establishment and persistence of the legume-grass mixtures. There was a significant effect ($P<0.05$) of alfalfa stage of development at harvest on the stem density in the spring of the first and second production years (Table 4.9). The stem densities were higher when plots were harvested at the early bud stage compared to early flower stage in spring 2018 and reverse was observed in spring 2019 with higher stem densities when plants were harvested at early flower stage compared to early bud stage. Alfalfa stage of development at harvest had no effect ($P>0.1$) on stem densities in both fall 2017 and fall 2018.

Differences ($P<0.1$) between mixtures treatments were observed in spring of both 2018 and 2019 (Table 4.9). In the spring of both 2018 and 2019, the average stem count was slightly higher with alfalfa – timothy mixtures (738 and 445 stems per m^2, respectively) compared to tall fescue mixtures (714 and 396 stems per m^2, respectively). The alfalfa stems were also slightly higher in reduced-lignin alfalfa treatments compared to standard ones in mixtures with both grasses, suggesting a better winter survival.

There was an overall decline in average alfalfa stem densities from fall 2018 (720 stems per m^2) to spring 2019 (420 stems per m^2) irrespective of the treatment. Harvesting with less than six weeks before the average first frost kill is not recommended to enable alfalfa build energy reserves to survive the winter (Bagg, 2009). The winter conditions with less than optimum snow cover over the winter (Figures 4.1 and 4.2) along with intensive management impacted the alfalfa persistence in the later production years in this experiment. Similar results where winterkill was associated with management decisions during the season were reported by Matteau (2018).

TABLE 4.1 Monthly precipitations (mm) from 2017 to 2018 along with the 30-year average (1971–2000) recorded at Sainte-Anne-de-Bellevue, QC, Canada and precipitations in 2019 recorded at Pierre Elliott Trudeau International Airport, Montreal, QC, Canada (located <10 km from Sainte-Anne-de-Bellevue).

Month	2017	2018	2019[a]	30-year average
January	64.7	86.9	90.7	67.8
February	89.5	62.2	73.9	58.4
March	77.3	42.1	59.3	71.4
April	143.5	98.3	114.8	69.6
May	133.6	44.9	89.7	71.4
June	88.9	70.8	80.8	88.6
July	104.5	60.2	38.8	93.6
August	72.2	52.5	56.1	104.2
September	37.5	91.6	99.8	96.0
October	113.6	65.6	259.6	77.2
November	78.8	115.5	53.5	86.4
December	52.3	87.6	64.0	78.2

[a]Data from 2019 **were unavailable at Sainte-Anne-de-Bellevue**

TABLE 4.2 Monthly mean temperature (°C) from 2017 to 2019 along with the 30-year average (1971-2000) recorded at Sainte-Anne-de-Bellevue, QC, Canada.

Month	2017	2018	2019	30-year average
January	-5.3	-10.2	-10.9	-10.1
February	-4.3	-5.1	-9.0	-8.5
March	-4.7	-1.7	-3.5	-2.3
April	7.7	3.5	5.0	6.3
May	12.9	15.1	11.5	14.1
June	18.4	18.4	17.6	19.1
July	20.1	23.4	22.8	21.9
August	19.1	22.3	19.8	21.0
September	17.8	17.2	15.2	16.5
October	12.8	6.6	9.5	9.3
November	1.1	-0.8	-1.3	1.9
December	-9.4	-5.8	-4.6	-7.5

TABLE 4.3 Annual total forage yield and contribution of alfalfa, grass and weeds in the first two production years of mixtures of alfalfa and grasses harvested at two alfalfa stages of development (Early bud and early flower) in Sainte-Anne-de-Bellevue, QC, Canada.

	Total		**Alfalfa**		**Grass**		**Weeds**	
	Year 1	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**
Alfalfa Stage	Mg ha^{-1}							
Early bud – intensive management	5.88b	5.81b	4.56b	4.11b	1.09	1.17b	0.23a	0.32a
Early flower – extensive management	7.94a	7.36a	6.59a	5.67a	1.25	1.51a	0.09b	0.18b
Mixture								
Standard alfalfa + Tall fescue	7.07	6.65ab	5.31	4.57	1.58a	1.84a	0.17	0.24
Standard alfalfa + timothy	7.22	5.73b	6.26	4.23	0.74b	1.16bc	0.22	0.33
Reduced-lignin alfalfa + Tall fescue	6.68	7.18a	4.97	5.35	1.58a	1.57ab	0.13	0.25
Reduced-lignin alfalfa + timothy	7.11	5.97b	6.18	4.70	0.78b	0.68c	0.15	0.18
S.E.M	0.60	0.48	0.60	0.48	0.16	0.18	0.04	0.05
	P-values							
Alfalfa stage (S)	0.0510*	0.024**	0.048**	0.013**	ns	0.031**	0.01**	0.036**
Mixture (M)	ns	0.021**	0.091*	ns	0.0002***	0.0040***	ns	ns
S×M	ns	ns	ns	ns	ns	ns	0.038**	0.0068***

*Significant at the 0.1 probability level

**Significant at the 0.05 probability level

***Significant at the 0.01 probability level

Means in a column followed by different letters are significantly different(P<0.05)

Table 4.4 Cut wise total forage yield and contribution of alfalfa, grass and weeds in the first production year of mixtures of alfalfa and grasses harvested at two alfalfa stages of development (Early bud and early flower) in Sainte-Anne-de-Bellevue, QC, Canada.

	Total	Alfalfa	Grass	Weeds
Cut 1				
Alfalfa Stage	Mg ha^{-1}			
Early bud – intensive management	2.86b	2.07	0.73	0.05
Early flower – extensive management	4.88a	3.78a	1.06	0.05
Mixture				
Standard alfalfa + Tall fescue	3.94	2.73	1.10a	0.10a
Standard alfalfa + timothy	3.68	3.00	0.66c	0.02b
Reduced-lignin alfalfa + Tall fescue	3.92	2.77	1.09ab	0.06a
Reduced-lignin alfalfa + timothy	3.94	3.20	0.73bc	0.01b
	P-values			
Alfalfa stage (S)	0.001***	0.007***	ns	ns
Mixture (M)	ns	ns	0.04**	0.002***
S×M	ns	ns	ns	ns
Cut 2				
Alfalfa Stage	Mg ha^{-1}			
Early bud – intensive management	1.08b	0.90	0.15	0.03a
Early flower – extensive management	1.72a	1.59	0.12	0.01b
Mixture				
Standard alfalfa + Tall fescue	1.46	1.20	0.24a	0.024
Standard alfalfa + timothy	1.48	1.41	0.03b	0.028
Reduced-lignin alfalfa + Tall fescue	1.28	1.01	0.24a	0.017
Reduced-lignin alfalfa + timothy	1.39	1.35	0.02b	0.012
	P-values			
Alfalfa stage (S)	0.02**	0.01**	ns	0.003***
Mixture (M)	ns	ns	<.0001	ns
S×M	ns	ns	ns	ns
Cut 3				
Alfalfa Stage	Mg ha^{-1}			
Early bud – intensive management	0.55b	0.47b	0.06	0.01
Early flower – extensive management	1.43a	1.35a	0.07	0.01
Mixture				
Standard alfalfa + Tall fescue	1.03	0.90	0.12a	0.01
Standard alfalfa + timothy	1.07	1.04	0.02b	0.01
Reduced-lignin alfalfa + Tall fescue	0.82	0.70	0.11a	0.01
Reduced-lignin alfalfa + timothy	1.02	0.99	0.01b	0.02
	P-values			
Alfalfa stage (S)	0.004***	0.004***	ns	ns
Mixture (M)	ns	ns	<.0001	ns
S×M	ns	ns	ns	ns
Cut 4[a]				
Mixture	Mg ha^{-1}			
Standard alfalfa + Tall fescue	0.80	0.63b	0.13a	0.04b
Standard alfalfa + timothy	1.12	0.92a	0.03b	0.17a
Reduced-lignin alfalfa + Tall fescue	0.75	0.57b	0.14a	0.04b

Reduced-lignin alfalfa + timothy	0.83	0.70ab	0.02b	0.11ab
	P-values			
Mixture	ns	0.08***	<0.0001	0.029
Cut 5[a]				
Mixture	Mg ha^{-1}			
Standard alfalfa + Tall fescue	0.48	0.33	0.11a	0.03b
Standard alfalfa + timothy	0.88	0.70	0.02b	0.15a
Reduced-lignin alfalfa + Tall fescue	0.57	0.40	0.13a	0.04b
Reduced-lignin alfalfa + timothy	0.69	0.58	0.02b	0.1ab
	P-values			
Mixture	ns	ns	0.0014***	0.026

a – only bud stage was harvested in the respective cut
*Significant at the 0.1 probability level
**Significant at the 0.05 probability level
***Significant at the 0.01 probability level
Means in a column followed by different letters are significantly different(P<0.05) ;

Table 4.5 Cut wise total forage yield and contribution of alfalfa, grass and weeds in the second production year of mixtures of alfalfa and grasses harvested at two alfalfa stages of development (Early bud and early flower) in Sainte-Anne-de-Bellevue, QC, Canada.

	Total	Alfalfa	Grass	Weeds
Cut 1				
Alfalfa Stage	Mg ha^{-1}			
Early bud – intensive management	2.40b	1.55b	0.76b	0.09
Early flower – extensive management	4.22a	2.86a	1.28a	0.08
Mixture				
Standard alfalfa + Tall fescue	3.22b	1.86b	1.27	0.1a
Standard alfalfa + timothy	3.20b	1.88b	1.03	0.08ab
Reduced-lignin alfalfa + Tall fescue	3.82a	2.55a	1.14	0.13a
Reduced-lignin alfalfa + timothy	3.21b	2.53a	0.64	0.03b
	P-values			
Alfalfa stage (S)	0.005***	0.016**	0.001***	ns
Mixture (M)	0.04	0.017**	0.07*	0.04
S×M	ns	ns	ns	ns
Cut 2				
Alfalfa Stage	Mg ha^{-1}			
Early bud – intensive management	1.24b	1.0b	0.21	0.03
Early flower – extensive management	1.65a	1.52a	0.12	0.01
Mixture				
Standard alfalfa + Tall fescue	1.52ab	1.21	0.30a	0.02
Standard alfalfa + timothy	1.33b	1.20	0.08b	0.04
Reduced-lignin alfalfa + Tall fescue	1.67a	1.43	0.22a	0.01
Reduced-lignin alfalfa + timothy	1.27b	1.19	0.07b	0.02
	P-values			
Alfalfa stage (S)	0.029**	0.0026***	ns	ns
Mixture (M)	0.03**	ns	0.0025***	0.08*
S×M	ns	ns	ns	0.047**
Cut 3				
Alfalfa Stage	Mg ha^{-1}			
Early bud – intensive management	0.90b	0.72b	0.10	0.06
Early flower – extensive management	1.49a	1.29a	0.11	0.08
Mixture				
Standard alfalfa + Tall fescue	1.31	1.04	0.20a	0.08
Standard alfalfa + timothy	1.04	0.90	0.05b	0.10
Reduced-lignin alfalfa + Tall fescue	1.25	1.05	0.15a	0.05
Reduced-lignin alfalfa + timothy	1.15	1.03	0.04b	0.08
	P-values			
Alfalfa stage (S)	0.008***	0.003***	ns	ns
Mixture (M)	ns	ns	<0.0031	ns
S×M	ns	ns	ns	ns
Cut 4[a]				
Mixture	Mg ha^{-1}			
Standard alfalfa + Tall fescue	1.20	0.93	0.17a	0.10
Standard alfalfa + timothy	0.76	0.52	0.02b	0.22

Reduced-lignin alfalfa + Tall fescue	0.88	0.64	0.13ab	0.12
Reduced-lignin alfalfa + timothy	0.68	0.50	0.04b	0.15
	P-values			
Mixture	ns	ns	0.06	ns

a – only bud stage was harvested in the respective cut
*Significant at the 0.1 probability level
**Significant at the 0.05 probability level
***Significant at the 0.01 probability level
Means in a column followed by different letters are significantly different(P<0.05)

Table 4.6 Botanical composition of alfalfa, grass and weeds in the first two production years of mixtures of alfalfa and grasses harvested at two alfalfa stages of development (Early bud and early flower) in Sainte-Anne-de-Bellevue, QC, Canada.

	Alfalfa		Grass		Weed	
	Year 1	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**
Alfalfa Stage	% (percentage contribution to total forage yield)					
Early bud – intensive management	79.6b	76.6b	15.1	16.1	5.3a	7.7a
Early flower – extensive management	87.7a	81.9a	11.4	15.0	0. 9b	3.1b
Mixture						
Standard alfalfa + Tall fescue	78.3a	73.2b	19.3a	22.5a	2.4b	4.3bc
Standard alfalfa + Timothy	89.4b	77.9ab	6.4b	13.6b	4.2a	8.5a
Reduced-lignin alfalfa + Tall fescue	76.7a	80.8a	21.1a	15.8b	2.2b	3.3c
Reduced-lignin alfalfa + Timothy	90.1b	82.9a	6.2b	11.5b	3.6a	5.6b
S.E.M	2.15	2.50	1.51	1.63	0.89	1.12
	P-values					
Alfalfa stage (S)	0.006***	0.04**	0.07*	ns	0.0002***	0.005***
Mixture (M)	<.0001***	0.007***	<.001***	0.0009***	0.001***	0.0001***
S×M	ns	ns	ns	ns	0.0002*	0.0007***

*Significant at the 0.1 probability level
**Significant at the 0.05 probability level
***Significant at the 0.01 probability level
Means in a column followed by different letters are significantly different(P<0.05)

Table 4.7 Forage nutritive value [neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude protein (CP)] of different alfalfa grass mixtures for two production years (2018, 2019) in Ste-Anne-de-Bellevue, QC, Canada.

	NDF		**ADF**		**ADL**		**CP**	
	Year 1	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**
Alfalfa Stage	g kg^{-1}							
Early bud – intensive management	421b	492b	246b	257b	43b	46b	197a	165a
Early flower – extensive management	501a	587a	309a	329a	60a	64a	166b	143b
Mixture								
Standard alfalfa + Tall fescue	494a	571a	285a	304	51	54	176bc	146c
Standard alfalfa + Timothy	440b	551ab	270ab	296	51	58	188a	154b
Reduced-lignin alfalfa + Tall fescue	469c	539bc	282ab	296	52	54	175c	151bc
Reduced-lignin alfalfa + Timothy	442b	516c	274b	287	51	56	186ab	160a
S.E.M	1.70	2.22	1.33	1.59	0.37	0.43	0.73	0.52
	P-values							
Alfalfa stage (S)	0.004***	0.003***	0.006***	0.001***	0.009***	0.008***	0.004***	0.004***
Mixture (M)	<.0001***	0.003***	0.0562*	0.089*	ns	ns	0.027**	0.0065***
S×M	ns	ns	ns	ns	0.0040***	ns	ns	ns

*Significant at the 0.1 probability level
**Significant at the 0.05 probability level
***Significant at the 0.01 probability level
Means in a column followed by different letters are significantly different(P<0.05) ;

Table 4.8 Silage nutritive value [neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude protein (CP)] of different alfalfa grass mixtures for a single harvest in second production year (2019) in Ste-Anne-de-Bellevue, QC.

	NDF	**ADF**	**ADL**	**CP**
Alfalfa Stage	g kg^{-1}			
Early bud – intensive management	403b	305b	52b	193.a
Early flower – extensive management	428a	345a	62a	189b
Mixture				
Standard alfalfa + Tall fescue	441a	336	56	184b
Standard alfalfa + Timothy	399b	320	59	191a
Reduced-lignin alfalfa + Tall fescue	421a	326	57	183b
Reduced-lignin alfalfa + Timothy	401b	317	58	192a
S.E.M	0.77	0.94	0.27	0.26
	P-values			
Alfalfa stage (S)	0.0023***	00013***	0.032**	0.038**
Mixture (M)	0.001***	ns	ns	0.0006***
S×M	ns	ns	ns	ns

*Significant at the 0.1 probability level
**Significant at the 0.05 probability level
***Significant at the 0.01 probability level
Means in a column followed by different letters are significantly different(P<0.05) ;

Table 4.9 Stem density of alfalfa in the fall and spring of following production years using two different cool season grasses in combination with two different alfalfa cultivars in Sainte-Anne-de-Bellevue, QC, Canada.

	2017-2018		**2018-2019**	
	Fall 2017	**Spring 2018**	**Fall 2018**	**Spring 2019**
Alfalfa Stage	Average stem/m^2			
Early bud	751	778a	447	400a
Early flower	691	674b	463	441b
Mixture				
Standard alfalfa + Tall fescue	657	644b	455	338b
Standard alfalfa + timothy	761	671ab	445	396ab
Reduced-lignin alfalfa + Tall fescue	732	785a	454	452ab
Reduced-lignin alfalfa + timothy	733	804a	466	495a
S.E.M	68.28	52.10	22.18	33.44
	P-values			
Alfalfa stage (S)	ns	0.04**	ns	0.047**
Mixture (M)	ns	0.058*	ns	0.073*
S×M	ns	ns	ns	ns

*Significant at the 0.1 probability level

**Significant at the 0.05 probability level

***Significant at the 0.001 probability level

Means in a column followed by different letters are significantly different(P<0.05)

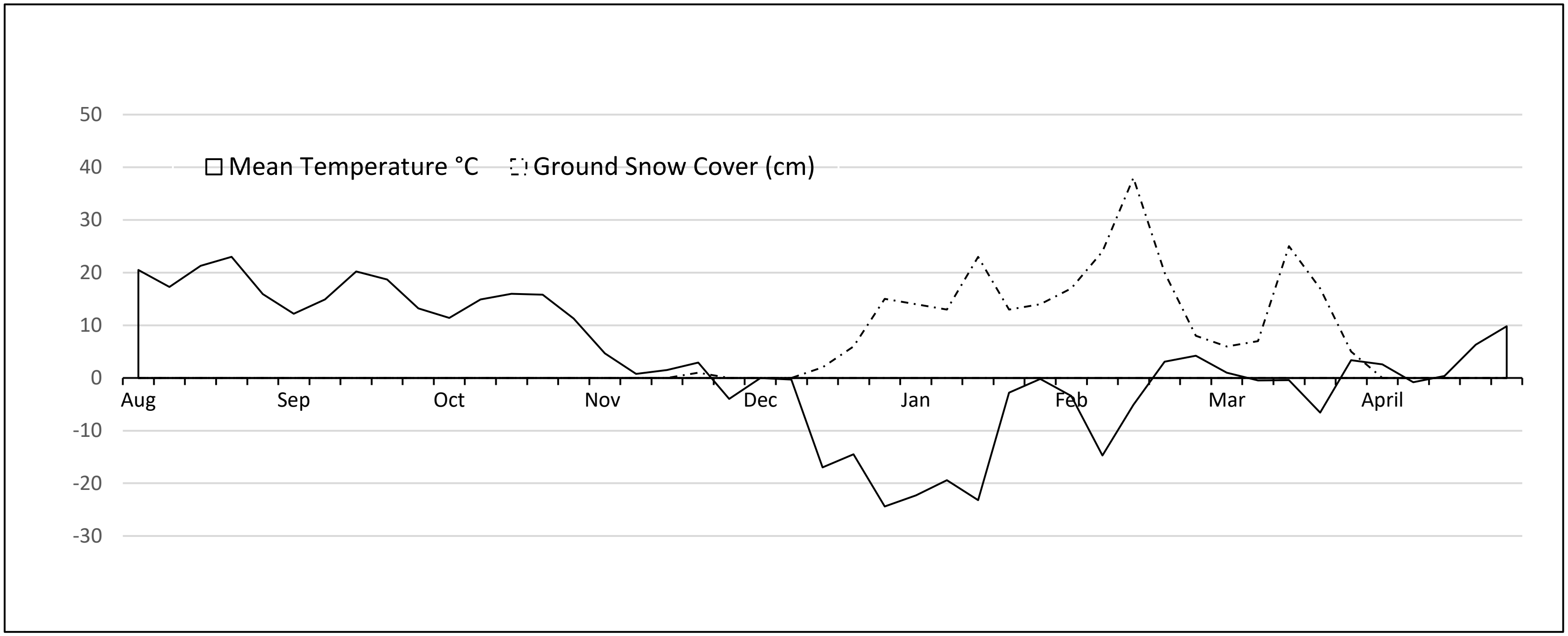


Figure 4.1 Daily average temperature (°C) and ground snow cover (cm) from Aug 2017 to April 2018 in Sainte-Anne-de-Bellevue, QC, Canada.

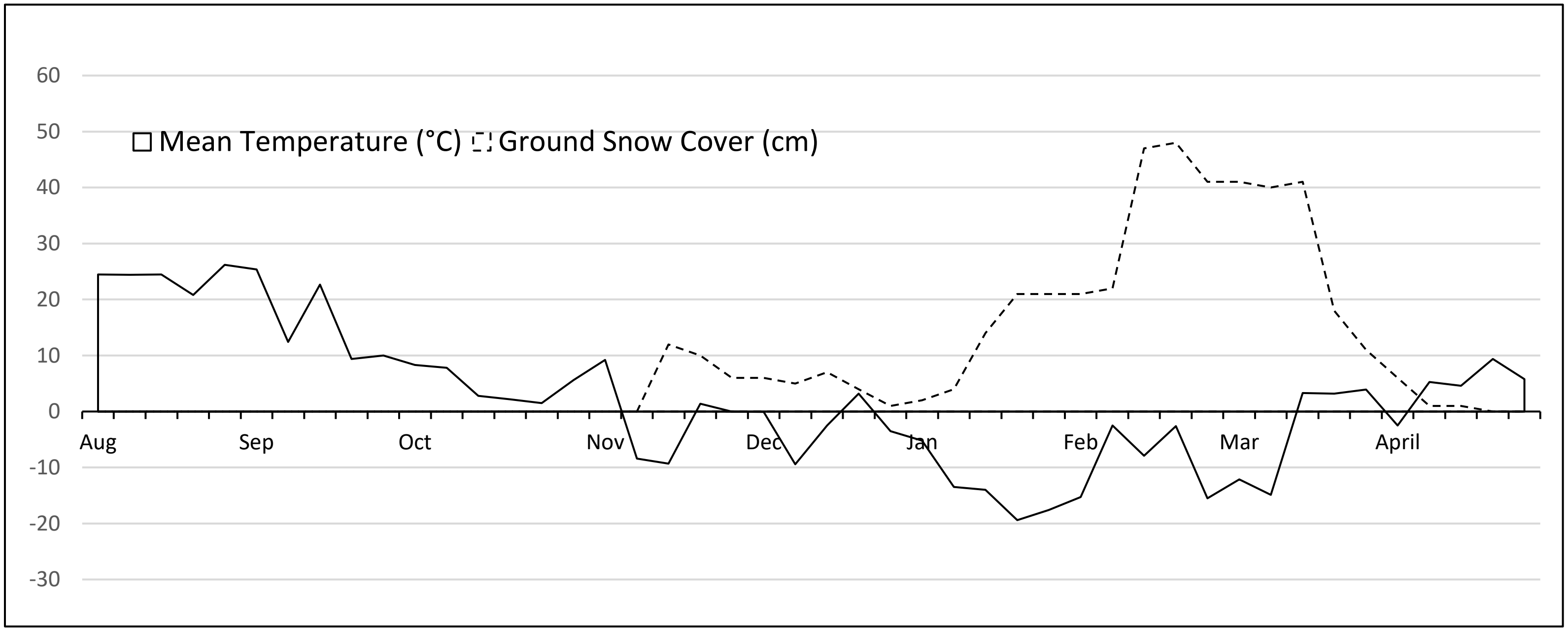


Figure 4.2 Daily average temperature (°C) and ground snow cover (cm) from Aug 2018 to April 2019 in Sainte-Anne-de-Bellevue, QC, Canada.

Chapter 5 GENERAL CONCLUSIONS

The warmer and dryer periods observed in both production years of experimentation compared to the 30-year average might have been responsible for the overall poor performance and regrowth of grasses observed in the mixtures with alfalfa we evaluated. In these conditions, tall fescue, overall, performed better than timothy in terms of yields under climatic conditions experienced in the present study. Alfalfa, however, dominated in all mixtures contributing up to 80% of the total annual forage yield. The higher forage dry matter yield of alfalfa-tall fescue mixtures compared to alfalfa-timothy mixtures compensated for their overall lower nutritive value.

The alfalfa stage of development at harvest had significance effects on both forage yield and quality of alfalfa-grass mixtures evaluated, but there were no interactions observed with the mixture treatments, suggesting they all responded similarly to the stage of development at harvest. Harvesting at the bud stage overall resulted in lower yields and higher nutritive value compared to harvesting at the early-flower stage.

Irrespective of the alfalfa stage of development at harvest or the grass it was associated with, the reduced-lignin alfalfa cultivar evaluated failed to significantly improve the nutritive value of alfalfa-grass mixtures compared to the standard alfalfa cultivar, an average 4% reduction (or 23 g kg^{-1} of NDF) in neutral detergent fiber concentration being observed across both production years, with no differences in acid detergent fiber, acid detergent lignin concentrations, or yields. The limited differences in forage nutritive value we observed between the two alfalfa cultivars occurred despite the fact that alfalfa contributed to an average of 80% of the biomass produced over the course of experimentation. The reduced-lignin non-GM alfalfa cultivar evaluated thus appears to have limited potential locally when used in mixtures with grasses.

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