Published December 20, 2018

Alternatives to Timothy Grown in Mixture with Alfalfa in Eastern Canada

Florence Pomerleau-Lacasse, Philippe Seguin,*
Gaëtan F. Tremblay, Gilles Bélanger, Julie Lajeunesse, and Édith Charbonneau

ABSTRACT

Timothy (*Phleum pratense* L.) is the main forage grass species cultivated with alfalfa (*Medicago sativa* L.) in eastern Canada, yet its regrowth under dry and warm conditions is poor. Air temperature and water stress are predicted to increase in the near future, which could further reduce timothy's regrowth. We evaluated six alfalfa–grass binary mixtures at three contrasted sites in eastern Canada to find potential alternatives to the alfalfa–timothy mixture under current climatic conditions. Timothy, tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort.), meadow fescue (*Schedonorus pratensis* [Huds.] P. Beauv.), festulolium (× *Festulolium* Asch. & Graebn), perennial ryegrass (*Lolium perenne* L.), and meadow bromegrass (*Bromus biebersteinii* Roem. & Schult.) were evaluated with harvests either at the early bud or early flower stage of alfalfa. Dry matter yield, nutritive attributes, and the yield contribution of each species were determined. Alfalfa mixtures with festulolium (cv. Spring Green) and perennial ryegrass (cv. Remington) had inferior grass yield contributions due to winter damages, as well as inferior forage yield and estimated milk production per hectare; these cultivars are not currently viable alternatives to timothy in eastern Canada. In contrast, alfalfa–meadow fescue and alfalfa–meadow bromegrass mixtures produced comparable yields, nutritive value, and estimated milk production per hectare and they are, therefore, possible alternatives to the alfalfa-timothy mixture. The alfalfa–tall fescue mixture also represents a possible alternative; its lower nutritive value was compensated by its slightly greater yield. Timothy, tall fescue, meadow fescue, and meadow bromegrass remained productive over the first three production years when cultivated in mixture with alfalfa.

Core Ideas

- Mixtures of alfalfa with meadow fescue, tall fescue, or meadow bromegrass have comparable yields and persistence to alfalfa-timothy.
- Mixtures with festulolium or perennial ryegrass had lower total seasonal DM yields than the alfalfa-timothy mixture.
- Harvesting mixtures at the alfalfa early flower stage maximizes the estimated milk production per ha.

Published in Agron. J. 111:314–327 (2019)
doi:10.2134/agronj2018.05.0309
Supplemental material available online
Available freely online through the author-supported open access option

Copyright © 2019 by the American Society of Agronomy
5585 Guilford Road, Madison, WI 53711 USA
This is an open access article distributed under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Alfalfa (*Medicago sativa* L.) is the main perennial forage legume species grown worldwide and is an important forage crop in Canada, being grown on millions of hectares (Statistics Canada, 2017). In eastern Canada, alfalfa is mostly grown in mixtures with perennial forage grasses (CFIA, 2012). Growing alfalfa or other forage legume species in mixtures with grasses increases forage dry matter (DM) yield (Berdahl et al., 2001; Bélanger et al., 2014) and reduces weed invasion (Sanderson et al., 2012; Sturludóttir et al., 2013; Bélanger et al., 2014), without decreasing forage digestibility compared with alfalfa or forage legumes grown alone (Kunelius et al., 2006; Sturludóttir et al., 2013; Bélanger et al., 2014).

In eastern Canada, the main forage grass species associated with alfalfa is timothy (*Phleum pratense* L.) due to its tolerance to adverse winter conditions (Bélanger et al., 2006), but its regrowth under prolonged warm and dry summer conditions is poor (Bertrand et al., 2008; Cosgrove, 2009; Virkajärvi et al., 2012b). Climate change is predicted in the future to result in warmer temperatures, more frequent drought stress events, and longer growing seasons (Qian et al., 2013). Despite temperature and precipitation variabilities observed in Canada, average seasonal and annual temperatures in the past 70 yr already illustrate a clear increase from the 1961 to 1990 climate normal (Government of Canada, 2016, 2017). In addition, there has been an increase in the duration of growing seasons (Barrow et al., 2004) and in the frequency of extreme warm days and nights and, conversely, a decrease in the frequency of extreme cold days and nights (Vincent and Mekis, 2006). An overall warming of the climate has therefore already started to take place across Canada, and this could negatively affect the regrowth and nutritive value of timothy (Jing et al., 2014; Piva et al., 2013), as well as the annual yield of the alfalfa–timothy mixture (Thivierge et al., 2016), especially in the currently warmer regions of southern

F. Pomerleau-Lacasse, P. Seguin, McGill Univ., Dep. of Plant Science, 21111 Lakeshore Road, Sainte-Anne-de-Bellevue, QC, H9X 3V9, Canada; G.F. Tremblay, G. Bélanger, Agriculture and Agri-Food Canada (AAFC), Québec Research and Development Centre, 2560 Hochelaga boul., Québec, QC, G1V 2J3, Canada; J. Lajeunesse, AAFC, Normandin Research Farm, 1468 Saint-Cyrille St., Normandin, QC, G8M 4K3, Canada; É. Charbonneau, Dep. des sciences animales, Univ. Laval, 2425 rue de l'Agriculture, Québec, QC, G1V 0A6, Canada. Received 8 May 2018. Accepted 4 Oct. 2018.
*Corresponding author (philippe.seguin@mcgill.ca).

Abbreviations: ADF, acid detergent fiber; aNDF, neutral detergent fiber assayed with a heat-stable amylase and sodium sulfite; CP, crude protein; DM, dry matter; GDD5, growing degree-days calculated using a 5 °C basis; IVTD, in vitro true digestibility of dry matter; NDFd, in vitro neutral detergent fiber digestibility; PCA, principal component analysis; TDN, total digestible nutrients; VNIRS, visible and near-infrared reflectance spectroscopy.

Quebec and Ontario. Current forage production practices must therefore be adapted to respond to ongoing climatic changes.

Tall fescue, meadow fescue, festulolium, perennial ryegrass, and meadow bromegrass are five grass species known to have better regrowth potential and drought tolerance than timothy (Cosgrove, 2009). Based on their growth characteristics, they are expected to have a better regrowth under current and predicted summer conditions but they are less tolerant to adverse winter conditions (Cosgrove, 2009). The potential of those species for use in binary mixtures with alfalfa under current eastern Canadian climate conditions is not well known. The objective of this study was therefore to evaluate five grass species for use in binary mixtures, as alternatives to timothy in eastern Canada under current climatic conditions, with mixtures being harvested at two alfalfa developmental stages.

MATERIALS AND METHODS

Sites and Treatments Description

The experiment was conducted at three climatically-contrasted sites in QC, Canada: Sainte-Anne-de-Bellevue (45°25′ N lat.; 73°55′ W long., 2100 cumulated growing degree days based on 5 °C [GDD5]), Saint-Augustin-de-Desmaures (46°43′ N lat. ; 71°29′ W long., 1700 cumulated GDD5), and Normandin (48°50′ N lat.; 72°33′ W long., 1350 cumulated GDD5). Plots were seeded on 21, 23, and 29 May 2014 at Saint-Augustin-de-Desmaures, Normandin, and Sainte-Anne-de-Bellevue, respectively, with binary mixtures of alfalfa and one of six cool-season grasses. Each mixture was harvested at two mean alfalfa developmental stages, specifically at the early bud (i.e., stage 3) and early flower (i.e., stage 5) (Fick and Muller, 1989; Pomerleau-Lacasse et al., 2017), resulting in a 2 × 6 factorial arrangement of 12 treatments. Treatments were assigned to a randomized complete block design with split-plot restriction and three replicates. The stage of development of alfalfa at harvest (hereafter called "Alfalfa stage") was assigned to main-plots and the six alfalfa-grass mixtures (hereafter called "Mixture") to subplots. Plot size varied at each site, but was a minimum of 1.3 × 5 m.

Alfalfa (cv. Calypso) was seeded at a rate of 9 kg ha^{-1} on a pure life seed basis (PLS) in mixture with one of six grass species: timothy (cv. AC alliance; 7 kg ha^{-1} PLS), tall fescue (cv. Carnival; 10 kg ha^{-1} PLS), meadow fescue (common seed; 10 kg ha^{-1} PLS), festulolium (cv. Spring Green; 10 kg ha^{-1} PLS), perennial ryegrass (cv. Remington; 12 kg ha^{-1} PLS), and meadow bromegrass (cv. Fleet; 12 kg ha^{-1} PLS). Cultivars and seeding rates were selected according to provincial recommendations (CRAAQ, 2013) or, when cultivar recommendations were unavailable for the province of Québec (i.e., meadow fescue, perennial ryegrass, festulolium), according to recommendations for the province of Ontario (Ontario Forage Crop Committee, 2013). Species, for which no seeding rate recommendations were locally available (i.e., meadow fescue, perennial ryegrass, festulolium, meadow bromegrass), were compared with other species with a similar number of seeds per kg to determine their seeding rates. Seeding was done at a targeted depth of 5 to 10 mm using a Fabro seven-row seeder (Swift Current, SK, Canada) at Sainte-Anne-de-Bellevue and a Carter five-row seeder (Brookston, IN) at Normandin, and by broadcast seeding immediately followed by a light raking for seed incorporation and rolling with a Brillion seeder at Saint-Augustin-de-Desmaures.

Phosphorus, K, and B fertilizers were applied before seeding and each year if needed based on soil tests and provincial recommendations (CRAAQ, 2010). In the establishment year (2014), plots were harvested once at Normandin and Saint-Augustin-de-Desmaures, and twice at Sainte-Anne-de-Bellevue, but no measurement was made. After the last harvest of the establishment year, plots were fertilized with 30 kg N ha^{-1} at Normandin and Sainte-Anne-de-Bellevue or 40 kg N ha^{-1} at Saint-Augustin-de-Desmaures. The insecticide Matador 120EC (Syngenta Crop Protection Canada, Guelph, ON, Canada), with the active ingredient lambda-cyhalothrin (120 g L^{-1}), was applied twice during the third production year at Sainte-Anne-de-Bellevue to control a severe outbreak of potato leafhopper (*Empoasca fabae)*, which affected alfalfa plants. The pesticide was applied on 4 and 19 July 2017 using a field sprayer at the recommended rate of 83 mL ha^{-1}. No herbicide was applied; weeds were clipped once above the seeded species in the establishment year to avoid seed dispersal.

Harvests and Data Collection

Plots were harvested two to four times in the first, second, and third production years (i.e., 2015, 2016, and 2017); the number of harvests depending on the developmental stage of alfalfa at harvest and the site. Half of the plots were harvested every time alfalfa reached the early bud mean stage of development, and the other half when it reached the early flower mean stage. Harvest dates are provided in Table 1. The average developmental stage of the associated grass in each plot (Moore et al., 1991; Pomerleau-Lacasse et al., 2017) was also visually estimated at each harvest. Climatic conditions at each harvest date were retrieved from the closest Environmental Canada weather station, and the GDD5, from 1 April to the first harvest and between harvests, were calculated (Table 1).

Each plot was harvested at a height of 7 cm from the ground using a self-propelled flail forage harvester, and the fresh weight was recorded. The area harvested varied at each site but was at least 0.6 × 4 m. A 500-g sample was collected, weighed, dried at 55 °C in a forced-air oven for 72 h, and weighed again to determine the DM concentration. The dry samples were then ground to pass 1-mm sieve using a Wiley mill (Standard model 4, Arthur H. Thomas Co., Philadelphia, PA) for laboratory analyses.

In each plot, immediately after each harvest and beside the previously harvested section, the forage in a distinct unharvested 0.25-m^2 fixed quadrat was cut using clippers at a 7-cm height from the ground, and later separated by hand into alfalfa, seeded grass, and weed components. Each component was then dried at 55 °C for 72 h to determine their yield contributions and DM yields.

Laboratory Analyses

Ground forage samples were scanned by visible and near infrared reflectance spectroscopy (VNIRS) using a NIRsystem DS 2500 monochromator (Foss, Silver spring, MD) to identify samples to be included in calibration and validation sets with the objective of using forage reflectance spectrum and correlate them against nutritive value attributes determined by laboratory analyses to predict nutritive attributes of all samples. Laboratory analyses were performed on calibration (n = 92) and validation (n = 24) sets of samples selected by the WinISI IV (version 4.0) software (Infrasoft International, LLC, Silver Spring, MD)

Table 1. Harvest dates and growing degree-days accumulated from 1 April to harvest 1 and between harvests of alfalfa-grass binary mixtures for two harvest regimes based on the developmental stage of alfalfa, at three sites in eastern Canada, and for three production years.

	2015		2016		2017	
	Early bud	Early flower	Early bud	Early flower	Early bud	Early flower
Normandin						
Harvest 1	June 9	June 22	June 7	June 27	June 13	June 22
GDD5†	255	372	236	428	281	374
Harvest 2	July 10	July 28	July 13	Aug. 1	July 12	July 26
GDD5	323	424	384	420	329	358
Harvest 3	Aug. 13	–	Aug. 11	–	Aug. 17	–
GDD5	418	–	358	–	369	–
Saint-Augustin-de-Desmaures						
Harvest 1	June 3	June 15	June 10	June 21	June 12	June 22
GDD5	336	461	356	487	341	476
Harvest 2	July 6	July 20	July 8	July 18	July 12	July 20
GDD5	376	420	363	397	391	379
Harvest 3	Aug. 7	Aug. 26	Aug. 2	Aug. 22	Aug. 7	Sept. 1
GDD5	434	561	385	532	341	503
Harvest 4	Sept. 3	–	Sept. 6	–	–	–
GDD5	411	–	527	–	–	–
Sainte-Anne-de-Bellevue						
Harvest 1	May 28	June 12	May 30	June 13	June 1	June 19
GDD5	375	535	313	468	340	559
Harvest 2	July 2	July 16	June 28	July 18	July 7	July 24
GDD5	419	480	391	554	450	530
Harvest 3	July 29	Aug. 24	July 26	Aug. 18	July 31	Sept. 6
GDD5	423	605	452	528	390	509
Harvest 4	Sept. 1	–	Aug. 22	–	Sept. 6	–
GDD5	517	–	456	–	419	–

†GDD5, cumulated growing degree-days calculated using a 5 °C basis from 1 April to harvest 1, and between harvests.

from each production year (2015, 2016, 2017) to determine the following nutritive attributes: neutral detergent fiber assayed with a heat-stable amylase and sodium sulfite (aNDF), acid detergent fiber (ADF), total nitrogen (TN), ash, crude fat, acid detergent lignin (ADL), neutral detergent insoluble nitrogen (NDIN), and acid detergent insoluble nitrogen (ADIN).

All laboratory analyses were performed as described in Simili da Silva et al. (2014). In summary, aNDF concentration was measured according to Mertens (2002) with the addition of a heat-stable α-amylase and sodium sulfite. The ADF concentration was determined following AOAC (1990). Both aNDF and ADF determinations were done using the ANKOM filter bag technique (ANKOM Technology, Macedon, NY; ANKOM Technology, 2017a; ANKOM Technology 2017b). The TN concentration was determined with an autoanalyser (QuikChem 8000 Lachat Zellweger Analytics Inc., Lachat Instruments, Milwaukee, WI) using the method 13–107–06–2-E (Lachat Instruments, 2011) following a mineralization in a mixture of sulfuric and selenious acids (Isaac and Johnson, 1976). The crude protein (CP) concentration was estimated from the TN concentration using the following formula: CP = TN × 6.25. The analytical DM and ash concentrations (Leco Corporation, 2009) were determined using a thermogravimetric analyzer (model TGA701, Leco Corporation, St. Joseph, MI). Crude fat (ether extract) was determined using Ankom xt15 Extractor Technology Method (AOCS, 2003). The NDIN and ADIN concentrations were also chemically determined (Licitra et al., 1996) for the calibration and validation sets of samples.

The in vitro true digestibility of DM (IVTD) and in vitro NDF digestibility (NDFd) were determined using the method of Goering and Van Soest (1970). Rumen fluid was collected from a rumen-fistulated dairy cow, and samples were incubated for 48 h in buffered rumen fluid in an Ankom Daisy II incubator (Ankom Technology, Macedon, NY). The IVTD (g kg^{-1} DM) and NDFd (g kg^{-1} aNDF) were calculated as below:

$$\text{IVTD} = (1 - \text{postdigestion dry weight following aNDF wash/predigestion dry weight}) \times 1000$$

$$\text{NDFd} = (1 - \text{postdigestion dry weight following aNDF wash/predigestion dry weight of aNDF}) \times 1000$$

The ADF, aNDF, IVTD, and NDFd determinations were followed by ashing of the fiber residue to provide results corrected for the ash content of the fiber residue. From the chemically-determined ADF, aNDF, CP, ash, crude fat, and NDIN concentrations, along with the NDFd value, the total digestible nutrient (TDN) concentration and the estimated milk production per kg of forage were calculated for the calibration and validation sets of forage samples using the Excel spreadsheet Milk2013 (Undersander et al., 2013).

The nutritive attributes described above were then predicted for all forage samples using VNIRS (WinISI IV ver. 4.0 software, Infrasoft International, LLC, Silver Spring, MD). The VNIRS predictions were considered excellent when the ratio of prediction to deviation (RPD), which is calculated by dividing the standard

Table 2. Seasonal forage dry matter yield and yield of individual components (alfalfa, seeded grass, others) for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Normandin (QC) along with the probabilities of fixed effects and their interactions.

	Total				Alfalfa				Seeded Grass				Others			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	Mg ha^{-1}															
Early bud	5.4	5.2	6.2	5.6	4.6	4.6	4.6	4.6	0.6	0.5	1.0	0.7	0.1	0.2	0.5	0.3
Early flower	4.9	5.7	5.7	5.4	4.0	5.2	5.3	4.8	0.8	0.5	0.2	0.5	0.1	0.0	0.2	0.1
SEM	0.3	0.1	0.2	0.1	0.5	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.0	0.0	0.1	0.0
Mixture†																
Alf + Tim	5.6a‡	5.6	5.7	5.6ab	4.4	5.0	5.1abc	4.8	1.1ab	0.5ab	0.3bc	0.6b	0.1	0.1	0.4	0.2
Alf + TF	5.2ab	5.7	6.2	5.7ab	4.7	4.7	4.5bc	4.6	0.4bc	0.9a	1.5a	0.9ab	0.1	0.1	0.2	0.1
Alf + MF	5.8a	5.4	6.0	5.7a	4.5	4.5	4.1c	4.4	1.3ab	0.9a	1.4ab	1.2a	0.0	0.1	0.4	0.2
Alf + Fest	4.0c	5.3	5.7	5.0c	3.8	5.1	5.2abc	4.7	0.0c	0.0b	0.1c	0.0c	0.2	0.2	0.5	0.3
Alf + Rye	4.3bc	5.4	6.1	5.3bc	4.2	5.3	5.6a	5.0	0.1c	0.0b	0.0c	0.0c	0.1	0.1	0.5	0.2
Alf + Bro	5.9a	5.4	6.1	5.8a	4.2	4.9	5.4ab	4.8	1.6a	0.4ab	0.5abc	0.8ab	0.1	0.1	0.2	0.1
SEM	0.3	0.2	0.2	0.2	0.4	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.1	0.0	0.1	0.1
ANOVA	*P*-value															
Year	–	–	–	***	–	–	–	*	–	–	–	ns§	–	–	–	***
Alfalfa stage	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mixture	***	ns	ns	***	ns	ns	**	ns	***	***	***	***	ns	ns	ns	ns
Year×stage	–	–	–	***	–	–	–	*	–	–	–	***	–	–	–	***
Year×mixture	–	–	–	***	–	–	–	**	–	–	–	***	–	–	–	ns
Stage×mixture	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	*	ns	ns	ns
Year×stage×mixture	–	–	–	ns	–	–	–	*	–	–	–	ns	–	–	–	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

‡ Within columns, and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

§ ns, nonsignificant.

deviation (SD) of the reference data used in the validation set by the standard error of prediction corrected for bias [SEP(C)] [RPD = SD/SEP(C)], was greater than 4, successful when the RPD was between 3 and 4, and moderately successful when it was between 2.25 and 3 (Nie et al., 2009). All attributes obtained excellent or successful predictions (RPD > 3.0) except NDFd (RPD = 2.86), for which the prediction was moderately successful.

Statistical Analysis

A two-way analysis of variance (ANOVA) was performed on DM yields and nutritive attributes using PROC mixed of the SAS software (version 9.4; SAS Institute Inc., 2013). Data were analyzed by experimental site. Replicates at each site were considered a random effect and the production years, mixtures, and harvest stages were considered fixed effects. Because the treatment × year interactions were often significant, data were further analyzed for each production year. A multiple comparisons adjustment using the simulation method on the least squares means was included in our analysis to account for our large number of treatments. Differences between treatment means were considered significant at $P \leq 0.05$; only such significant effects are later discussed.

A principal component analysis (PCA) was also performed on the least squares means of the 12 mixture × harvest stage treatment combinations using the correlation matrix method of the SAS software (version 9.4; SAS Institute Inc., 2013); equal weight was given to all variables. This PCA allowed us to characterize the relationship among DM yields, NDFd, IVTD, concentrations of CP, aNDF, and TDN, and the estimated milk production per hectare, and to observe how treatments related to these variables.

RESULTS AND DISCUSSION

The analyses of variance indicated that there were generally no significant interactions between the mixtures and alfalfa stages at harvest for seasonal DM yields and nutritive attributes (Tables 2–7), thus the emphasis in the presentation and discussion of the results is on the main effects of mixtures and alfalfa developmental stages at harvest.

Alfalfa-Grass Mixtures

Seasonal Dry Matter Yield

At Normandin, alfalfa–grass mixtures differed in total, alfalfa, and grass DM yields in at least one production year (Table 2). In the first production year (2015), mixtures with tall fescue, meadow fescue, or meadow bromegrass had similar total, alfalfa, and grass DM yields to the alfalfa–timothy mixture. Mixtures with festulolium and perennial ryegrass, however, had lower total DM yields due to lower grass DM yields. Indeed, the grass DM yields of the alfalfa–festulolium and alfalfa–perennial ryegrass mixtures ranged from 0.0 to 0.1 Mg ha^{-1} in all production years (2015–2017); festulolium and perennial ryegrass were nearly absent from their respective mixtures because of their poor survival in the first winter following seeding. In the second and third production years (2016, 2017), the alfalfa DM yields of the alfalfa–festulolium and alfalfa-perennial ryegrass mixtures were

Table 3. Seasonal forage dry matter yield and yield of individual components (alfalfa, seeded grass, others) for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Saint-Augustin-de-Desmaures (QC) along with the probabilities of fixed effects and their interactions.

	Total				Alfalfa				Seeded Grass				Others			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	Mg ha^{-1}															
Early bud	10.2	8.3	5.8b‡	8.1	7.4	5.8b	3.6	5.6b	2.7	2.0	1.7	2.1	0.1a	0.5	0.5	0.4
Early flower	12.2	11.4	9.8a	11.1	9.9	9.1a	7.8	8.9a	2.3	2.1	1.8	2.1	0.0b	0.2	0.3	0.2
SEM	1.6	1.3	1.1	1.3	1.2	1.2	0.8	1.0	0.4	0.3	0.4	0.3	0.0	0.1	0.1	0.1
Mixture†																
Alf + Tim	11.2	10.2	7.6	9.7	9.7a	8.0ab	5.8ab	7.9ab	1.3c	1.5bc	1.1ab	1.3b	0.2a	0.6ab	0.7ab	0.5a
Alf + TF	11.8	10.1	8.3	10.1	8.9abc	6.5b	5.6ab	7.0bc	2.8ab	3.3a	2.5a	2.9a	0.0b	0.2bc	0.2ab	0.2b
Alf + MF	10.6	9.8	7.8	9.4	8.1bc	7.2ab	5.4ab	6.9c	2.4abc	2.3ab	2.1a	2.3a	0.1b	0.3abc	0.3ab	0.2b
Alf + Fest	11.0	9.3	7.8	9.4	7.6c	7.0b	5.6ab	6.7c	3.4a	2.1abc	2.0a	2.5a	0.0b	0.2c	0.3ab	0.2b
Alf + Rye	11.4	9.7	7.6	9.6	8.1bc	7.4ab	5.2b	6.9c	3.3a	2.1bc	2.3a	2.6a	0.0b	0.1c	0.2b	0.1b
Alf + Bro	11.2	10.0	7.8	9.6	9.4ab	8.6a	6.6a	8.2a	1.6bc	0.8c	0.4b	0.9b	0.2a	0.6a	0.7a	0.5a
SEM	1.5	1.3	1.0	1.2	1.1	1.2	0.6	0.9	0.5	0.3	0.5	0.3	0.0	0.1	0.2	0.1
ANOVA	*P*-values															
Year	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	***
Alfalfa stage	ns§	ns	*	ns	ns	*	ns	**	ns	ns	ns	ns	*	ns	ns	ns
Mixture	ns	ns	ns	ns	***	***	*	***	***	***	**	***	***	***	*	***
Year×stage	–	–	–	***	–	–	–	***	–	–	–	ns	–	–	–	ns
Year×mixture	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns
Stage×mixture	ns	ns	**	ns	ns	***	*	ns	ns	ns	ns	ns	*	ns	ns	ns
Year×stage×mixture	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

‡ Within columns, and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

§ ns, nonsignificant.

comparable with or greater than those of other mixtures, including the alfalfa–timothy mixture. Therefore, despite the absence or near absence of festulolium and perennial ryegrass, the numerically greater alfalfa DM yields in these two mixtures allowed for similar total DM yields of all mixtures in the second and third production years (2016, 2017). In addition, the grass DM yield of the alfalfa–timothy mixture in the third production year (0.3 Mg ha^{-1}) was less than that of the alfalfa–tall fescue mixture (1.5 Mg ha^{-1}), indicating a better persistence of tall fescue over the three production years, perhaps due to its greater competitive ability.

At Saint-Augustin-de-Desmaures, total DM yields of all six mixtures did not differ in the three production years (2015–2017; Table 3), although differences in alfalfa and grass DM yields were observed. On average, across the three production years, mixtures containing meadow fescue, festulolium, or perennial ryegrass had lower alfalfa DM yields and greater grass DM yields than mixtures with timothy or meadow bromegrass. Furthermore, meadow bromegrass generally had a lower grass DM yield than all grasses except timothy (2015–2017). At this site, the cumulative precipitation for March and April, when water infiltration in the frozen soil and evaporation are still limited, was well above 200 mm in the establishment year (2014), and in 2016 and 2017 (Supplemental Table S2); the expected precipitation for this period based on the monthly 30-yr average (1971–2000) is 168 mm at Saint-Augustin-de-Desmaures (Government of Canada, 2018a). This excess water early in the growing season may have negatively affected the emergence and spring regrowth of meadow bromegrass, a forage species reportedly sensitive to flooding (Ogle et al., 2006), resulting in lower grass seasonal DM yields of the alfalfa-meadow bromegrass mixture.

At Sainte-Anne-de-Bellevue, the mixtures differed in total, alfalfa, and grass DM yields averaged across the three production years (Table 4). Mixtures containing tall fescue, meadow fescue, or meadow bromegrass had similar total DM yields to the alfalfa–timothy mixture. Mixtures containing festulolium or perennial ryegrass, however, consistently had lower total DM yields (2015–2017) due to lower alfalfa DM yields in 2015 and lower grass DM yields in 2016 and 2017. This result probably reflects a poor survival of festulolium and perennial ryegrass during the second winter after seeding.

Winter conditions at Normandin and Sainte-Anne-de-Bellevue may have contributed to the poor performance of festulolium and perennial ryegrass at these two sites, two species known to be prone to winterkill (Cosgrove, 2009). At Normandin, festulolium and perennial ryegrass had initially established successfully according to visual observations of plant density in the fall of 2014. However, conditions in the first winter after seeding, which included a shallow snow cover (avg. Dec.-March: 5.8 cm) combined with particularly low sub-freezing temperatures for extended periods of time (supplemental Fig. S1), could have been lethal to festulolium and perennial ryegrass. The historical average snow cover at this site is 35 cm (1971–2000; Government of Canada, 2018a). These two grasses were, indeed, already nearly absent from their respective

Table 4. Seasonal forage dry matter yield and yield of individual components (alfalfa, seeded grass, others) for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Sainte-Anne-de-Bellevue (QC) along with the probabilities of fixed effects and their interactions.

	Total				Alfalfa				Grass				Other			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	Mg ha^{-1}															
Early bud	6.2	3.3	5.4	5.0b	2.4a	2.1	2.6b	2.3a	3.5	1.2	2.4	2.4	0.3	0.0	0.4	0.3
Early flower	8.4	6.2	10.0	8.2a	3.7b	4.3	7.0a	5.0b	4.3	1.9	2.8	3.0	0.4	0.0	0.2	0.2
SEM	0.9	0.6	0.6	0.7	0.6	0.6	0.4	0.5	0.4	0.2	0.2	0.2	0.0	0.0	0.0	0.0
Mixture†																
Alf + Tim	8.1a‡	5.2a	8.4a	7.2a	4.0a	3.7	4.8	4.2a	3.7ab	1.5bc	3.3ab	2.8b	0.4abc	0.0	0.3bc	0.2abc
Alf + TF	8.2a	5.4a	8.8a	7.5a	3.3a	2.9	4.5	3.6ab	4.5a	2.5a	4.2a	3.7a	0.4abc	0.0	0.1c	0.2c
Alf + MF	7.6a	5.4a	7.6ab	6.9a	3.2ab	3.3	4.6	3.7ab	3.9ab	2.2ab	2.7bc	2.9b	0.5ab	0.0	0.3b	0.3ab
Alf + Fest	6.1b	3.7b	6.4b	5.4b	1.9c	3.0	5.0	3.3b	3.9ab	0.7c	0.7d	1.8c	0.3bc	0.1	0.7a	0.3a
Alf + Rye	5.8b	3.4b	6.7b	5.3b	2.2bc	2.8	5.0	3.3b	3.4b	0.6c	1.4cd	1.8c	0.2c	0.0	0.3bc	0.2bc
Alf + Bro	8.2a	5.5a	8.3a	7.3a	3.7a	3.6	4.9	4.0ab	4.0ab	1.8ab	3.2ab	3.0b	0.5a	0.0	0.3bc	0.3ab
SEM	0.9	0.6	0.6	0.7	0.7	0.6	0.5	0.5	0.3	0.2	0.3	0.2	0.1	0.0	0.1	0.0
ANOVA	*P*-values															
Year	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	***
Alfalfa stage	ns§	ns	ns	*	*	ns	**	**	ns	ns	ns	ns	ns	ns	ns	ns
Mixture	***	***	***	***	***	ns	ns	**	*	***	***	***	**	ns	***	***
Year×stage	–	–	–	***	–	–	–	***	–	–	–	ns	–	–	–	**
Year×mixture	–	–	–	ns	–	–	–	*	–	–	–	***	–	–	–	***
Stage×mixture	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year×stage×mixture	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

‡ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

§ ns, nonsignificant.

mixtures in the first harvest of the first production year (2015; supplemental Fig. S4). At Sainte-Anne-de-Bellevue, these two grasses had similar DM yields to grasses of other mixtures in the first production year (2015), but their DM yields decreased from 2015 to 2016 (Table 4), and this trend was already observed at the first harvest of 2016 (supplemental Fig. S6). During the 2015 to 2016 winter, multiple periods of above zero temperatures (supplemental Fig. S3) possibly dehardened plants (Sakai and Larcher, 1987), reduced the insulating snow cover, and led to the formation of ice sheets above the plants. Indeed, the snow cover recorded at this site (Avg. Dec.-Apr.: 2.6 cm) was less than half what is usually expected for this region (10 cm; Government of Canada, 2018b). This site also experienced prolonged periods with no snow on the ground during which the air temperature reached values below –15 °C (supplemental Fig. S3). These combined detrimental climatic conditions could have resulted in the mortality of winter-sensitive forage species (Bélanger et al., 2006) and, consequently, in inferior DM yields of the alfalfa–festulolium and alfalfa–perennial ryegrass mixtures at Normandin (Table 2) and Sainte-Anne-de-Bellevue (Table 4).

Our results thus suggest the winter susceptibility of the cultivars of festulolium (cv. Spring Green) and perennial ryegrass (cv. Remington) when grown in eastern Canada. Perennial ryegrass generally has poor winter hardiness (Ogle et al., 2008; Cosgrove, 2009), yet different cultivars may vary in performance and competitiveness at given locations (Jung et al., 1996). The cultivar Remington used in our experiment is a tetraploid ryegrass with improved winter hardiness and drought tolerance relative to traditional tetraploid perennial ryegrass cultivars. Festulolium, a hybrid grass between meadow fescue or tall fescue, and annual ryegrass or perennial ryegrass, has been developed for an enhanced nutritive value and winter hardiness relative to its parental species (DLF International Seeds, 2013). However, the specific genetic background of festulolium cultivars strongly affects their DM productivity, winter hardiness, and persistence, as demonstrated by Opitz von Boberfeld and Banzhaf (2006). The festulolium cultivar 'Spring Green' used in our experiment is a selection from hybrids of meadow fescue with perennial ryegrass or Italian ryegrass (Casler et al., 2001). The festulolium 'Spring Green', like the perennial ryegrass 'Remington', was developed for enhanced winter hardiness (Casler et al., 2001; Goslee et al., 2017). However, it appeared to be insufficient for them to thrive at Normandin and Sainte-Anne-de-Bellevue. While other cultivars of festulolium and perennial ryegrass could have potentially performed better under current eastern Canadian climatic conditions, the two cultivars used in this project may be, in some years, risky alternatives to timothy when grown in mixture with alfalfa in eastern Canada.

At Normandin and Saint-Augustin-de-Desmaures, starting in the first production year (2015), the grass contribution to the DM yield of all mixtures was less than expected (Tables 2 and 3; supplemental Fig. S4 and S5). Indeed, the grass contribution averaged 18% (ranging between 0 and 32%), which is below the

Table 5. Nutritive attributes and estimated milk production per hectare for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Normandin (QC) along with the probabilities of fixed effects and their interactions.

	CP†				aNDF				NDFd				IVTD				TDN				Milk Production‡			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
	g kg^{-1} DM								g kg^{-1} aNDF				g kg^{-1} DM								Mg ha^{-1}			
Alfalfa stage																								
Early bud	194a§	216	209	206a	355b	356	384	365	660	662a	685a	669a	870a	874a	874a	873a	633a	631a	627	630a	9.7	9.4	11.1	10.1
Early flower	164b	184	192	180b	405a	406	391	400	618	593b	604b	605b	829b	828b	835b	831b	600b	595b	604	600b	8.4	9.7	9.9	9.3
SEM	4.1	5.7	6.5	5.2	5.1	11.3	10.0	7.2	12.4	6.3	6.3	7.3	6.2	3.9	2.7	1.7	4.6	5.7	4.8	2.2	0.42	0.21	0.37	0.26
Mixture¶																								
Alf + Tim	177abc	201	207a	195ab	396bc	374ab	376bc	382b	659a	616ab	637ab	637b	854a	848	853ab	851ab	620ab	617ab	624a	620ab	9.9a	9.8	10.3	10.0a
Alf + TF	182ab	195	186b	188bc	365cd	403a	424a	397ab	616b	646a	670a	644b	841b	854	858ab	851ab	616ab	599b	592c	602c	9.1ab	9.7	10.4	9.8ab
Alf + MF	165bc	196	196ab	186c	419ab	392ab	397ab	402ab	669a	649a	669a	662a	853ab	859	865a	859a	602bc	610ab	617ab	609bc	10.0a	9.4	10.5	10.0a
Alf + Fest	195a	204	209a	203a	329d	361b	358c	349c	619b	612b	621b	617c	854ab	849	850ab	851ab	640a	622ab	628a	630a	7.3c	9.3	10.3	9.0b
Alf + Rye	194a	202	210a	202a	332d	358b	356c	349c	617b	605b	632b	618c	853ab	846	856ab	852ab	636a	624a	632a	631a	8.0bc	9.6	11.1	9.5ab
Alf + Bro	161c	199	196ab	185c	439a	399a	412a	417a	654a	636ab	639ab	643b	842ab	851	845b	846b	585c	605ab	600bc	597c	9.9a	9.3	10.5	9.9a
SEM	5.3	5.5	6.8	5.0	8.8	11.2	10.6	6.9	9.8	8.7	8.4	6.4	5.4	4.8	4.3	2.8	6.5	6.2	6.1	3.5	0.44	0.31	0.37	0.29
ANOVA												*P*-values												
Year	–	–	–	***	–	–	–	ns	–	–	–	***	–	–	–	ns#	–	–	–	ns	–	–	–	***
Alfalfa stage	*	ns	ns	*	*	ns	ns	ns	ns	**	**	**	*	**	**	**	*	*	ns	**	ns	ns	ns	ns
Mixture	***	ns	***	***	***	**	***	***	***	***	***	***	*	ns	*	ns	***	*	***	***	***	ns	ns	**
Year×stage	–	–	–	***	–	–	–	***	–	–	–	*	–	–	–	ns	–	–	–	ns	–	–	–	***
Year×mixture	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	ns	–	–	–	**	–	–	–	***
Stage×mixture	ns	ns	**	ns	ns	ns	**	ns	**	ns	ns	*	**	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns
Year×stage×mixture	–	–	–	**	–	–	–	**	–	–	–	ns	–	–	–	ns	–	–	–	*	–	–	–	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† CP, crude protein; aNDF, neutral detergent fiber; NDFd, in vitro neutral detergent fiber digestibility; IVTD, in vitro true digestibility; TDN, total digestible nutrients. The CP, NDF, and TDN concentrations, as well as the NDFd and IVTD were adjusted for the weight of each harvest on the seasonal DM yield.

‡ Estimated milk production per hectare of forage calculated using the Excel spreadsheet Milk2013 (Undersander et al., 2013).

§ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

¶ Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

\# ns, nonsignificant.

optimal minimal value of 40% typically expected for alfalfa–grass mixtures (Drapeau et al., 2005). An optimal grass contribution to total biomass of alfalfa-based mixtures has been associated with a better persistence of all seeded species (Thomas, 1992), while reducing bloat risk, a potentially fatal condition that may occur when ruminants pasture a legume-rich diet (Mouriño et al., 2003; Burggraaf et al., 2008). Because all six grass species had poor DM yield contributions at these two sites, environmental factors may have contributed to giving an advantage to alfalfa over the grass in the mixtures. Indeed, there was a prolonged period with limited precipitation in June and July of the establishment year (2014) at Normandin and Saint-Augustin-de-Desmaures. Precipitation was about 12 mm over a period of 23 d at Normandin, and 22 mm over 31 d at Saint-Augustin-de-Desmaures, while the 30-yr monthly precipitation averages (June-July) at these two sites are 93 and 116 mm, respectively. These dry periods in the establishment year could have negatively affected the growth and survival of forage grasses, and potentially have given alfalfa, a relatively drought-tolerant species, a competitive advantage over the seeded grasses in the mixtures, even in subsequent years.

At all three sites and three production years, no difference in total DM yields was observed among alfalfa–timothy, alfalfa–tall fescue, alfalfa–meadow fescue, and alfalfa–meadow bromegrass mixtures (Tables 2–4). Some differences in total DM yields among these four mixtures were reported by Bélanger et al. (2018) in an experiment where mixtures were frequently harvested when timothy reached about 33 cm

Table 6. Nutritive attributes and estimated milk production per hectare for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Saint-Augustin-de-Desmaures (QC) along with the probabilities of fixed effects and their interactions.

	CP†				aNDF				NDFd				IVTD				TDN				Milk Production‡			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	$g\ kg^{-1}$ DM								$g\ kg^{-1}$ aNDF				$g\ kg^{-1}$ DM								$Mg\ ha^{-1}$			
Early bud	178	183	164	175	367b§	404b	433	401b	678a	682a	671a	677a	883a	864a	843	863a	629a	599a	587	605a	18.1	14.0	9.6b	13.9
Early flower	166	162	163	164	411a	435a	438	428a	602b	603b	604b	603b	834b	818b	814	822b	594b	576b	575	582b	20.4	18.5	16.0a	18.3
SEM	2.6	4.4	3.6	2.3	9.5	5.1	9.7	7.6	6.7	8.4	11.5	8.2	5.4	5.0	8.7	6.1	8.7	7.1	13.2	9.4	2.47	1.96	1.41	1.93
Mixture¶																								
Alf + Tim	179ab	176b	167b	174b	371bc	416b	427c	405c	627b	643ab	637b	636bc	855	839ab	827b	840bcd	623a	596ab	591b	603ab	19.8	17.2	12.8	16.6
Alf + TF	174ab	158c	155b	162c	392b	460a	467a	440a	638b	641ab	639b	639b	856	830b	821b	836d	606ab	557c	556d	573d	20.0	15.7	13.0	16.2
Alf + MF	169bc	164bc	150b	161c	395ab	430ab	461ab	429a	639ab	642ab	637b	639b	856	837ab	820b	838dc	609ab	588ab	565cd	587c	181	16.3	12.3	15.6
Alf + Fest	158c	168bc	156b	161c	419a	415b	441bc	425ab	664a	657a	645ab	655a	862	848a	831ab	847ab	597b	591ab	579bc	589c	18.3	15.5	12.8	15.5
Alf + Rye	168bc	174bc	166b	169bc	392b	419b	428c	413bc	646ab	643ab	656a	648ab	863	844a	843a	850a	610ab	584b	586b	594bc	19.6	15.8	12.6	16.0
Alf + Bro	185a	194a	188a	189a	363c	378c	389d	377d	627b	630b	612ab	623c	860	846a	831ab	846abc	625a	609a	608a	614a	19.7	17.1	13.3	16.7
SEM	3.4	4.9	4.6	2.7	10.7	7.6	10.7	8.2	7.8	8.8	10.0	7.9	6.3	5.5	8.3	6.2	9.6	8.4	13.1	9.7	2.29	1.90	1.27	1.78
ANOVA												*P*-values												
Year	–	–	–	***	–	–	–	***	–	–	–	ns#	–	–	–	***	–	–	–	***	–	–	–	***
Alfalfa stage	ns	ns	ns	ns	**	*	ns	*	**	**	*	**	**	**	ns	**	**	*	ns	**	ns	ns	*	ns
Mixture	***	***	***	***	***	***	***	***	**	**	***	***	ns	**	***	***	ns	***	***	***	ns	ns	ns	ns
Year×stage	–	–	–	***	–	–	–	***	–	–	–	ns	–	–	–	***	–	–	–	***	–	–	–	***
Year×mixture	–	–	–	*	–	–	–	***	–	–	–	ns	–	–	–	ns	–	–	–	**	–	–	–	ns
Stage×mixture	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
Year×stage×mixture	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† CP, crude protein; aNDF, neutral detergent fiber; NDFd, in vitro neutral detergent fiber digestibility; IVTD, in vitro true digestibility; TDN, total digestible nutrients. The CP, NDF, and TDN concentrations, as well as the NDFd and IVTD were adjusted for the weight of each harvest on the seasonal DM yield.

‡ Estimated milk production per hectare of forage calculated using the Excel spreadsheet Milk2013 (Undersander et al., 2013).

§ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

¶ Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

ns, nonsignificant.

in height to simulate a grazing scenario. The proportion of the seeded grasses in the last three production years of that five-year experiment averaged 55% whereas, in our experiment, grass DM yield contributions to the total DM yield averaged only 24% for the three production years (2015–2017). If the grass species had contributed to a greater proportion of total DM yields, differences in yields among these four mixtures might have been more pronounced.

Although one of the main concerns with timothy is its limited regrowth under warm and dry conditions after the first harvest (Jing et al., 2014; Piva et al., 2013), in the present experiment, tall fescue, meadow fescue, and meadow bromegrass had similar regrowth patterns to timothy over the season at the three sites and the two alfalfa developmental stages at harvest (supplemental Fig. S4-S6). Festulolium and perennial ryegrass also had similar regrowth patterns to other grasses at Saint-Augustin-de-Desmaures. The regrowth of these two grasses was difficult to evaluate at Normandin and Sainte-Anne-de-Bellevue due to their relatively poor DM yields at the first harvest (supplemental Fig. S4 and S6). Therefore, despite the poor regrowth potential and drought susceptibility of timothy (Cosgrove, 2009), its regrowth does not seem to differ significantly from that of tall fescue, meadow fescue, and meadow bromegrass when grown in mixture with alfalfa under the current climatic conditions prevailing in eastern Canada.

Differences in DM yields of non-seeded species (i.e., weeds) were observed among the six alfalfa–grass mixtures at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Tables 3 and 4), but these differences were not consistent from one production year to the other, and between the two sites. More importantly, at the three sites, the DM yield contribution of non-seeded species ranged only between 2 and 6% of the total DM yield of mixtures, with an average of 3%. Our result are consistent with other experiments reporting that mixing legumes with one or many grasses reduces weed invasion compared to monocultures (e.g., Sanderson et al., 2012; Sturludóttir et al., 2013; Bélanger et al., 2014).

Nutritive Attributes

Alfalfa–grass mixtures differed for most nutritive attributes at all sites and in most years (Tables 5–7). Averaged across the three production years, the TDN and CP concentrations of the alfalfa–tall fescue mixture were less and the aNDF concentration was greater than that of the alfalfa–timothy mixture at the three sites, while there was no or little difference in NDFd and IVTD. Similar results were reported by Bélanger et al. (2018) from a study of 18 legume–grass binary mixtures under grazing or frequent cutting in eastern Canada. Previous field studies in eastern Canada (Pelletier et al., 2010) and Finland (Virkajärvi et al., 2012a) reported that the NDF concentration of tall fescue tended to be lower than that of timothy when grown in pure stands, yet our study demonstrated that the alfalfa-tall fescue mixture generally had a greater aNDF concentration than the alfalfa–timothy mixture. The alfalfa–tall fescue mixture had a greater proportion of grasses than the alfalfa–timothy mixture at the three sites (16 vs. 11% at Normandin; 49 vs. 39% at Sainte-Anne-de-Bellevue; 29 vs. 13% at Normandin). Because grasses have greater NDF concentrations than

Table 7. Nutritive attributes and estimated milk production per hectare for the main effects of six alfalfa-grass binary mixtures and two alfalfa developmental stages for each production year and averaged across the first three production years at Sainte-Anne-de-Bellevue (QC) along with the probabilities of fixed effects and their interactions.

	CP†				aNDF				NDFd				IVTD				TDN				Milk Production‡			
	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.	2015	2016	2017	Avg.
Alfalfa stage	g kg⁻¹ DM								g kg⁻¹ aNDF				g kg⁻¹ DM								Mg ha⁻¹			
Early bud	137a§	184a	160a	160a	437b	328b	406b	390b	755a	719a	722a	732a	889a	905a	888a	894a	617a	668a	621a	635a	11.0	6.3	9.5b	8.9
Early flower	115b	159b	146b	140b	498a	411a	465a	458a	697b	624b	603b	641b	842b	833b	812b	829b	573b	603b	561b	579b	13.8	10.7	15.9a	13.5
SEM	5.7	3.4	2.8	3.6	5.9	7.4	4.2	4.8	10.4	12.2	6.2	9.0	3.9	6.7	3.1	4.2	2.9	5.8	3.0	2.8	1.57	1.07	1.00	1.16
Mixture¶																								
Alf + Tim	135a	177abc	152bc	155abc	454b	367b	440b	420c	710cd	669	673	684ab	857c	866b	852ab	858b	608ab	641b	598bc	616b	14.1a	9.5a	14.1a	12.6a
Alf + TF	123bc	138d	135d	132d	484a	454a	489a	476a	729bc	681	668	693a	856c	846c	837b	847c	575c	579d	555d	570d	13.3a	8.7ab	13.6a	11.9a
Alf + MF	129ab	165c	149c	148c	457b	387b	442b	429c	709cd	680	669	686a	862c	872ab	850ab	861b	595b	630bc	590c	605b	13.0a	9.7a	12.5ab	11.7a
Alf + Fest	116c	186ab	170a	157ab	467ab	303c	382c	384d	746ab	676	655	692a	877b	889a	859a	875a	598ab	673a	623a	631a	10.4b	7.0b	11.2b	9.5b
Alf + Rye	123bc	191a	164ab	159a	452b	298c	389c	380d	759a	668	655	694a	890a	887a	860a	879a	612a	675a	616ab	634a	10.1b	6.5b	11.6b	9.4b
Alf + Bro	132ab	171bc	148c	150bc	487a	410b	470ab	456b	701d	655	656	671b	851c	855bc	840b	849c	580c	614c	566d	587c	13.5a	9.5a	13.3a	12.1a
SEM	6.1	4.4	3.6	3.9	7.1	10.3	7.2	5.3	10.7	12.3	7.5	9.2	4.4	7.3	4.2	4.5	3.8	6.7	5.2	3.3	1.52	1.01	1.04	0.11
ANOVA	*P*-values																							
Year	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	***
Alfalfa stage	**	*	*	**	**	*	**	**	*	**	**	**	**	**	**	**	**	*	**	**	ns#	ns	*	*
Mixture	***	***	***	***	***	***	***	***	***	ns	ns	***	***	***	**	***	***	***	***	***	***	***	**	***
Year×stage	–	–	–	**	–	–	–	*	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	***
Year×mixture	–	–	–	***	–	–	–	***	–	–	–	***	–	–	–	*	–	–	–	***	–	–	–	ns
Stage×mixture	ns	ns	**	***	***	ns	**	***	*	ns	ns	*	**	ns	ns	*	**	ns	*	***	ns	ns	ns	ns
Year×stage×mixture	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns	–	–	–	ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† CP, crude protein; aNDF, neutral detergent fiber; NDFd, in vitro neutral detergent fiber digestibility; IVTD, in vitro true digestibility; TDN, total digestible nutrients. The CP, NDF, and TDN concentrations, as well as the NDFd and IVTD were adjusted for the weight of each harvest on the seasonal DM yield.

‡ Estimated milk production per hectare of forage calculated using the Excel spreadsheet Milk2013 (Undersander et al., 2013).

§ Within columns and for a given main treatment effect (Alfalfa stage and Mixture), means followed by the same letter are not significantly different according to LSD (0.05).

¶ Alf, alfalfa; Tim, timothy; TF, tall fescue; MF, meadow fescue; Fest, festulolium; Rye, perennial ryegrass; Bro, meadow bromegrass.

ns, nonsignificant.

legumes (Ball et al., 2001), the greater aNDF concentrations observed with the alfalfa-tall fescue mixture could to a large extent be explained by its greater grass proportion relative to the alfalfa-timothy mixture, rather than by the grass species themselves. When considering the nutritive value of alfalfa–grass mixtures at a given alfalfa developmental stage, the grass stage of development should be taken into account. Tall fescue was not more advanced in phenological development than the other grass species when mixtures were harvested at either of the two alfalfa developmental stages and at the three sites (data not presented). Therefore, the lower nutritive value of the alfalfa–tall fescue observed in our experiment cannot be explained by a difference in the relative stages of development of tall fescue and timothy, and differences among mixtures was most likely related to the grass contribution to the total forage DM yield.

The alfalfa–festulolium and alfalfa–perennial ryegrass mixtures tended to have a greater TDN concentration and a lower aNDF concentration than that of the alfalfa–timothy mixture at Normandin and Sainte-Anne-de-Bellevue, while there was no or little difference in NDFd and IVTD, and CP concentration for values averaged across the three production years. Because grasses typically have a greater fiber concentration and a lower TDN concentration than legumes (Ball et al., 2001), differences in aNDF and TDN concentration can be partly explained by the lower proportion of the grasses in the alfalfa–festulolium and alfalfa–perennial ryegrass mixtures than in the alfalfa–timothy mixture (0 and 0 vs. 11% at Normandin; 33 and 34 vs. 39% at Sainte-Anne-de-Bellevue).

Averaged across the three production years, the alfalfa-–meadow bromegrass mixture had a greater aNDF concentration than the alfalfa–timothy mixture at the three sites, and a lower TDN concentration at Normandin and Sainte-Anne-de-Bellevue with no or little difference in CP concentration, and IVTD and NDFd. Bélanger et al. (2018) also observed a greater aNDF concentration and a lower TDN concentration of the alfalfa-meadow bromegrass mixture compared to the alfalfa–timothy mixture. The proportion of grasses in the alfalfa–meadow bromegrass mixture did not differ much from that in the alfalfa–timothy mixture (14 vs. 11% at Normandin; 41 vs. 39% at Sainte-Anne-de-Bellevue; 13 vs. 9% at Saint-Augustin-de-Desmaures). It appears, therefore, that meadow bromegrass might have had a lower TDN concentration and a greater aNDF concentration than timothy.

The estimated milk production per hectare was generally less with the alfalfa–festulolium and alfalfa–perennial ryegrass mixtures than with the alfalfa–timothy mixture at Normandin and Sainte-Anne-de-Bellevue (Tables 5–7). The lower total DM yields of the alfalfa–festulolium and alfalfa–perennial ryegrass mixtures had more impact on the estimated milk production per hectare than their TDN concentration. The mixtures with tall fescue, meadow bromegrass, or meadow fescue had similar estimated milk production per hectare to the alfalfa–timothy mixture.

Alfalfa Developmental Stages at Harvest

At the three sites and three production years, the total seasonal forage and alfalfa DM yields of the six alfalfa-grass mixtures were similar or superior when mixtures were harvested at the early flower stage of alfalfa than at the early bud stage (Tables 2–4), and this, even if an additional cut was taken when mixtures were harvested at the early bud stage (Normandin and Sainte-Anne-de-Bellevue: 2015–2017; Saint-Augustin-de-Desmaures: 2015 and 2016). The grass DM yields, however, did not differ between the two stages of development of alfalfa at harvest (Tables 2–4). Grass DM accumulation was, therefore, negligible between the early bud and early flower stages of alfalfa.

Alfalfa–grass mixtures harvested at the early flower stage of alfalfa had an improved persistence over mixtures harvested at the early bud stage (Tables 2–4). Total DM yields between the first and third production years decreased less or increased more with harvests at the early flower stage than at the early bud stage of alfalfa at Saint-Augustin-de-Desmaures (–19% vs. −43%) and Sainte-Anne-de-Bellevue (+19% vs. –14%), with little difference at Normandin (+17% vs. +16%). Similarly, the alfalfa DM yields between the first and third production years increased more or decreased less with harvests at the early flower stage than at the early bud stage of alfalfa at Saint-Augustin-de-Desmaures (–21% vs. −51%), Sainte-Anne-de-Bellevue (+89% vs. +8%), and Normandin (+33% vs. 0%). Consequently, the difference in DM yields between mixtures harvested at the early bud and early flower stages of alfalfa generally became more pronounced in the third production year. The persistence of alfalfa–grass mixtures is, therefore, greater with harvests at the early flower stage than at the early bud stage of alfalfa. Waiting for alfalfa to flower allows carbohydrate reserves to replenish and reduces the occurrence of winterkill (Vignau-Loustau and Huyghe, 2008).

For the three production years and at the three sites, alfalfa–grass mixtures harvested at the early flower stage of alfalfa generally had a greater aNDF concentration, and lower CP and TDN concentrations, as well as lower NDFd and IVTD than mixtures harvested at the early bud stage of alfalfa (Tables 5–7). Although the year × stage interaction was sometimes significant, similar trends were observed in the three production years. Averaged across the three production years, the aNDF concentration in the alfalfa-grass mixtures was greater (+6 to +15%), and the CP (−7 to –13%) and TDN (−4 to −9%) concentrations along with NDFd (−10 to –12%) and IVTD (−5 to −7%) were lower with harvests taken at the early flower stage compared with the early bud stage of alfalfa. Decreases in nutritive value with advancing stages of development are well known for pure stands of alfalfa and timothy (Bélanger et al., 2001; Yu et al., 2003). In alfalfa–grass mixtures, however, this decrease in nutritive value could be affected or mitigated by the relative contribution of alfalfa and the grass species to DM yield and how this relative contribution changes with advancing stages of development. In our experiment, the relative contribution of alfalfa to total DM was greater when mixtures were harvested at the early flower stage than at the early bud stage of alfalfa (88% vs. 82% at Normandin; 80% vs. 69% at Saint-Augustin-de-Desmaures; 61% vs. 46% at Sainte-Anne-de-Bellevue; Tables 2–4). Because alfalfa has a lower fiber concentration, and greater CP and TDN concentrations, and IVTD than grasses (Yu et al., 2003), the greater presence of alfalfa when mixtures were harvested at the early flower stage of alfalfa mitigated the benefits of harvesting alfalfa-grass mixtures at an earlier stage of development in terms of nutritive value. It is known from previous experiments that the net energy available for lactation (NE_L) and the TDN concentration in a forage sample is negatively correlated to plant maturity (Yu et al., 2003) and to grass DM yield contributions in the mixtures (Yu et al., 2003; Johansen et al., 2018).

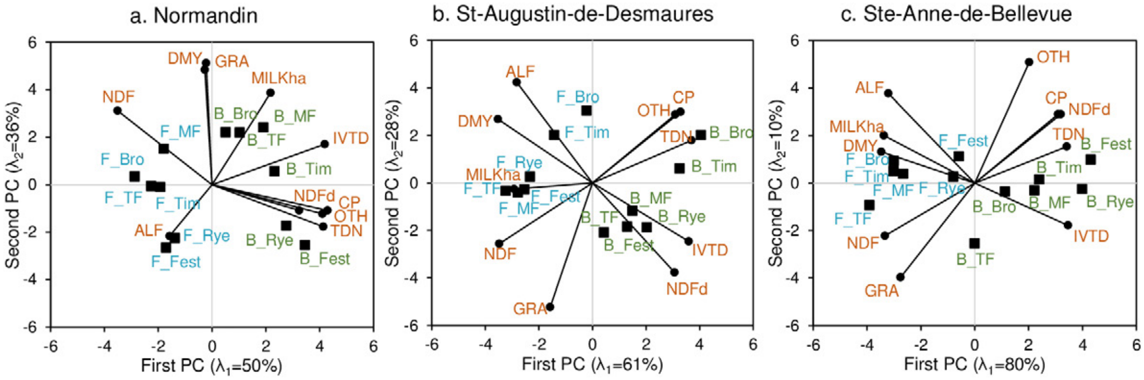

Fig. 1. Diagram of the first two principal component (PC) scores calculated for each of the twelve combinations of six alfalfa and grass binary mixtures (■) harvested at two alfalfa developmental stages, and for each of the nine attributes (●), on average for three production years, and at three study sites in eastern Canada. The contribution of each PC score to the total covariation (λ) appears in parenthesis on each axis identification. DMY, seasonal dry matter yield; ALF, alfalfa DMY; GRA, grass DMY; OTH, DMY of non-seeded species; CP, crude protein concentration; aNDF, neutral detergent fiber concentration; NDFd; in vitro NDF digestibility; IVTD, in vitro true digestibility of DM; TDN; total digestible nutrient concentration; MILKha; estimated milk production per hectare of forage; B_, alfalfa early bud stage; F_, alfalfa early flower stage; Tim; alfalfa-timothy mixture; TF, alfalfa-tall fescue mixture; MF, alfalfa-meadow fescue mixture; Fes; alfalfa-festulolium mixture; RG, alfalfa-perennial ryegrass mixture; Bro, alfalfa-meadow bromegrass mixture.

Alfalfa–grass mixtures harvested at the early flower stage of alfalfa generally were associated with a similar or greater estimated milk production per hectare than those harvested at the early bud stage, in the three production years and at the three sites (Tables 5–7). On average across the three production years, harvesting mixtures at the early flower stage of alfalfa increased the estimated milk production per hectare by 32% at Saint-Augustin-de-Desmaures and 51% at Sainte-Anne-de-Bellevue relative to mixtures harvested at the early bud stage of alfalfa; the total DM yields of mixtures harvested at the early flower stage were also numerically greater by 38% at Saint-Augustin-de-Desmaures and 65% at Sainte-Anne-de-Bellevue (Tables 3 and 4). At Normandin, the estimated milk production per hectare was comparable for the two alfalfa developmental stages at harvest (Table 5), along with their DM yields (Table 2). Therefore, the greater estimated milk production per hectare of forage mixtures harvested at the early flower stage of alfalfa seems to be related primarily to greater total DM yields.

Relationship Among Nutritive Attributes and Dry Matter Yield

Three PCAs, one for each site, were performed to characterize the relationship among seasonal values of DM yield and selected nutritive attributes averaged across the three production years (Fig. 1). The first two principal components (PC) accounted for 86% ($\lambda_1 + \lambda_2$) of the total variation at Normandin, 89% at Saint-Augustin-de-Desmaures, and 90% at Sainte-Anne-de-Bellevue. For each component, variables on the same side of the axis were positively correlated, and variables on opposite sides were negatively correlated. At the three sites, the first component was positively correlated to the CP and TDN concentrations, IVTD, and NDFd of the mixtures, and the DM yield of non-seeded species. On the negative side, the first component was mostly defined by the total, alfalfa, and grass DM yields, and by the aNDF concentration (Fig. 1). This first component was also defined on the negative side by the estimated milk production per hectare at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 1b and 1c). The first component at the three sites was primarily driven by the two alfalfa developmental stages at harvest (Fig. 1). Our results suggest that forage DM yield and nutritive attributes and that their relationship is more affected by the stage of development at harvest than by the alfalfa–grass mixture.

Harvesting alfalfa–grass mixtures at the early flower stage of alfalfa, as compared with the early bud stage, resulted in greater total, alfalfa, and grass DM yields, and estimated milk production per hectare at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 1b and 1c; Tables 3 and 4), while, at Normandin, harvesting at the early flower stage had negligible effects on total and grass DM yields (Fig. 1a; Table 2), and resulted in lower estimated milk production per hectare (Fig. 1a), although not significantly (Table 5). At all sites, harvesting the mixtures at the alfalfa early flower stage also resulted in lower forage nutritive value with higher aNDF concentration, and lower CP and TDN concentrations, IVTD, and NDFd than harvesting at the early bud stage of alfalfa (Fig. 1; Tables 5–7). The first component of the PCA, therefore, confirms that harvesting alfalfa–grass mixtures at the early flower stage of alfalfa results in similar or greater DM yields and estimated milk production per hectare, despite a lower nutritive value, than harvesting at the early bud stage of alfalfa (Yu et al., 2003).

The second component of the PCAs was primarily driven by the six alfalfa–grass mixtures, but with different patterns at the three sites (Fig. 1). At Normandin, mixtures with festulolium and perennial ryegrass were associated with low grass DM yield, and opposed to the mixtures with other grasses, which had high grass DM yields (Fig. 1a). At Saint-Augustin-de-Desmaures, mixtures with meadow bromegrass and timothy were associated with low grass DM yields, and opposed to the mixtures with most other grasses, which had high grass DM yields (Fig. 1b). At Sainte-Anne-de-Bellevue, the mixture with tall fescue was associated with a high grass DM yield, and opposed the mixture with festulolium (Fig. 1c). This second component confirms the generally poor performance of festulolium and perennial ryegrass at Normandin and Sainte-Anne-de-Bellevue (Fig. 1a and 1c; Tables 2 and 4), and a performance of the mixtures with tall fescue, meadow fescue, and meadow bromegrass similar to that of the alfalfa–timothy mixture.

In the first component of the PCAs at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 1b and 1c), and in the second component of the PCA at Normandin (Fig. 1a), the total DM yield was positively correlated with the grass DM yield, aNDF concentration, and estimated milk production per hectare, while being negatively correlated with the TDN and CP concentrations, the NDFd, and the DM yield from non-seeded species (Fig. 1). These correlations confirm that the estimated milk production per hectare associated with our twelve treatments was mostly driven by the yield of alfalfa–grass mixtures rather than their nutritive value. Our results (Fig. 1) also confirm those of other studies (e.g., Yu et al., 2003) showing that, regardless of the grass species in the alfalfa-grass mixtures, greater grass DM yields are associated with lower CP and TDN concentrations, but a greater aNDF concentration.

As expected, grass DM yields and the alfalfa DM yields were opposed on the second component, a component mostly driven by the alfalfa–grass mixtures (Fig. 1). Mixtures with low grass DM yields (e.g., festulolium and perennial ryegrass at Normandin; meadow bromegrass at Saint-Augustin-de-Desmaures) tended to have greater alfalfa DM yields than those with high grass DM yields because of compensation between alfalfa and the seeded grass. The second component also indicates that total DM yield was positively correlated to the alfalfa DM yield at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue (Fig. 1b and 1c), but negatively correlated at Normandin (Fig. 1a). These correlations demonstrate that greater total DM yields of the alfalfa–grass mixtures were due to greater alfalfa yields at Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue. At Normandin, however, all mixtures had similar alfalfa DM yields, yet the alfalfa–festulolium and alfalfa–perennial ryegrass had lower total and grass DM yields (Table 2), resulting in the alfalfa DM yield being negatively correlated to the total and grass DM yields at this site (Fig. 1a).

CONCLUSIONS

The alfalfa–meadow fescue, alfalfa–meadow bromegrass, and alfalfa–tall fescue mixtures generally performed as well as the alfalfa–timothy mixture, and they represent valuable alternatives. The alfalfa–tall fescue mixture generally had a lower nutritive value than the alfalfa–timothy mixture, but it was compensated by a generally greater total DM yield. Timothy, tall fescue, meadow fescue, and meadow bromegrass remained productive over the first three production years when cultivated in mixture with alfalfa. The alfalfa–grass mixtures with the 'Spring Green' festulolium or the 'Remington' perennial ryegrass had lower total DM yields than the alfalfa-timothy mixture, due most likely to poor winter survival of the two grass species. The cultivars used for these two grass species, therefore, do not seem to be viable alternatives to timothy in mixture with alfalfa grown in eastern Canada. Harvesting alfalfa–grass mixtures at the early flower stage of alfalfa rather than the early bud stage maximizes the persistence of the mixtures, their DM yields, and their estimated milk production per hectare.

SUPPLEMENTAL MATERIAL

Three tables and six figures are provided as supplemental material. The three tables provide monthly precipitations from 2014 to 2017, and the 30-year average (1971–2000), at Normandin, Saint-Augustin-de-Desmaures and Sainte-Anne-de-Bellevue, QC, Canada. The first three figures illustrate the average daily air temperature and snow cover on the ground from September to June in 2014–2015, 2015–2016, and 2016–2017 at the three sites. The last three figures represent the alfalfa and grass dry matter yields of the six alfalfa-grass binary mixtures at each harvest made at two alfalfa developmental stages, for three production years and three sites.

ACKNOWLEDGMENTS

This study was funded by the concerted action FRQNT-MAPAQ-NOVALAIT as part of the "Programme projet de recherche en partenariat orienté: Innovation en production et en transformation laitières–VI (3^{e} concours) (2014–2018)". P. Seguin research program is funded by the Natural Sciences and Engineering Research Council of Canada through a Discovery Grant. Florence Pomerleau-Lacasse was recipient of scholarships from the FRQNT and NSERC (2014–2018). The authors express their gratitude to the research assistants from AAFC and McGill University including: Andrée-Dominique Baillargeon, Geneviève Bégin, Camille Lambert-Beaudet, Jean-Noël Bouchard, Lucie Lévesque, Danielle Mongrain, and Shimin Fan.

REFERENCES

AOCS. 2003. Rapid determination of oil/fat utilizing high temperature solvent extraction. Official methods and recommended practices of the AOCS (5th ed. 2nd printing). In: D. Firestone, editor, Method AM 5-04. AOCS, Champaign, IL.

AOAC. 1990. Method 973.18: Determination of acid detergent fiber by refluxing. In: Official method of analysis. 15th ed. AOAC International, Gaithersburg, MC.

ANKOM Technology. 2017a. Method 12: Acid detergent fiber in feeds-Filter bag technique (for A2000 and A2000I). ANKOM Technology, Macedon, NY. https://www.ankom.com/sites/default/files/document-files/Method_13_NDF_A2000.pdf (accessed 13 July 2018).

ANKOM Technology. 2017b. Method 13: Neutral detergent fiber in feeds- Filter bag technique (for A2000 and A2000I). ANKOM Technology, Macedon, NY. https://www.ankom.com/sites/default/files/document-files/Method_13_NDF_A2000.pdf (accessed 13 July 2018).

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

Barrow, E., B. Maxwell, and P. Gachon. 2004. Climate variability and change in Canada: Past, present and future. ACSD Science Assessment Series No. 2, Meteorological Service of Canada, Environment Canada, Ontario, Toronto.

Bélanger, G., Y. Castonguay, A. Bertrand, C. Dhont, P. Rochette, L. Couture, R. Drapeau, D. Mongrain, F.-P. Chalifour, and R. Michaud. 2006. Winter damage to perennial forage crops in eastern Canada: Causes, mitigation, and prediction. Can. J. Plant Sci. 86:33–47. doi:10.4141/P04-171

Bélanger, G., Y. Castonguay, and J. Lajeunesse. 2014. Benefits of mixing timothy with alfalfa for forage yield, nutritive value, and weed suppression in northern environments. Can. J. Plant Sci. 94:51–60. doi:10.4141/cjps2013-228

Bélanger, G., R. Michaud, P.G. Jefferson, G.F. Tremblay, and A. Brégard. 2001. Improving the nutritive value of timothy through management and breeding. Can. J. Plant Sci. 81:577–585. doi:10.4141/P00-143

Bélanger, G., G.F. Tremblay, Y.A. Papadopoulos, J. Duynisveld, J. Lajeunesse, C. Lafrenière, and S.A.E. Fillmore. 2018. Yield and nutritive value of binary legume-grass mixtures under grazing of frequent cutting. Can. J. Plant Sci. 98:395–407.

Berdahl, J.D., J.F. Karn, and J.R. Hendrickson. 2001. Dry matter yields of cool-season grass monocultures and grass–alfalfa binary mixtures. Agron. J. 93:463–467. doi:10.2134/agronj2001.932463x

Bertrand, A., G.F. Tremblay, S. Pelletier, Y. Castonguay, and G. Bélanger. 2008. Yield and nutritive value of timothy as affected by temperature, photoperiod and time of harvest. Grass Forage Sci. 63:421–432. doi:10.1111/j.1365-2494.2008.00649.x

Burggraaf, V., G. Waghorn, S. Woodward, and E. Thom. 2008. Effects of condensed tannins in white clover flowers on their digestion in vitro. Anim. Feed Sci. Technol. 142:44–58. doi:10.1016/j.anifeedsci.2007.07.001

Canadian Food Inspection Agency (CFIA). 2012. The biology of *Medicago sativa* L. (Alfalfa). Canadian Food Inspection Agency, Ottawa, ON. http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive 94-08/biology-documents/medicago-sativa-l-/eng/1330981151254/1330981232360#b1 (accessed 13 July 2018).

Casler, M.D., P.G. Pitts, C. Rose-Fricker, P.C. Bilkey, and J.K. Wipff. 2001. Registration of 'Spring Green' festulolium. Crop Sci. 41:1365–1366. doi:10.2135/cropsci2001.4141365x

Centre de Référence en Agriculture en Agroalimentaire du Québec (CRAAQ). 2010. Guide de référence en fertilisation, 2[e] édition. CRAAQ, QC, Canada.

Centre de Référence en Agriculture en Agroalimentaire du Québec (CRAAQ). 2013. Recommandations de plantes fourragères 2013-2014. Le Producteur de Lait Québécois 33(9):17–21.

Cosgrove, D. 2009. Species selection for pastures. In: Proceedings of the 2009 Wisconsin Crop Management Conference, Madison, WI. 13–15 Jan. 2009. University of Wisconsin-Extension, Madison, WI. p. 21–24.

Drapeau, R., G. Bélanger, G. Tremblay, and L. Couture. 2005. Le semis des plantes fourragères. In: G. Bélanger, L. Couture, and G. Tremblay, editors, Les plantes fourragères. Centre de Reference en Agriculture et Agroalimentaire du Québec, Québec, QC. p. 62–73.

DLF International Seeds. 2013. Festulolium hybrid grass. DLF International Seeds, Halsey, OR. http://millbafs.com/wp-content/uploads/2017/08/Festulolium-white-paper-Final.pdf (accessed 13 July 2018).

Fick, G.W., and S.C. Muller. 1989. Alfalfa: Quality, maturity, and mean stage of development. Department of Agronomy, College of Agriculture and Life Sciences, Cornell University. Cornell Cooperative Extension, Ithaca, NY.

Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analysis (Apparatus, reagents, procedures and some applications). USDA Agriculture Handbook 379. US. Gov. Print Office, Washington, D.C.

Goslee, S.C., J.M. Gonet, and R.H. Skinner. 2017. Freeze tolerance of perennial ryegrass and implications for future species distribution. Crop Sci. 57:2875–2880. doi:10.2135/cropsci2017.02.0135

Government of Canada. 2016. Climate change and variation bulletin. Catalogue No. En81-23E-PDF. Environment and Climate Change Canada, Ottawa, ON.

Government of Canada. 2017. Climate change and variation bulletin. Catalogue No. En81-23E-PDF, Environment and Climate Change Canada, Ottawa, ON.

Government of Canada. 2018a. Canadian climate normals or averages 1971 to 2000. Government of Canada, Ottawa, ON. http://climate.weather.gc.ca/climate_normals/index_e.html (accessed 13 July 2018).

Government of Canada. 2018b. Past weather and climate: Historical data. Government of Canada, Ottawa, ON. http://climate.weather.gc.ca/historical_data/search_historic_data_e.html (accessed 13 July 2018).

Isaac, R.A., and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue, using a block digestor. J. Assoc. Off. Anal. Chem. 59:98–100.

Jing, Q., G. Bélanger, B. Qian, and V. Baron. 2014. Timothy yield and nutritive value with a three-harvest system under the projected future climate in Canada. Can. J. Plant Sci. 94:213–222. doi:10.4141/cjps2013-279

Jung, G.A., J.A. Shaffer, and J.R. Everhart. 1996. Harvest frequency and cultivar influence on yield and protein of alfalfa-ryegrass mixtures. Agron. J. 88:817–822. doi:10.2134/agronj1996.00021962008800050022x

Johansen, M., P. Lund, and M.R. Weisbjerg. 2018. Feed intake and milk production in dairy cows fed different grass and legume species: A meta-analysis. Animal 12:66–75. doi:10.1017/S1751731117001215

Kunelius, H.T., G.H. Dürr, K.B. McRae, and S.A.E. Fillmore. 2006. Performance of timothy-based grass/legume mixtures in cold winter region. J. Agron. Crop Sci. 192:159–167. doi:10.1111/j.1439-037X.2006.00195.x

Lachat Instruments. 2011. Methods list for automated ion analyzers (flow injection analyses, ion chromatography). Lachat Instruments, Loveland, CO.

Leco Corporation. 2009. Moisture and ash determination in flour. Organic application note. Leco Corporation, St. Joseph, MI.

Licitra, G., T.M. Hernandez, and P.J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347–358. doi:10.1016/0377-8401(95)00837-3

Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217–1240.

Moore, K.J., L.E. Moser, K.P. Vogel, S.S. Waller, B.E. Johnson, and J.F. Pedersen. 1991. Describing and quantifying growth stages of perennial forage grasses. Agron. J. 83:1073–1077. doi:10.2134/agronj1991.00021962008300060027x

Mouriño, F., K.A. Albrecht, D.M. Schaefer, and P. Berzaghi. 2003. Steer performance on kura clover–grass and red clover–grass mixed pastures. Agron. J. 95:652–659. doi:10.2134/agronj2003.0652

Nie, Z., G.F. Tremblay, G. Belanger, R. Berthiaume, Y. Castonguay, A. Bertrand, and J. Han. 2009. Near-infrared reflectance spectroscopy prediction of neutral detergent-soluble carbohydrates in timothy and alfalfa. J. Dairy Sci. 92:1702–1711. doi:10.3168/jds.2008-1599

Ogle, D.G., L. St. John, L.K. Holzworth, and K.B. Jensen. 2006. Plant guide for meadow brome (*Bromus biebersteinii*). USDA-Natural Resources Conservation Service, Idaho State Office, Boise, ID.

Ogle, D.G., S. Engle, and G. Shewmmaker. 2008. Plant guide for perennial ryegrass (*Lolium perenne*). USDA-Natural Resources Conservation Service, Idaho State Office, Boise, ID.

Ontario Forage Crop Committee. 2013. Ontario forage crop variety performance. Ontario Forage Crop Committee, University of Guelph, Guelph, ON. https://www.plant.uoguelph.ca/sites/www.plant.uoguelph.ca/files/forages/documents/OFCC%20Brochure%202013.pdf (accessed 13 July 2018).

Opitz von Boberfeld, W., and K. Banzhaf. 2006. Yield and forage quality of different ×Festulolium cultivars in winter. J. Agron. Crop Sci. 192:239–247. doi:10.1111/j.1439-037X.2006.00214.x

Pelletier, S., G.F. Tremblay, G. Bélanger, A. Bertrand, Y. Castonguay, D. Pageau, and R. Drapeau. 2010. Forage nonstructural carbohydrates and nutritive value as affected by time of cutting and species. Agron. J. 102:1388–1398. doi:10.2134/agronj2010.0158

Piva, A., A. Bertrand, G. Bélanger, Y. Castonguay, and P. Seguin. 2013. Growth and physiological response of timothy to elevated carbon dioxide and temperature under contrasted nitrogen fertilization. Crop Sci. 53:704–715. doi:10.2135/cropsci2012.07.0436

Pomerleau-Lacasse, F., P. Seguin, G.F. Tremblay, and D. Mongrain. 2017. Developmental stages of timothy and alfalfa (AAFC No. 12606E). Catalogue No. A72-135/2017E-PDF. Agriculture and Agri-Food Canada, Ottawa, ON. p. 1–23.

Qian, B., R. de Jong, S. Gameda, T. Huffman, D. Neilsen, R. Desjardins, H. Wang, and B. McConkey. 2013. Impact of climate change scenarios on Canadian agroclimatic indices. Can. J. Soil Sci. 93:243–259.

Sakai, A., and W. Larcher. 1987. Frost survival of plants: Responses and adaptation to freezing stress. New York, Springer-Verlag, Berlin, NY. doi:10.1007/978-3-642-71745-1

Sanderson, M.A., G. Brink, L. Ruth, and R. Stout. 2012. Grass–legume mixtures suppress weeds during establishment better than monocultures. Agron. J. 104:36–42. doi:10.2134/agronj2011.0130

SAS Institute Inc. 2013. SAS/STAT 13.1 User's Guide. SAS Institute Inc., Cary, NC.

Simili da Silva, M., C.C. Jobim, G.F. Tremblay, G. Bélanger, J. Lajeunesse, Y.A. Papadopoulos, and S.A.E. Fillmore. 2014. Forage energy to protein ratio of several legume-grass complex mixtures. Anim. Feed Sci. Technol. 188:17–27. doi:10.1016/j.anifeedsci.2013.11.006

Statistics Canada. 2017. Census of Agriculture, hay and field crops. Statistics Canada, Ottawa, ON. http://www5.statcan.gc.ca/cansim/pick-choisir?lang=eng&p2=33&id=0040213 (accessed 13 July 2018).

Sturludóttir, E., C. Brophy, G. Bélanger, A.M. Gustavsson, M. Jørgensen, T. Lunnan, and Á. Helgadóttir. 2013. Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. Grass Forage Sci. 69:229–240. doi:10.1111/gfs.12037

Thivierge, M.-N., G. Jégo, G. Bélanger, A. Bertrand, G.F. Tremblay, C.A. Rotz, and B. Qian. 2016. Predicted yield and nutritive value of an alfalfa-timothy mixture under climate change and elevated atmospheric carbon dioxide. Agron. J. 108:585–603. doi:10.2134/agronj2015.0484

Thomas, R.J. 1992. The role of the legume in the nitrogen cycle of productive and sustainable pastures. Grass Forage Sci. 47:133–142. doi:10.1111/j.1365-2494.1992.tb02256.x

Undersander, D., D. Combs, R. Shaver, and P. Hoffman. 2013. University of Wisconsin alfalfa/grass evaluation system- Milk2013. Forage Research and Extension, University of Wisconsin, Madison, WI.

Vignau-Loustau, L., and C. Huyghe. 2008. Strateìgies fourrageÌres. France Agricole Editions, Paris.

Vincent, L.A., and E. Mekis. 2006. Changes in daily and extreme temperature and precipitation indices for Canada over the twentieth century. Atmos.-ocean 44:177–193. doi:10.3137/ao.440205

Virkajärvi, P., M. Hyrkäs, K. Pakarinen, and M. Rinne. 2012a. Timotein ja ruokonadan erot sadontuottoprosessissa. [*In Finnish*]. In: Nurmen kasvu- ja kehitysprosessit: NURFYS-hankkeen 2006–2011 loppuraportti, MTT Report 56. Natural Resources Institute Finland, Helsinki, Finland. p. 22–46.

Virkajärvi, P., K. Pakarinen, M. Hyrkäs, M. Seppänen, and G. Bélanger. 2012b. Tiller characteristics of timothy and tall fescue in relation to herbage mass accumulation. Crop Sci. 52:970–980. doi:10.2135/cropsci2011.01.0039

Yu, P., D. Christensen, J. McKinnon, and J. Markert. 2003. Effect of variety and maturity stage on chemical composition, carbohydrate and protein subfractions, in vitro rumen degradability and energy values of timothy and alfalfa. Can. J. Anim. Sci. 83:279–290. doi:10.4141/A02-053