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ARTICLE

Evaluation of sainfoin (*Onobrychis viciifolia*) for forage yield and persistence in sainfoin–alfalfa (*Medicago sativa*) mixtures and under different harvest frequencies

Bill Biligetu, Paul G. Jefferson, Herbert A. Lardner, and Surya N. Acharya

Abstract: Condensed tannins in sainfoin (*Onobrychis viciifolia*) improve forage digestion and reduce the risk of ruminant bloat caused by grazing alfalfa (*Medicago sativa*). The objectives of this study were to evaluate the forage dry matter yield (DMY) and persistence of newer sainfoin cultivars in mixtures with alfalfa, and to determine the impact of harvest frequency on the persistence of sainfoin. Sainfoin cultivars 'AAC Mountainview', 'AAC Glenview', 'Delaney', 'Shoshone', and six experimental populations were compared with 'Nova' and 'Melrose' at Lanigan, SK, from 2016 to 2018. Field plots were seeded in either monocultures of sainfoin at 33 $kg \cdot ha^{-1}$ or sainfoin–'AC Grazeland' alfalfa mixtures at 16:9 $kg \cdot ha^{-1}$ in alternate rows. Forage DMY was greater ($P = 0.001$) in mixtures than in sainfoin monocultures in all harvests. The proportion of sainfoin in mixtures at Cut 1 declined from 4.1% to 1.3% of total DMY from 2016 to 2018, and 19.0% to 4.8% in Cut 2, which was less than the recommended level to eliminate ruminant bloat risk. A second field trial was established in 2017 to compare responses of 'AAC Mountainview', 'Nova', and 'Shoshone' sainfoin under one-, two- or three-harvest frequencies in 2018 and 2019. The increase of harvest frequency did not reduce sainfoin stand (%). Stand percentage of 'AAC Mountainview' (91%) was greater ($P = 0.01$) than 'Nova' sainfoin (62%). Further agronomic studies focusing on weed control in sainfoin stands and the optimum seeding ratios of sainfoin–alfalfa within the Parkland region of Saskatchewan are needed.

Key words: sainfoin, forage dry matter yield, botanical composition, adaptation.

Résumé : La forte concentration de tanins dans le sainfoin (*Onobrychis viciifolia*) améliore la digestion des fourrages et réduit les risques de météorisme quand les bêtes paissent de la luzerne (*Medicago sativa*). Les auteurs voulaient évaluer le rendement en matière sèche et la persistance des nouveaux cultivars de sainfoin poussant avec la luzerne, ainsi que déterminer les conséquences du nombre de coupes sur la persistance de la culture. De 2016 à 2018, les auteurs ont comparé les cultivars de sainfoin AAC Mountainview, AAC Glenview, Delaney et Shoshone ainsi que six populations expérimentales aux variétés Nova et Melrose, à Lanigan, en Saskatchewan. Les parcelles ont été ensemencées soit uniquement avec du sainfoin (33 kg par ha), soit avec un mélange de sainfoin et de luzerne AC Grazeland (16 kg et 9 kg par hectare, respectivement, en rangs alternés). Les mélanges ont donné un fourrage dont le rendement en matière sèche était supérieur ($P = 0,001$) à celui de la monoculture, peu importe la coupe. Entre 2016 et 2018, la proportion de sainfoin dans le mélange est passée de 4,1 à 1,3 % du rendement total en matière sèche lors de la première coupe, et de 19,0 à 4,8 % lors de la deuxième, ce qui est inférieur à la proportion recommandée pour prévenir le météorisme chez les ruminants. En 2017, les auteurs ont procédé à un deuxième essai sur le terrain pour comparer la réaction des variétés de sainfoin AAC Mountainview, Nova et Shoshone à une, deux ou trois coupes en 2018 et 2019. Augmenter la fréquence des coupes ne réduit pas le peuplement de l'herbacée (en %). Le peuplement d'AAC Mountainview (91 %) était plus important ($P = 0,01$) que celui de Nova (62 %). Il faudrait entreprendre d'autres études agronomiques pour préciser la lutte contre les adventices dans les peuplements de sainfoin et la densité de semis optimale des mélanges sainfoin-luzerne dans la région des prairies-parcs de la Saskatchewan. [Traduit par la Rédaction]

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Mots-clés : sainfoin, rendement des fourrages en matière sèche, composition botanique, adaptation.

Introduction

Sainfoin (*Onobrychis viciifolia* Scop. ssp. *viciifolia*) is a non-bloat causing perennial forage legume that is native to Europe and Central Asia. It is cultivated for hay in Europe, from Turkey in the south to Sweden in the north (Bhattarai et al. 2016). The non-bloating characteristic of this forage legume is due to the condensed tannins (CTs), a phenolic compound, which binds with soluble plant protein in the rumen (Gea et al. 2011; Scharenberg et al. 2007). When fed in combination with bloat-prone forages such as alfalfa (*Medicago sativa* L.), sainfoin reduces or prevents frothy bloat of ruminant (McMahon et al. 1999; Acharya et al. 2013).). Li et al. (1996) reported that about 1.0 mg CT·g^{-1} dry matter (DM) was needed to prevent the frothy bloat associated with alfalfa. Other benefits of sainfoin for grazing animals is a reduction in parasite load in the digestive tract (Hoste et al. 2012).

Adoption of sainfoin by producers has been limited since its introduction in the 1960s in Canada because it yielded only 85% of the forage production of alfalfa (Goplen et al. 1991). In recent years, there has been a renewed interest in growing sainfoin in mixtures with alfalfa to provide high quality legume mixtures for greater weight gain in cattle without the risk of ruminant bloat caused by alfalfa (Acharya et al. 2013; Bhattarai et al. 2016; Iwaasa et al. 2018). In a recent grazing study, Sottie et al. (2014) found that if a mixture of sainfoin and alfalfa contained at least 30% sainfoin by dry weight, then bloat incidences in grazing cattle were reduced by 98%. Sainfoin growth rate in spring is similar to alfalfa, but regrowth after grazing is slower than alfalfa (Hanna et al. 1972), increasing the bloat risk during the regrowth period (Acharya et al. 2013). New sainfoin cultivars with improved regrowth were developed under alfalfa competition in Canada (Acharya et al. 2013, 2018).

Though sainfoin cultivars released in the 1980s were more winter hardy than the cultivars from the 1960s, they still exhibited winter kill in Canada (Goplen et al. 1991). Sainfoin may require an uninterrupted growth period prior to the first killing fall frost to prevent winter kill (Saskatchewan Forage Council 2007), and harvest frequency may negatively affect its winter survival. In addition, the demand for sainfoin combined with a lack of commercial seed production for the current cultivars resulted in importation of sainfoin cultivars from western USA to Canada (Gray et al. 2006). However, previous studies indicate that sainfoin cultivars imported from other countries and regions may lack winter hardiness (Hanna et al. 1972; Goplen et al. 1991).

The objectives of this research were to (1) evaluate the forage dry matter yield (DMY) and persistence of new sainfoin cultivars and breeding populations from Canadian and USA breeding programs in monocultures and mixtures with alfalfa, and (2) evaluate the impact of harvest frequency on forage DMY and persistence of sainfoin cultivars.

Materials and Methods

Experiment 1: Forage dry matter yield and botanical composition of mixtures

Sainfoin seeds of 'AAC Mountainview', 'AAC Glenview', 'Nova', 'Melrose', and experimental breeding populations LRC05 3900, LRC053901, LRC 3519, LRC3432, LRC 4498, and LRC 4500 were obtained from Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, and seeds of 'Delaney' and 'Shoshone' were obtained from the Wyoming Seed Certification Service at Powell, WY. For sainfoin–alfalfa mixtures, alfalfa cultivar 'AC Grazeland' was used. Germination data were used to calculate seeding rates of sainfoin and alfalfa to the recommended rate for monoculture sainfoin at 33 kg·ha^{-1} and alternate row, sainfoin–alfalfa mixtures at a ratio of 16:9 kg·ha^{-1}.

The soil at the Lanigan, SK (51°51′N, 105°02′W) site is classified as an Orthic Black Chernozem, Meota-Hamlin association of loamy sand to very fine sandy loam texture (Saskatchewan Soil Survey, 1992) within the Thin Black Chernozem soil zone (Padbury et al. 2002). The experimental design was a randomized complete block design with 24 treatments (12 sainfoin monocultures, 12 sainfoin–alfalfa binary mixtures) in four replications. 'AC Grazeland' monoculture was also seeded as a comparison. The 12 sainfoin entries (populations) were seeded as either monocultures or in alternate rows with 'AC Grazeland' alfalfa. Individual plots were 1.8 m × 6.0 m in size. This location was used for cereal crop production for two years followed by one year of fallow prior to this trial. Soil contained adequate soil nutrients (P, K, and S) for legume production based on a soil test. Prior to seeding, 0.4 L·ha^{-1} glyphosate was applied to control weeds. Seeding was done on 4 June 2015 using a six-row plot drill with 20 cm row spacing. Weeds such as foxtail barley (*Hordeum jubatum* L.), narrow leaf hawksbeard (*Crepis tectorum* L.) and sow thistles (*Sonchus* spp.) were observed throughout the plots after seeding. To control the weeds, Odyssey (Imazamox, imazethapyr) herbicide was applied at recommended rate of 42 g·ha^{-1} on 26 June 2015. Additional weed invasion occurred after the initial weed control, which was removed by mowing the whole plot areas on 25 Sept. 2015 before they produced seed. Emergence was slowed by dry weather in June 2015, but stand establishment was visually determined to be adequate for future data collection in Sept. 2015. No fertilizer nor irrigation was applied at the Lanigan site.

On 8 July 2016, 6 July 2017, and 5 July 2018 all plots were harvested with a Wintersteiger forage plot harvester CiBus F (WinterSteiger, Salt Lake, UT) for forage DMY. A 500 g subsample of forage was collected, weighed, dried for 72 h at 60 °C, and re-weighed to determine DM content. Then, the DMY was calculated using the DM content. An additional 1 kg subsample was collected from the harvested biomass, separated into sainfoin, alfalfa and weeds by hand, then each component was dried for 72 h at 60 °C and weighed to determine botanical composition of the alfalfa and sainfoin by dry weight. On 23 Aug. 2016, 29 Aug. 2017, and 27 Aug. 2018 one random 0.5 m^2 quadrat within each plot was hand clipped and separated into sainfoin, alfalfa, and weed subsamples. The regrowth was harvested at the full bloom stage of the alfalfa. The subsamples were dried for 72 h at 60 °C and weighed to determine regrowth DMY and botanical composition. Relative DMY of each plot was calculated from the DMY of the plot and 'AC Grazeland' alfalfa DMY in each replication.

Experiment 2: Effect of harvest frequency on sainfoin stand persistence

This experiment was conducted at the University of Saskatchewan, Saskatoon (52°04′N, 108°08′W), SK. The soil at the site is classified as an Orthic Dark Brown Chernozem (Saskatchewan Soil Survey, 1999). No fertilizer was applied as soil analyses on 29 Apr. 2017 indicated that total available N concentration was 115.9 $kg{\cdot}ha^{-1}$, available P was 104.4 $kg{\cdot}ha^{-1}$, K was > 1000 $kg{\cdot}ha^{-1}$, and S was 38.2 $kg{\cdot}ha^{-1}$, which were adequate for forage legume establishment. The plots were seeded on 16 May 2017 at a seeding rate of 17 $kg{\cdot}ha^{-1}$ of sainfoin seed. The individual plots were 1.2 m × 6 m in size and consisted of four rows, spaced 30 cm apart. The experiment was a split-plot design with four replications. The main plot factor was harvest frequency (one-, two-, and three-harvest per season) and the subplot factor was sainfoin cultivar ('AAC Mountainview', 'Nova', and 'Shoshone'). Weed control was done by a preseeding application of glyphosate at 1.2 $L{\cdot}ha^{-1}$ on 11 May 2017. The plots were mowed on Aug. 2017 to remove weeds. No weed control was carried out in 2018 or 2019. On 10 Oct. 2018, 11:52:0 fertilizer was applied to all plots at 108 $kg{\cdot}ha^{-1}$.

The plots were harvested on 13 June,17 July, and 14 Sept. in 2018 and 24 June, 29 July, and 16 Sept. in 2019 for the three-harvest treatment; 25 June and 15 Aug. in 2018, and 2 July and 20 Aug. in 2019 for the two-harvest treatment; and 29 June 2018 and 29 June 2019 for the single-harvest treatment. The plots were harvested with a Wintersteiger forage plot harvester CiBus F (WinterSteiger, Salt Lake, UT). A 500 g subsample was collected from the harvested forage biomass, weighed, dried at 60 °C for 48 h, and re-weighed to determine dry matter (DM) content. The DM content, plot area, and forage mass recorded were used to calculate forage DMY.

Sainfoin samples from Cut 1 of the all treatments were analyzed for crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF). The dried plant samples were ground in a Wiley mill (Thomas-Wiley, Philadelphia, PA) and then passed through a 1-mm mesh screen (Cyclone Mill, UDY Mfg., Fort Collins, CO). For CP determination, nitrogen content in the ground samples was analyzed using a Leco 628 Element Analyzer (Leco Corporation, St. Joseph, MI). Neutral detergent fiber and ADF concentrations were analyzed using Ankom^{2000} automated fiber analyzer (ANKOM Technology Corporation, NY) according to the manufacturer's instruction. In 2019, visual rating of the stand density (0 to 100% scale) was done by two independent and experienced observers. The average visual stand rating was calculated and used for statistical analysis.

Statistical analysis

For Experiment 1 at the Lanigan site, analysis of variance (ANOVA) was conducted for Cut 1, Cut 2, and total yield parameters using year as a repeated measurement over time. The UNIVARIATE procedure was used to test data for normality and homogeneity of variance.

'AC Grazeland' alfalfa monoculture data were excluded from this analysis. Thus, the model reflected 12 sainfoin entries (cultivars and experimental populations) grown in two mixtures (alternate sainfoin–alfalfa vs. sainfoin monoculture). The final statistical model was:

$$Yijkl = \mu + \beta_i + \alpha_j + \tau_k + \alpha\tau_{jk} + \eta'_{ijk} + \zeta_l + \alpha\zeta_{jl} + \tau\zeta_{jl} + \alpha\tau\zeta_{jkl} + \varepsilon_{ijkl}$$

where the observation is sum of the mean (μ), the i replication (β), the j mixture (α), the k entry (τ), the jk mixture × entry interaction ($\alpha\tau$), the ijk main plot error (η') the l year (ζ), the kl entry × year interaction ($\alpha\zeta$), jl mixture × year interaction ($\tau\zeta$), jkl entry × mixture × year interaction ($\alpha\tau\zeta$), and the $ijkl$ error (ε) terms. When the F test was significant ($P \leq 0.05$) for a source, means were compared with Tukey's HSD test. The ANOVA was determined using JMP software version 13 (SAS Inc., Cary, NC).

In experiment 2 at the Saskatoon site, year was considered as a repeated measure in time in the combined ANOVA analysis with harvest frequency and sainfoin cultivar as fixed effects. The mixed model for the split plot design was:

$$Yijk = \mu + \beta_i + \alpha_j + \eta'_{ij} + \tau_k + \alpha\tau_{jk} + \zeta_l + \zeta\alpha_{jk} + \zeta\tau_{kl} + \zeta\alpha\tau_{jkl} + \varepsilon_{iikl}$$

where the observation is the sum of the mean (μ), the ith replication (β), the jth harvest frequency (α), ijth main

Table 1. Probability of *F* tests in ANOVA by variable for 12 sainfoin cultivars and experimental populations (entries) grown in alternate sainfoin–alfalfa rows or in sainfoin monoculture and harvested twice from 2016 to 2018 at Lanigan, SK (Experiment 1).

Source	Df	Total DMY (kg·ha^{-1})	DMY% of alfalfa	Legume DMY (kg·ha^{-1})	Sainfoin DMY (kg·ha^{-1})	Sainfoin (%)	Alfalfa (%)	Weeds (%)
		Cut 1						
Entry (E)	11	0.4143	0.9182	0.4202	0.0339	0.0235	0.3438	0.1199
Mixture (M)	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
E × M	11	0.2124	0.8152	0.4154	0.5396	0.2337	0.3831	0.2921
Year (Y)	2	0.0001	0.0010	0.0001	0.0001	0.0001	0.5132	0.0001
E × Y	22	0.8551	0.9973	0.4340	0.2488	0.0344	0.0854	0.3963
M × Y	2	0.0001	0.0012	0.0001	0.0001	0.0001	0.0129	0.0001
E × M × Y	22	0.9935	0.9999	0.6753	0.8787	0.7812	0.7017	0.5854
		Cut 2						
Entry (E)	11	0.6920	0.7327	0.7156	0.0171	0.0608	0.0609	—
Mixture (M)	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—
E × M	11	0.2963	0.9822	0.2964	0.4600	0.0662	0.0662	—
Year (Y)	2	0.0001	0.0145	0.0001	0.0001	0.0001	0.0001	—
E × Y	22	0.6718	0.6627	0.6737	0.0354	0.3909	0.3911	—
M × Y	2	0.0001	0.2488	0.0001	0.0001	0.0001	0.0001	—
E × M × Y	22	0.1392	0.3804	0.1577	0.6986	0.4154	0.4156	—
		Total						
Entry (E)	11	0.8958	0.9154	0.1806	0.0117	—	—	—
Mixture (M)	1	0.0001	0.0001	0.0001	0.0001	—	—	—
E × M	11	0.0350	0.5522	0.1449	0.5631	—	—	—
Year (Y)	2	0.0001	0.2774	0.0001	0.0001	—	—	—
E × Y	22	0.9028	0.8799	0.0899	0.0883	—	—	—
M × Y	2	0.0001	0.1886	0.0001	0.0001	—	—	—
E × M × Y	22	0.0442	0.8196	0.0184	0.6227	—	—	—

Note: DMY, dry matter yield; total DMY: alfalfa, sainfoin, and weed biomass; legume DMY: alfalfa and sainfoin biomass; DMY% of alfalfa, DMY as a percentage of 'AC Grazeland' alfalfa; Df, degrees of freedom.

plot error (η'), the*k*th cultivar (τ), the *jk*th harvest frequency × cultivar interaction ($\alpha\tau$), the *l*th year (ζ), the *kl*th harvest frequency × year ($\zeta\alpha$), the *kl*th cultivar × year ($\zeta\tau$), the *ijk*th harvest frequency × cultivar × year ($\zeta\alpha\tau$), and the *ijkl*th error (ε) terms. For Cut 2 and Cut 3 DMY, the number of harvest frequency treatments declined, so these data were analyzed separately.

Results and Discussion

Experiment 1

Forage dry matter yield

The sainfoin–alfalfa mixtures produced significantly ($P < 0.01$) greater total DMY (including weeds), legume DMY, and contained less weeds at both Cut 1 and Cut 2 than the sainfoin monocultures (Tables 1 and 2). A greater DMY was expected for mixtures containing alfalfa than for sainfoin monocultures (Goplen et al. 1991), but the difference in this study was more than 50%. This large yield difference may be in part due to less vigorous seedlings of sainfoin resulting in higher weed density than the alfalfa stand. However, weed control by the application of Odyssey and mowing improved sainfoin seedling growth, and stand establishment was visually determined to be adequate in Sept. 2015. The droughts in 2015 and 2017 reduced the DMY of both sainfoin and alfalfa, but the impact was higher for sainfoin. This was reflected by a significant year effect ($P < 0.0145$) for all variables except total annual relative yield (as % of 'AC Grazeland' alfalfa) and alfalfa proportion at Cut 1 (Table 1). Sainfoin seems less drought tolerant than alfalfa, resulting in a lower yield. In a six-year study, Biligetu et al. (2014) found that sainfoin regrowth was slow after a drought stress, while alfalfa recovered to about 40% of pre-drought yield. Total DMY, legume DMY, and sainfoin DMY declined from 2016 to 2018 for both Cut 1, Cut 2, and annual forage DMY except for Cut 1 DMY of sainfoin from 2017 to 2018 (Tables 3 and 4).

Sainfoin cultivars differed for yield parameters (Table 1). The differences at Cut 1 were small and Tukey's test was unable to indicate a significant mean separation (Table 5). At Cut 2, DMY of 'AAC Mountainview' was greater ($P = 0.0171$) than that of 'Delaney' and 'LRC 3432', while the other entries did not differ (Table 5). The total annual sainfoin DMY of 'AAC Mountainview' and 'Nova' were greater ($P = 0.0117$)

Table 2. Forage dry matter yield (DMY) (kg·ha^{-1}) and proportion (% dry matter) of sainfoin, weed, and alfalfa of 12 sainfoin grown in alternate row with alfalfa or in sainfoin monoculture and harvested twice from 2016 to 2018 at Lanigan, SK (Experiment 1).

Factor	Total DMY (kg·ha^{-1})	DMY% of alfalfa	Legume DMY (kg·ha^{-1})	Sainfoin DMY (kg·ha^{-1})	Sainfoin (%)	Weeds (%)	Alfalfa (%)
Mixture	Cut 1						
Alternate row	4395a	123.4a	3770a	135b	2.6b	12.9b	84.4b
Monoculture	1952b	50.2b	721b	605a	24.9a	75.1a	0b
SE	92	4.3	112	42	1.1	1.6	1.3
	Cut 2						
Alternate row	2322a	95.6a	2322a	233b	10.5b	—	89.4a
Monoculture	591b	24.2b	593b	591a	100.0a	—	0b
SE	78	3.1	78	27	0.9	—	1.5
	Total						
Alternate row	6611a	109.2a	6019a	368b	—	—	—
Monoculture	2247b	34.6b	1192b	1079a	—	—	—
SE	143	2.9	131	54	—	—	—

Note: Means within a column and cut followed by different letters differ ($P < 0.05$) by Tukey's HSD; total DMY: alfalfa, sainfoin, and weed biomass; legume DMY: alfalfa and sainfoin biomass; DMY% of alfalfa: DMY as a percentage of 'AC Grazeland' alfalfa; SE, standard error.

Table 3. Change (2016–2018) of forage dry matter yield (DMY) (kg ha^{-1}) and proportion (% dry matter) of sainfoin, weed, and alfalfa of 12 sainfoin grown in alternate row with alfalfa or in sainfoin monoculture and harvested twice at Lanigan, SK (Experiment 1).

Factor	Total DMY (kg ha^{-1})	DMY% of alfalfa	Legume DMY (kg ha^{-1})	Sainfoin DMY (kg ha^{-1})	Sainfoin (%)	Weeds (%)	Alfalfa (%)
Year	Cut 1						
2016	5765a	91.9a	4103a	873a	20.6a	34.5c	44.9
2017	2153b	96.7a	1584b	175b	13.9b	42.8b	43.2
2018	1586c	71.7b	1048c	60b	6.8c	50.8a	42.4
SE	118	5.5	144	54	1.5	2.1	1.7
	Cut 2						
2016	2439a	66.0a	2439a	871a	59.5a	—	40.6b
2017	1477b	50.9b	1480b	275b	54.0b	—	46.0a
2018	452c	62.8ab	452c	90c	52.4b	—	47.6a
SE	95	3.8	95	34	1.1	—	1.1
	Total yield						
2016	7616a	74.5	6253a	1569a	—	—	—
2017	3630b	73.8	3062b	450b	—	—	—
2018	2039c	67.3	1502c	151c	—	—	—
SE	175	3.5	161	66	—	—	—

Note: Means within a column and cut followed by different letters differ ($P < 0.05$) by Tukey's HSD; total DMY: alfalfa, sainfoin, and weed biomass; legume DMY: alfalfa and sainfoin biomass; DMY % of alfalfa, DMY as a percentage of 'AC Grazeland' alfalfa; SE, standard error.

Table 4. Mean total dry matter yield (DMY), relative DMY of alfalfa, legume DMY, sainfoin DMY, and botanical composition (% dry matter) interaction of alternate sainfoin–alfalfa rows or in sainfoin monoculture from 2016 to 2018 at Lanigan SK. (Experiment 1).

Mixture and year	Total DMY (kg ha^{-1})	DMY% of alfalfa	Legume DMY (kg ha^{-1})	Sainfoin DMY (kg ha^{-1})	Sainfoin (%)	Weeds (%)	Alfalfa (%)
	Cut 1						
Alternate 2016	7606a	116.8b	6395a	288b	4.1d	13.9d	82.0a
Alternate 2017	3253c	148.2a	2906b	88b	2.6d	10.9d	86.5a
Alternate 2018	2331d	105.3b	2007c	29b	1.3d	13.9d	84.8a
Mono 2016	3923b	60.5c	1811c	1457a	45.0a	55.0c	0b
Mono 2017	1052e	45.3c	263d	263b	25.3b	74.7b	0b
Mono 2018	841e	38.2c	89d	92b	12.2c	87.8a	0b
SE	178	7.2	224	84	2.3	3.2	2.6
	Cut 2						
Alternate 2016	3718a	99.8	3718a	583b	19.0b	—	81.0b
Alternate 2017	2494b	83.3	2494b	89c	7.9c	—	92.1a
Alternate 2018	753cd	103.6	753cd	28c	4.8c	—	95.2a
Mono 2016	1160c	32.1	1160c	1160a	100a	—	0c
Mono 2017	460de	18.6	460de	460b	100a	—	0c
Mono 2018	152e	22.0	152e	152c	100a	—	0c
SE	134	5.4	136	48	1.6	—	1.6
	Total yield						
Alternate 2016	11000a	107.3	9897a	869b	—	—	—
Alternate 2017	5748b	115.7	5401b	177c	—	—	—
Alternate 2018	3084d	104.5	2760c	57c	—	—	—
Mono 2016	4233c	41.7	2609c	2609a	—	—	—
Mono 2017	1513e	31.9	723d	723b	—	—	—
Mono 2018	993e	30.1	244d	244c	—	—	—
SE	248	5.0	228	94	—	—	—

Note: Means within a column and cut followed by different letters differ ($P < 0.05$) by Tukey's HSD; total DMY: alfalfa, sainfoin, and weed biomass; legume DMY: alfalfa and sainfoin biomass; DMY% of alfalfa, DMY as a percentage of 'AC Grazeland' alfalfa; SE, standard error.

than that of 'Delaney', but it was similar to the other 10 entries (Table 5). There was a significant ($P = 0.035$) cultivar × year interaction for Cut 2 sainfoin DMY (Table 1). 'AAC Mountainview' yielded significantly more forage DMY than 'Melrose', 'Delaney', and three experimental populations at Cut 2 in 2016, but there was no difference among them in 2017 and 2018 (Table 6). 'AAC Mountainview' was selected for improved regrowth (Acharya 2015), but its regrowth may have limited in 2017 due to a drier than normal year. The growing season of 2017 received 70 mm of precipitation from May to Aug. (26% of the long-term average) (Fig. 1). Total annual sainfoin DMY in monocultures declined by 72% from 2016 to 2017 (2609 vs. 723 kg ha^{-1}) and by 66% from 2017 to 2018 (723 vs. 244 kg ha^{-1}). In addition, low winter temperature in Feb. (–17.7 °C vs. –12 °C long-term average) and March 2018 (–10.3 °C vs. –5.3 °C long-term average) may also have had a negative impact on growth in 2018, though we did not record winter survival (Fig. 1). Ditterline and Cooper (1975) recommended that sainfoin be seeded in areas with rainfall amounts of 330 mm.

Proportion of sainfoin in mixtures with alfalfa

In the present study, the proportion of sainfoin in sainfoin–alfalfa mixtures was 2.6% (of DM) at Cut 1 and 10.5% at Cut 2 (Table 2). The proportion of sainfoin at Cut 1 declined annually from 2016 to 2018, while the weed proportion increased each year (Table 3). At Cut 2, the proportion of sainfoin declined from 2016 to 2018, while the proportion of alfalfa increased. The percentage of sainfoin in this study was lower than the 20% to 30% (of DM) needed to avoid ruminant bloat of grazing cattle as reported by Iwaasa et al. (2018). Alfalfa dominated ($P = 0.001$) the botanical composition of the sainfoin–alfalfa mixtures (Table 2), especially during the regrowth period ($P = 0.001$), increasing from 81% in 2016 to 95% in 2018 (Table 4). This suggests that the impact of alfalfa competition on sainfoin growth increased over time. Alternate row seeding did not show significant increase in sainfoin proportion under drought in this study, due to high weed pressure. Though it was not tested in this study, the same row seeding of sainfoin and alfalfa, a common practice, may further reduce the sainfoin proportion compared with an alternative row seeding

Table 5. Mean total dry matter yield (DMY), relative DMY, legume DMY, sainfoin DMY, and botanical composition for 12 sainfoin cultivars and experimental populations at Lanigan, SK (Experiment 1).

Entry	Total DMY (kg ha^{-1})	DMY% of alfalfa	Legume DMY (kg ha^{-1})	Sainfoin DMY (kg ha^{-1})	Sainfoin (%)	Alfalfa (%)	Weeds (%)
				Cut 1			
AAC Glenview	3321	92.8	2225	207a	8.3b	46.1	45.8
AAC Mountainview	3556	94.2	2467	466a	16.4ab	41.9	41.7
Delaney	3412	93.6	2284	165a	7.7b	42.3	49.9
LRC 3432	3024	78.9	2398	290a	10.2ab	46.8	43.0
LRC 3519	2907	77.5	2233	357a	13.9ab	44.3	41.8
LRC 4498	2909	72.8	2144	185a	12.1ab	48.1	39.8
LRC 4500	3477	85.7	1830	427a	11.8ab	39.1	49.0
LRC05 3900	3357	92.4	2568	554a	16.0ab	45.7	38.6
LRC05 3901	3076	90.8	2185	463a	16.3ab	43.5	40.2
Melrose	2911	86.4	1676	320a	15.3ab	37.2	47.4
Nova	3116	88.7	2474	581a	21.4a	45.2	33.4
Shoshone	3244	87.7	2458	425a	16.0ab	41.8	42.3
SE	212	10.4	307	115	3.2	3.1	4.4
				Cut 2			
AAC Glenview	1379	55.5	1379	471ab	57.9	42.1	—
AAC Mountainview	1432	59.5	1432	645a	61.1	38.8	—
Delaney	1342	56.0	1342	258b	52.7	47.3	—
LRC 3432	1490	54.6	1490	322b	53.4	46.6	—
LRC 3519	1650	64.2	1650	492ab	55.6	44.4	—
LRC 4498	1396	65.3	1396	347ab	52.2	47.7	—
LRC 4500	1202	50.8	1213	388ab	61.0	39.0	—
LRC05 3900	1281	54.9	1281	356ab	53.4	46.6	—
LRC05 3901	1336	57.2	1336	437ab	54.4	45.6	—
Melrose	1712	74.1	1712	358ab	53.1	46.9	—
Nova	1594	64.0	1594	420ab	55.3	44.7	—
Shoshone	1660	62.6	1660	447ab	53.5	46.5	—
SE	190	7.6	190	67	2.3	2.3	—
				Total			
AAC Glenview	4192	75.1	3320	680ab	—	—	—
AAC Mountainview	4553	75.8	3784	990a	—	—	—
Delaney	4464	71.6	3552	345b	—	—	—
LRC 3432	4378	63.9	3838	562ab	—	—	—
LRC 3519	4429	68.2	3834	791ab	—	—	—
LRC 4498	4306	69.0	3493	513ab	—	—	—
LRC 4500	4116	62.8	2813	727ab	—	—	—
LRC05 3900	4638	73.6	3849	911ab	—	—	—
LRC05 3901	4038	73.4	3314	715ab	—	—	—
Melrose	4414	77.4	3284	569ab	—	—	—
Nova	4711	76.4	4068	1002a	—	—	—
Shoshone	4904	75.1	4118	873ab	—	—	—
SE	350	7.0	322	133	—	—	—

Note: Means within a column and cut followed by different letters differ ($P < 0.05$) by Tukey's HSD; total DMY: alfalfa, sainfoin, and weed biomass; legume DMY: alfalfa and sainfoin biomass; DMY% of alfalfa, DMY as a percentage of 'AC Grazeland' alfalfa; SE, standard error.

because it would increase plant competition (Hanna et al. 1977; Jefferson et al. 1994).

'Nova' sainfoin exhibited greatest sainfoin proportion at Cut 1 (21%), but was significantly ($P = 0.0235$) greater than only 'Delaney' (8%) (Table 6). 'AAC Mountainview' had 61% sainfoin proportion at Cut 2 compared with about 54% for cultivars 'Nova', 'Melrose', 'Shoshone', and 'Delaney'. This numerical trend suggests that the selection of sainfoin to persist in alfalfa stands has at least helped new populations to maintain their

Table 6. Mean sainfoin dry matter yield (kg ha^{-1}) × entry and year interaction at Cut 2 at Lanigan, SK (Experiment 1).

Entry	2016	2017	2018
AAC Glenview	1140ab	228e–h	45h
AAC Mountainview	1456a	347d–h	131gh
Delaney	527b–h	176gh	71h
LRC 3432	593b–h	307d–h	65h
LRC 3519	1077ab	335d–h	63h
LRC 4498	681b–h	245e–h	116gh
LRC4500	895a–d	212f–h	61h
LRC05 3900	749b–g	249e–h	72h
LRC05 3901	850a–f	370c–h	93h
Melrose	637b–h	296d–h	141gh
Nova	857a–e	275d–h	129gh
Shoshone	997abc	256d–h	90h
SE	117		

Note: Means followed by different letters differ ($P < 0.05$) by Tukey's HSD.

proportions, if not improved it. 'AAC Mountainview' sainfoin had been selected for persistence in competition with alfalfa for grazing (Acharya 2015). However, the improved performance of 'AAC Mountainview' sainfoin compared with 'Nova' for grazing in mixtures with alfalfa as demonstrated by Sottie et al. (2014) in southern Alberta was less evident in the Parkland region of Saskatchewan in this study due to differences in climatic and soil conditions. In this study, the proportion of sainfoin was undoubtedly reduced by weed competition. Weed control, therefore, is an important aspect for sainfoin establishment and stand performance over time. While we did not test the effect of seeding rate, an alfalfa seeding rate of 9 kg·ha^{-1}, which is close to the full recommended seeding rate in the region, might be too high. The reduction of alfalfa seeding rate may be an important factor for improving sainfoin establishment and performance.

Weed density

The proportion of weeds was greater ($P = 0.0001$) in the sainfoin monocultures at Cut 1 than in sainfoin–alfalfa mixtures in all three years, increasing from 55% in 2016 to 88% in 2018 (Table (4). In the first year of stand establishment, Moyer (1985) found weeds made up more than 90% of DMY in sainfoin fields without any weed control measures. This suggests that effective weed control that promote sainfoin seedling establishment in the presence of alfalfa competition is essential to establish satisfactory stands of sainfoin–alfalfa mixtures. Bhattarai et al. (2016) described more than 10 herbicides registered for use in sainfoin stand. The application of glyphosate has a 10-fold greater negative impact on alfalfa DM yield than it does on sainfoin (Peel et al. 2013), which may be an option for weed control in the mixture.

Experiment 2

Forage dry matter yield

'AAC Mountainview' produced more Cut 1 DMY ($P = 0.0003$) and total DMY ($P = 0.0187$) than 'Nova' (Table 8). It also exhibited lower NDF and ADF concentrations ($P = 0.004$ and 0.0006, respectively) than 'Nova' sainfoin and similar CP concentration. 'AAC Mountainview' sainfoin also had higher visual stand rating in 2019 than 'Nova' sainfoin (Table 8). 'Shoshone' sainfoin was intermediate in stand rating between 'AAC Mountainview' and 'Nova'. The decline in 'Nova' sainfoin stand rating in 2019 may be attributable to its low adaptation to multiple- cut management coupled with low Feb. 2019 temperature (−24.1 °C compared with long-term average temperature of −11.4 °C) with below average snow cover for insulation (Fig. 2). Jefferson et al. (1994) reported that low winter temperature stress can result in reduced plant survival and production in the following growing season and that 'Nova' sainfoin was susceptible to low winter temperature stress at Swift Current, SK.

'AAC Mountainview' sainfoin was selected for persistence in mixtures with alfalfa and improved regrowth yield (Acharya 2015). We observed greater Cut 1 and total annual forage DMY and persistence of 'AAC Mountainview' compared with 'Nova'. Bhattarai et al. (2018) reported that 'Nova' sainfoin was similar in winter survival to 'Shoshone' (88% vs 80% survival) and superior to many other germplasm. They harvested forage once per year followed by seed production from the regrowth, which was a more lenient harvest regime than was used in the current study. 'Nova' sainfoin had been selected for improved winter survival compared with other cultivars available in the 1980s (Hanna 1980). 'Nova' was also reported to exhibit lower regrowth yield compared with multi-cut cultivars from other countries (Hanna 1980). 'Shoshone' sainfoin was selected in Wyoming for improved persistence through increased resistance to root-knot nematode (Gray et al. 2006). It appears to have some potential adaptation in regions where nematodes have not been reported to reduce the persistence of sainfoin.

Harvest frequency and year

The year effect was significant ($P < 0.05$) for all variables (Table 7). The harvest frequency significantly ($P = 0.0024$) affected total forage DMY of sainfoin, where two and three harvests per year yielded more total forage DMY than one harvest (Table 8). Two harvests per year yielded more Cut 2 DMY than three harvests per year. There was no difference in stand rating due to harvest frequency ($P = 0.42$, Table 7) despite the low temperature observed in Feb. 2018 (Fig.2). In fact, total forage DMY was increased in 2019, mainly because of an increase in Cut 2 and Cut 3 DMY compared with 2018 (Table 8). The above average precipitation in June and July 2019 (Fig. 2) contributed to increased regrowth and

Fig. 1. Monthly mean air temperature (°C) and monthly total rainfall (mm) during the growing season from 2015 to 2018 at Watrous, SK (Source: Environment Canada's weather database).

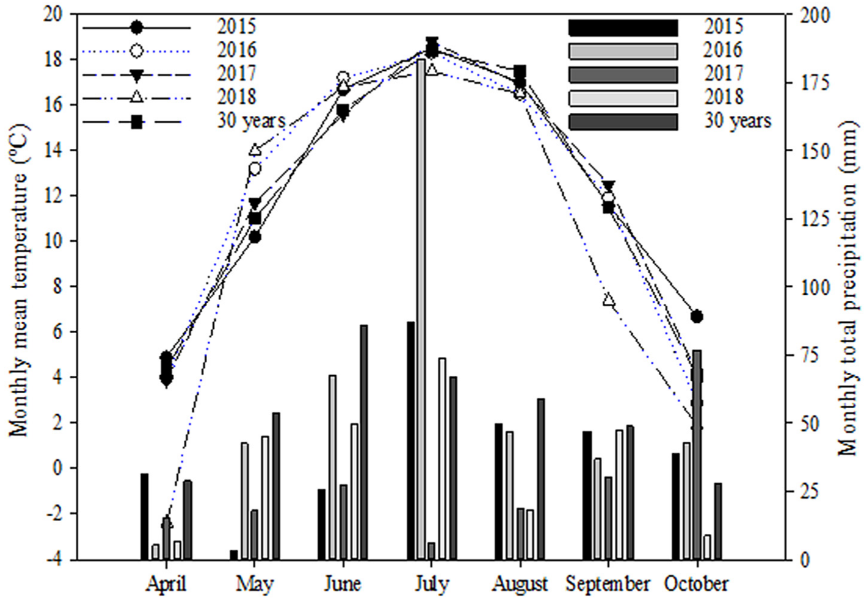
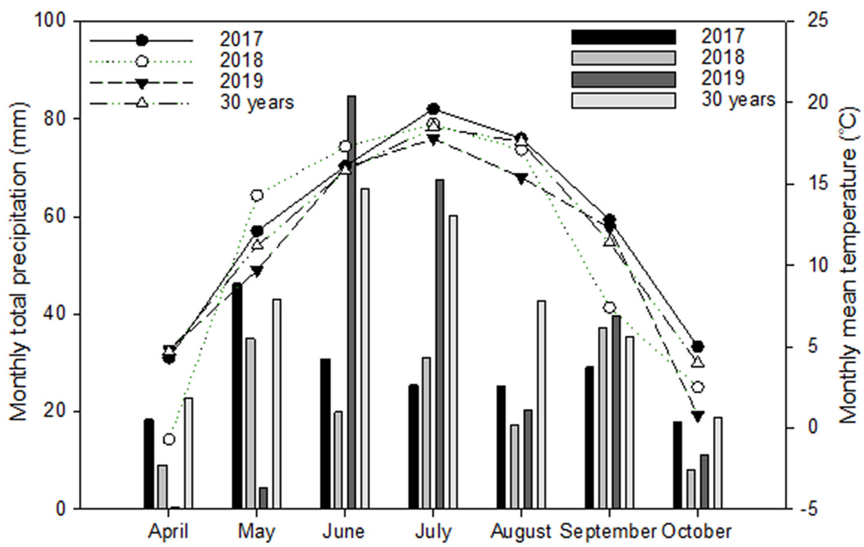


Fig. 2. Monthly mean air temperature (°C) and monthly total precipitation (mm) during the growing season from 2017 to 2019 at Saskatoon, SK (Source: Environment Canada's weather database).



forage DMY. Similarly, CP concentration was greater ($P = 0.0001$) and ADF and NDF concentrations were lower ($P = 0.001$ for both) in 2019 compared with 2018. There was no significant ($P = 0.8844$) cultivar × harvest frequency interaction for stand rating in 2019. In our study, the three harvest per year treatment was harvested in Sept. 2018 and there was a 'killing frost' on 29 Sept. (−4.9 °C), but there was no evident reduction in stand rating in 2019 due to harvest frequency. As has been reported for alfalfa, the importance of critical fall harvest period may depend on the winter hardiness of the cultivar for the region (Jefferson and Gossen 1992). However, our study was conducted at one location over three years. Therefore, a multi-location study over a longer period of time will be necessary to further verify fall harvest management of sainfoin stands for persistence and winter survival.

Conclusions

Sainfoin productivity and persistence in mixtures with alfalfa are required traits to provide successful bloat-safe grazing of sainfoin-alfalfa pastures by cattle. A sufficient proportion of sainfoin biomass must be present in the mixture to ensure sufficient intake of

Table 7. Probability of F tests in ANOVA by variable for three sainfoin cultivars under three harvest frequencies in 2018 and 2019 at Saskatoon, SK (Experiment 2).

		Forage dry matter yield (kg ha^{-1})				Nutritive value (g ha^{-1})			
Source	Df	Cut 1	Cut 2	Cut 3	Total	CP	NDF	ADF	Stand (%)
Harvest Frequency (HF)	2	0.0628	0.0127	—	0.0024	0.0037	0.0281	0.0355	0.4239
Cultivar (C)	2	0.0003	0.5952	0.1519	0.0187	0.5206	0.004	0.0006	0.0096
HF × C	4	0.9084	0.693	—	0.656	0.8837	0.4139	0.5392	0.8844
Year (Y)	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	—
Y × HF	2	0.0658	0.0055	—	0.0002	0.0001	0.0001	0.0001	—
Y × C	2	0.4119	0.9979	0.16	0.4585	0.9278	0.7516	0.6347	—
Y × HF × C	4	0.7516	0.4759	—	0.7166	0.0129	0.0518	0.0611	—

Note: Degrees of freedom (Df) for 2-harvest and 3-harvest treatments are 1, 2, 1, and 2 for harvest frequency (HF), HF × cultivar (C), year (Y) × HF, and Y × HF × C, respectively.

Table 8. Forage dry matter yield, nutritive value, and stand percentage of three sainfoin cultivars under different harvest managements in 2018 and 2019 at Saskatoon, SK (Experiment 2).

	Forage dry matter yield (kg ha^{-1})				Nutritive value (g kg^{-1})			
	Cut 1	Cut 2	Cut 3	Total	CP	NDF	ADF	Stand (%)
				Cultivar				
Mountainview	2174a	1354	1280	3504a	156b	320b	230b	91.7a
Nova	1644b	1197	691	2673b	150b	340a	252a	62.2b
Shoshone	2105a	1187	1157	3282ab	171a	343a	252a	80.6ab
SEM	83	122	212	207	3	6	6	5.7
				Harvest frequency				
1	2115	—	—	2115b	159	344ab	255a	77.2
2	1965	1579a	—	3594a	157	349a	252ab	84.7
3	1844	912b	1043	3800a	161	309b	227b	72.5
SEM	63	88	122	207	3	8	6	5.7
				Year				
2018	2205a	430b	96b	2523b	145b	363b	273a	—
2019	1744b	2062a	1990a	3783a	173a	306a	216b	—
SEM	64	97	173	169	2	6	4	—

Note: Means within a column and source of variation followed by different letters differ ($P < 0.05$) by Tukey's HSD. SEM, standard error of the mean.

condensed tannins to bind the soluble protein from alfalfa in the anaerobic digestion process of the rumen and reduce the production of stable foam that traps methane gas and causes bloat. The minimum proportion of sainfoin in sainfoin–alfalfa mixtures has been reported to be 20% to 30% of DM, yet none of the tested sainfoin–alfalfa mixtures in the study met this requirement at Lanigan, SK, from 2016 to 2018. Weed control and low alfalfa seeding rate that promote sainfoin seedling establishment in the presence of alfalfa competition is essential to establish satisfactory stands of sainfoin–alfalfa mixtures. The selection for persistence of sainfoin in competition with alfalfa has improved in 'AAC Mountainview', but further genetic improvement for the Parkland region of Saskatchewan is needed. Sainfoin stand declined both at Lanigan and Saskatoon under different growth environments can be attributed to weed and alfalfa competitions, water stress, low winter temperature stress, or their combination. 'AAC Mountainview' sainfoin was more persistent than the current standard, 'Nova' sainfoin, but this was not related to harvest frequency at the Saskatoon site. We conclude that more testing of 'AAC Mountainview' and 'AAC Glenview' sainfoin is needed in the Parkland region of Saskatchewan to confirm their adaptation and use for bloat-safe alfalfa grazing.

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