



Canadian Journal of Plant Science

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Journal:	<i>Canadian Journal of Plant Science</i>
Manuscript ID	CJPS-2020-0131.R3
Manuscript Type:	Article
Date Submitted by the Author:	24-Nov-2020
Complete List of Authors:	Biliget, Bill; University of Saskatchewan, Department of Plant Sciences Jefferson, Paul; Western Beef Development Centre Lardner, Herbert; University of Saskatchewan College of Agriculture and Bioresources, Animal and Poultry Science Acharya, Surya; AAFC, Lethbridge Research Centre
Keywords:	sainfoin, botanical composition, adaptation, alfalfa-sainfoin mixture
Is the invited manuscript for consideration in a Special Issue?:	Not applicable (regular submission)

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Short title: persistence of sainfoin in alfalfa-sainfoin mixtures

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

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Abstract

Condensed tannins in sainfoin (*Onobrychis viciifolia*) improves 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 persistence of sainfoin. Sainfoin cultivars, 'AAC Mountainview', 'AAC Glenview', 'Delaney', 'Shoshone' and six experimental populations were compared with 'Nova' and 'Melrose' at Lanigan, SK Canada from 2016 to 2018. Field plots were seeded in either monocultures of sainfoin at 33 kg ha⁻¹ or sainfoin - 'AC Grazeland' alfalfa mixtures at 16 kg ha⁻¹: 9 kg ha⁻¹ in alternate rows. Forage DMY was greater ($P=0.001$) in mixtures than 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 optimum seeding ratios of sainfoin-alfalfa within the Parkland region of Saskatchewan are needed.

KEYWORDS: sainfoin, forage dry matter yield, botanical composition, adaptation

Introduction

Sainfoin (*Onobrychis viciifolia* 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 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 1960's 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 by 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 regrowth period (Acharya et al. 2013). New sainfoin cultivars with improved regrowth were developed under alfalfa competition in Canada (Acharya 2013, 2018).

Though sainfoin cultivars released in 1980s were more winter hardy than the cultivars from 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 I: 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

Lethbridge Research and Development Centre, and seeds of ‘Delaney’ and ‘Shoshone’ were 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 16 kg ha⁻¹: 9 kg ha⁻¹.

The soil at Lanigan, Saskatchewan (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 × 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 June 4, 2015 using a six-row plot drill with 20 cm row spacing. Weeds such as foxtail barley (*Hordeum jubatum*), narrow leaf hawksbeard (*Crepis tectorum*) 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 June 26, 2015. Additional weed invasion occurred after the initial weed control, which was removed by mowing the whole plot areas on September 25, 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 September 2015. No fertilizer nor irrigation was applied at Lanigan site.

On July 8 2016, July 6 2017, and July 5 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 sub-sample 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 August 23 2016, August 29 2017, and

August 27 2018 one random 0.5 m² quadrat within each plot was hand clipped and separated into sainfoin, alfalfa and weed sub-samples. The regrowth was harvested at full bloom stage of the alfalfa. The sub-samples 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 II: Effect of harvest frequency on sainfoin stand persistence

This experiment was conducted at the University of Saskatchewan, Saskatoon (52°04'N, 108°08'W), Saskatchewan. 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 April 29 2017 indicated that total available N concentration was 115.9 kg ha⁻¹, available P was 104.4 kg ha⁻¹, K was >1000 kg ha⁻¹ and S was 38.2 kg ha⁻¹, which were adequate for forage legume establishment. The plots were seeded on May 16 2017 at a seeding rate of 17 kg ha⁻¹ of sainfoin seed. The individual plots were 1.2 × 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 sub-plot factor was sainfoin cultivar ('AAC Mountainview', 'Nova' and 'Shoshone'). Weed control was done by a pre-seeding application of glyphosate at 1.2 L ha⁻¹ on May 11 2017. The plots were mowed on August 2017 to remove weeds. No weed control was carried out in 2018 or 2019. On October 10 2018, 11:52:0 fertilizer was applied to all plots at 108 kg ha⁻¹.

The plots were harvested on June 13 July 17 and September 14 in 2018 and June 24 July 29 and September 16 in 2019 for the three-harvest treatment; June 25 and August 15 in 2018 and July 2 and August 20 in 2019 for the two-harvest treatment; and June 29 2018 and June 29 2019 for single harvest treatment. The plots were harvested with a Wintersteiger forage plot harvester CiBus F (WinterSteiger, Salt Lake, UT). A 500 g sub-sample 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, USA) and then passed through a 1-

mm mesh screen (Cyclone Mill, UDY Mfg., Fort Collins, CO, USA). For CP determination, nitrogen content in the ground samples was analyzed using a Leco 628 Element Analyzer (Leco Corporation, St. Joseph, MI, USA). Neutral detergent fiber and ADF concentrations were analyzed using Ankom²⁰⁰⁰ automated fiber analyzer (ANKOM Technology Corporation, NY, USA) according to 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 Exp. I at Lanigan site, Analysis of Variance (ANOVA) was conducted for Cut 1, Cut 2, and total yield parameters using year as 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:

$$Y_{ijkl} = \mu + \beta_i + \alpha_j + \tau_k + \alpha\tau_{jk} + \eta_{ijk} + \zeta_l + \alpha\zeta_{jl} + \tau\zeta_{jl} + \alpha\tau\zeta_{jkl} + \varepsilon_{ijkl}$$

where the observation is sum of the mean (μ), the i replication (β), the j mixture (α), the k entry (τ), the jk mixture $\times$ entry interaction ($\alpha\tau$), the ijk main plot error (η) the l year (ζ), the kl entry $\times$ year interaction ($\alpha\zeta$), jl mixture $\times$ year interaction ($\tau\zeta$), jkl entry $\times$ mixture $\times$ 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 v.13 (SAS Inc., Cary NC USA).

In Exp. II at 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:

$$Y_{ijk} = \mu + \beta_i + \alpha_j + \eta_{ij} + \tau_k + \alpha\tau_{jk} + \zeta_l + \zeta\alpha_{jk} + \zeta\tau_{kl} + \zeta\alpha\tau_{jkl} + \varepsilon_{iikl},$$

where the observation is sum of the mean (μ), the i th replication (β), the j th harvest frequency (α), ij th main plot error (η), the k th cultivar (τ), the jk th harvest frequency $\times$ cultivar interaction ($\alpha\tau$), the l th year (ζ), the kl th harvest frequency $\times$ year ($\zeta\alpha$), the kl th cultivar $\times$ year ($\zeta\tau$), the ijk

the harvest frequency $\times$ cultivar $\times$ year ($\zeta\alpha\tau$) and the $ijklth$ 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 I

Forage dry matter yield

The sainfoin-alfalfa mixtures produced significantly ($P < 0.01$) greater total DMY (including weeds), legume DMY and contained less weed 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 percent. 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 application of Odyssey and mowing improved sainfoin seedling growth, and stand establishment was visually determined to be adequate in September 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 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 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 ($P = 0.0117$) than that of 'Delaney', but it was similar to the other 10 entries (Table 5). There was significant ($P = 0.035$) cultivar $\times$ 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 August (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 February (−17.7 °C vs −12 long-term average) and March 2018 (−10.3 °C vs −5.3 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 to 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 to an alternative row seeding 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 to about 54% for ‘Nova’, ‘Melrose’, ‘Shoshone’ and ‘Delaney’ cultivars. This numerical trend suggests that the selection of sainfoin to persist in

alfalfa stand has at least helped new populations to maintain their proportions, if not improved. '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 to '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 difference 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 II

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 February 2019 temperature (-24.1°C compared to 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, Saskatchewan.

'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 to '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 to other cultivars available in the 1980s (Hanna 1980). 'Nova' was also reported to exhibit lower regrowth yield compared to 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-harvest per year yielded more Cut 2 DMY than three-harvest per year. There was no difference in stand rating due to harvest frequency ($P=0.42$, Table 7) despite the low temperature observed in February 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 to 2018 (Table 8). The above average precipitation in June and July 2019 (Fig. 2) contributed to increased regrowth and 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 to 2018. There was no significant ($P=0.8844$) cultivar $\times$ harvest frequency interaction for stand rating in 2019. In our study, the three-harvest per year treatment was harvested in September 2018 and there was a 'killing frost' on 29 September (-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 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 Saskatchewan 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 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.

Acknowledgements

Funding for this research was provided the Agricultural Development Fund of Saskatchewan, Saskatchewan Ministry of Agriculture. Technical support by Dashnyam Byambatseren of Department of Plant Sciences and Leah Pearce of the Livestock and Forage Centre of Excellence, University of Saskatchewan (formerly Western Beef Development Centre) is gratefully acknowledged.

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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 I)

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.

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 I)

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	4395 ^a	123.4 ^a	3770 ^a	135 ^b	2.6 ^b	12.9 ^b	84.4 ^b
Monoculture	1952 ^b	50.2 ^b	721 ^b	605 ^a	24.9 ^a	75.1 ^a	0 ^b
s.e.	92	4.3	112	42	1.1	1.6	1.3
	----- Cut 2 -----						
Alternate row	2322 ^a	95.6 ^a	2322 ^a	233 ^b	10.5 ^b	--	89.4 ^a
Monoculture	591 ^b	24.2 ^b	593 ^b	591 ^a	100.0 ^a	--	0 ^b
s.e.	78	3.1	78	27	0.9	--	1.5
	----- Total -----						
Alternate row	6611 ^a	109.2 ^a	6019 ^a	368 ^b	--	--	--
Monoculture	2247 ^b	34.6 ^b	1192 ^b	1079 ^a	--	--	--
s.e.	143	2.9	131	54	--	--	--

Note: ^{a-b}Means within a column and cut with 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.

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 I)

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	5765 ^a	91.9 ^a	4103 ^a	873 ^a	20.6 ^a	34.5 ^c	44.9
2017	2153 ^b	96.7 ^a	1584 ^b	175 ^b	13.9 ^b	42.8 ^b	43.2
2018	1586 ^c	71.7 ^b	1048 ^c	60 ^b	6.8 ^c	50.8 ^a	42.4
s.e.	118	5.5	144	54	1.5	2.1	1.7
Year	----- Cut 2 -----						
2016	2439 ^a	66.0 ^a	2439 ^a	871 ^a	59.5 ^a	--	40.6 ^b
2017	1477 ^b	50.9 ^b	1480 ^b	275 ^b	54.0 ^b	--	46.0 ^a
2018	452 ^c	62.8 ^{ab}	452 ^c	90 ^c	52.4 ^b	--	47.6 ^a
s.e.	95	3.8	95	34	1.1	--	1.1
Year	----- Total Yield -----						
2016	7616 ^a	74.5	6253 ^a	1569 ^a	--	--	--
2017	3630 ^b	73.8	3062 ^b	450 ^b	--	--	--
2018	2039 ^c	67.3	1502 ^c	151 ^c	--	--	--
s.e.	175	3.5	161	66	--	--	--

Note: ^{a-b}Means within a column and cut with 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.

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 I)

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

Note: ^{a-d}Means within a column and cut with 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.

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 I)

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

Note: ^{a-b}Means within a column and cut with 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.

Table 6. Mean sainfoin dry matter yield (kg ha⁻¹) by entry and year interaction at Cut 2 at Lanigan SK. (Experiment I)

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

Note: ^{a-f}Means with different letters differ ($P < 0.05$) by Tukey's HSD.

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 II)

Source	Df	Forage dry matter yield (kg ha ⁻¹)				Nutritive value (g kg ⁻¹)			
		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: Degree of freedoms (Df) for 2-harvest and 3-harvest treatments are 1, 2, 1, and 2 for harvest frequency (HF), HF × cultivar (C), and year (Y) × HF, Y × HF × C.

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 II)

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

Note: ^{a-b}Means within a column and source of variation with different letters differ ($P < 0.05$) by Tukey's HSD.

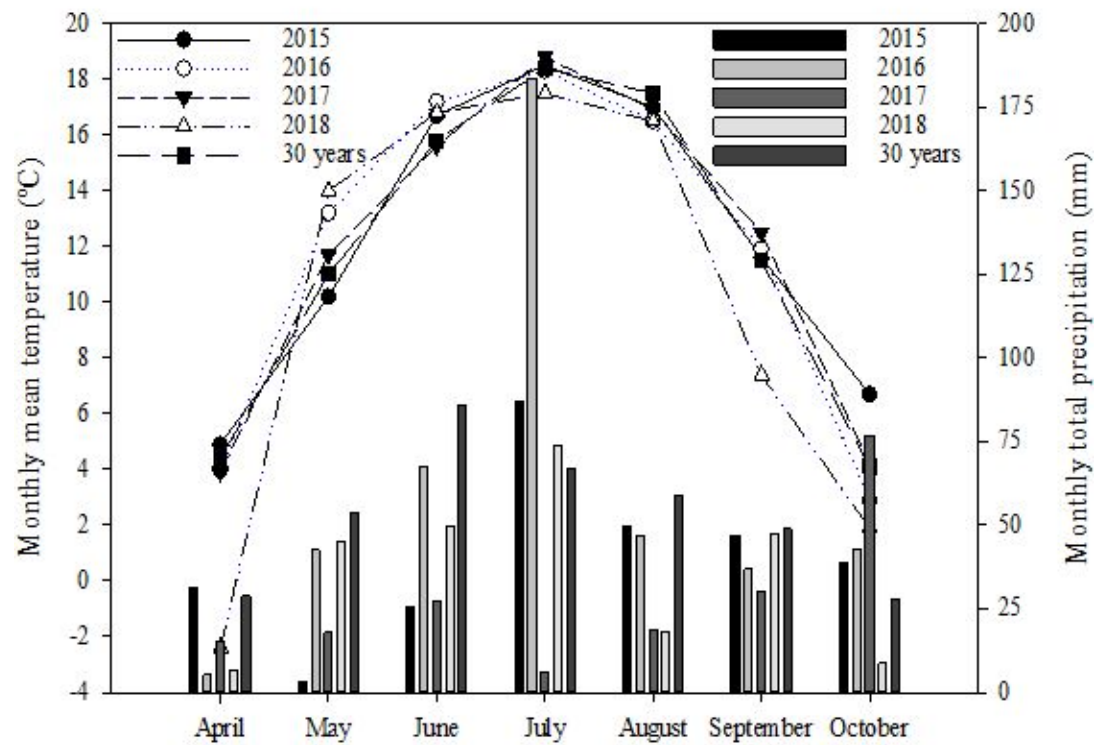


Figure 1. Monthly mean air temperature (°C) and monthly total rainfall (mm) during the growing season from 2015 to 2018 at Watrous SK. (Source: Environment Canada's weather database)

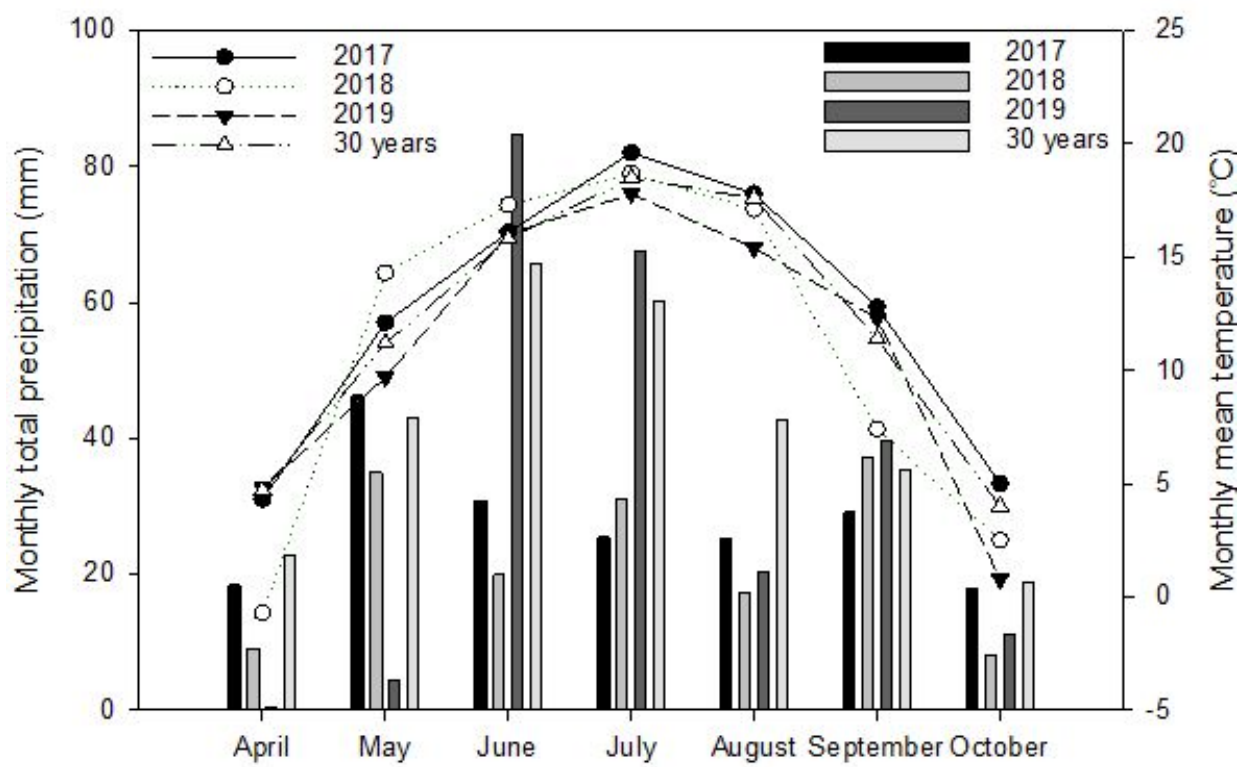


Figure 2. Monthly mean air temperature (°C) and monthly total precipitation (mm) during the growing season from 2017 to 2019 at Saskatoon, SK. (Source: Environment Canada’s weather database)