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Compatibility of new sainfoin populations as forage mixtures with alfalfa and orchardgrass in Alberta

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Abstract

Sainfoin is a highly nutritious non-bloating leguminous forage crop of temperate regions. Despite non-bloating properties, its use in pastures was limited due to low dry matter yield (DMY), lack of persistence in the mixed pasture, and slow regrowth. The paradigm then shifted when new sainfoin cultivars planted in mixtures with alfalfa reduced 98% bloat incidence in ruminants. Two experiments were conducted to determine the compatibility of sainfoin with grass and alfalfa. Experiment (Exp.) I was established by drilling alfalfa cv. Longview and orchardgrass cv. Kayak with new sainfoin populations in alternate or cross-seeding rows under irrigated and unirrigated conditions in Lethbridge, AB. Exp. II was established by drilling sainfoin populations in mixture with alfalfa cultivars in alternate rows under irrigation. DMY and botanical composition (dry matter basis) were observed for both experiments. In Exp. I, monoculture orchardgrass produced the least DMY compared with the mixtures under both growing conditions and over all growing seasons. Alfalfa–sainfoin mixture yielded higher under irrigatated and less under unirrigated conditions relative to alfalfa monoculture. The percentage DMY contribution of sainfoin in alfalfa–sainfoin mixtures decreased but increased in sainfoin–orchardgrass mixtures over successive harvests under both environments. In Exp. II, we observed that new sainfoin populations mixed with alfalfa cvs. Beaver and Longview yielded greater ($P < 0.05$) than their corresponding monocultures and this increase was not associated with the proportion of the species in the mixture suggesting new sainfoin populations are as productive as alfalfa.

Key words: sainfoin–alfalfa mixture, sainfoin–orchardgrass mixture, perennial forage, bloat-free forage, sustainable forage production

Résumé

Le sainfoin est une légumineuse fourragère extrêmement nourrissante qui ne cause pas de météorisme. On la cultive dans les régions tempérées. Bien qu'elle ne ballonne pas, on ne l'utilisait guère dans les pâturages à cause de son faible rendement en matière sèche, de sa piètre persistance dans les prairies mixtes et de la lenteur de la repousse. Le paradigme a cependant changé avec l'arrivée de nouveaux cultivars qui, semés avec la luzerne, réduisent l'incidence du météorisme de 98 % chez les ruminants. Les auteurs ont réalisé deux expériences pour vérifier la compatibilité du sainfoin avec les graminées et la luzerne. Dans la première, ils ont semé en ligne le cultivar de luzerne Longview et le cultivar de dactyle pelotonné Kayak avec les nouvelles populations de sainfoin, en alternance ou par passages croisés, sur des terres irriguées ou pas, à Lethbridge (Alberta). Lors de la deuxième expérience, les peuplements de sainfoin ont été établis par semis en ligne avec un mélange de cultivars de luzerne, par rangs alternés, avec irrigation. Les auteurs ont noté le rendement en matière sèche et déterminé la composition botanique des peuplements lors des deux expériences. Dans la première, c'est la monoculture de dactyle pelotonné qui a produit le rendement en matière sèche le plus faible, comparativement aux mélanges, dans les deux régimes de culture, pour toutes les périodes végétatives. Le mélange luzerne-sainfoin donne un meilleur rendement sous irrigation, mais produit moins que la monoculture de luzerne en conditions arides. Proportionnellement, l'apport du sainfoin au rendement en matière sèche des mélanges luzerne-sainfoin diminue avec les récoltes successives dans les deux environnements, alors qu'elle augmente dans les mélanges sainfoin-dactyle. Dans la deuxième expérience, les auteurs ont remarqué que les nouveaux cultivars de sainfoin mélangés aux cultivars de luzerne Beaver et Longview donnent un meilleur rendement ($P < 0,05$) que les monocultures correspondantes, une hausse qu'on n'associe pas à la part de l'espèce dans le mélange, ce qui pourrait indiquer que les nouvelles variétés de sainfoin sont aussi productives que la luzerne. [Traduit par la Rédaction]

Mots-clés : mélange sainfoin-luzerne, mélange sainfoin-dactyle pelotonné, vivaces fourragères, plantes fourragères sans météorisme, production fourragère durable

Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) is a non-bloating perennial forage legume adapted to semi-arid areas of the world and to western Canada with mean annual precipitation of >300 mm (Goplen et al. 1991). Despite the non-bloating nature of sainfoin (Tanner et al. 1994; Wang et al. 2006), its use in pasture is limited worldwide because of the lower dry matter yield (DMY) compared with alfalfa and the lack of persistence when mixed with other forages. The yield potential of sainfoin is considered to be about 80% of alfalfa in monoculture (Goplen et al. 1991; Frame et al. 1998; Carbonero et al. 2011; Bhattarai et al. 2016) and the dry matter (DM) contribution in the mixture progressively declined over few years of establishment (Acharya et al. 2013; Acharya2015). Moreover, the older sainfoin cultivars released in western Canada are single-cut type and their cultivation in monoculture or primary mixture with other forage species is not considered a sustainable forage production strategy (Kielly et al. 1994).

Sainfoin contains high concentration of condensed tannins such that as little as 15% of sainfoin in mixture with alfalfa can make alfalfa bloat-safe (McMahon et al. 1999). This undoubtedly is a huge boon to the cattle industry as loss due to alfalfa pasture bloat is a main deterrent for ranchers. Despite being bloat-free, farmers were reluctant to establish alfalfa–sainfoin pasture because of uncertainty associated with low persistence of sainfoin cultivars with alfalfa. The paradigm then shifted when the AAFC forage breeding program at Lethbridge released two sainfoin cvs. AAC Mountainview (Acharya 2015) and AAC Glenview (Acharya 2018) with better compatibility and persistence with alfalfa. These multicut cultivars were selected for growing together with alfalfa and are considered bloat-safe (Acharya et al. 2013; Sottie et al. 2014). These cultivars and their sister populations were also reported to have increased persistence for growing with alfalfa when planted in alternate rows.

The DMYs of new sainfoin cvs. AAC Glenview and AAC Mountainview were higher than or equal to those of Longview alfalfa under both irrigated and unirrigated conditions. These cultivars retained significant proportion of sainfoin in alfalfa mixed pasture (at least 15%) for 4 years after seeding when seeded in alternate rows and the stand was sufficiently bloat-safe for grazing cattle (Acharya 2015, 2018). However, the potential of these cultivars has not been tested with other alfalfa cultivars and grass species. In this study, we reported results from two separate experiments. In Experiment (Exp.) I, we tested the compatibility of the improved sainfoin populations with alfalfa (cv. Longview) and orchardgrass (cv. Kayak) using different seeding methods under irrigated and unirrigated environments at Lethbridge, AB. Orchardgrass among several other grasses was selected because of its higher competitiveness with alfalfa (Casler 1988; Bi et al. 2019). In Exp. II, we investigated the compatibility of these new sainfoin populations with five commercially available alfalfa cultivars. The yield potential and percentage of sainfoin in the mixture were evaluated to determine whether the primary mixture of sainfoin with alfalfa or orchardgrass can be a sustainable option for direct grazing or hay making in western Canada. We hypothesize that newer sainfoin populations are better adapted to the mixture of both alfalfa and orchardgrass compared with old sainfoin cv. Nova and newer sainfoin population in the mixture will retain at least 15% suitable for bloat-free grazing.

Table 1. The origin and parental designation of the new sainfoin populations used in Exp. I and Exp. II.

Population	Parentage	Experiment (s)
LRC-3519	300 individual plants selected from cultivar Splendid from Romania after three spring applications of 1.1 L glyphosate ha^{-1}	I and II
LRC-3900	230 individuals from LRC-3432, 35%; LRC-3519, 19%; Melrose, 13%; Perly, 13%; LRC 3401,8%; Eski, 5%; Chinese accessions, 5%; and Kazakhstan, 2%	I and II
LRC-4012	Original unirrigated population from China, kindly contributed by Yun Jin Feng, Inner Mongolia Agricultural University in 2007	I
LRC-3432	300 individual plants selected from Remont from Montana, USA after three spring applications of 1.1 L glyphosate ha^{-1}	II

Materials and methods

Experiment I (mixture of sainfoin with alfalfa and orchardgrass in irrigated and unirrigated environments)

Exp. I consisted of binary mixtures of orchardgrass (cv. Kayak (Acharya et al. 2007)) and alfalfa (cv. Longview (Acharya and Huang 2000)) with each of the four sainfoin cultivars or improved populations (Nova, LRC-3432, LRC-3519, and LRC-3900; hereinafter called populations) including alfalfa–orchardgrass mixture and monoculture alfalfa. The parentage of the LRC-designated populations (developed at Lethbridge Research and Development Center, Agriculture and Agri-Food Canada) is presented in Table 1. The seeds were seeded in randomized complete design with four blocks into four independent trials: (*i*) alternate row seeding, irrigated; (*ii*) cross-seeding, irrigated; (*iii*) alternate row seeding, unirrigated; and (*iv*) cross-seeding, unirrigated.

All four trials were seeded in May 2012 at Lethbridge, AB using custom-built Great Plains Drill (Great Plains AG, Salina, KS, USA) containing two separate seed bins for alternate row seeding. The seeding rates were 17 kg pure live seeds (PLS) ha^{-1} for sainfoin and 4 kg PLS ha^{-1} for alfalfa or 11 kg PLS ha^{-1} for orchardgrass in the mixture. In monoculture, the seeding rates were 8 kg PLS ha^{-1} for alfalfa or 22 kg PLS ha^{-1} for orchardgrass. Plot dimensions were 1.8 × 8 m^2. Seeds were drilled in rows at the row spacing of 0.18 m for alternate row seeding and 0.36 m for cross-seeding such that a plot in the alternate seeding method consisted of 10 rows, while the cross-seeded plots consisted of 5 rows and 5 columns.

The establishment year growth was mowed without taking DMY data. For all production years, the forage biomass was harvested using a customized harvester three times per year in irrigated plots and two times per year in unirrigated plots for three production years. Plots were harvested when at least 10% of the sainfoin plants were in bloom. Before each harvest, botanical composition was determined by hand separating hand-clipped herbage mass within a randomly placed quadrant (0.5 m × 0.5 m) from each plot. Those samples were hand separated by species, dried in an oven for 3 days at 60 °C, and weighted for DMY.

Data analysis was performed for each trial separately. DMY recorded across harvests within a year was added to generate yearly DMY for each treatment. Data for DMY was analysed using generalized mixed models consisting of factorial design with two levels of species (alfalfa and orchardgrass) and six levels of cultivar (four sainfoin cultivars and two monocultures) as implemented in SAS 9.4. Species, cultivar, year, and their interactions were considered fixed factors, while block, block × species, block × cultivar, and block × species × cultivar were considered random factors. The denominator degrees of freedom were corrected using the Satterthwaite approximation. Year was considered a repeated measure factor. The random residual variance was accounted for by considering autoregressive-1 variance–covariance structure for unirrigated trials and heterogeneous compound symmetry for irrigated trials based on the lowest Akaike information criterion (AIC) values. Fisher's protected least significant difference (LSD) test was used to evaluate differences among means (Saxton 1998).

The percentage composition was calculated as the ratio of DMY of sainfoin to total DMY if the mixture contained a sainfoin component and ratio of DMY of alfalfa to the total DMY in the orchardgrass–alfalfa mixture. Botanical composition was analysed as a regression to harvest time for each species × cultivar without accounting for the effect of seeding method. Simple linear models were fitted for all except for irrigated trials consisting of alfalfa in which quadratic models had better fits (based on AIC values).

Experiment II (comparison of sainfoin in monoculture vs. in mixture with alfalfa)

Exp. II consisted of seven sainfoin populations planted either in monoculture or in mixture with five commercially available cultivars of alfalfa. The experiment was set up into 7 (sainfoin populations) × 6 (5 alfalfa + sainfoin monocultures) factorial design with three blocks. The seven sainfoin populations included Nova (Hanna 1980), Melrose (Cooke et al. 1971), Nova-161, RM-Remnot (Carlton and Delaney 1972), LRC-3519, LRC-3900, and LRC-4012. Nova-161 is a modified Nova population selected for multicut sainfoin. The last three LRC-designated populations are the newly developed populations at the Lethbridge Research and Development Center whose origin and parentage are described in Table 1. The five alfalfa cultivars included Beaver (Bolton et al. 1963), Nordic (Goplen and Gossen 1994), Rangelander, Yellowhead, and Longview (Acharya and Huang 2000). Yellowhead is a falcata-type, yellow-flowered alfalfa cultivar, which is tolerant to heavy grazing and mixed swards with grasses and is suitable for unirrigated areas (McLeod et al. 2009). Rangelander is a creeping-rooted alfalfa cultivar suitable for unirrigated areas of western Canada (Heinrichs et al. 1979).

The test was seeded in May 2012 at Lethbridge, AB. The seeding rates were 17 kg PLS ha^{-1} of sainfoin and 4 kg PLS ha^{-1} of alfalfa for the mixture. For monoculture sainfoin, seeding rate was 34 kg PLS ha^{-1}. Each plot was 1.8 m × 8 m. Seeds were drilled in rows at the row spacing of 0.18 m using Great Plains Drill (Great Plains AG, Salina, KS, USA). The mixture plot consisted of 5 rows of sainfoin and 5 rows of alfalfa alternatively placed, while the monoculture plot consisted of 10 rows of sainfoin alone. Plots were supplemented with irrigation as needed.

The forage biomass was harvested three times a year for four consecutive production years (after the establishment year) using a customized harvester as described in Exp. I. The sample herbage mass within the quadrant and estimation of total DMY were similar to those in Exp. I. DMYs recorded from three harvests within a year were summed to generate yearly yield, while the contribution to DMY (% of sainfoin = 100 × sainfoin DMY/total DMY) was averaged across harvests within a year.

Data were analysed using a generalized mixed model consisting of factorial design with seven levels of sainfoin population and six levels of alfalfa cultivars (five alfalfa cultivars and one monoculture) as implemented in SAS 9.4 for both DMY and % sainfoin composition. Sainfoin, alfalfa, year, and their all-way interactions were considered fixed factors, while block, block × sainfoin, block × mixture, and block × sainfoin × mixture were considered random factors. The denominator degrees of freedom were corrected using the Satterthwaite approximation. Production year was considered a repeated factor. The random residual variance was accounted for by using heterogeneous compound symmetry variance–covariance structure based on the lowest AIC value. Fisher's protected LSD test was used to evaluate differences among means between sainfoin population and alfalfa cultivars using SAS macro (Saxton 1998). Effects were considered significant at $P < 0.05$ unless otherwise specified.

In both the experiments (Exp. I and II), the relationship between DMY and % sainfoin contribution was assessed for each population and year using simple linear regression. This approach was used to determine the relative gain or loss as a result of mixture. A positive regression coefficient suggests positive mixture effects, while a negative regression coefficient means negative mixture effects in terms of DMY.

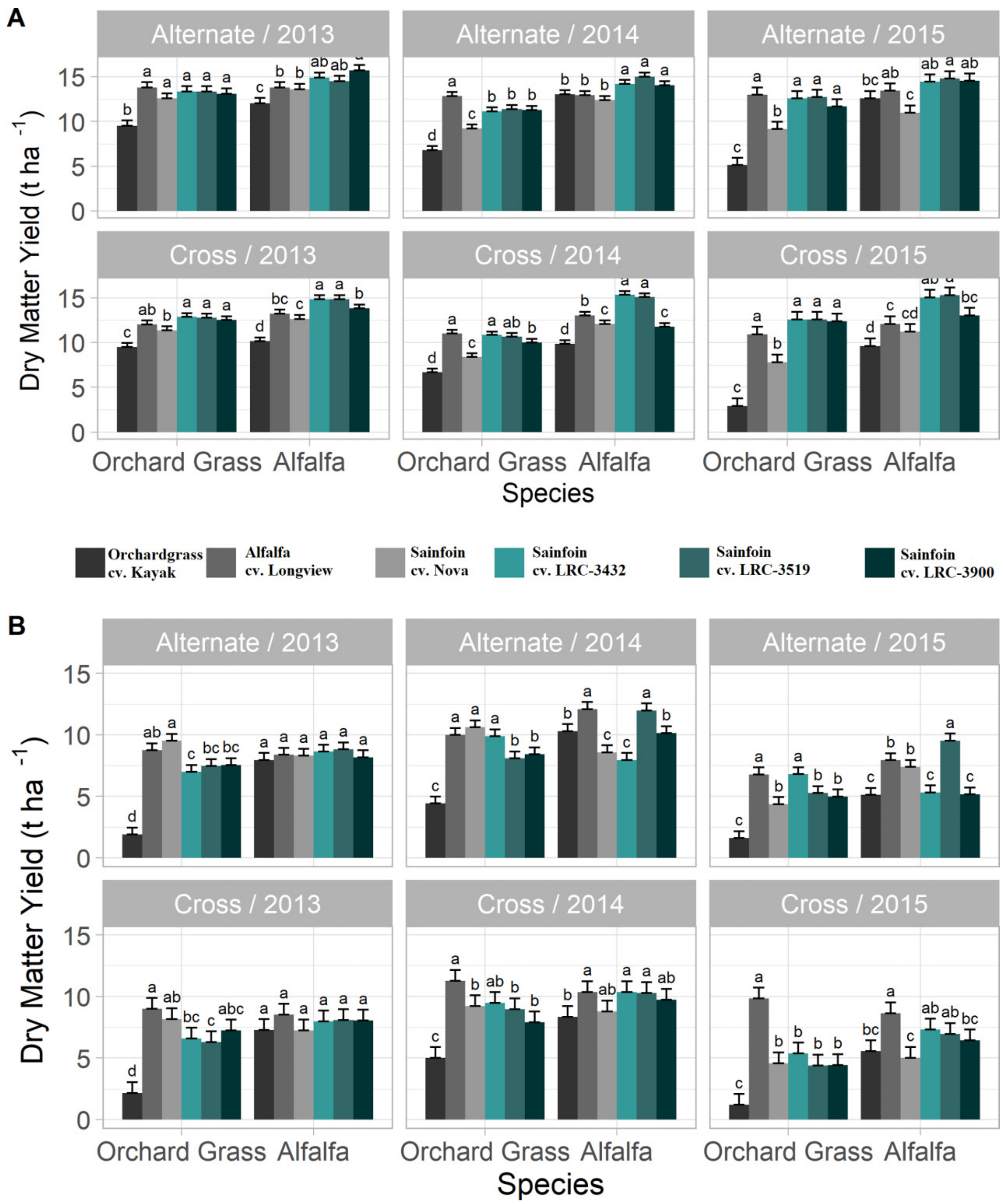
Results

Experiment I (mixture of sainfoin with alfalfa and orchardgrass in irrigated and unirrigated environments)

Dry matter yield

Sainfoin populations, species mixtures, year, sainfoin × species, and sainfoin × year were significant for both seeding methods and environments ($P < 0.05$, Table S1, Fig. 1).

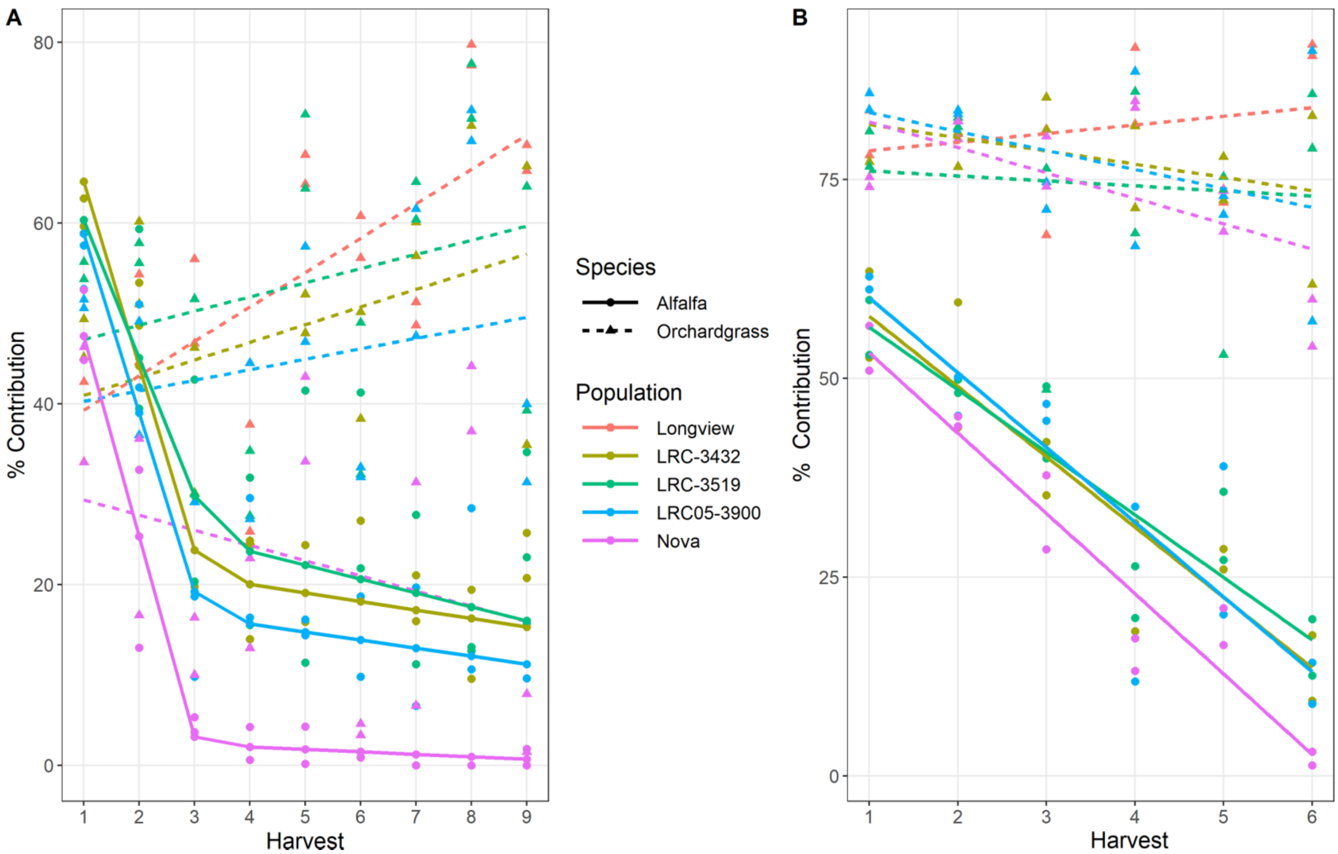
Fig. 1. DMY of mixed stand of sainfoin with either orchardgrass or alfalfa and pure stand of orchardgrass or alfalfa measured using combined harvester by year for Experiment I: (A) irrigated and (B) unirrigated. Kayak in orchardgrass and Longview in alfalfa represent the monoculture. The DMY is the sum of herbage yield across multiple cuts within a year (three cuts in irrigated land and two cuts in unirrigated land).



Alternate row seeding had higher DMY compared with the cross-seeded row in irrigated environment (paired t test, diff = 0.8 t ha^{-1}, $P < 0.05$) but not in unirrigated environment (paired t test, diff = 0.25 t ha^{-1}, $P = 0.08$). Alfalfa–sainfoin mixture produced 2.5 t ha^{-1} more compared with the orchardgrass–sainfoin mixture in irrigated areas, but the difference was only 1.5 t ha^{-1} in unirrigated areas ($P < 0.05$).

Orchardgrass monoculture (cv. Kayak) had significantly lower DMY than orchardgrass–alfalfa mixture in both environments (irrigated and unirrigated) and both seeding methods (alternate rows and cross-seeded) across all years (Fig. 1). Alfalfa monoculture had higher DMY than alfalfa–orchardgrass mixture in 8 out of 12 combinations of environment, seeding methods, and years (Fig. 1). In most cases, mixtures of sainfoin cv. Nova with Kayak orchardgrass and Longview alfalfa produced lower than all three new sainfoin populations (LRC-3432, LRC-3519, LRC-3900) suggesting newer sainfoin populations had better compatibility to both alfalfa and grass than Nova sainfoin. In general, the DMYs of three new sainfoin populations in mixture with alfalfa or or-

Fig. 2. Linear regression of % sainfoin (DM basis) on the harvest events for each species separated by sainfoin population for Experiment I. (A) Linear segmented regression in the irrigated plot. The harvest events ranged from 1 to 9 in the irrigated plot (three harvests per year in 2013, 2014, and 2015). (B) Simple linear regression in unirrigated environment. The harvest events ranged from 1 to 6 in unirrigated environment (two harvests per year in 2013, 2014, and 2015).



chardgrass did not differ among themselves when averaged across all years.

from other new sainfoin populations while mixed with either alfalfa or orchardgrass suggesting its compatibility in drier conditions was similar to that of new sainfoin populations.

Percentage sainfoin composition (DM basis)

In irrigated conditions, the % contribution of sainfoin in alfalfa mixtures decreased sharply over the three successive harvest periods (1–3) and then plateaued (Fig. 2A, Table S2). The % contribution in orchardgrass mixed sward increased linearly over successive harvests with alfalfa ($P < 0.05$), but the increase was not significant with new sainfoin populations ($P > 0.05$, Fig. 2A, Table S2). However, the percentage of sainfoin populations in the orchardgrass mixture increased with stand age ($P < 0.05$) except for Nova sainfoin suggesting improved compatibility of new sainfoin populations with orchardgrass. The % contribution of Nova sainfoin decreased in orchardgrass mixture as was the case with alfalfa suggesting poor compatibility of orchardgrass in legume mixtures.

Under unirrigated condition, the % sainfoin decreased linearly with increase in stand age under mixed alfalfa sward, while % sainfoin in orchardgrass mixture did not change with the age of the sward (Fig. 2B, Table S2). The % contribution of Nova sainfoin in unirrigated environment did not differ

Relationship between DMY and % sainfoin contribution

Year has significant effect on DMY and there was significant interaction between production year and species. Therefore, the relationship between DMY and % sainfoin contribution was reported by year (Fig. 3). In general, the DMY of mixed sward increased with increase in % sainfoin (7 out of 12 regression, Fig. 3). It was noticeable that % sainfoin contribution with orchardgrass in unirrigated plots was greater than that in irrigated plots and with alfalfa in all three years, suggesting poor performance of orchardgrass in dry environments. In the irrigated plots, the DMY of orchardgrass–sainfoin mixture increased with increase in % sainfoin composition. It was also interesting that DMY of alfalfa–sainfoin mixed sward increased as percentage of sainfoin increased in irrigated conditions, which suggests the combination of sainfoin with alfalfa resulted in complementary yield advantage when grown in mixture.

Fig. 3. Relationship between DMY and % sainfoin composition for unirrigated and irrigated environments by species and by year for Experiment I. Regressions not indicated with *P* values are not significant at $P < 0.05$.

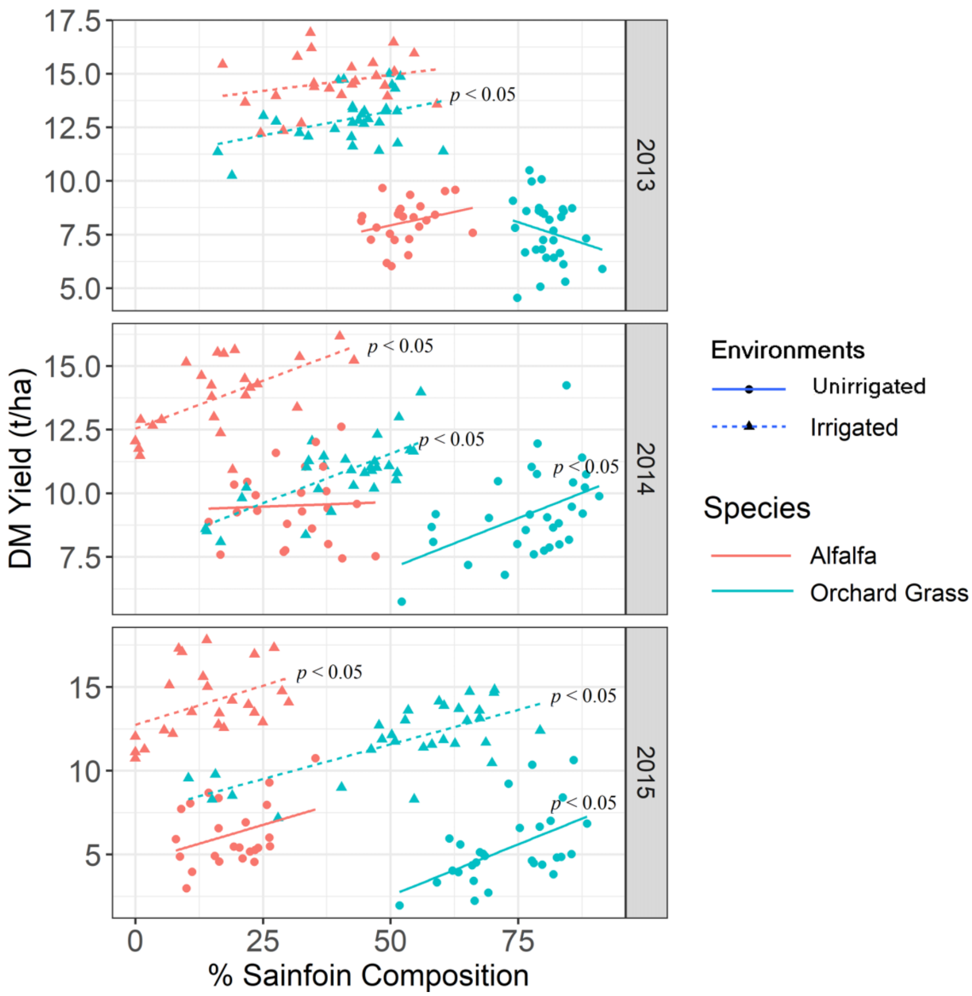


Table 2. DMY of the mixed and monoculture sward averaged across years (2013–2016) for Experiment II. DMY represents sum of three harvests per year. The significance is based on the one-sided *P* value compared with the Nova-monoculture mixture. The value within parenthesis represents % DMY of Nova monoculture.

Alfalfa cultivar	Sainfoin population						
	Nova ($t\,ha^{-1}$)	Nova-161 ($t\,ha^{-1}$)	RM-Remont ($t\,ha^{-1}$)	Melrose ($t\,ha^{-1}$)	LRC-3900 ($t\,ha^{-1}$)	LRC-4012 ($t\,ha^{-1}$)	LRC-3519 ($t\,ha^{-1}$)
Monoculture	11.7 (100)	13.3 (113)*	13.2 (112)*	13.2 (112)*	12.5 (107)	12.9 (110)	13.4 (114)**
Beaver	12 (103)	12.8 (109)	13.6 (116)**	12.8 (109)	13.9 (118)**	13.9 (119)**	13.9 (119)**
Longview	12.7 (108)	14.4 (123)***	14.8 (126)***	14.5 (124)***	15.1 (128)***	15.4 (131)***	15.5 (132)***
Nordic	11.5 (98)	12 (102)	13.7 (117)**	12 (103)	12.9 (110)	12.6 (108)	13.3 (114)*
Rangelander	11.1 (95)	12.9 (110)	13.4 (114)*	12.7 (108)	12.5 (107)	12.7 (108)	12.8 (110)
Yellowhead	11.3 (96)	12.3 (105)	10.9 (93)	11.7 (100)	11.9 (102)	12.5 (107)	13.4 (114)*

Note: DMY represents sum of three harvests per year. The significance is based on the one-sided *P* value compared with the Nova-monoculture mixture. The value within parentheses represents % DMY of Nova monoculture. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

Experiment II (comparison of sainfoin in monoculture vs. in mixture with alfalfa)

Dry matter yield

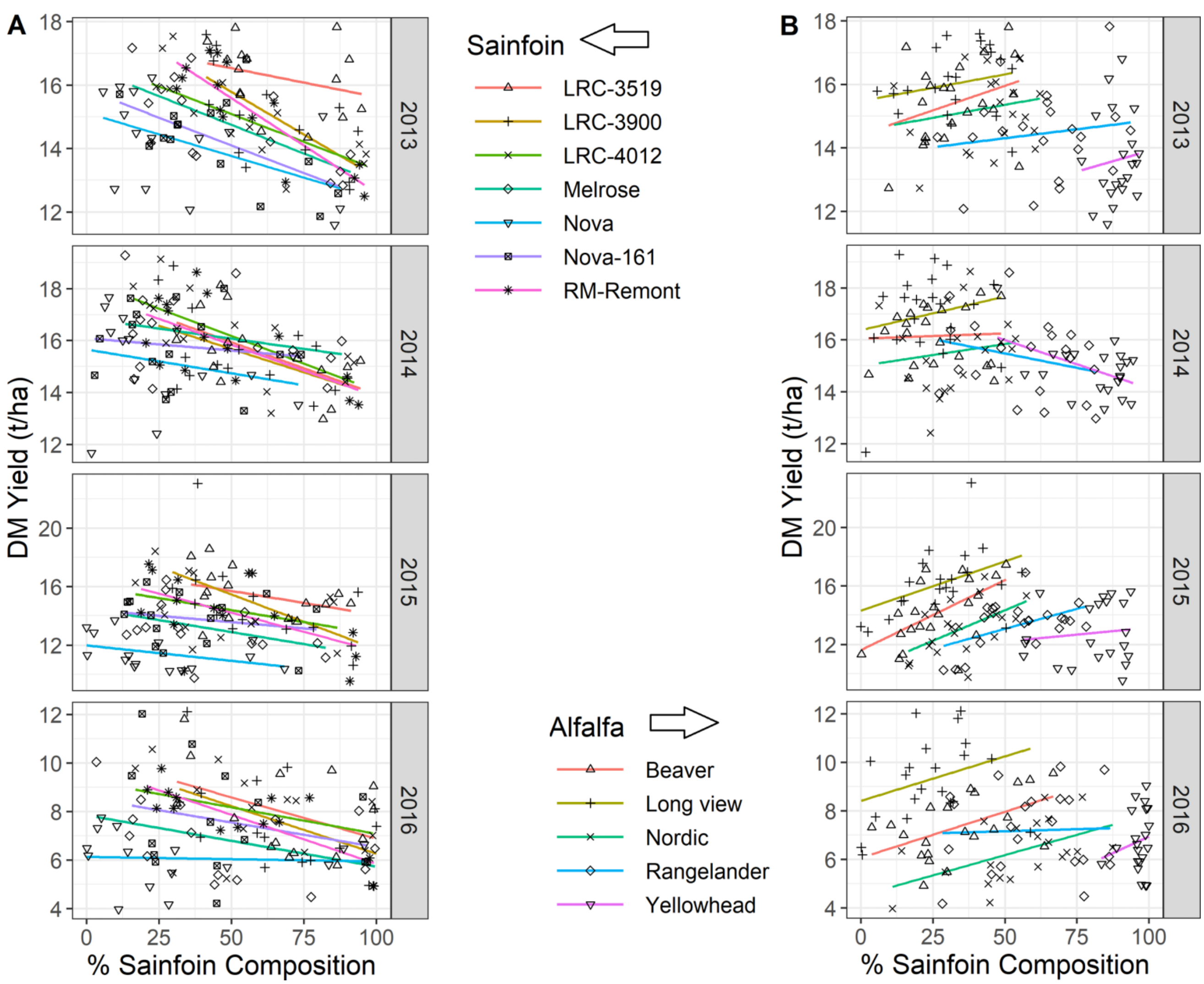
The effects of sainfoin population, alfalfa cultivar, year, and their interactions were significant ($P < 0.05$), except sainfoin × alfalfa and the three-way interaction for DMY (Table S3). Year had high variability in DMY suggesting that the analysis should be performed separately by year. The second production year (third year of establishment) was most productive ($15.6\,t\,ha^{-1}$ DM) and the DMY decreased to $7.5\,t\,ha^{-1}$ in the fourth production year (Fig. S1). Sainfoin population LRC-

Table 3. Sainfoin composition [% DM basis (% Nova–Beaver)] of the mixed sainfoin–alfalfa sward averaged across cuts and all years (2013–2016) for Experiment II.

Alfalfa cultivar	Sainfoin population						
	Nova	Nova-161	RM-Remont	Melrose	LRC-3900	LRC-4012	LRC-3519
Beaver	13 (100)	20.5 (157)*	31.8 (244)***	20 (154)	42.9 (329)***	31 (238)***	50.8 (389)***
Longview	6.2 (48)	21.1 (162)	31.9 (244)***	21.6 (166)*	40.5 (311)***	26.2 (201)**	40.6 (311)***
Nordic	17.3 (132)	36.2 (277)***	48.7 (374)***	36.9 (283)***	53.7 (412)***	47.7 (366)***	54.5 (418)***
Rangelander	35.6 (273)***	45.5 (349)***	53.9 (413)***	59.5 (456)***	76 (583)***	62.8 (482)***	80.1 (615)***
Yellowhead	75.5 (579)***	77.9 (598)***	94 (721)***	85.7 (657)***	90.1 (691)***	92.3 (708)***	91.2 (699)***

Note: The significance is based on the one-sided *P* value compared with the Nova–Beaver mixture. The value within parentheses represents % sainfoin composition relative to Nova–Beaver mixture. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

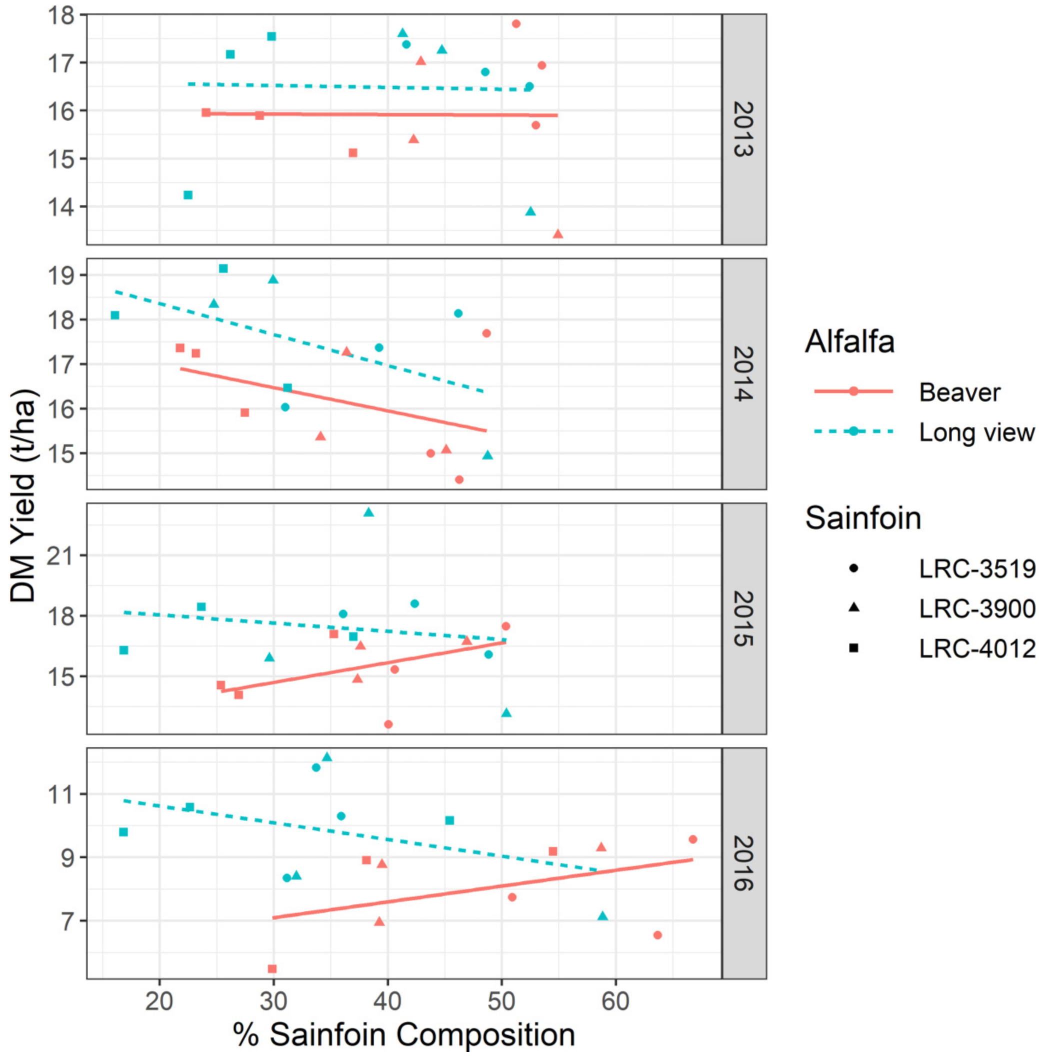
Fig. 4. Linear regression of DMY with % sainfoin composition (DM basis) for Experiment II: (A) for sainfoin populations averaged across alfalfa cultivars and (B) for alfalfa cultivars averaged across sainfoin populations. The percentage of sainfoin composition represents average of % sainfoin composition by harvests within a year.



3519 notably exhibited higher compatibility with all alfalfa cultivars (Fig. S1 and Table S2) and the selected progeny from this population had been released as a high-yielding sainfoin cultivar with high compatibility to alfalfa (cv. Mountainview; Acharya 2018).

All three new sainfoin populations mixed with alfalfa cvs. Beaver, RM-Remont, and Longview produced greater herbage mass than sainfoin cv. Nova in pure stand (Table 2). None of the sainfoin populations mixed with alfalfa cvs. Nordic, Rangelander, and Yellowhead produced significantly

Fig. 5. Linear regression of DMY with % sainfoin contribution for alfalfa cultivars (Beaver and Longview) averaged across selected new sainfoin populations for Experiment II.



greater DMY compared with their respective monocultures ($P > 0.05$).

Percentage contribution by sainfoin (% sainfoin DM)

The effects of sainfoin population, alfalfa cultivar, and year, and their interactions were significant ($P < 0.05$, Table S2). The percentage of sainfoin across all years on all sainfoin populations except Nova was greater (>162%) compared with Nova–Beaver mixture with a significantly higher proportion of sainfoin on alfalfa cvs. Rangelander and Yellowhead (>273% of Nova–Beaver). All sainfoin populations had higher proportion of sainfoin in mixture with Yellowhead alfalfa (>0.6) across each years.

The compatibility of sainfoin cultivar is considered to be good when its percentage contribution in the mixture is between 20% and 50%, which is necessary for the pasture to be bloat-free without compromising DMY. Yellowhead and Rangelander alfalfa were considered incompatible with any of the new sainfoin populations as these mixtures had significantly lower biomass yield, rendering their proportion in mixture lower than the critical level to prevent bloat. Nova–Longview mixture was considered incompatible with only 6.2% sainfoin in the mixture (Table 3), which may not be sufficient to make the pasture bloat-free. Rangelander alfalfa had significantly lower DM contribution with new sainfoin populations but had higher proportion with older sainfoin cultivar Nova (Fig. S2) suggesting that it is the only compatible alfalfa cultivar with Nova.

Relationship between DMY and % sainfoin contribution

Year had a significant role in DMY. Therefore, the relationship between DMY and % sainfoin contribution was reported by year (Fig. 4). Generally, the DMY of mixed sward decreased (13 out of 28 regressions, $P < 0.05$) with increase in % sainfoin contribution when regressed across the average of alfalfa cultivars (Fig. 4A). Conversely, in one-third of the cases, the DMY of mixed sward increased with increase in % sainfoin contribution when regressed across average of the sainfoin populations (7 out of 20, Fig. 4B, Table S4). This inconsistency was due to huge alfalfa × sainfoin interaction (Table S3).

The regression fitted by selecting new sainfoin populations on alfalfa cvs. Beaver and Longview indicated that the DMY of the mixed sward did not change with the change in % of sainfoin (Fig. 5). The results suggested that new sainfoin populations can be used in the mixture with Longview or Beaver alfalfa without compromising DMY. This relationship remained consistent in the results obtained from Exp. I.

Discussion

Our objective was to determine whether new sainfoin populations can be used for mixed stands with alfalfa and grass for improved productivity and bloat control in western Canada. From these experimental results, the answer can be "yes". We can infer this based on the higher DMY and % sainfoin composition in the mixture compared with the monoculture of each species (Figs. 1 and 4). Such positive mixture effect of sainfoin on yield and weed suppression has been reported with other grass and forage legumes (Acharya et al. 2013; Malisch et al. 2017; Biligetu et al. 2021), but the information on orchardgrass is novel. The positive mixture effects on DMY could be attributed to the maximum utilization of available resources both above and below ground owing to the differential root depth of different species (Skinner and Comas 2010), provided there is no allelopathy effect. The combination of the deep taproot of sainfoin with the relatively shallow-rooted orchardgrass may lead to spatial complementarity in nutrient uptake that leads to the increased DMY of the mixture. Even though grasses have a lower water requirement and use water more efficiently than forage legumes, the DMY of orchardgrass in our experiment was poor in water-limiting environments because of their shallow roots compared with deep taproots of the sainfoin.

Tall fescue is a potential alternative to orchardgrass for irrigated forage production in monoculture and mixtures with alfalfa in southern British Columbia because of its higher biomass yield and better survival in mixed stands (Thompson 2013). However, tall fescue is less drought-tolerant compared with orchardgrass due to its lower root mass and root length in stressed conditions (Skinner and Comas 2010). This suggests that orchardgrass is better adapted than tall fescue in Canadian prairies.

Major challenges with sainfoin include lack of persistence in monoculture or mixtures (Kilcher 1982; Kielly et al. 1994; Sheppard et al. 2019) and 20% lower biomass yield compared with alfalfa (Goplen et al. 1991; McMahon et al. 1999; Sheppard et al. 2019). The sainfoin persistence can be improved by selecting compatible cultivars and (or) by adjusting intercropping arrangements. For example, in Swift Current, SK, sainfoin did not persist beyond 2 years when seeded in the mixture with Russian wildryegrass (*Psatlryrosnchys iuncea* Fisch. Nevski), but its persistence was increased to 4 years by seeding in alternate rows (Kilcher 1982). In another study by Kielly et al. 1994, the alfalfa–sainfoin mixed swards were dominated by alfalfa as early as 2 years of seeding due to drought stress. The stand persistence of sainfoin with alfalfa improved in new cultivars directly selected for improved persistence in alfalfa mixed stand at Lethbridge, AB. These new sainfoin cultivars when seeded in alternate rows with alfalfa retained at least 15% sainfoin after 3 or 4 years of establishment (Acharya et al. 2013; Acharya 2015, 2018). These new sainfoin populations also had increased ability for sod seeding with alfalfa in irrigated environments (Khatiwada et al. 2020). Khatiwada et al. (2020) reported greater performance of new sainfoin populations for sod seeding compared with the cicer milkvetch cultivars. These mixtures significantly reduced alfalfa pasture bloat in grazing ruminants (McMahon et al. 1999; Sottie et al. 2014) adding more value to the sainfoin. In addition, the sainfoin–alfalfa mixture provides bloat-free pasture for grazing, controls weeds, and has antiparasitic activity (Häring et al. 2008). We also noticed a non-negative correlation between % sainfoin and the total DMY in the mixtures (Exp. I and II). These observations indicate complementary effect of mixtures and further suggest new sainfoin populations have better compatibility with alfalfa compared with cv. Nova.

There was 64% reduction in the percentage of sainfoin when grown with alfalfa (in both irrigated and unirrigated trials in Exp. I), but the overall DMY reduced by only 4% in irrigated and 17% in unirrigated trials at the end of 3 years. The higher yield reduction under unirrigated condition could be due to lower than average precipitation (234 mm) in 2015 compared with long-term average (380 mm) (Fig. S3). In Exp. II, reduction in percentage of sainfoin in the mixtures was not observed for new populations, but yield reduction was similar to that of Nova (~50%) in the fourth production year. This was consistent with the previous study by Acharya et al. (2013), which suggested sainfoin–alfalfa/grass mixture had stable productivity for at least 3 years in both irrigated and unirrigated environments of Lethbridge, AB and Saskatoon, SK. Therefore, it would be advisable to re-establish the alfalfa–sainfoin or sainfoin–orchardgrass pasture every 3 or 4 years to maximize productivity. The yield reduction in third production year was also observed in alfalfa monoculture at Lethbridge even though some reports suggest alfalfa stands last up to more than 10 years without significant yield loss at several locations, including Nova Scotia (Suzuki 1991). However, Atlantic region of Canada is not suitable for sainfoin as the present cultivars are not adapted to high soil moisture. Yield decline is challenge in the semi-arid regions of western Canada where severe drought episodes debilitate sainfoin stand reducing its longevity. It is also to be noted that in all of these tests in western Canada, there were usually 3 cuts/year in irrigated locations and 2 cuts/year in unirrigated trials. The lack of persistence in unirrigated areas could be due to drought episodes and in irrigated areas could be due to 3 cuts/year limiting nutrient reserve in the crown for overwintering. In western Canada, third cut of alfalfa is extremely critical as it determines the rate of winter survival (Bélanger et al. 1999).

Western Canada requires extremely winter hardy alfalfa cultivars that are fall dormant. Winter hardy alfalfa plants generally are fall dormant but less productive (Schwab et al. 1996; Brouwer et al. 2000; Brummer et al. 2000; Castonguay et al. 2006; Wang et al. 2009). Even though the two traits are related, the genes regulating the two traits are not necessarily the same (Adhikari et al. 2018). This is reflected in a study by Weishaar et al. (2005), where selection for winter hardiness decreased autumn plant height without change in the DMY. In newer cultivars, the relationship between fall dormancy and winter hardiness has been partially broken by plant breeding efforts such that fall dormant genotypes with intermediate ratings (3–6) are more winter hardy and these relatively non-dormant genotypes contribute to higher productivity. In a recent study by Claessens et al. (2022), one-unit fall dormancy ratings and significant DMY increase were achieved within one cycle of selection without compromising winter survival in alfalfa cv. Peace. A standard rating system of cold hardiness and fall dormancy similar to alfalfa has not been developed for sainfoin. Development of a similar fall dormancy rating and its association with winter hardiness in sainfoin may help standardize approach in this crop.

Conclusions

The performance of monoculture orchardgrass was lower compared with the mixture in both irrigated and unirrigated environments. The low DMY of monoculture orchardgrass under unirrigated environment for all 3 years suggests that orchardgrass is not successful without irrigation in semi-arid regions of western Canada. However, orchardgrass in mixture with sainfoin had a huge advantage over monoculture orchardgrass in both the environments. Additionally, use of newer sainfoin populations with orchardgrass mixture can supplement the productivity of forages in dry years.

In general, newer sainfoin populations had good compatibility to alfalfa and comparable yield to the best alfalfa cultivar in monoculture under irrigated conditions. The DMY of alfalfa–sainfoin mixture was higher than or at least not lower than any of the sainfoin monocultures. DMY of new sainfoin population was consistently higher with Beaver and Longview alfalfa and may be the recommended choice in western Canada. Conversely, sainfoin composition was consistently higher with Rangelander and Yellowhead alfalfa that may be suitable for high-ratio sainfoin pastures/hay. In drier areas, sainfoin–alfalfa mixture produced low herbage mass compared with pure Longview alfalfa and could be attributed to lower drought tolerance of sainfoin. Future breeding programs should focus on improving drought tolerance of sainfoin for widening its adaptability to the semi-arid regions in western Canada.

Author contributions

Hari Prasad Poudel: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review and editing. Surya N. Acharya Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review and editing.

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Supplementary material

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