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Agronomic Characteristics and Nutritive Value of Purple Prairie Clover (*Dalea purpurea* Vent) Grown in Irrigated and Dryland Conditions in Western Canada †

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Abstract

Three purple prairie clover (PPC; *Dalea purpurea* Vent.) varieties, namely Common seed (CS), AC Lamour (ACL) and Bismarck (BIS), were established in plots of irrigated land (rain-fed plus irrigation, Lethbridge, AB) and dryland (rain-fed only, Swift Current, SK) to assess its agronomic characteristics and nutritive value under different ecoclimate and growing conditions in Western Canada. Each seed source was replicated in four test plots arranged as a randomized complete block design at each experimental site. Forage mass on dry matter (DM) basis, canopy height, proportions of leaf and stem and nutritive value were determined at vegetative (VEG), full flower (FF) and late flower (LF) phenological stages. The forage masses of the three PPC varieties were similar ($p < 0.05$) at each phenological stage with the mean values for VFG, FF and LF being 4739, 4988 and 6753 kg DM/ha under the Lethbridge irrigated conditions, and 1423, 2014 and 2297 kg DM/ha under the Swift Current dryland conditions. The forage mass was higher ($p < 0.001$) under Lethbridge irrigation than under Swift Current dryland conditions and increased ($p < 0.05$) with maturity. The three varieties had similar concentrations of organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and crude protein (CP) and in vitro DM digestibility (DMD) at each phenological stage, but CP concentration and in vitro DMD decreased ($p < 0.001$) whilst NDF and ADF concentration increased ($p < 0.001$) with maturity. Purple prairie clover grown at Lethbridge irrigated land had higher ($p < 0.001$) DMD, OM and CP, but lower ($p < 0.001$) NDF, ADF and condensed tannin concentrations than that grown at Swift Current dryland conditions. These results indicate that PPC has great potential as an alternative legume forage for the cattle industry.

Keywords: agronomic traits; growing conditions; nutritive value; purple prairie clover

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1. Introduction

Alfalfa (*Medicago Sativa* L.) is the most common legume forage grown in Canada, but its propensity to cause bloat limits its use in grazed pastures. Condensed tannin (CT) containing forages are considered to be bloat-safe and possess beneficial antioxidant, antimicrobial, antiparasitic, anti-methanogenic and improved protein utilization properties [1,2]. However, only sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus*)

are currently offered as legumes with these properties in Western Canada. Therefore, increasing the availability and diversity of CT-containing legume forages with high nutritive value has the potential to increase the sustainability of ruminant production [1].

The native legume, purple prairie clover (PPC; *Dalea purpurea* Vent.) is distributed throughout the North American prairies. It is considered an important palatable component of prairie hay [3,4] and was used by indigenous North Americans to treat various health conditions as it contains bioactives including terpenoids, flavonols and isoflavones [5–7]. Previous studies showed that PPC contains a high level of CT [8–10] with strong anti-*Escherichia coli* O157:H7 activity [11,12]. The high concentration (as high as 9.5% in the whole plant has been reported) of CT in PPC was shown to decrease ruminal protein degradation, with minimum negative effects on ruminal fermentation due to its specific CT structure of the predominancy of procyanidins [13–15]. Compared to alfalfa hay, PPC hay had been found to have greater dry matter (DM; 67.7 vs. 59.8%), organic matter (OM; 66.9 vs. 60.8%) and protein (65.5 vs.61.0%) digestibility, but did not improve the growth of lambs [16]. Therefore, PPC could potentially be a valuable forage, but there is limited information on its basic agronomic characteristics and nutritive value. In addition, because PPC is a native legume species and normally co-exists with other species in native pastures, there is a lack of knowledge on farming and crop management under improved grassland conditions.

The hypothesis of this study was that PPC could potentially grow under different geoclimatic conditions in Western Canada to be used as alternative legume forage. The objective of this study was to determine the forage mass, nutritional composition and agronomic characteristics of different PPC varieties growing at different climate and soil conditions.

2. Materials and Methods

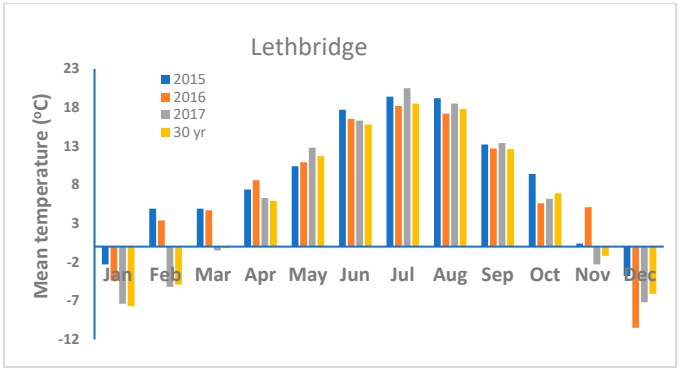
2.1. Experimental Arrangement and Test Plot Establishment

Three PPC varieties, namely Common seed (CS), AC Lamour (ACL) and Bismarck (BIS), were selected and seeded in May of 2015 into irrigated test plots (2 × 6 m; rain-fed plus irrigation) at Lethbridge, AB, and dryland (2 × 6 m; rain-fed only) plots at Swift Current, SK. The soil type at both sites was Swinton silt loam soil (Orthic Brown Chernozem soil; [17]). Common seed (B8019; variety not stated) was obtained from Sunshine Seeds Ltd., Magrath, AB, Canada, whilst ACL was from ecovar^{TM} of Nutrien Ag Solutions Ltd., Lethbridge, AB, Canada, and BIS from USDA NRCS-Bismarck Plant Materials Center, Bismarck, ND, USA. Each of the three seed sources was replicated in four test plots and all the plots were arranged as a randomized complete block design at each location. The Lethbridge plots were seeded using a small plot drill seeder (John Deere, Moline, IL) at a seeding rate of 4 kg/ha with 18 cm row spacing. The Swift Current plots used a small plot drill seeder (self-propelled hydrostatic with double disc opener, AAFC-SCRDC) at the same seeding rate with 30.5 cm row spacing. The row spacing seeded in the Lethbridge irrigated plots (18 cm) and Swift Current dryland plots (30.5 cm) was according to the common practice normally used under these two geoclimatic conditions. Fertilizer (34-17-0; Terrico, Parrish & Heimbecker Ltd., Watrous, SK, Canada) was applied at 112 kg/ha at the time of seeding. The plots were sprayed with glyphosate [N-(phosphonomethyl) glycine] (Cheminova Canada Inc., Oakville, ON, Canada) before seeding for controlling perennial and grassy weeds and annual weeds were controlled by mowing plots twice during the year of establishment. The plots in irrigated land received approximately 75 mm of water per month during the growing season whilst the plots in dryland received only natural precipitation.

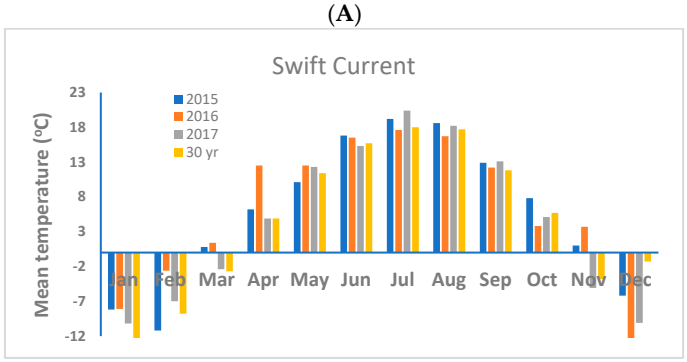
2.2. Weather

The monthly mean temperature and total precipitation (rainfall + snowfall) for both experimental sites in 2015 (plot establishment year) and 2016–2017 (measurement year) are

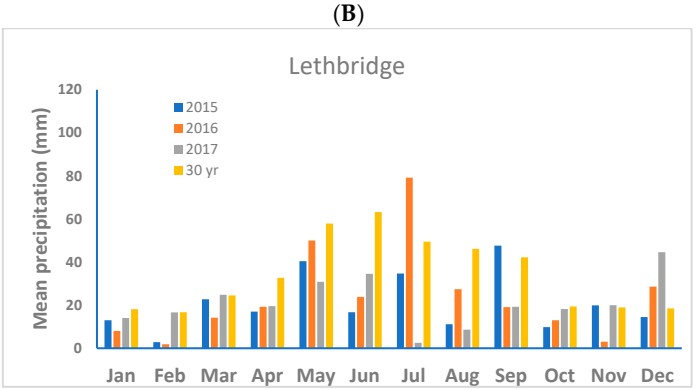
shown in Figure 1. The average monthly temperatures for 2016 and 2017 were variable but followed the same pattern as the long-term (30 years) average for the two locations (Figure 1A,B). Temperatures during the growing season (May-September) in Lethbridge were higher than that in Swift Current, averaging 15.3 and 14.9 °C, respectively. Temperatures in July of 2017 (20.5 °C) were much higher at both sites than the long-term average (18.3 °C), as well as that observed in 2016 (17.9 °C).



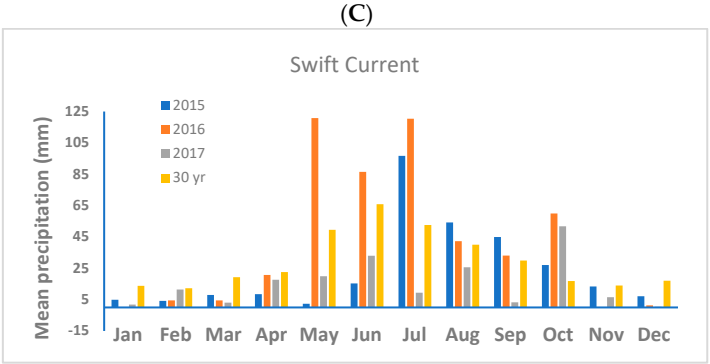
(**A**)



(**B**)



(**C**)



(**D**)

Figure 1. Mean monthly temperature (**A**,**B**) and total monthly precipitation (**C**,**D**) for 2015–2017 and long-term (30 years) average for Lethbridge, AB, and Swift Current, SK, Canada.

The total monthly precipitations were below the long-term (30 years) average (34.0 mm) for both 2016 (23.9 mm) and 2017 (21.1 mm) at Lethbridge (Figure 1C). This was particularly noticeable for the months of May-Sept, which were 39.9, 19.1 and 51. 8 mm for 2016, 2017 and long-term average, respectively. For Swift Current (Figure 1D), the total precipitation was higher in 2016 (41.3 mm), but lower in 2017 (15.3 mm). This was particularly noticeable during the May to Sept growing seasons with a long-term monthly average of 47.5 mm as compared to 80.5 and 18.2 mm in 2016 and 2017, respectively.

2.3. Determination of Agronomic Characteristics

The test plots were sampled during the 2016 (2nd year) growing season to determine forage mass, canopy height and proportions of leaf, stem and flower, with forage mass also determined in 2017 (3rd year). Two rows of whole-plant PPC in each replicate plot were harvested by cutting to 5 cm stubble height using a forage harvester with sickle bar mower (Lethbridge) or a hand sickle (Swift Current) at the vegetative (VEG; 20% flower), full flower (FF; 80% flower) and late flower (LF; or seedpod) stages and immediately weighed. The flower buds (inflorescences) of PPC were cylindrical-shaped spikes located at the stem ends, which are first formed in vegetative PPC. Thus VEG PPC includes flower buds. Harvested forage from each plot was divided into three samples. One sample was freeze-dried for the determination of nutritive value. The second sample was oven-dried at 105 °C for 48 h to determine DM concentration, and forage mass (kg DM/ha) was calculated based on the size of the plot. The third sample was manually dissected to determine leaf, stem and flower proportions. Canopy height was manually measured with a ruler from the ground to the apical meristem for at least 20 plants per plot and averaged.

2.4. Determination of Nutritive Value

The freeze-dried whole plant PPC harvested in 2016 and 2017 was ground through a 1 mm screen using a Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia, PA, USA). Organic matter was analyzed by AOAC method [18]. Neutral detergent fibre (NDF) was determined with the addition of sodium sulfite and α-amylase, while acid detergent fibre (ADF) was analyzed as per AOAC [18]. Both procedures were modified for use in an Ankom 200 system (Ankom Technology Corporation, Fairport, NY, USA). The samples were analyzed for total N by elemental analysis (NA1500 Nitrogen/Carbon analyzer, Carlo Erba Instruments, Milan, Italy) and multiplied by 6.25 to estimate crude protein (CP) concentration. Ground samples (5 g) were combined with 95 g of de-ionized H_2O, blended for 30 s, boiled for 10 min and then centrifuged at 5000× *g* for 15 min to obtain supernatant for analysis of water-soluble carbohydrate (WSC; glucose equivalent) as described by Zahiroddini et al. [19]. Extractable CT was extracted from the whole plant samples and determined as described by Terrill et al. [20] using CT purified from the whole PPC plants as a standard [21].

For mineral analysis (2016 samples only), the ground PPC samples (250 mg) were digested in a mixture of nitric acid (HNO_3), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) in an open vessel digestion tube according to the EPA 3050B method [22]. This procedure dissolves all minerals except silica. A Spectro ARCOS MV Inductively Coupled Plasma-Optical Emission Spectrometer (Spectro Analytical Instruments GmbH, Boschstr, Kleve, Germany) in the radial view was used to identify and quantify minerals in the digest.

Whole-plant samples were randomly selected from 3 of 4 plots for each variety at each experimental site and used to determine 48 h in vitro DM digestibility (DMD). Anaerobic incubations (48 h) were conducted by placing ground PPC (0.5 g) in acetone-washed, pre-weighted F57 filter bags (pore size of 25 µm; ANKOM Technology Corp., Macedon, NY, US) with 60 mL ruminal inoculum that contained one part of rumen fluid and two

parts of mineral buffer [23] in 150 mL serum vials. Rumen fluid was collected from three healthy and rumen cannulated non-lactating cows (523 ± 5.58 kg body weight) fed a diet containing 50% alfalfa hay, 45% barley silage and 5% mineral and vitamin supplement (DM basis) for two weeks prior to rumen fluid collection. Cattle were fed twice daily, had free access to water and were cared for in accordance with the standards of the Canadian Council on Animal Care [24]. The animal care protocol and experimental procedure were approved by the Lethbridge Research and Development Centre Animal Care Committee (Protocol # 1710; 2017). Rumen content was collected with a plastic cup via the rumen cannula from five locations within the rumen, strained through four layers of cheesecloth, combined in equal portions from the three cattle and transported to the lab in a thermos container. The cattle were returned to the normal herd after the experiment. The procedure of the in vitro incubation was according to that described by Peng et al. [14]. Ankom bags were retrieved after 48 h of incubation and rinsed with the running tap water until the water became clear and the residual DM was determined after oven-drying at 60 °C to constant weight. The 48 h DMD was calculated by the weight difference in DM before and after incubation. Triplicate incubations of each sample were conducted within two weeks with duplicate vials per incubation. Therefore there were 18 measurements for each variety at each harvest stage.

2.5. Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (version 9.3.1) [25] by analysis of variance. The initial analysis including variety, experimental site, plant phenological stage and growth year as main effects in the model showed significant differences between two years and two experimental site samples. Therefore, separate analysis for each year and experimental site was subsequently conducted and reported in the results. For each analysis, the PPC variety and maturity stage were the main effects with the forage plot as a random factor. Differences were determined using LSMEANS with the PDIFF option in SAS [25] and significance was declared at $p \leq 0.05$.

3. Results

3.1. Agronomic Characteristics

Only one cut was obtained for all three PPC varieties which reached FF from late July to late Aug depending on the experimental site and weather conditions. Regrowth was slow and was unable to reach the flower stage by the end of the growing season at both experimental sites. Purple prairie clover grown at Lethbridge irrigation plots were harvested on 5 August, 24 August and 20 September in 2016 for the VEG, FF and LF stages, respectively. However, in 2017, the harvest dates were earlier (7 and 18 July and 11 August) due to the hot and dry conditions. Harvest time for the VEG and FF stages was similar between 2016 (14 July and 2 August) and 2017 (12 July and 3 August) at Swift Current. However, PPC at the LF stage was harvested on 21 September 2016 and on 28 August 2017 due to the extremely hot weather in 2017. In general, PPC grown under irrigation had higher ($p < 0.001$) forage mass in 2016 and 2017, but lower ($p < 0.05$) canopy height irrespective of variety or maturity.

3.1.1. Irrigated Plots

In 2016, there was no variety × maturity interaction for any of the agronomic traits measured (Table 1). The three PPC varieties had similar average forage mass (kg DM/ha) at the three growth stages with forage mass increasing as the plant matures. For all the varieties, forage mass (kg DM/ha) was considerably higher ($p < 0.001$) at LF (8030) than at the VEG (3622) and FF (4051) stages. In 2017, however, although the CS variety produced

less ($p < 0.05$) forage mass than that of BIS and ACL, there was no difference among the three maturities of averaged three varieties, with 5854, 5923 and 5500 kg DM/ha for VEG, FF and LF stages, respectively.

Canopy height varied ($p < 0.05$) among the PPC varieties. On average, the canopy height increased from VEG (36 cm) to FF (40 cm) and LF (41 cm). However, CS had a lower ($p < 0.01$) canopy height than BIS for all three maturity stages with ACL being intermediate. All three varieties had similar proportions of leaf and stem. As the plant matured the leaf proportion decreased ($p < 0.05$) from VEG (47.9%) to FF (32.3%) and LF (26.7%) and the corresponding stem proportion increased.

3.1.2. Dryland Plots

Unlike with the irrigation plots in Lethbridge, variety × maturity interaction was observed that affected ($p < 0.05$) the forage mass of PPC in 2016 but not in 2017 in the Swift Current dryland plots. In general, BIS had higher ($p < 0.01$) DM yield than ACL and CS, but no difference was observed between ACL and CS in 2016. However, in 2017, CS had a higher ($p < 0.05$) forage mass than ACL. Similarly to that observed with Lethbridge irrigated plots, irrespective of the variety forage mass (kg DM/ha) increased ($p < 0.05$) with plant maturity with 1010, 1786 and 2482 in 2016 and 1853, 2241 and 2111 in 2017 for VEG, FF and LF, respectively.

There was no variety × maturity interaction for canopy height or proportions of leaf and stem for PPC grown on dryland in Swift Current. Common seed had higher ($p < 0.05$) canopy height than BIS at the VEG and FF stages and than ACL and BIS at the LF stage, with all three varieties having similar proportions of leaf and stem. With advancing maturity, canopy height increased ($p < 0.01$) and leaf proportion decreased ($p < 0.001$).

3.2. Nutritive Value

The initial analysis showed that regardless of variety, maturity, or year, PPC grown at the Lethbridge irrigated plots contained higher ($p < 0.05$–0.001) concentrations of DM, OM and CP, but lower ($p < 0.001$) concentrations of ADF, NDF and CT than that grown at the Swift Current dryland plots (Tables 2 and 3). The PPC grown in Lethbridge also had greater ($p < 0.001$) DMD than that grown in the Swift Current dryland, irrespective of growth stage. For the mineral concentration, the Lethbridge irrigated land samples had higher ($p < 0.01$) concentrations of K, Mo and Na (Table 4), but lower ($p < 0.05$~0.001) concentrations of Al, Cr, Cu, Fe, Mn, Ni and P than the Swift Current dryland samples (Table 5). Samples from both experimental sites had similar concentrations of B, Ca, Mg, Mn, S and Zn.

3.2.1. Irrigated Plots

No variety × maturity interaction was observed for 2016 except for OM concentration (Table 2). The three PPC varieties contained similar DM irrespective of growth stage; however, the DM concentration in the whole plant increased ($p < 0.001$) with maturity from the VEG (33.3%), FF (37.6%) to LF (41.7%) stages, respectively. Interaction between the PPC variety and maturity was observed for OM ($p < 0.05$) in that CS had higher ($p < 0.05$) OM concentration than ACL at the FF stage but not at the other stages. No difference was observed among the three varieties in CP, NDF, ADF, WSC, CT or 48 h DMD. However, with advancing maturity, CP concentration decreased (18.5 vs. 15.8 vs. 13.1%; $p < 0.001$) whilst NDF (38.0 vs. 42.0 vs. 43.4%) and ADF (28.6 vs. 35.2 vs. 34.5%) concentrations increased ($p < 0.001$), irrespective of PPC variety. The 48 h DMD was higher for PPC harvested at VEG (61.8%) as compared to FF and LF (56.9 and 56.9%). However, WSC and CT were not affected by variety or maturity. Average CT concentrations across three varieties were 5.74, 6.51 and 6.36% for the VEG, FF and LF stages, respectively.

Table 1. Agronomic characteristics of three varieties of purple prairie clover grown in irrigated land at Lethbridge, AB, and dryland at Swift Current, SK (2016, 2017; $n = 4$).

	AC Lamour (ACL)			Bismarck (BIS)			Common Seed (CS)			SEM	*p* Values		
	VEG	FF	LF	VEG	FF	LF	VEG	FF	LF		Variety (V)	Maturity (M)	V × M
Irrigated plots													
Forage mass, kg/ha, 2016	2557.0 ^{c}	3294.4 ^{c}	8450.9 ^{a}	4806.2 ^{abc}	4295.3 ^{bc}	7752.2 ^{ab}	3503.1 ^{c}	4564.9 ^{abc}	7886.9 ^{ab}	825.84	0.457	<0.001	0.456
Forage mass, kg/ha, 2017	6038.9	6757.4	5776.3	6045.3	7050.7	6115.6	5480.9	3962.8	4309.9	813.18	0.021	0.794	0.570
Canopy height, cm	37.0 ^{ab}	40.7 ^{a}	39.75 ^{a}	41.8 ^{a}	45.1 ^{a}	43.5 ^{a}	29.6 ^{b}	38.6 ^{ab}	37.0 ^{ab}	1.97	0.001	0.011	0.532
Leaf, %	46.7 ^{abc}	32.7 ^{bcd}	23.8 ^{d}	47.5 ^{ab}	33.9 ^{abcd}	26.4 ^{d}	49.7 ^{a}	30.3 ^{cd}	30.0 ^{bc}	3.39	0.713	<0.001	0.737
Stem,%	53.3 ^{bcd}	67.3 ^{abc}	76.22 ^{a}	52.5 ^{cd}	66.1 ^{abcd}	73.6 ^{a}	50.3 ^{d}	69.7 ^{ab}	70.0 ^{cd}	3.39	0.713	<0.001	0.737
Dryland plots													
Forage mass, kg/ha, 2016	863.2 ^{b}	1476.6 ^{b}	1465.6 ^{b}	1163.1 ^{b}	2047.6 ^{b}	4145.2 ^{a}	1004.6 ^{b}	1834.3 ^{b}	1836.7 ^{b}	393.26	0.003	<0.001	0.021
Forage mass, kg/ha, 2017	1623.0	2057.5	2053.3	1851.5	2134.7	2079.1	2031.1	2531.6	2199.6	197.32	0.114	0.052	0.897
Canopy height, cm	41.8 ^{bc}	45.0 ^{abc}	46.8 ^{abc}	39.5 ^{c}	42.7 ^{bc}	47.5 ^{abc}	46.3 ^{abc}	50.1 ^{a}	53.7 ^{a}	1.84	<0.001	0.001	0.915
Leaf, %	46.1 ^{a}	31.5 ^{c}	_	49.4 ^{a}	30.7 ^{c}	_	42.7 ^{ab}	33.0 ^{bc}	_	2.24	0.629	<0.001	0.163
Stem, %	54.0 ^{c}	68.5 ^{a}	_	50.6 ^{c}	69.3 ^{a}	_	57.3 ^{bc}	67.0 ^{ab}	_	2.24	0.629	<0.001	0.163

FF, full flower stage; LF, late flower stage; SEM, standard of means; VEG, vegetative stage. $^{a–d}$, means within a row without the same letter differ at $p < 0.05$. Canopy height and other plant characteristics were measured in 2016 only.

Table 2. Chemical composition (DM basis) of purple prairie clove grown on irrigated land at Lethbridge, AB, and on dryland at Swift Current, SK (2016, $n = 4$).

	AC Lamour (ACL)			Bismarck (BIS)			Common Seed (CS)			SEM	*p* Values		
	VEG	FF	LF	VEG	FF	LF	VEG	FF	LF		Variety (V)	Maturity (M)	V × M
Irrigated plots													
DM, %	34.2 ^{cd}	36.7 ^{abcd}	43.4 ^{a}	33.7 ^{cd}	35.9 ^{bcd}	42.1 ^{ab}	32.1 ^{d}	40.0 ^{abc}	39.7 ^{abc}	1.50	0.729	<0.001	0.147
OM, %	95.8 ^{ab}	94.6 ^{b}	96.1 ^{ab}	95.9 ^{ab}	95.7 ^{ab}	97.1 ^{ab}	95.6 ^{ab}	98.0 ^{a}	96.7 ^{ab}	0.51	0.022	0.168	0.019
CP, %	19.1 ^{a}	15.9 ^{bcd}	13.0 ^{c}	17.8 ^{ab}	16.7 ^{abc}	13.3 ^{e}	18.6 ^{ab}	14.7 ^{cde}	13.0 ^{de}	0.57	0.435	<0.001	0.172
NDF, %	38.0 ^{c}	41.2 ^{abc}	43.4 ^{abc}	38.2 ^{bc}	41.0 ^{abc}	44.6 ^{a}	37.7 ^{c}	43.9 ^{ab}	42.1 ^{abc}	1.18	0.889	<0.001	0.253
ADF, %	29.0 ^{bc}	35.0 ^{ab}	34.1 ^{ab}	29.7 ^{bc}	33.0 ^{abc}	33.6 ^{abc}	27.0 ^{c}	37.5 ^{a}	35.8 ^{ab}	1.41	0.510	<0.001	0.173
WSC, %	13.4	11.6	12.8	13.0	12.6	14.0	12.8	11.6	12.0	1.14	0.522	0.445	0.923
CT, %	6.0	6.5	6.9	4.9	6.8	5.3	6.3	6.4	6.9	0.44	0.060	0.110	0.117
DMD, %	60.52 ^{ab}	54.79 ^{b}	54.13 ^{b}	60.08 ^{ab}	59.83 ^{ab}	57.75 ^{ab}	64.85 ^{a}	56.19 ^{ab}	57.14 ^{ab}	1.94	0.127	0.002	0.297

Table 2. *Cont.*

	AC Lamour (ACL)			Bismarck (BIS)			Common Seed (CS)			SEM	*p* Values		
	VEG	FF	LF	VEG	FF	LF	VEG	FF	LF		Variety (V)	Maturity (M)	V × M
						Dryland plots							
DM, %	25.4 ^{d}	33.4 ^{c}	52.7 ^{a}	25.4 ^{d}	34.8 ^{c}	44.1 ^{b}	24.8 ^{d}	34.2 ^{c}	52.0 ^{a}	0.81	0.002	<0.001	<0.001
OM, %	89.4 ^{cd}	91.7 ^{ab}	91.4 ^{abc}	89.3 ^{d}	92.3 ^{ab}	92.5 ^{a}	90.3 ^{bcd}	92.0 ^{ab}	92.6 ^{a}	0.50	0.101	<0.001	0.485
CP, %	17.4 ^{a}	13.9 ^{bcd}	13.0 ^{cde}	14.5 ^{bc}	12.6 ^{de}	13.0 ^{cde}	17.6 ^{a}	15.1 ^{b}	12.0 ^{e}	0.43	<0.001	<0.001	<0.001
NDF, %	45.9 ^{ab}	46.9 ^{ab}	46.1 ^{ab}	50.4 ^{a}	48.6 ^{ab}	48.2 ^{ab}	47.3 ^{ab}	44.6 ^{b}	50.0 ^{a}	1.17	0.151	0.250	0.025
ADF, %	39.8 ^{abc}	37.8 ^{bc}	39.7 ^{abc}	37.7 ^{bc}	39.4 ^{abc}	41.7 ^{ab}	37.5 ^{bc}	36.7 ^{c}	43.3 ^{a}	1.13	0.788	<0.001	0.025
WSC, %	12.6	13.0	12.6	12.0	12.4	12.8	13.4	12.8	11.6	1.38	0.794	0.676	0.288
CT, %	7.36	7.48	6.95	7.20	7.37	6.88	7.53	7.57	7.11	0.308	0.053	0.087	0.999
DMD, %	57.11 ^{abc}	56.14 ^{abc}	51.83 ^{bc}	61.73 ^{a}	53.13 ^{abc}	50.17 ^{bc}	58.70 ^{ab}	53.71 ^{abc}	48.39 ^{c}	2.37	0.632	<0.001	0.412

ADF, acid detergent fibre; CP, crude protein; CT, condensed tannins; DM, dry matter; DMD, 48 h in vitro dry matter digestibility; FF, full flower stage; LF, late flower stage; NDF, neutral detergent fibre; OM, organic matter; SEM, standard error of means; VEG, vegetative stage; WSC, water-soluble carbohydrates. $^{a–e}$, means within a row without the same letter differ at $p < 0.05$.

Table 3. Chemical composition (DM basis) of purple prairie clove grown on irrigated land at Lethbridge, AB, and on dryland at Swift Current, SK (2017, $n = 4$).

	AC Lamour (ACL)			Bismark (BIS)			Common Seed (CS)			SEM	*p* Values		
	VEG	FF	LF	VEG	FF	LF	VEG	FF	LF		Variety (V)	Maturity (M)	V × M
						Irrigated plots							
DM, %	29.3 ^{c}	37.9 ^{b}	40.7 ^{b}	30.12 ^{c}	38.8 ^{b}	39.0 ^{b}	37.9 ^{b}	39.2 ^{b}	45.2 ^{a}	0.84	<0.001	<0.001	0.001
OM, %	91.8 ^{abc}	92.5 ^{abc}	93.1 ^{ab}	91.4 ^{bc}	92.6 ^{abc}	93.3 ^{ab}	91.0 ^{c}	93.5 ^{a}	93.3 ^{ab}	0.40	0.873	<0.001	0.275
CP, %	12.8 ^{abc}	13.6 ^{a}	11.4 ^{bc}	12.5 ^{ab}	13.2 ^{ab}	10.2 ^{cd}	11.7 ^{bc}	14.0 ^{a}	9.3 ^{d}	0.33	0.018	<0.001	0.022
NDF, %	27.0 ^{c}	41.4 ^{a}	51.3 ^{a}	25.2 ^{c}	40.2 ^{b}	51.6 ^{a}	26.5 ^{c}	36.4 ^{b}	49.9 ^{a}	1.04	0.047	<0.001	0.130
ADF, %	24.5 ^{c}	35.8 ^{b}	41.5 ^{a}	22.1 ^{c}	33.4 ^{b}	41.2 ^{a}	23.0 ^{c}	32.4 ^{b}	42.0 ^{a}	0.75	0.045	<0.001	0.110
						Dryland plots							
DM, %	32.4 ^{d}	38.7 ^{c}	49.9 ^{ab}	31.6 ^{d}	38.9 ^{c}	45.9 ^{b}	30.8 ^{d}	40.2 ^{c}	50.9 ^{ab}	0.98	0.065	<0.001	0.042
OM, %	91.8	93.3	93.2	91.9	92.8	93.4	91.6	92.1	93.1	0.44	0.356	0.001	0.754
CP, %	13.5	11.8	10.9	12.7	11.4	9.8	12.3	11.6	11.9	0.52	0.615	0.083	0.696
NDF, %	49.8	51.6	53.3	48.0	46.9	50.7	53.7	47.4	51.3	1.73	0.104	0.093	0.216
ADF, %	43.0	44.2	46.6	41.6	41.0	45.4	45.6	41.0	44.3	1.32	0.219	0.014	0.166

ADF, acid detergent fibre; CP, crude protein; DM, dry matter; FF, full flower stage; LF, late flower stage; NDF, neutral detergent fibre; OM, organic matter; SEM, standard error of means; VEG, vegetative stage. $^{a–d}$, means within a row without the same letter differ at $p < 0.05$.

Table 4. Mineral element concentrations (dry matter basis) of three varieties of purple prairie clover grown on irrigated land in Lethbridge, AB, and harvested at vegetative (VEG), full flower (FF) and late flower (LF) stages (2016; $n = 4$).

	AC Lamour (ACL)			Bismarck (BIS)			Common Seed (CS)			SEM	*p* Values		
Mineral	**VEG**	**FF**	**LF**	**VEG**	**FF**	**LF**	**VEG**	**FF**	**LF**		**Variety (V)**	**Maturity (M)**	**V × M**
Ca, %	2.10 ^{a}	1.74 ^{abc}	1.62 ^{c}	1.95 ^{abc}	1.62 ^{abc}	1.58 ^{bc}	2.08 ^{abc}	1.55 ^{c}	1.46 ^{c}	0.105	0.333	<0.001	0.780
K, %	1.66	1.54	1.23	1.66	1.61	1.22	1.6	1.44	1.26	0.096	0.710	<0.001	0.853
P, %	0.26 ^{ab}	0.24 ^{abc}	0.16 ^{c}	0.25 ^{abc}	0.26 ^{ab}	0.18 ^{abc}	0.27 ^{a}	0.23 ^{abc}	0.17 ^{bc}	0.019	0.765	<0.001	0.744
S, %	0.20 ^{ab}	0.18 ^{bbc}	0.14 ^{c}	0.20 ^{ab}	0.18 ^{bc}	0.14 ^{c}	0.24 ^{a}	0.20 ^{ab}	0.17 ^{bc}	0.011	0.003	<0.001	0.956
Mg, %	0.31 ^{abc}	0.28 ^{abc}	0.24 ^{bc}	0.27 ^{abc}	0.25 ^{abc}	0.19 ^{c}	0.37 ^{a}	0.31 ^{ab}	0.27 ^{abc}	0.024	0.002	0.003	0.929
Al, mg/kg	158.5	362.9	206.5	151.9	151.0	114.0	218.0	169.2	122.1	67.80	0.186	0.365	0.430
B, mg/kg	31.3 ^{a}	28.2 ^{abc}	26.9 ^{abc}	28.7 ^{abc}	26.2 ^{abc}	23.0 ^{c}	30.1 ^{ab}	28.1 ^{abc}	23.9 ^{bc}	1.41	0.074	0.001	0.885
Cr, mg/kg	8.1	20.0	15.0	6.4	13.0	6.6	14.0	11.7	5.4	3.28	0.124	0.076	0.184
Cu, mg/kg	7.4 ^{a}	7.3 ^{a}	5.7 ^{abc}	7.1 ^{ab}	6.5 ^{abc}	4.6 ^{c}	7.8 ^{a}	7.3 ^{a}	4.9 ^{bc}	0.47	0.113	<0.001	0.783
Fe, mg/kg	261.2	567.8	369.6	241.4	288.1	198.7	364.0	294.3	201.0	92.30	0.129	0.243	0.360
Mn, mg/kg	43.4 ^{a}	41.5 ^{ab}	33.7 ^{abc}	39.0 ^{abc}	34.8 ^{abc}	28.6 ^{abc}	35.4 ^{abc}	24.4 ^{bc}	21.7 ^{c}	3.61	0.002	0.005	0.783
Mo, mg/kg	1.9	2.9	2.1	2.1	2.6	2.1	2.8	3.2	2.5	0.52	0.402	0.262	0.971
Na, mg/kg	31.0	40.3	54.6	28.6	25.8	31.7	39.2	61.4	65.8	10.01	0.027	0.145	0.756
Ni, mg/kg	1.7 ^{b}	2.0 ^{a}	1.7 ^{ab}	1.6 ^{ab}	1.8 ^{ab}	1.3 ^{b}	1.8 ^{ab}	1.7 ^{ab}	1.2 ^{b}	0.15	0.058	0.006	0.518
Zn, mg/kg	23.0 ^{abc}	23.5 ^{ab}	16.7 ^{bcd}	22.1 ^{abcd}	21.4 ^{abcd}	15.2 ^{d}	24.1 ^{a}	22.2 ^{abcd}	16.1 ^{cd}	1.44	0.420	<0.001	0.942

FF, full flower stage; LF, late flower stage; SEM, standard error of means; VEG, vegetative stage. $^{a–d}$, means within a row without the same letter differ at $p < 0.05$.

Table 5. Mineral element concentrations (dry matter basis) of three varieties of purple prairie clover grown on dryland in Swift Current, SK and harvested at vegetative (VEG), full flower (FF) and late flower (LF) stages (2016; $n = 4$).

	AC Lamour (ACL)			Bismarck (BIS)			Common Seed (CS)			SEM	*p* Values		
Minerals	**VEG**	**FF**	**LF**	**VEG**	**FF**	**LF**	**VEG**	**FF**	**LF**		**Variety (V)**	**Maturity (M)**	**V × M**
Ca, %	1.90 ^{ab}	1.71 ^{abc}	1.68 ^{abc}	1.91 ^{a}	1.47 ^{bc}	1.4 ^{c}	1.82 ^{ab}	1.63 ^{abc}	1.6 b^{abc}	0.096	0.08	<0.001	0.370
K, %	1.32 ^{ab}	1.28 ^{ab}	1.16 ^{b}	1.39 ^{a}	1.28 ^{ab}	1.25 ^{ab}	1.38 ^{a}	1.3 ^{ab}	1.16 ^{b}	0.039	0.189	<0.001	0.516
P, %	0.28 ^{ab}	0.23 ^{cd}	0.22 ^{de}	0.24 ^{bcd}	0.22 ^{de}	0.22 ^{de}	0.28 ^{a}	0.26 ^{abc}	0.19 ^{e}	0.008	0.005	<0.001	<0.001
S, %	0.19 ^{b}	0.16 ^{bcd}	0.17 ^{bcd}	0.22 ^{a}	0.17 ^{bcd}	0.18 ^{bc}	0.19 ^{b}	0.16 ^{cd}	0.15 ^{d}	0.006	<0.001	<0.001	0.021
Mg, %	0.28 ^{abc}	0.26 ^{bc}	0.30 ^{abc}	0.35 ^{ab}	0.31 ^{abc}	0.36 ^{a}	0.26 ^{bc}	0.23 ^{c}	0.28 ^{abc}	0.024	<0.001	0.042	0.990
Al, mg/kg	449.7 ^{a}	123.1 ^{ab}	305.1 ^{ab}	448.8 ^{a}	129.9 ^{ab}	187.9 ^{ab}	318.6 ^{ab}	122.1 ^{b}	165.5 ^{ab}	75.38	0.267	<0.001	0.690
B, mg/kg	25.4 b^{c}	23.2 ^{bc}	31.8 ^{a}	26.6 ^{abc}	21.6 ^{c}	28.8 ^{ab}	25.5 ^{abc}	22.4 ^{c}	30.0 ^{ab}	1.53	0.605	<0.001	0.622
Cr, mg/kg	63.0 ^{a}	22.4 ^{ab}	28.8 ^{ab}	53.7 ^{ab}	15.7 ^{b}	19.0 ^{b}	37.2 ^{ab}	21.5 ^{ab}	21.2 ^{ab}	10.48	0.317	<0.001	0.660
Cu, mg/kg	8.3 ^{ab}	6.8 ^{abc}	6.9 ^{bc}	7.6 ^{abc}	6.2 ^{c}	6.3 ^{c}	8.3 ^{a}	6.8 ^{c}	6.2 ^{c}	0.34	0.048	<0.001	0.600
Fe, mg/kg	1012.5 ^{a}	316.5 ^{b}	628.9 ^{ab}	923.8 ^{ab}	282.1 ^{b}	393.0 ^{ab}	686.0 ^{ab}	319.8 ^{b}	402.3 ^{ab}	155.04	0.277	<0.001	0.705
Mn, mg/kg	73.3 ^{a}	53.2 ^{abc}	72.9 ^{abc}	63.5 ^{abc}	40.1 ^{c}	46.1 ^{bc}	69.4 ^{ab}	53.9 ^{abc}	63.6 ^{abc}	5.69	0.002	<0.001	0.452
Mo, mg/kg	0 ^{b}	0.1 ^{ab}	0.4 ^{a}	0 ^{b}	0 ^{b}	0.4 ^{a}	0.1 ^{b}	0.1	0.3 ^{ab}	0.06	0.504	<0.001	0.342
Na, mg/kg	49.3	25.1	25.4	34.3	21.5	29.6	42.5	19.4	20.9	7.33	0.531	0.002	0.609
Ni, mg/kg	4.7 ^{abc}	5.9 ^{a}	3.9 ^{abc}	4.1 ^{abc}	3.9 ^{abc}	2.9 ^{c}	4.0 ^{abc}	5.3 ^{ab}	3.1 ^{bc}	0.53	0.020	<0.001	0.577
Zn, mg/kg	25.1 ^{a}	19.2 ^{ab}	21.1 ^{ab}	22.1 ^{ab}	17.9 ^{b}	18.6 ^{b}	25.3 ^{a}	21.5 ^{b}	18.3 ^{b}	1.04	0.010	<0.001	0.083

FF, full flower stage; LF, late flower stage; SEM, standard error of means; VEG, vegetative stage. $^{a–e}$, means within a row without the same letter differ at $p < 0.05$.

In contrast, variety × maturity interaction was found for DM ($p < 0.001$) and CP ($p < 0.05$) concentrations for the PPC harvested in 2017 (Table 3). The DM concentration increased ($p < 0.001$) with maturity by LF > FF > VEG ($p < 0.05$) for ACL, LF and FF > VEG ($p < 0.05$) for BIS and LF > FF and VEG ($p < 0.01$) for CS. Crude protein concentration was the highest at FF and lowest at LF for all three varieties, with the ranks of VEG and FF > LF for ACL, FF > VEG > LF for BIS and FF > VEG > LF for CS ($p < 0.05$), respectively. The averaged CP concentrations across the three varieties were 12.3, 13.6 and 10.3% for the PPC harvested at VEG, FF and LF, respectively. No difference in CP concentration was observed among the three varieties at the VEG and FF stages, but ACL had a higher ($p < 0.05$) CP concentration than CS at the LF stage. Across the three maturities, NDF and ADF concentrations ranked ACL > BIS > CS with the difference being significant between ACL and CS ($p < 0.05$). As the plant matured from VEG, FF to LF, concentrations of ADF (23.2, 33.9 and 41.9%) and NDF (26.2, 39.3 and 50.9%) increased ($p < 0.001$).

No interaction between the PPC variety and plant maturity was observed for all 15 analyzed mineral elements (Table 4). Most minerals were similar among the three varieties, except for S, Mg, Mn and Na. Common seed had higher ($p < 0.05$) S, Mg and Na, but lower ($p < 0.01$) Mn concentrations than ACL and BIS, respectively. In contrast, most mineral elements were affected by maturity with the exception of Al, Cr, Fe, Mo and Na. For all 11 minerals affected by maturity, concentrations at VEG were higher ($p < 0.05$) than those at LF and intermediate at FF.

3.2.2. Dryland Plots

Unlike irrigation, variety ($p < 0.01$), plant maturity ($p < 0.001$) and their interaction ($p < 0.001$) all affected the DM concentration of the PPC grown on dryland in 2016, but only maturity ($p < 0.001$) and variety × maturity interaction ($p < 0.05$) affected it in 2017 (Tables 2 and 3). In general, BIS had lower ($p < 0.05$) DM concentration than the other two varieties in 2016. The average DM concentration of the three varieties at the VEG, FF and LF stages were 25.2, 34.1 and 49.6% in 2016, and 31.6, 39.2 and 48.9% in 2017.

In 2016, concentrations of OM, NDF, ADF, WSC and CT and 48 h DMD were similar among the three varieties. However, BIS had lower ($p < 0.05$) CP concentration (13.3%) than that of ACL (14.8%) and CS (14.9%). Compared with that harvested at LF, PPC harvested at VEG had generally higher ($p < 0.01$) CP and 48 h DMD, but lower ($p < 0.05$) OM and ADF concentrations. Plant maturity had no effect on NDF, WSC and CT concentration. In 2017, only OM and ADF concentrations were affected by plant maturity.

Variety × maturity interaction affected only P and S concentration (Table 5). The two minerals were higher ($p < 0.05$) in VEG than in FF and LF for ACL and BIS, whereas they ranked as VEG > FF > LF ($p < 0.05$) for CS. Differences were observed for seven minerals (P, S, Mg, Cu, Mn, Ni and Zn) among the three varieties, with S and Mg being higher ($p < 0.05$) but the other five minerals (P, Cu, Mn, Ni and Zn) being lower ($p < 0.05$) in BIS than in ACL and CS. Regardless of variety, the majority of the minerals decreased with maturity. Concentrations of 11 of the mineral elements out of 15 analyzed were higher ($p < 0.05$) in the PPC harvested at VEG than at FF and LF with no difference between FF and LF. However, concentrations of B and Mo were higher ($p < 0.05$) for LF than VEG.

4. Discussion

4.1. Agronomic Characteristics of Three Varieties of PPC Grown at Different Geoclimatic Conditions

Overall, the three PPC varieties grown at Lethbridge under irrigation produced more forage mass than that grown on dryland at Swift Current. This was expected as the growing conditions at Swift Current were less favourable for growth [26] due to limited precipitation

as compared to irrigation at Lethbridge. The larger row spacing used in the Swift Current dryland plots than that used in the Lethbridge irrigated plots also partially contributed to the forage mass difference between the two experimental sites.

The results from both sites showed the forage mass in both years increased as the plant matured and reached the highest yield for all the varieties at the late flower stage. The increase in forage mass as the plant matured was in part attributable to the higher DM concentration of the plant at LF as compared to that at the VEG stage. This conclusion is supported by the similar canopy height for all the varieties across all three stages of maturity. However, the decreasing leaf percentage with maturity suggests that the increased forage mass was mostly a result of an increase in the stem fraction. Li et al. [10] also observed that the percentage of leaves decreased as PPC matured from the VEG to the LF stage.

In 2016, the forage mass of three varieties performed with the rank of BIS > CS > ACL consistently across the three growth stages in dryland conditions, whereas although ACL ranked third in forage mass at the VEG and FF stages it was the highest at LF, with no difference observed among the three varieties under Lethbridge irrigated land. These results showed that although all three varieties could be successfully grown under these two conditions, BIS and CS generally produced more forage mass than ACL at both Swift Current and Lethbridge environmental conditions.

The higher forage mass obtained in 2017 than in 2016 at both the experimental sites except for CS at the LF stage at Lethbridge may indicate a better-developed sward in the 3rd year of establishment. The average monthly temperature during the growing season was also higher in 2017 than in 2016 for Lethbridge (16.3 vs. 15.1 °C) and Swift Current (15.9 vs. 15.1 °C). This was particularly the case for July, when the majority of PPC growth occurred at both Lethbridge (20.5 vs. 18.2 °C) and Swift Current (20.4 vs. 17.6 °C). It is commonly regarded that temperature and precipitation are the most important factors affecting forage mass and quality because they directly influence plant growth [27,28]. Therefore, the warmer weather in 2017 also contributed to the higher forage mass at both experimental sites. However, it must be noted that there were great variations in forage mass among replicate plots. This was mainly due to the uneven distribution of PPC plants among rows in each plot as a result of random invasion of pests (e.g., Richardson Ground Squirrels, deer, rabbit, etc.) and weeds. Weed control was key to the establishment of PPC as it starts to grow in mid-June, which is later than most common weeds.

The forage masses of the three varieties assessed in this study are similar to those of first-cut alfalfa and sainfoin, the two most popular legume forages in Western Canada [26,29,30]. Purple prairie clover (CS and ACL variety) has been successfully grown in native pastures in Swift Current [9,10,31,32] and under irrigation in Lethbridge [33,34]. The three tested PPC varieties were also successfully established in dryland at Panoka in Central Alberta in 2017 and had a yield in 2018 that was comparable to that of Swift Current [35]. These studies demonstrated PPC can be established under both the irrigated and dryland conditions in Western Canada. However, further research is needed to develop effective weed control strategies during the establishment year and in the early spring before PPC starts to grow. Irrigation can also facilitate the establishment of PPC if precipitation in dryland between May and July is below normal levels. Furthermore, although the yield of the first-cut PPC was comparable to that of first-cut alfalfa and sainfoin, research is needed to improve its regrowth agronomic characteristics in the efforts to increase its yearly forage mass.

4.2. Nutritive Value of Three Varieties of PPC Grown Under Different Geoclimatic Conditions

The fact that the three PPC varieties grown at Lethbridge had similar OM, CP, ADF, NDF, CT and WSC concentrations at the same maturity suggests that their feed value is

similar. This is supported by the observation that the three varieties had similar 48 h DMD at each maturity. The same trend was also observed for PPC grown at Swift Current. The results showed that as the PPC plant matured, concentrations of NDF and ADF increased but CP decreased whilst OM, CT and WSC remained relatively constant irrespective of the PPC variety and growing condition. This resulted in the decreasing DMD as plant maturity progressed from VEG to LF. Comparisons in nutrient composition and DMD between the PPC grown at the two experimental sites showed that the PPC grown at the Lethbridge irrigated land had higher OM and CP concentrations and DMD, but lower concentrations of NDF, ADF and CT than that grown at the Swift Current dryland. This is mainly due to the different growing conditions between the two experimental sites. The weather and growing conditions at the Swift Current dryland are more stressful than those at the Lethbridge irrigated land [26]. It is well known that dry and warm weather promotes the accumulation of tannins [36,37] and fibre [28,38] in plants. Melo et al. [39] reported that ADF, NDF and lignin concentrations of forage positively correlate with temperature. It has been estimated that for every 1 °C rise in ambient temperature, the NDF concentration of plants increases by 0.4% [40]. However, the similar chemical composition among the three PPC varieties suggests they responded similarly to different growing conditions. The CP, OM, NDF and ADF concentrations in the three PPC varieties were numerically similar to that of alfalfa and sainfoin grown under similar conditions [29,30,34]. We previously showed that CS harvested at the FF stage had similar nutritive and feeding value to that of alfalfa harvested at the same phenological stage [34].

Our previous research showed that PPC contained a high concentration of CT with unique biological activity and strong anti-*E. Coli* activity [11,12,32,33] but limited effects on rumen fermentation [14,15,34] as compared to other sources of CT. Concentrations of CT were similar among the three varieties grown at each experimental site and harvested at different stages. However, PPC grown at Swift Current had a higher CT concentration than that grown at Lethbridge. This observation is consistent with the hypothesis that drought conditions may promote CT synthesis.

Mineral concentration is an important factor affecting the nutritional value of forages. To our knowledge this is the first study to report the mineral concentration of PPC. At least 15 mineral elements were found in PPC regardless of the phenological stage or growing conditions in this study. However, as plant maturity progresses from the VEG to LF stage, the concentrations of most of the minerals decrease irrespective of the variety or growing conditions. This is consistent with the observation for alfalfa [41,42]. However, there was variation between the PPC growing at the Lethbridge and Swift Current growing conditions, with seven minerals (Al, Cr, Cu, Fe, Mn, Ni and P) being higher but K, Mo and Na being lower in the PPC growing at Lethbridge. These variations may reflect the growing conditions of the two sites. Nevertheless, the concentrations of these minerals in PPC are similar to that reported for alfalfa at a similar penological stage [41–43].

Overall, this study demonstrated that the three tested PPC varieties could be established at the Lethbridge (irrigated) and Swift Current (dryland) growth conditions with comparable first-cut forage mass to that of first-cut alfalfa and sainfoin under similar growing conditions. However, the establishment of PPC under dryland depended on there being precipitation during the May-July period. Regrowth after the first cut was slow at both the experimental sites and a second cut was not achievable. The three PPC varieties had similar nutritive values irrespective of the phenological stage, which were comparable to alfalfa and sainfoin. Therefore, PPC has great potential as an alternative legume forage for the ruminant industry. However, further research is needed to improve its agronomic characteristics such as regrowth potential for increasing forage mass and to improve its competitiveness against weeds.

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Abbreviations

The following abbreviations are used in this manuscript:

AAFC-SCRDC	Agriculture and Agri-Food Canada-Swift Current Research and Development Centre
ACL	AC Lamour
ADF	Acid detergent fibre
AOAC	Association of Official Agricultural Chemists
BIS	Bismarck
CP	Crude protein
CS	Common seed
CT	Condensed tannin
DM	Dry matter
DMD	Dry matter disappearance
FF	Full flower
ha	hectare
LF	Late flower
NDF	Neutral detergent fibre
OM	Organic matter
PPC	Purple prairie clover
SCRDC	Swift Current Research and Development Centre
SEM	Standard error of means
VEG	Vegetative
WSC	Water-soluble carbohydrates

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