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Effects of clipping frequency on tiller development of crested wheatgrass and hybrid brome grass at vegetative and reproductive stages

Ru-Yue Fan, David MacTaggart, Hu Wang, Ravindra N. Chibbar, Qing Feng Li, and Bill Biligetu

Abstract: Information on the tiller development of hybrid brome grass (*Bromus inermis* × *Bromus riparius*) is limited. The objective of this study was to evaluate the effect of clipping frequency at the vegetative and reproductive stages on the tiller development and concentrations of sugars of c.v 'AC Knowles' hybrid brome grass compared with c.v 'Kirk' crested wheatgrass (*Agropyron cristatum* L.). This experiment was conducted in a greenhouse using the tillers of the vernalized plants dug from the field. The experimental design was a randomized complete block design (RCBD). Grasses were clipped once or four times at either vegetative or reproductive stage. Regardless of the growth stage, four clippings reduced the tiller number of hybrid brome grass by 25.9% compared with the undefoliated control, while single clipping had no impact. The four clippings had no impact on the tiller number of crested wheatgrass, while single clipping increased its tiller number on average by 50.4% at both growth stages. Crested wheatgrass produced 16.6% more tillers than hybrid brome grass under the single clipping. The two grass species had a similar number of axillary buds under different clipping treatments. The axillary bud size of crested wheatgrass was larger than hybrid brome grass. All axillary buds were viable under the two clipping treatments for both grasses. Four clippings significantly decreased the stem base glucose concentration of the two grasses and the root sucrose concentration of crested wheatgrass. Hybrid brome grass was less tolerant to frequent clippings than crested wheatgrass. Therefore, intensive grazing of hybrid brome grass pasture may result in a thin stand.

Key words: tiller biomass, tiller number, bud number, bud size, glucose, sucrose.

Résumé : On sait peu de choses sur le développement des talles du brome hybride (*Bromus inermis* × *Bromus riparius*). La présente étude devait établir quels effets la fréquence de la tonte aux stades végétatif et reproductif a sur le développement des talles et sur la concentration des sucres chez le brome hybride AC Knowles, comparativement à la variété Kirk d'agropyre à crête (*Agropyron cristatum* L.). L'expérience, en blocs aléatoires complets, a été réalisée en serre sur des talles de plants vernalisés, recueillis sur le terrain. Les graminées ont été coupées une ou quatre fois au stade végétatif ou reproductif. Comparativement au témoin non défolié, quatre tontes réduisent le nombre de talles du brome hybride de 25,9 %, peu importe le stade de croissance, alors qu'on n'observe aucun impact après une seule tonte. En revanche, les quatre tontes n'ont eu aucune incidence sur le nombre de talles de l'agropyre à crête, mais une tonte en a augmenté le nombre de talles d'en moyenne 50,4 %, aux deux stades. L'agropyre à crête produit 16,6 % plus de talles que le brome hybride après une tonte. Les deux graminées comptaient un nombre analogue de bourgeons axillaires à la suite des traitements. Les bourgeons axillaires de l'agropyre à crête étaient plus gros que ceux du brome hybride. Les bourgeons axillaires des deux graminées étaient tous viables après les deux traitements. Quatre tontes diminuent sensiblement la concentration de glucose à la base de la tige des deux graminées et celle de sucrose dans les racines de l'agropyre à crête. Le brome hybride tolère moins des tontes fréquentes que l'agropyre à crête. Par conséquent, la paissance intensive des prés de brome hybride pourrait entraîner un éclaircissement du peuplement. [Traduit par la Rédaction]

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Mots-clés : biomasse des talles, nombre de talles, nombre de bourgeons, taille des bourgeons, glucose, sucrose.

Introduction

Perennial grasses are important components of hay and pasture production in western Canada. After defoliation, perennial grasses initiate new growth through tiller development (Van Staaldin and Anten 2005) from apical meristems, intercalary meristems, and axillary buds (Briske and Richards 1995; Klimešová and Klimeš 2007). Bud bank demography is important for compensatory growth as tiller recruitment following the removal of the apical meristem relies on the activation and outgrowth of axillary buds (Briske and Richards 1995; Barbier et al. 2017). An understanding of tiller dynamics after clipping is important for grazing strategy and pasture management, especially for newer grass species like hybrid brome grass as the information is lacking.

The response to clipping varies greatly among perennial grass species due to their different tiller recruitment strategies (Hendrickson et al. 2005; Biligetu and Coulman 2010; VanderWeide and Hartnett 2015) as well as the size and viability of the below-ground bud banks (Busso et al. 2011). Wheatgrass (*Agropyron* sp.) species with similar phenological and physiological traits differed markedly in their ability to produce new tillers following defoliation (Caldwell et al. 1981). In smooth brome grass (*Bromus inermis* Leyss), tillering ceased at the stem elongation stage, and did not resume again until the anthesis stage unless apical dominance was removed by defoliation (Eastin et al. 1964). Tall fescue [*Festuca arundinacea* (Schreb.)] ecotypes with longer rhizomes were less affected by defoliation because they can uptake more nutrients from the surrounding areas following defoliation compared with ecotypes with shorter rhizomes (Bryant et al. 2015). Some studies also found that increasing clipping frequency caused a reduction in the bud viability without significantly changing the total bud number (Newton and Hay 1996; Busso et al. 2011). However, Dalgleish et al. (2008) found that grazing decreased bud bank density and increased the number of outgrowing tiller numbers when compared with no grazing in a tallgrass prairie. As the size of the bud bank and the rate of tiller recruitment fluctuate over the growing season (Klimešová and Klimeš 2007; Ott 2009; Ott and Hartnett 2012), the effects of clipping frequency on these two factors would differ between the vegetative and reproductive growth stages. In brome grass (*Bromus* sp.) species, the total bud number was similar while the number of successfully outgrown tillers varied among species after defoliation at different growth stages (Biligetu 2009). Generally, more tillers were recruited after defoliation in the vegetative stage because of a high number of active meristems compared with the stem elongation and reproductive stages (Briske and Richards 1995).

Traditionally, the concept of apical dominance was used to explain tiller initiation in perennial grasses (Murphy and Briske 1992). Several mechanisms were proposed for apical dominance such as the hormonal (Phillips 1975), and nutritional hypotheses (Gregory and Veale 1957). In recent years, Mason et al. (2014) suggested that the mechanism of axillary bud activation after apical meristem removal is regulated by different sugars rather than hormones because as a form of energy, sugars were transferred from sink organs to adjacent stem tissues immediately after defoliation. The sugars consist of small sugars (glucose, fructose, sucrose, trehalose), sucrose-derived oligosaccharides (fructans in grasses), starch and its breakdown products (Van den Ende 2013). Sucrose is a major storage form of soluble carbohydrates, and it is used to maintain cellular metabolism, cell wall biosynthesis, maintenance and growth respirations (Ohashi et al. 2018). Ohashi et al. (2018) demonstrated that a reduction in sucrose during early growth caused tiller outgrowth cessation in rice (*Oryza sativa* L.). Similarly, Kebrom et al. (2012) indicated that in wheat (*Triticum aestivum* L.), the suppression of axillary bud outgrowth was associated with a reduction in sucrose content. Defoliation immediately decreases the total leaf area, rate of photosynthesis, and the sugars available for new tiller recruitment in defoliated plants (Painter and Detling 1981). The organic reserves are mobilized to support the new growth immediately after clipping (Davidson and Milthorpe 1966; Gonzalez et al. 1989).

Crested wheatgrass (*Agropyron cristatum* L.) is a drought tolerant and winter hardy grass due to an extensive root system. It is one of the most important grasses for early spring grazing, offering highly palatable and nutritious forage on the Canadian Prairies (Baral et al. 2020). Hybrid brome grass (*Bromus inermis* Leyss × *Bromus riparius* Rehm) is an inter-species hybrid between meadow brome grass (*Bromus riparius* Rehm) and smooth brome grass (Coulman 2006). It has a short rhizome with upright tillering, making it well suited for both hay production and pastures on the Canadian Prairies. Hybrid brome grass can produce a tiller density greater than smooth brome grass, but lower than meadow brome grass after clipping at both the vegetative and stem elongation stages (Biligetu and Coulman 2010). The hypothesis tested in this study was that tiller recruitment differs greatly between the two grasses after defoliation, because of variance in tiller size, axillary bud number, and soluble sugar contents in the tissues. The objectives of this study were to (1) compare the tiller recruitment and bud bank demography of two grasses following two clipping frequencies at vegetative and reproductive growth stages, and (2) identify the

concentrations of glucose and sucrose of the two grasses under two clipping frequencies at the two growth stages.

Materials and Methods

Experimental design

In this study, hybrid brome grass, c.v. AC Knowles (Coulman 2004), and crested wheatgrass, c.v. Kirk were used. Fifteen crested wheatgrass plants and 15 hybrid brome grasses plants were randomly dug from a spaced breeding nursery at the Agriculture and Agri-Food Canada Saskatoon Research Farm on 14 May 2019 after being fully vernalized during the winter. These plants were then hand separated into more than 200 single tillers per species for the study. Each individual tiller was transferred to a one gallon pot (15 × 18 cm, Sun Gro Horticulture) with propagation soil mix containing 70%–80% sphagnum peat moss, vermiculite and dolomite (SS#3, Sunagro Horticulture Ltd.) at the College of Agriculture and Bioresources greenhouse of the University of Saskatchewan. In the greenhouse, the light period was 18-h, supplemented with high pressure sodium halogen lamps to a total of 490–550 $\mu\text{M}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PAR. The air temperature was 27 °C and 22 °C, for day/night, respectively. Relative humidity was maintained at 48.0 %. All tillers were grown for two weeks prior the experiment at two greenhouse benches (shaded vs. direct sun). The majority of tillers in the bench with direct sun developed into reproductive tillers with spikelet fully emerged. During the synchronization process, plants were constantly moved between the two benches to achieve a uniform growth stages. Then, the tillers were grouped into vegetative and reproductive tillers for the study.

The experimental design was a randomized complete block design (RCBD) with a split-split plot arrangement with five replications. The experimental unit was three pots (or three tillers) per replication. The main plot factor was species (crested wheatgrass and hybrid brome grass), the sub-plot factor was clipping frequency (no clipping, single clipping to 5 cm stubble height, and four clippings to 5 cm stubble height), and the sub-subplot factor was the growth stage (vegetative and reproductive stages). Thus, there were 12 treatment combinations (180 total pots). Each experimental period lasted 12 weeks and was repeated twice with 5-d intervals between them.

Sampling and measurement

Tiller number and biomass, bud viability and size

For the single clipping treatment, grasses were clipped once at either the vegetative (V2) or reproductive stage (R2) as described by Moore and Moser (1995). For four clippings, grasses were clipped at either V2 or R2 stages followed by three consecutive clippings at two wk intervals, irrespective of the growth stages. Tiller number per plant was counted prior to each clipping. For each clipping, the tiller biomass of each plant was

measured after drying the samples in a forced air dryer at 60 °C for 48 h. In the four clippings treatment, tiller biomass represents the sum of four cuts.

One tiller per pot was randomly selected and measured for the viability, size, and number of axillary buds per tiller after the final clipping. Bud viability was tested following the double staining procedure (Busso et al. 1989). Briefly, tillers were immersed in a tetrazolium chloride (TTC) solution (Thermo Fisher Scientific, Heysham, UK) at 0.6 % (w/v) in the dark for 15 h. Buds stained with either red or pink colors were considered metabolically active. Unstained buds were further tested with the vital stain Evan's blue (Sigma Aldrich, Oakville, Canada) at 0.25% (w/v). The unstained buds were considered dormant while the dark blue stained buds were considered dead. The number of buds per tiller was the sum of the active and dormant buds. Finally, bud size was determined by measuring from the prophyll tip to the lowest attachment on the tiller using a Zeiss Stemi 508 stereomicroscope (Carl Zeiss AG, Oberkochen, Germany) at 10× magnification.

Determination of soluble sugar

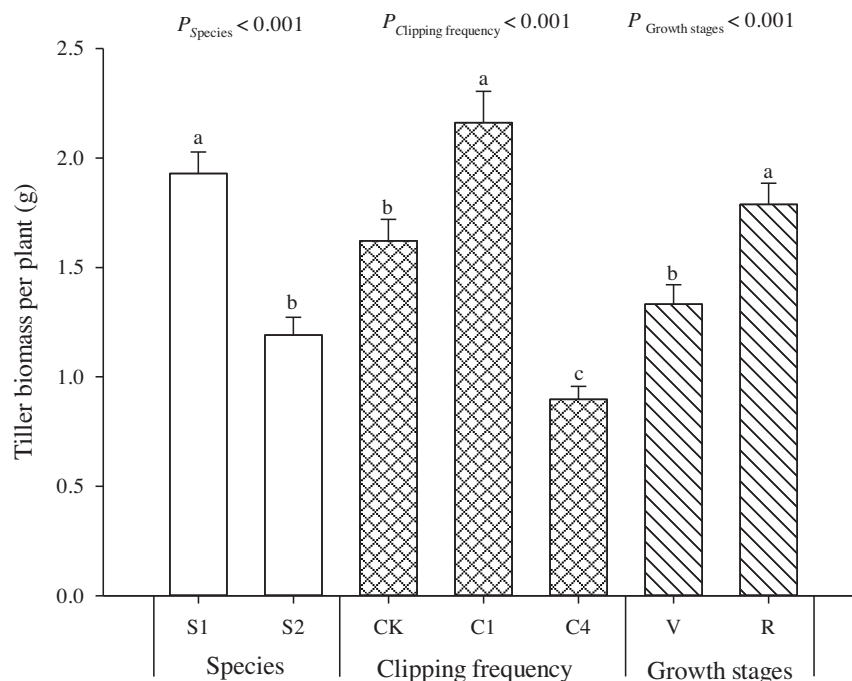
Due to the insufficient amount of stem base and root samples for soluble sugar analysis, the tillers from the two experimental runs were bulked by treatment to form three replication per treatment. Leaves, stem bases, and root samples were separately collected into 2 mL centrifuge tubes from each treatment immediately after the final clipping. The samples were flash frozen in liquid nitrogen, and then transferred to a –80 °C freezer for temporary storage. All samples were freeze-dried at –52 °C and < 0.08 Pa for 72 h. Then, the individual freeze-dried samples were ground by a ball mill (Retsch MM400, Retsch GmbH, Haan, Germany) with 2.38 mm metal beads (Qiagen Inc., Toronto, Canada). The powdered samples were then analyzed for the concentrations of sucrose and glucose with a Raffinose/Sucrose/Glucose Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Sugar was extracted with ethanol (95% v/v), sodium acetate buffer (50 mM, pH 4.5), and chloroform. The sodium acetate buffer and invertase were then added separately to the liquid extract. This was followed by a glucose oxidase/peroxidase reagent (GOPOD) to produce a red colored quinoneimine. The concentration of D-glucose was determined at A510 nm using a spectrophotometer. Calculations were as follows:

$$(1) \quad \text{D-glucose (mmol/100 g)} = \Delta A \times F \times 50$$

$$(2) \quad \text{sucrose (mmol/100 g)} = (\Delta B - \Delta A) \times F \times 50$$

where ΔA is GOPOD absorbance for D-glucose, ΔB is GOPOD absorbance for D-glucose + sucrose, and $F = 0.556$ ($\mu\text{moles of glucose}/\text{GOPOD absorbance for } 0.556 \mu\text{moles of glucose}$).

Fig. 1. The main effects of species, clipping frequency and growth stage on tiller biomass per plant. Error bars indicate standard error of the mean. Means within each treatment with different lowercase letters are significantly different ($P \leq 0.05$). Species: S1 = hybrid bromegrass and S2 = crested wheatgrass. Clipping frequency: CK = control, C1 = single clipping and C4 = four clippings (the sum of four cuts). Growth stages: V = vegetative stage and R = reproductive stage.



Data analysis

Statistical analyses were performed using the MIXED procedure in SAS (version 9.4; SAS Institute, Cary, NC) as a RCBD with a split-split plot arrangement of the treatments. The model included species, clipping frequency, growth stage, and their interactions as fixed effects, and experimental run, replication, replication \times species, and replication \times species \times clipping frequency were considered as random effects. For glucose and sucrose concentrations analysis, replication, replication \times species, and replication \times species \times clipping frequency were treated as random effects. Because of the large difference in bud size between the two species, and species \times clipping frequency, bud size data were re-analyzed for each species. Means were separated using the least square means of the MIXED procedure. Multiple linear regression analysis (Minitab 18.1, Minitab, Inc., PA) was conducted to determine the effects of tiller biomass, axillary bud number, and axillary bud size on tiller number of each species. Statistical significance was set at $P \leq 0.05$.

Results

Tiller biomass and tiller number

The tiller biomass per plant of hybrid bromegrass was greater than that of crested wheatgrass ($P < 0.001$, Fig. 1). Total tiller biomass was greater under the single clipping treatment, while four clippings reduced biomass regardless of species and the growth stage

($P \leq 0.05$, Fig. 1). Moreover, tiller biomass was greater in the reproductive stage than in the vegetative stage ($P < 0.001$, Fig. 1). There was a significant clipping frequency \times growth stage effect on tiller biomass ($P < 0.001$, Fig. 2A, Table 1), with four clippings at vegetative stage produced least tiller biomass while single clipping at reproductive stage produced the highest tiller biomass.

Tiller number was affected by the species \times clipping frequency ($P \leq 0.05$, Table 1, Fig. 2B). Four clippings greatly reduced tiller number in hybrid bromegrass compared with the undefoliated control and the single clipping. By contrast, four clippings had no impact on the tiller number of crested wheatgrass in comparison to the undefoliated control, while a single clipping significantly increased its tiller number. In addition, tiller number was affected by the species \times growth stage ($P \leq 0.05$, Table 1, Fig. 2C). Hybrid bromegrass maintained similar number of tillers between the vegetative and reproductive stages. However, crested wheatgrass produced more tillers in the reproductive stage compared with the vegetative stage ($P \leq 0.05$, Table 1, Fig. 2C). There was a significant growth stage and clipping frequency interaction effect on tiller number, both single and four clippings had no effect on tiller number of the two grasses at the vegetative stage, while tiller number increased after single clipping at the reproductive stage ($P \leq 0.05$, Table 1, Fig. 2D).

Fig. 2. Effects of clipping frequency \times growth stage on tiller biomass (A), species \times clipping frequency (B), species \times growth stage (C) and growth stage \times clipping frequency on tiller number per plant (D). Error bars indicate standard error of the mean. Means within each figure with a common lower case letter are not significantly different ($P > 0.05$). Clipping frequency: CK = undefoliated control, C1 = single clipping, and C4 = four clippings. Growth stage: V = vegetative stage and R = reproductive stage. (Note: in Fig. 2A, C4 biomass represents the sum of four cuts).

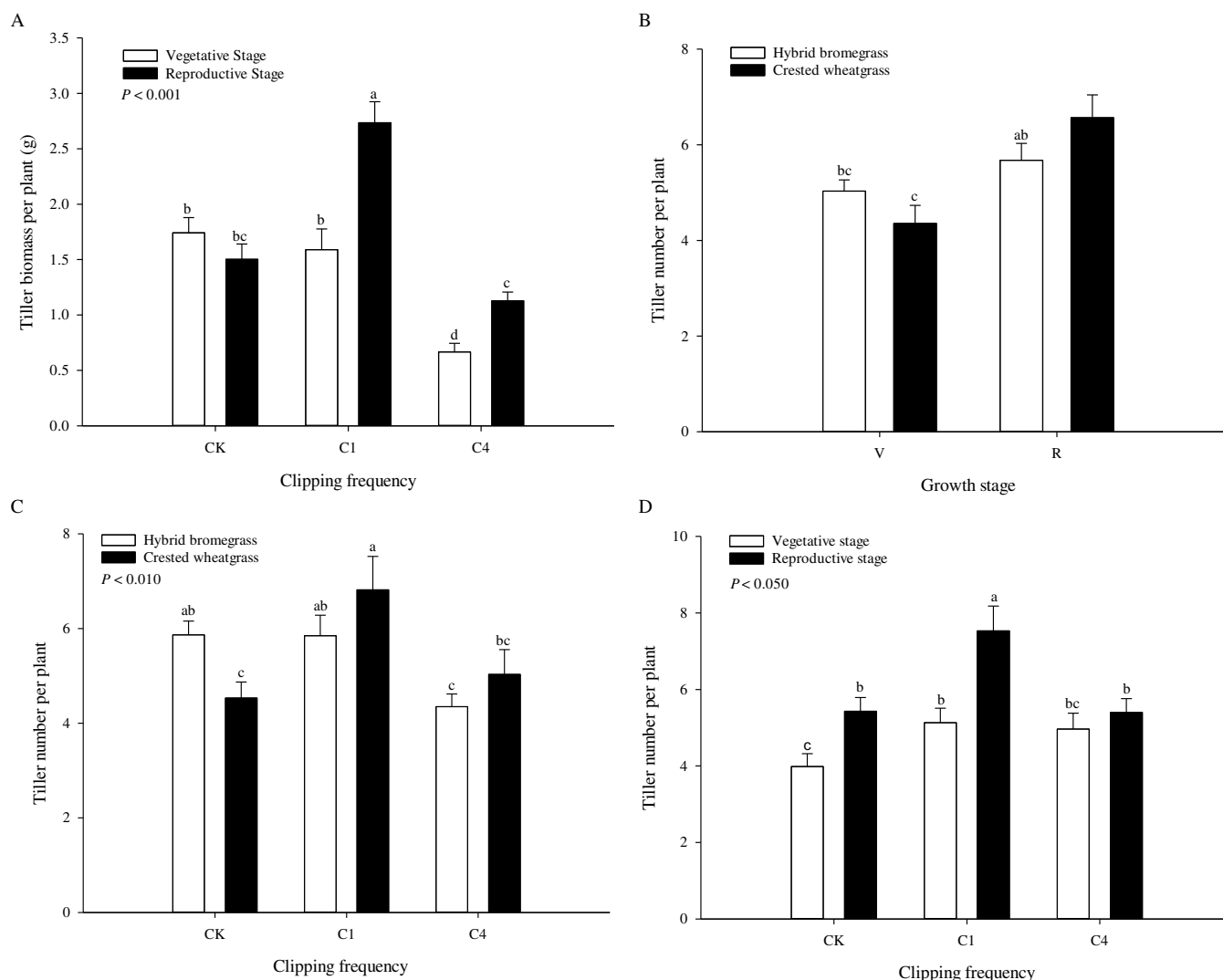
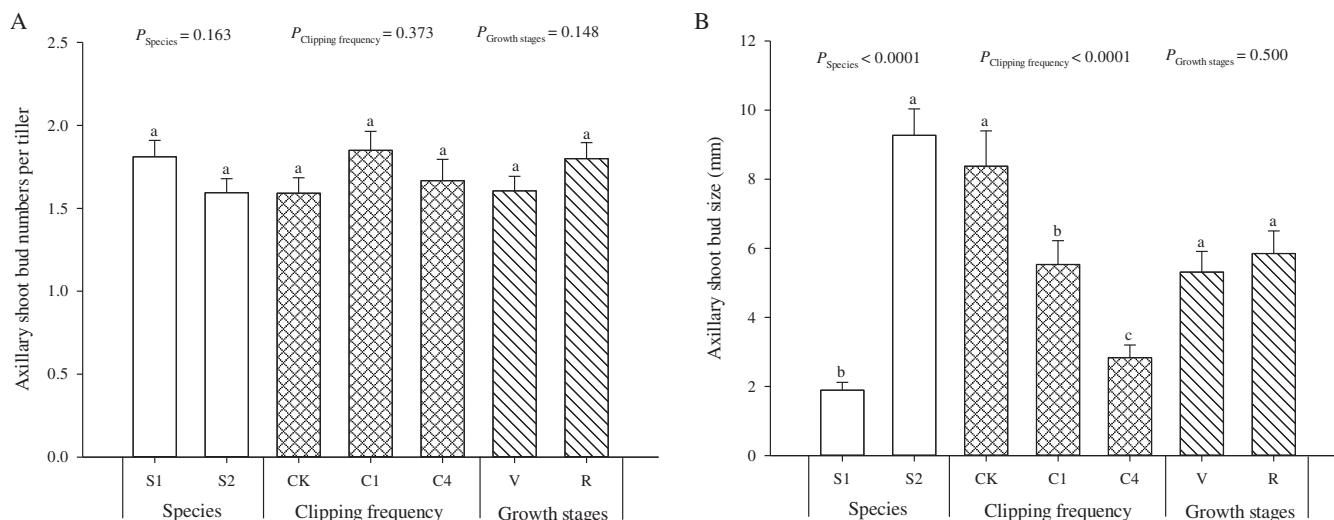


Table 1. Source of variation of species (hybrid bromegrass and crested wheatgrass), clipping frequency (undefoliated control, single clipping and four clipping), growth stages (vegetative stage and reproductive stage) and their interactions on tiller biomass, tiller number, axillary bud size and axillary bud number.

Source	df	Tiller biomass	Tiller number	Bud number	Bud size
Species	1	***	NS	NS	***
Clipping frequency	2	***	***	NS	***
Growth stage	1	***	***	NS	NS
Species \times Clipping frequency	2	NS	*	NS	***
Species \times Growth stage	1	NS	*	NS	NS
Clipping frequency \times Growth stage	2	***	*	NS	NS
Species \times Clipping frequency \times Growth stage	2	NS	NS	NS	NS

Note: df, degree of freedom; NS, not significantly different at $P > 0.05$; *significant treatment effect at $P \leq 0.05$, ***significant treatment effect at $P \leq 0.001$.

Fig. 3. The main effects of species, clipping, and growth stage on axillary bud numbers per tiller (A) and axillary bud size (B). Error bars indicate standard error of the mean. Means within each figure with a common lower case letter are not significantly different ($P > 0.05$). Species: S1 = hybrid bromegrass; S2 = crested wheatgrass. Clipping frequency: CK = undefoliated control, C1 = single clipping, and C4 = four clippings. Growth stage: V = vegetative stage and R = reproductive stage.



Bud bank demography

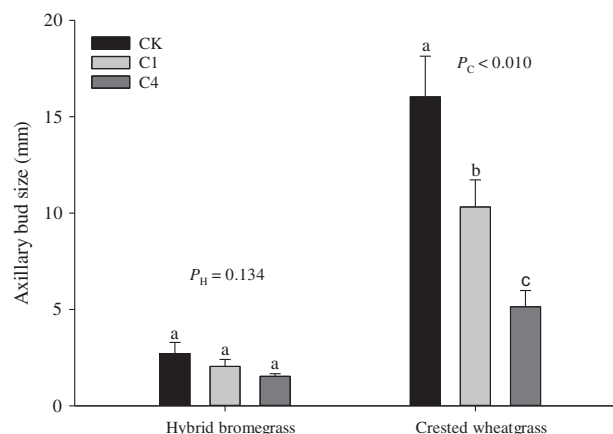
There was no significant variation of axillary bud number between the two grasses, growth stage or clipping frequency (Fig. 3A). Axillary bud size was about four times larger ($P \leq 0.05$) in crested wheatgrass than in hybrid bromegrass (Fig. 3B). All axillary buds were viable for the two grasses under different growth stages and clipping treatments. Interestingly, there was a significant species \times clipping frequency, increasing clipping frequency gradually reduced axillary bud size of crested wheatgrass ($P \leq 0.05$, Table 1, Figs. 4, 5A, 5B for crested wheatgrass). However, repeated clipping did not reduce the bud size of hybrid bromegrass, although new tiller number was reduced in this species after four clippings ($P \leq 0.05$, Table 1, Figs. 4, 5C, 5D for hybrid bromegrass).

According to the regression analysis, no significant correlations existed between tiller number and axillary bud number or tiller number and bud size for the two grass species ($P > 0.05$, Table 2). However, a positive correlation existed between tiller number and tiller biomass for hybrid bromegrass ($R^2 = 0.51$) and crested wheatgrass ($R^2 = 0.39$). Inclusions of axillary bud number or bud size did not increase the R^2 values for hybrid bromegrass, but tiller biomass and bud size together explained greater variation of tiller number ($R^2 = 0.45$) for crested wheatgrass (Table 2).

Concentrations of glucose and sucrose

Hybrid bromegrass contained higher glucose and lower sucrose concentrations than crested wheatgrass in the leaves, stem bases, and roots. Four clippings increased the leaf sucrose concentration, but decreased stem base glucose concentration for both species

Fig. 4. Effect of clipping frequency on axillary bud size of hybrid bromegrass and crested wheatgrass. Error bars indicate standard error of the mean. Means within each species with a common lower case letter are not significantly different ($P > 0.05$). Clipping frequency: CK = undefoliated control, C1 = single clipping, and C4 = four clippings.



($P < 0.001$, Table 3). Regardless of species and clipping frequency, the stem base sucrose concentration in the vegetative stage was greater than in the reproductive stage (Table 3).

There was a significant species and clipping frequency interaction for the root sucrose and stem base glucose concentrations ($P < 0.05$, Table 3). Compared with the undefoliated control, both clipping frequencies reduced the concentration of root sucrose in crested wheatgrass ($P \leq 0.05$, Fig. 6A). Compared with the undefoliated control, the root sucrose concentration of crested wheatgrass decreased by 40.3% and 37.3% under a single clipping and

Fig. 5. Axillary shoot bud images of crested wheatgrass [undefoliated control (A) and four clippings (B)] and hybrid bromegrass [undefoliated control (C) and four clippings (D)]. [Colour online.]



Table 2. Multiple regression equations of tiller number (TN) affected by tiller biomass (TB), bud number (BN), or bud size (BS) for hybrid bromegrass and crested wheatgrass following defoliation either at vegetative or reproductive stage in greenhouse.

Species	n	Model	R ²	P-value
Hybrid bromegrass	60	TN = 2.93 + 1.26 TB	0.51	<0.001
	60	TN = 4.52 + 0.46 BN	0.04	0.068
	60	TN = 4.96 + 0.21 BS	0.04	0.069
	60	TN = 2.58 + 1.22 TB + 0.23 BN	0.51	<0.001
	60	TN = 2.83 + 1.23 TB + 0.08 BS	0.50	<0.001
	60	TN = 4.39 + 0.36 BN + 0.16 BS	0.06	0.072
Crested wheatgrass	60	TN = 3.12 + 1.97 TB	0.39	<0.001
	60	TN = 4.34 + 0.70 BN	0.02	0.138
	60	TN = 5.22 + 0.026 BS	0.00	0.584
	60	TN = 2.95 + 1.94 TB + 0.13 BN	0.38	<0.001
	60	TN = 3.58 + 2.41 TB - 0.11 BS	0.45	<0.001
	60	TN = 4.28 + 0.68 BN + 0.01 BS	0.00	0.328

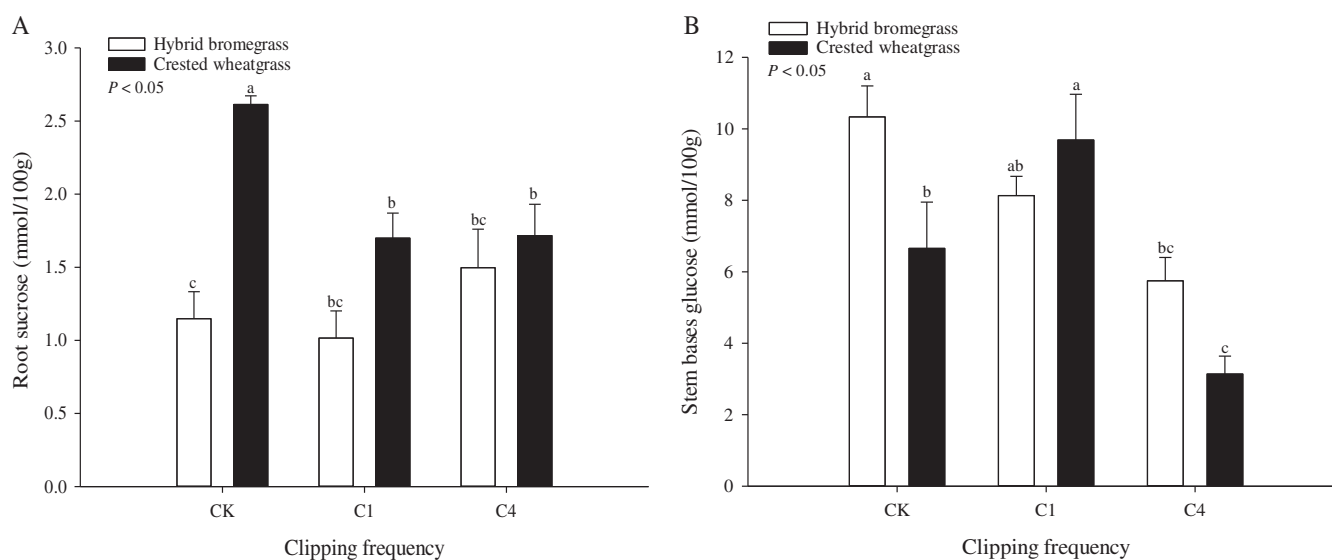
four clippings, respectively (Fig. 6A). Clipping had no effect on the root sucrose concentration of hybrid bromegrass. Increasing clipping frequency lowered the glucose concentration in the stem bases of hybrid bromegrass with a 44% decrease relative to the undefoliated control

after four clippings ($P < 0.05$, Fig. 6B). For crested wheatgrass, the concentration of stem base glucose increased by 45.5% following a single clipping, but then decreased by 52.9% following four clippings relative to the undefoliated control ($P < 0.05$, Fig. 6B).

Table 3. The concentrations (mmol/100 g) of glucose and sucrose in leaves, stem bases and roots of two grasses as affected by clipping frequency, growth stage and their interactions.

Treatment	Leaves		Stem bases		Roots	
	glucose	sucrose	glucose	sucrose	glucose	sucrose
Species						
Hybrid bromegrass	5.95a	5.59b	8.07a	1.21b	1.47a	1.22b
Crested wheatgrass	3.27b	12.99a	6.50b	2.91a	0.69b	2.01a
SEM	0.43	0.46	0.54	0.30	0.14	0.12
Clipping frequency						
Control	4.02a	8.36b	8.50a	2.15a	1.14a	1.88a
Single clipping	5.42a	8.13b	8.91a	2.25a	1.11a	1.36a
Four clippings	4.40a	11.38a	4.44b	1.78a	0.99a	1.61a
SEM	0.49	0.56	0.66	0.34	0.14	0.14
Growth Stage						
Vegetative	4.29a	9.81a	7.08a	2.43a	1.13a	1.66a
Reproductive	4.93a	8.77a	7.49a	1.69b	1.03a	1.57a
SEM	0.43	0.46	0.54	0.29	0.13	0.12
P-value						
Species	<0.001	<0.0001	<0.050	<0.001	<0.050	<0.010
Clipping frequency	0.090	<0.001	<0.001	0.468	0.612	0.056
Growth stage	0.200	0.124	0.602	<0.050	0.452	0.622
Species × Clipping frequency	0.511	0.254	<0.050	0.900	0.556	<0.050
Species × Growth stage	0.287	0.579	0.508	0.623	0.782	0.646
Clipping frequency × Growth stage	0.883	0.415	0.242	0.671	0.933	0.623
Species × Clipping frequency × Growth stage	0.552	0.848	0.575	0.722	0.460	0.618

Note: Means within a column at each treatment level with a common lower case letter are not significant different ($P > 0.05$). SEM indicates standard error of the means.

Fig. 6. The effects of clipping frequency on sucrose concentration in roots (A) and glucose concentration (B) in stem bases of hybrid bromegrass and crested wheatgrass. Error bars indicate standard error of the means. Means within each figure with a common lower case letter are not significantly different ($P > 0.05$). Clipping frequency: CK = undefoliated control, C1 = single clipping, and C4 = four clippings.

Discussion

This study was designed to understand the tiller development after clipping of two forage grasses widely used in western Canada. Compared with crested wheatgrass, hybrid brome grass had greater tiller biomass, smaller axillary bud size, and similar tiller and axillary bud numbers per tiller. Hybrid brome grass had higher concentrations of glucose and lower concentration of sucrose than crested wheatgrass in the stem bases in our study.

Tiller biomass was not affected by species \times clipping frequency in the study. However, a single clipping at the reproductive stage increased the tiller biomass of both species but not at the vegetative stage. This was also seen in other grasses such as in little bluestem (*Andropogon scoparius* Michx.), big bluestem (*A. gerardi* Vitman), and Indian grass [*Sorghastrum nutans* (L.) Nash] where the tiller biomass and tiller number both increased after a single clipping during the reproductive stage (Vogel and Bjergstad 1968). The low total tiller biomass at the vegetative stage was in part because of a lower initial tiller biomass compared with the reproductive stage. In addition, active meristems for regrowth differed between the two growth stages after defoliation. Before the internode elongation, vegetative tillers continue to elongate after defoliation to ensure tiller density (Jewiss 1972). Following defoliation at the reproductive stage, apical meristems are often removed, and new tiller comes from axillary buds (Briske and Richards 1995). A single clipping at the reproductive stage stimulated more tiller growth from the fully developed axillary buds and increased the regrowth biomass in grasses (Mullahey et al. 1991). However, in our study, four clippings greatly reduced tiller biomass for both species at the two growth stages compared with their undefoliated controls. This results could be expected because frequent clippings often reduced the regrowth by restraining the photosynthetic capacity of grasses and reducing nutrients uptake (Arredondo and Johnson 1998; Zhao et al. 2008; Xu et al. 2013). In addition, the root organic reserves for the new growth are generally low under frequent clippings, further reducing regrowth biomass (Davidson and Milthorpe 1966).

The main difference between the two grasses was their tiller development in response to clipping. Four clippings greatly reduced the tiller number of hybrid brome grass, but it had no impact on the tiller number of crested wheatgrass. A single clipping increased the tiller number of crested wheatgrass. New tiller recruitment mainly depends on the activation and subsequent outgrowth of axillary buds after internode elongation (Klimešová and Klimeš 2007). Interestingly, the total axillary bud number was similar between the two grasses, which was not affected by clipping frequency and growth stage in our study. In addition, the number of tillers per plant was not correlated to the total axillary bud

number in these two grasses based on our regression analysis. In a previous study, Biligetu (2009) reported that axillary bud number was similar among three brome species after clipping, although the tiller number was significantly different during regrowth. Wheatgrass species differed in their ability to produce new tillers following defoliation, but the total axillary bud number per tiller was similar (Richards and Caldwell 1985). Thus, factors affecting the outgrowth of the axillary bud are critical for tiller development than the total number of available axillary buds.

Busso et al. (2011) found that increasing clipping frequency caused a reduction in the bud viability without significantly changing the total bud number. However, axillary bud was viable for both grasses, but axillary bud size was about four times larger in crested wheatgrass than in hybrid brome grass. Therefore, a larger bud size of crested wheatgrass might suggest higher energy for growth compared with hybrid brome grass. We also found that increasing clipping frequency gradually reduced the axillary bud size of crested wheatgrass but it had no effect on the bud size of hybrid brome grass. Similar to crested wheatgrass, defoliation caused a decrease in the bud size of two caespitose grasses, desert wheatgrass (*Agropyron desertorum*) and bluebunch wheatgrass (*Agropyron spicatum* Scribn.) (Busso et al. 1989).

Mason et al. (2014) suggested that the mechanism of axillary bud activation after apical meristem removal is regulated by different sugars, therefore, the sucrose translocation may play functions other than energy supply for axillary bud outgrowth. However, a continuous reduction of sucrose might cause tiller outgrowth cessation (Kebrom et al. 2012; Ohashi et al. 2018). The single and four clippings reduced the concentration of root sucrose in crested wheatgrass, but it had no effect on hybrid brome grass in our study. Beside regulation of apical dominance, the reduction of sucrose suggested an immediate use of sucrose in crested wheatgrass. Rapid production of new leaves immediately after defoliation is considered a critical trait in response to defoliation, because it allows the plant to assimilate carbohydrates to meet the need of future growth and respiration (Caldwell et al. 1981). Four clippings caused significant decreases in stem base glucose concentrations relative to the control for both grasses, while a single clipping increased glucose concentration of crested wheatgrass. The increase of glucose might be one of the factors affecting axillary bud outgrowth in crested wheatgrass after single clipping. Glucose as a readily available simple sugar may provide energy for new leaf area development in defoliated plants (Painter and Detling 1981).

This study demonstrated that hybrid brome grass may not be adapted to pasture systems under frequent defoliation because of its reduced tiller development in these conditions. Although tiller number was similar between vegetative and reproductive stages for hybrid

bromegrass, grazing at the reproductive stage would maximize the total forage biomass.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

R.-Y.F., B.B. conceived the project; R.-Y.F., B.B., R.N.C. designed experiments; B.B. prepared the study materials; R.-Y.F. performed experiments; R.-Y.F. analyzed data; R.-Y.F. and B.B. wrote the manuscript and D.M., R.N.C., H.W., Q.F.L., B.B. substantially contributed to the final version of the manuscript. All authors read and approved the final manuscript.

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