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Water-Soluble Carbohydrate Recovery in Pastures of Perennial Ryegrass (*Lolium perenne* L.) and Pasture Brome (*Bromus valdivianus* Phil.) Under Two Defoliation Frequencies Determined by Thermal Time

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Abstract: The objectives of the experiment were to (i) examine the dynamics of WSC use and the recovery of leaf sheaths and blades of *Bromus valdivianus* Phil. and *Lolium perenne* L. subjected to two defoliation frequencies (DFs) determined by thermal time (TT); (ii) evaluate how DF influenced regrowth and accumulated herbage mass (AHM) during fall. Defoliation was carried out at frequencies of 135 and 270 accumulated growing degree days (AGDDs) for both species. Twelve plots were arranged in a three-block design. All plots had a conditioning period to establish the assigned DF prior to sampling. From the start of the experiment, "cores" were collected from each plot every three days until the DF was reached. Every core was separated into leaf and sheath material before measuring the WSC concentration. *Lolium perenne* had concentrated more WSCs than *B. valdivianus*. Both species adapted their WSC recovery according to the DF. The recovery of WSC was faster under a DF of 135 AGDDs than that of 270 AGDDs. Leaf sheaths contained more WSCs than leaf blades and were identified as WSC storage organs. This period can be used as the optimal defoliation interval in *B. valdivianus* and *L. perenne* grazing systems.

Keywords: accumulate growing degree days; phyllochron; grass regrowth; leaf sheaths; blades

1. Introduction

Along with soil fertility and environmental conditions, defoliation frequency (DF) is one of the main factors related to pasture management that influences the rate of regrowth and accumulated herbage mass (AHM). However, when days are used to assign the interval between defoliations, changes in plant phenology are not considered. Temperate forage grows differently depending on the season. Therefore, using days as a criterion for DF can reduce pasture growth and longevity. In some perennial crops, such as sorghum and oats growing under field conditions, the thermal time, expressed as accumulated growing degree days (AGDDs), has been widely used to determine sowing data [1,2] because there is a positive relation between plant development and temperature [3]. The thermal time (TT) was used as a tool to determine the best DF in *Lolium perenne* L. and *Bromus valdivianus*

Phil. grass [4], because they are two species with different growth habits growing under the same environmental conditions.

Water-soluble carbohydrates are also important for DF because leaf growth is related to a decrease in WSC concentration [5,6]. When carbohydrate synthesis is greater than its utilization, WSCs are temporarily stored in the base of leaf sheaths with a lower proportion stored in leaf blades [7]. Water-soluble carbohydrates are an immediate energy source for plant growth after defoliation; therefore, the quantity of stored WSC pre-defoliation is associated directly with the speed of pasture regrowth [8].

In pastures, the amount of stored WSCs is associated with the plant's phenological stage [9]. *Lolium perenne* uses WSCs as an energy source immediately following defoliation until approximately one leaf has expanded completely and can generate enough energy via photosynthesis to cover maintenance requirements and tissue development [10,11]. Once the third leaf has emerged, it can be assumed that the plant has accumulated enough WSC reserves to tolerate subsequent defoliation.

Defoliation frequency influences the mobilization and storage of WSCs in grass pastures. Short intervals between defoliation do not allow plants to recover enough WSCs, reducing pasture persistence, whereas longer intervals permit plants to synthesize enough carbohydrates to support post-defoliation regrowth [9,12,13]. The season also influences WSC recovery. Most studies have been conducted during spring or in controlled environments because it is known that the concentration of WSCs in grass increases during daylight as a result of the positive balance between photosynthesis and respiration [10,14]. In C_3 species, the accumulation and mobilization of stored WSCs, predominantly fructans, are of great importance in the synthesis of new tissues during regrowth [15]. Hence, the concentration of WSCs in plants varies through the day and from season to season [16,17], playing an important role in the balance between photosynthesis, carbon contributions, and C requirements for plant growth and development [18].

Some studies have concluded that *L. perenne* has a higher concentration of carbohydrates than *B. valdivianus.* The concentration of WSCs in species such as *L. perenne* and *B. valdivianus* increases in the afternoon because of increased photosynthetic activity in the presence of full light [19,20]. In fall, post-defoliation regrowth is slow compared to spring because the temperature drops and luminosity is lower [9]. However, fall is an important season because, along with spring, it provides the most forage during the year. Therefore, the two objectives of this study were (1) to examine the dynamics of WSC use and the recovery of leaf sheaths and blades of *L. perenne* and *B. valdivianus* subjected to two DFs; (2) to evaluate how DF influences regrowth and accumulated herbage mass (AHM) during fall.

2. Materials and Methods

2.1. Site Description

This study was carried out at the Austral University of Chile (UACh) in the Austral Agriculture Research Station (39°46′ S, 73°13′ W, 12 m a.s.l.) in Valdivia, southern Chile, during the fall of 2017, from 20 March to 23 June. The precipitation during the study period (March to June) was 677 mm with a mean temperature of 11 °C (Figure 1), and the annual precipitation and mean temperature at the experimental site was 2210 mm and 11.7 °C, respectively. Climate information for the experiment was collected daily via a meteorological station (Agromet-Inia) located 5 m from the study site. The topography was flat with a slope of less than 2%. The soil corresponded to a Duric Hapludand Andisol, in the Valdivia series [21], with a pH of 5.4, organic matter level of 14.6%, 17.4 mg kg^{-1} P_{olsen}, and 4.9% aluminum saturation (Soil Lab, Institute of Agricultural Engineering and Soils, Faculty of Agriculture, Universidad Austral de Chile, Valdivia, Chile). The fertilizer application was 150 kg ha^{-1} $year^{-1}$ of nitrogen, 44 kg ha^{-1} $year^{-1}$ of phosphorous, and 50 kg ha^{-1} $year^{-1}$ of potassium. The soil was suitable for perennial species.

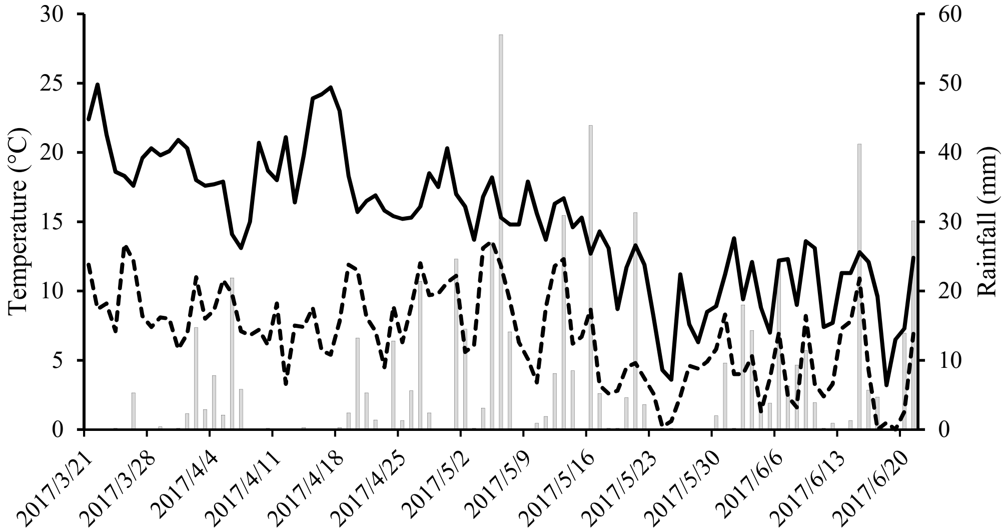

Figure 1. Daily maximum (solid line) and minimum (dashed line) temperature and rainfall (bars) for Valdivia, Chile, during the fall of 2017.

2.2. Experimental Design

The experiment included 12 plots of 15 m^2 (3 m wide × 5 m long) randomly distributed into three blocks. In March 2015, six plots were sown with 30 kg ha^{-1} *L. perenne* cv. Alto and six with 45 kg ha^{-1} *B. valdivianus* Phil. Each plot was defoliated with one of the following fixed DFs based on AGDDs: DF1 = 135 AGDDs that corresponded to the 1.5 leaf stage (LS) for *L. perenne* and 1.7 for *B. valdivianus*; DF2 = 270 AGDDs that corresponded to the 3 and 3.4 LS for *L. perenne* and *B. valdivianus*, respectively, using a rotary mower equipped with a collection bag and with a residual height of 5 cm left.

Each block comprised four plots, and each plot corresponded to the interaction between species (*L. perenne* and *B. valdivianus*) and DFs (135 and 270 AGDDs). The interaction between these two factors was considered the treatment (TTm). All plots were defoliated according to the assigned DF during the plot conditioning period from October 2016 to March 2017. Sampling was conducted from 21 March to 21 June 2017.

The following equation was used to calculate accumulated thermal time:

$$\mathrm{AGDD} = \sum [(T_{\mathrm{max}} + T_{\mathrm{min}})/2] - T_{\mathrm{base}}, \quad (1)$$

where T_{max} is the daily maximum air temperature, T_{min} is the daily minimum air temperature, and T_{base} is the temperature below which the observed process does not occur. A base temperature of 5 °C was used for both species, and the temperature accumulated from day 1 to day n [22,23]. The air temperature was measured at 150 cm high above the ground level, and it was taken from the meteorological station close to the field experiment.

2.3. Evaluated Variables and Sampling

Three cores (90 cm in diameter and 10 cm deep) were collected from each plot at 08:00 every third day, until each plot reached the assigned AGDDs. Each core corresponded to a sample composed of soil with root, leaf blades, and leaf sheaths which were stored on ice to avoid WSC losses until they were transported to the Animal Nutrition Laboratory of the UACh, where they were washed to remove soil residue. The root was discarded, and the grass blades were separated from the sheaths.

The blade and sheath materials were measured separately for green weight and then dried in a forced-air oven at 65 °C for 72 h to measure dry matter (DM) content and dry weight. Dried samples were ground to 1 mm using a Wiley mill (Model Digital ED-5, Thomas Scientific, Swedesboro, NJ, USA) and stored in plastic containers at ambient temperature for subsequent WSC analysis.

Water-soluble carbohydrate concentration was determined in the blade and sheath by near infrared reflectance spectroscopy (NIRS) with a FOSS-NIRSystems Model 6500 and Version 4.4 FOSS-ISIScam software and equations developed in the UACh Animal Nutrition Laboratory. The equation for WSC concentration was calibrated using the anthrone method described by Yemm and Willis [24], where WSCs are expressed as glucose and determined using spectroscopy based on the blue–green color created when carbohydrates are heated with anthrone in sulfuric acid. The standard errors of cross-validation and R^2 for WSCs were 6.99 and 0.96, respectively.

The AHM was estimated every six days using a rising plate meter (Ashgrove Plate Meter, Hamilton, New Zealand). Each value was calculated using the average height of 10 samples per plot. To estimate production per hectare (kg DM ha^{-1}), an equation specific to fall pastures in southern Chile was used [25]:

$$Y = 120X + 350\ (r^2 = 0.74), \quad (2)$$

where Y = AHM in kg DM ha^{-1}, and X = compressed average height.

When each plot accumulated the assigned AGDDs, it was defoliated again for further sampling. Therefore, during the fall, plots with 135 AGDDs were defoliated twice, and plots with 270 AGDDs were defoliated once. The growth was calculated from AHM at the defoliation time minus the residue that was measured in the past defoliation and calculated at 1200 kg DM ha^{-1}, and the growth of 135 AGDDs was adding up the growth for both periods.

2.4. Statistical Analysis

Response variables included WSC concentration (g kg^{-1}) in blades and sheaths and AHM production (kg DM ha^{-1}). Data were analyzed using the MIXED procedure of SAS (SAS Institute, V9.0, 2008) in a complete randomized block design with a factorial arrangement of treatments (two species and two DF), where AGDDs and species were fixed effects, and the field block and their interaction were random effects. Sampling time was included as a repeated measure in the model. The covariance structure [26] was based on the probability test and the Akaike information criterion, test according to (a) no structure, (b) composite symmetry (CS), (c) heterogeneity of compound symmetry (HCS), (d) Toeplitz (TOEP), and (e) Toeplitz heterogeneity (HTOEP). Prior to analysis, all data were checked for normality and homogeneity of variances. When there were significant differences ($p < 0.05$), a multiple mean comparison test (LSMEANS) was performed with the PDIFF command.

3. Results

3.1. Effect of Defoliation Frequency on WSC Concentration in Leaf Sheaths and Blades

Lolium perenne L. and *B. valdivianus* defoliated after 135 AGDDs had similar WSC levels ($p > 0.05$, Figure 2a,b) in both the blades and sheaths. When the DF was extended to 270 AGDDs, significant differences ($p < 0.05$, Figure 3a,b) were found between the two species. This change became apparent after 195 AGDDs, at which point *L. perenne* regrowth was able to accumulate approximately 33% more WSCs than *B. valdivianus*. This difference remained constant between the two species from 195 to 270 AGDDs.

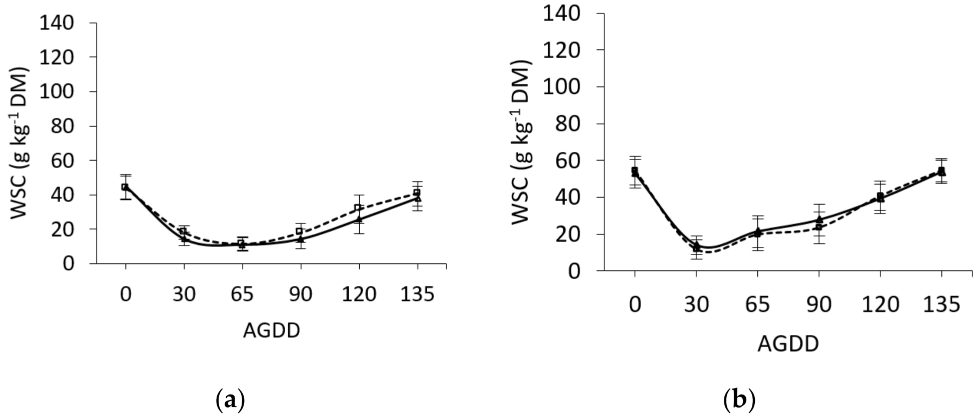

Figure 2. Mean of two growth cycles for water-soluble carbohydrate (WSC) concentration (g kg^{-1} dry matter (DM)) in leaf blades (**a**) and leaf sheaths (**b**) for *L. perenne* (–□–) and *B. valdivianus* Phil. (-△-) plots defoliated at 135 accumulated growing degree days (AGDDs). Bars indicate standard error.

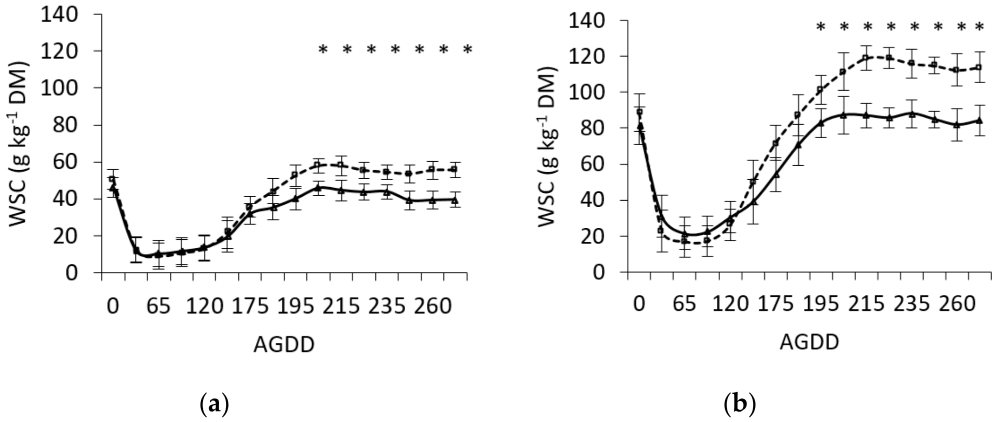

Figure 3. Mean water-soluble carbohydrate (WSC) concentration (g kg^{-1} DM) post-defoliation in leaf blades (**a**) and leaf sheaths (**b**) for *L. perenne* (–□–) and *B. valdivianus* Phil. (-△-) plots defoliated at 270 accumulated growing degree days (AGDDs). * Indicates significant differences ($p < 0.05$) between species. Bars indicate standard error.

It was observed that in both *L. perenne* and *B. valdivianus*, the concentration of WSCs in the blades and sheaths significantly decreased following defoliation until 30 AGDDs ($p < 0.05$, Figures 2 and 3), (**a**) and (**b**) when plants began to accumulate WSCs. This process was similar for both blades and sheaths.

After both DFs, leaf blade WSC concentration was reduced by approximately 80% in *L. perenne* and 74% in *B. valdivianus*. For both species, plants began to recover WSCs after 65 to 90 AGDDs post-defoliation when defoliated every 135 AGDDs. For plants defoliated every 270 AGDDs, the recovery of WSCs was initiated after 90 to 120 AGDDs had been reached. On the other hand, the recovery rate of WSCs in leaf sheath was different between species defoliated at 270 AGDDs. The *L. perenne* recovered faster than the *B. valdivianus* using 1.5 AGDDs per 1 g of WSC, whereas *B. valdivianus* needed more TT (2.3 AGDDs) to accumulate the same amount of WSCs. When the species were defoliated at 135 AGDDs, the recovery rate was the same for *L. perenne* and *B. valdivianus* with 2.5 AGDDs per 1 g of WSCs.

3.2. Water-soluble Carbohydrates and Pasture Regrowth

After the initial defoliation (day 0) and during the subsequent growth cycle of 135 AGDDs DF, there were no significant differences in WSC levels between species for both leaf blades and sheaths

($p > 0.05$, Figure 4), except at the end of the experiment, when the leaf blade WSC concentration of both species had not fully recovered. In contrast, leaf sheaths recovered WSCs to the pre-defoliation levels measured on day 0 ($p < 0.05$, Figure 4). Mean concentrations of WSC were 25% and 28% greater in leaf sheaths than in leaf blades for *L. perenne* and *B. valdivianus*, respectively, when defoliation occurred every 135 AGDDs (Figure 4a; $p < 0.05$).

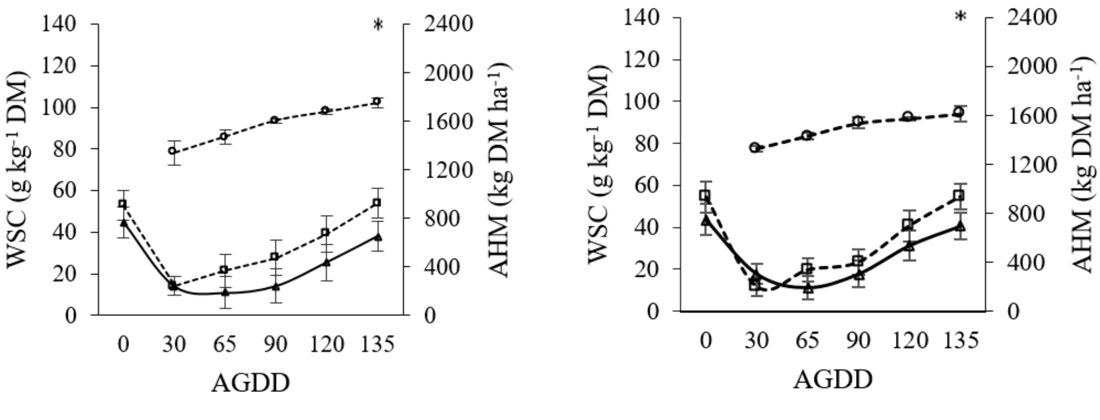

Figure 4. Mean of two growth cycle for water soluble carbohydrates (WSCs, g kg^{-1} DM) and accumulated herbage mass (AHM, kg DM ha^{-1}) in *Bromus valdivianus* Phil. (**a**) and *Lolium perenne* L. (**b**) in: leaf blades (-△-) and leaf sheath (–□–) defoliated at 135 accumulated growing degree days (AGDDs) and AHM (–o–). * Indicates significant differences ($p < 0.05$) between leaf sheaths and blades for the same species. Bars indicate the standard error.

When plants were defoliated after 270 AGDDs (Figure 5), the WSC concentration was significantly greater for leaf sheaths than for leaf blades at day 0 and after 135 AGDDs ($p < 0.05$, Figure 5). Between 30 and 120 AGDDs, the WSC concentration was similar for both leaf portions (Figure 5). The same tendency was observed in pasture plots defoliated every 135 AGDDs, where the WSC concentration reduced from day 0 to 30 AGDDs (Figures 2–4).

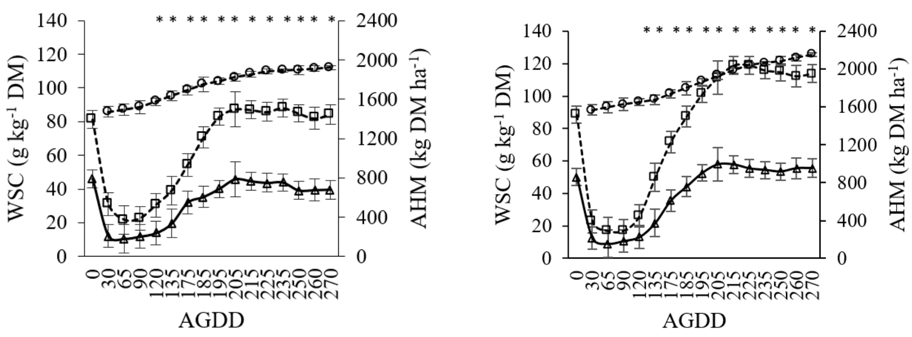

Figure 5. Mean water soluble carbohydrates (WSCs, g kg^{-1} DM) and accumulated herbage mass (AHM, kg DM ha^{-1}) in *Bromus valdivianus* Phil. (**a**) and *Lolium perenne* L. (**b**) in: leaf blade (-△-) and leaf sheath (–□–) defoliated at 270 accumulated growing degree days (AGDDs) and AHM (–o–). * Indicates significant differences ($p < 0.05$) between leaf sheaths and blades for the same species. Bars indicate the standard error.

In *L. perenne* plots defoliated every 270 AGDDs, the WSC concentration was reduced by 85% compared with the pre-defoliation levels (day 0) and did not recover until approximately 215 AGDDs, when plants reached the WSC concentration used for regrowth. In *B. valdivianus* plots defoliated with the same DF, the WSC concentration levels decreased by 75% post-defoliation and required 205 AGDDs to completely recover the WSC reserves used during regrowth (Figure 5a,b).

Similar patterns in the movement of WSCs throughout the regrowth cycle were observed in both *L. perenne* and *B. valdivianus* plots defoliated after 135 and 270 AGDD. Plants in both DFs utilized WSC immediately after defoliation, but the replenishment of WSC levels before the next defoliation was slower at 270 AGDDs than at 135 AGDDs (Figures 4 and 5).

Plots defoliated at 270 AGDDs had time to accumulate greater concentrations of WSC compared to those defoliated at 135 AGDDs. In both forage species and at both DFs, the concentration of WSC was higher in leaf sheaths than in leaf blades during the entire regrowth cycle. When considering the combined effect of DF and species, the highest concentration of WSCs corresponded to leaf sheaths of *L. perenne* plots defoliated at 270 AGDDs, followed by sheaths of *B. valdivianus* defoliated at the same frequency.

The water-soluble carbohydrate concentration in leaf sheaths did not significantly differ between species when defoliated at 135 AGDDs ($p > 0.05$, Table 1). The highest leaf blade WSC concentration levels were found in the interaction among *L. perenne* plots defoliated at 270 AGDDs, but there were no other significant differences in leaf blade WSC concentration level interactions ($p > 0.05$, Table 1). A significant interaction between species and DF was observed for both blades and sheaths. In *B. valdivianus*, WSC in blades was similar in both DF, whereas for *L. perenne*, a greater WSC concentration was observed for DF 270 compared with 135. In the case of sheaths, the interaction showed a greater increase from DF 135 to DF 270 for *L. perenne* (+58 g kg^{-1} DM) compared with *B. valdivianus* (+31 g kg^{-1} DM). The same trend was obtained for WSCs per hectare, where *L. perenne* produced 110 kg more than *B. valdivianus*, both defoliated at 270 AGDDs, while *L. perenne* and *B. valdivianus*, which were defoliated at 135 AGDDs, did not show a significant difference.

Table 1. Mean water-soluble carbohydrate (WSC) content (kg kg^{-1} DM) in leaf sheaths and leaf blades and production per hectare (kg WSC ha^{-1}) for *Bromus valdivianus* Phil. and *Lolium perenne* L. defoliated at 135 and 270 accumulated growing degree days (AGDDs).

Species	AGDDs	Blade	Sheath	Hectare
B. valdivianus	135	37.95 ^{b}	53.70 ^{c}	193 ^{c}
	270	39.63 ^{b}	84.28 ^{b}	238 ^{b}
L. perenne	135	40.72 ^{b}	54.65 ^{c}	192 ^{c}
	270	55.61 ^{a}	113.93 ^{a}	348 ^{a}
SEM		1.481	1.923	36.7
p-Value		0.001	0.001	0.001

Means within a column with different superscripts differ ($p < 0.05$); SEM = standard error of the mean.

3.3. Defoliation Frequency and Growth

The increase in growth followed the same trend as WSC replenishment levels. As shown in Figures 4 and 5, growth began to increase after 30 AGDDs in both DF treatments. In contrast, WSCs did not begin to recover until 90 AGDDs in plots defoliated at 135 AGDDs (Figure 4) and 45 AGDDs in plots defoliated at 270 AGDDs (Figure 5). Table 2 shows that the average value of the interaction between DF and species for growth was not significant ($p > 0.05$, Table 2), illustrating that similar growth occurs in fall grazing with intervals of 135 and 270 AGDDs. However, *B. valdivianus* defoliated at 135 AGDDs produced 52 (6%) kg DM ha^{-1} more than *L. perenne* defoliated at 270 AGDDs; that in one hectare is not significant but in several hectares it could be significative. In addition, pastures defoliated to 135 AGDDs have a better nutritive value in terms of crude protein and metabolizable energy than defoliated pastures at 270 AGDDs.

Table 2. Mean growth (kg DM ha^{-1}) of *Bromus valdivianus* Phil. and *Lolium perenne* L. defoliated after 135 and 270 accumulated growing degree days (AGDDs).

Species	AGDDs	Growth
B. valdivianus	135	906 [x]
	270	723
L. perenne	135	816 [x]
	270	854
SEM		17.319
p-Value		0.415

[x] Accumulated value of two growth cycles each one at 135 AGDDs; SEM = standard error of the mean.

4. Discussion

The present field study highlights the effect of DF, determined by AGDDs, on the utilization, concentration levels, and recovery of WSC concentrations in *L. perenne* and *B. valdivianus* pastures during fall. More frequent defoliation (135 AGDDs) reduced the levels of WSCs in both leaf sheaths and blades, but reserves were replaced faster. When defoliated with a lower frequency (270 AGDDs), WSC levels were higher but recovery was slower.

4.1. Defoliation Frequency and WSCs

During most of the growth cycle, the WSC level and recovery rate werer greater in leaf sheaths than in leaf blades, supporting previous studies that report the sheath as the main WSC storage component in grass species [7]. Moraes et al. [27] evaluated 24 *poa* species and reported that the WSC concentration was greater in sheaths than in blades, with the exception of *Echinolaena inflexa*. The storage of WSCs in leaf sheaths is probably a survival mechanism given that the reserves are located close to the growth points and allow for efficient use. If WSCs were predominately stored in the leaf blades, they would be removed by grazing activity and the plant would lose this resource for regrowth in the subsequent cycle [28].

In most of terrestrial plants, the use of WSCs begins immediately after a plant is defoliated and after recovery process the reserves are restored to the original concentration [29]. In the present study, plants defoliated with the lower DF of 270 AGDDs recovered the original concentration (between 100 to 120 g kg^{-1} DM) after approximately 195 AGDDs. Similar results were reported by Donaghy and Fulkerson [5], who measured 125 g kg^{-1} DM of WSC for *L. perenne* pastures defoliated at the three-leaf stage with 5 cm of residue. Turner et al. [6] reported that *L. perenne, B. willdenowwii* Kunth., and *Dactylis glomerata* L. recovered WSCs after 2.5 to 3 leaves had appeared. Many studies have shown that AGDDs are directly related to the phyllochron and the leaf stage [30]. The new leaf appearance in *L. perenne* takes 88 AGDDs, while for *B. valdivianus* it is only 77 AGDDs [4]. After 195 AGDDs, pasture grass has between two and three leaves per tiller and some 80% of WSC reserves are restored, which supports recovery after the next grazing cycle [31].

Berone et al. [32] evaluated the impact of DF on WSC concentrations in winter pastures of *L. perenne* and *B. stamineus* in Argentina using leaf stage as a grazing criterion. They reported that the WSC concentration was greater in plants defoliated with less frequency (5 leaf stage) than in plants defoliated more frequently (3 leaf stage), and the WSC mean values were 25 g kg^{-1} DM for *B. stamineus* and 15 g kg^{-1} DM for *L. perenne*. The response to different DFs is consistent with the trend measured in the present study; however, they reported lower WSC values. This may be due to the fact that the two studies were conducted under different seasons and climate conditions.

The measurements of WSC concentration following defoliation at intervals of 135 and 270 AGDDs in *L. perenne* and *B. valdivianus* indicated that WSC recovery was initiated at the same time in both leaf sheaths and blades, with a consistently greater concentration in the sheaths. This supports the conclusion that, regardless of the DF or species, leaf sheaths are the main organ for WSC reserves [10].

The levels of WSC in sheaths of *L. perenne* defoliated at 135 and 270 AGDDs measured in the present study (54.65 and 113.93 g kg^{-1} DM, respectively) were lower than values reported by Loaiza et al. [33] with more than 150 g kg^{-1} DM for *L. perenne* plants defoliated at the three-leaf stage during fall. This difference between WSC levels could be explained by N fertilization, as N supply induces sucrose cleavage to release hexoses capable of supporting regrowth during fall growing conditions [15]. The season is a main factor affecting pasture growth, because weather changes modify the physiological stages of each species (water requirements, soil type, water absorption, and transpiration); therefore, the season determines how WSCs are stored [34,35]. In spring and summer, plants maintain greater concentrations of WSCs as well as DM production because of the increased photosynthesis activity from higher temperatures and luminosity [7]. Water-soluble carbohydrates are only accumulated when the synthesis of carbohydrates exceeds their use, which generally occurs when plants have sufficient photosynthetically active leaf blades expanded to support the energy requirements to continue the growth cycle [36,37].

4.2. Defoliation Frequency and AHM

The higher AHM with defoliation every 270 AGDDs compared to 135 AGDDs demonstrates that there is a relationship with WSC recovery, given that higher WSC concentrations were measured with higher AHM (20% and 27% more than 135 AGDDs, respectively). This is supported by both Donaghy and Fulkerson [5] and Lee et al. [38], who reported that there is a positive linear relationship between these variables (r^2 = 0.52, leaf blade (g kg^{-1} DM) = 1.04 + 0.99 WSCs). This behavior is replicated in the majority of forage species, corroborating the close relationship between WSC concentration and AHM [39,40].

In addition to their role in pasture regrowth, WSCs are also important at the nutritional level, in particular the portion stored in the leaf blade, since this is the plant part consumed by grazing animals. Cajarville et al. [9] measured WSC concentrations of 39.1 g kg^{-1} DM in *Festuca arundinacea* pastures in fall when harvested in the morning and also found that there was a positive relationship between in vitro gas production and WSC concentration. Beltran [41] observed that *L. perenne* pastures had higher WSC concentrations in the afternoon compared to the morning (57 g and 82 g kg^{-1} DM, respectively), but this did not significantly affect milk production. The average WSC concentration for the two species measured in the present study were 40 g and 100 g kg^{-1} DM when defoliated at 135 and 270 AGDDs, respectively.

5. Conclusions

The present study confirmed that the leaf sheath was the principal storage organ for WSC reserves, having higher concentrations than leaf blades in *L. perenne* and *B. valdivianus* fall pastures. Water-soluble carbohydrates are easily accessible energy sources that support plant physiological requirements immediately after defoliation. Approximately 80% of total WSC was used during the regrowth process before WSC storage recommenced. Defoliation frequency affected WSC concentration, with longer intervals between defoliation (270 AGDDs) being preferred, because the plants could recover 99% of WSC reserves and could tolerate another grazing event better. Defoliation with greater frequency (135 AGDDs) diminished the synthesis and storage of WSCs in *L. perenne* and *B. valdivianus* and led to slower regrowth. We encourage further research under other seasons and with others defoliation frequencies.

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