Sward and tiller growth dynamics of *Lolium perenne* L. as affected by defoliation frequency during autumn

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Abstract. A mini-sward study was undertaken between January and September 2008 in Valdivia, Chile, to quantify and compare changes in the herbage production, growth dynamics and nutritive quality of a *Lolium perenne* sward during autumn in response to different nitrogen (N) application regimes and defoliation frequencies. The N levels used in the present study corresponded to the equivalent of an autumnal application of 0, 25 or 75 kg N/ha. The defoliation frequency was based on leaf regrowth stage, and corresponded to the time taken for 1.5, 2.5 or 3.5 leaves per tiller to expand. The growth dynamic measurements were also related to temperature. The results showed that the level of N had no effect on the studied variables. Plants defoliated at the 3.5-leaf stage showed a higher herbage production, higher tillering and a more balanced nutritional profile than plants cut at the 1.5-leaf stage. The defoliation interval had no effect on leaf elongation or leaf appearance. The leaf elongation rate was linearly related to the mean daily temperature ($P < 0.01$), and the accumulated leaf and tiller number showed a significant linear relationship ($P < 0.01$) with the thermal time expressed as growing degree days. From the defoliation frequencies tested here, we can conclude that the best time for grazing *L. perenne* swards during autumn is at the 3.5-leaf stage due to a higher herbage production, higher tillering rate, more balanced nutritive quality and higher reserve levels of the plant, which provide a more favourable background for subsequent growth.

Additional keywords: autumn, defoliation, leaf stage, pasture quality, pasture utilisation, tiller growth dynamics.

Introduction

Soil nitrogen (N) availability and herbage defoliation are potentially two major sources of pasture stress (Tilman 1999). Thus, N fertiliser applications are likely to increase pasture growth, depending on the environmental limitations imposed by the local environment (Jarvis *et al*. 1995). Defoliation may stimulate growth for some plant species, whereas it may diminish the population of other species (Silvertown *et al*. 1994).

It has been well established that when N fertilisation is increased, sward herbage production increases (Hopkins *et al*. 1990; Schils 1997), as does the N content [and, therefore, the crude protein (CP)] of the harvested material (Belanger and McQueen 1998; Duru 2003). However, N has very little or no effect on the digestibility of the forage (Duru 2003). Regarding tiller growth dynamics, N has a strong positive influence on leaf elongation (Ryle 1964; Kavanová *et al*. 2008) and tillering initiation (Ryle 1964; Harris *et al*. 1996; McKenzie 1998). However, contradictory results have been published concerning N addition and leaf initiation. Pearse and Wilman (1984) showed that N addition stimulated leaf initiation. However, Duru *et al*. (1999), Duru and Ducrocq (2000) and Hirata (2000) observed that N diminished leaf initiation, whereas other investigators have reported no effect of N on leaf initiation (Sampaio and Beaty 1976; Wilman *et al*. 1977).

In a rotational grazing system, the defoliation interval is managed by the farmer. It is related to the amount of feed on offer, and to a given stocking intensity, it sets the rate of feed utilisation (Fulkerson and Slack 1994). The defoliation interval, defined as the time between one defoliation event and the next, is frequently based on day rotations, sward surface height or targeted pregrazing herbage mass. An alternative to these criteria is to base the defoliation frequency on leaf regrowth stage, which reflects the physiological status of the plant in terms of energy levels and appropriateness of herbage quality for ruminant nutrition (Fulkerson and Donaghy 2001). Donaghy and Fulkerson (1997) investigated the effect of defoliation, based on leaf stage, on the water-soluble carbohydrate (WSC) reserves of *Lolium perenne* L. They found that plants defoliated before the 2-leaf stage grew less than those defoliated at the 2- or 3-leaf stages. This was largely a result of the reduced WSC reserves. An earlier study by Fulkerson and Slack (1994) had reported that *L. perenne* accumulated reserves of WSC until the plant had four fully emerged leaves.

Studies on defoliation frequencies based on the leaf stage have shown that longer intervals between consecutive herbage defoliations have a positive effect on herbage production (Fulkerson and Slack 1995; Turner *et al*. 2006*a*), a negative effect on CP (Sinclair *et al*. 2006; Donaghy *et al*. 2008) and, in some species, a negative effect on neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Turner *et al*. 2006*b*; Donaghy *et al*. 2008). The defoliation frequency also affects tiller growth dynamics, such that longer grazing intervals

stimulate leaf elongation (Volenec and Nelson 1983; Donaghy and Fulkerson 1998). However, the role of the length of time between consecutive defoliations on tiller initiation is unclear. It has been reported that increasing the defoliation interval stimulates leaf initiation (Hume 1991; Donaghy and Fulkerson 1998), but no effect of defoliation frequency on leaf initiation has also been described (Korte and Watkin 1985; Velasco *et al.* 2007).

In regions of temperate humid climate with a warm summer and a rainfall greater than 1000 mm per year, non-irrigated permanent pastures increase their growth rate to 30–50 kg DM ha^{-1} day^{-1} during autumn compared to 5–20 kg DM ha^{-1} day^{-1} during summer. In autumn, grass development is centred in vegetative growth; however, during spring, grass species change from the vegetative development to reproductive growth. This means that grass species, during autumn, develop only new tillers and leaves. There is currently little information available regarding tiller growth and development, and no research has been published regarding the effects of defoliation interval, based on leaf stage, and N application during this season. The present study analysed the effects of defoliation frequency, based on leaf stage, and N supply on the herbage production, growth dynamics and nutritive quality of a *L. perenne* sward during autumn in Chile.

Materials and methods

Experimental site

The trial was conducted between January and September 2008 at the Universidad Austral de Chile, Valdivia, Chile (elevation 12 m a.s.l., longitude 73°15′W, latitude 39°48′S, annual rainfall 2500 mm).

The mean daily temperature and rainfall for the studied period are shown in Fig. 1. Pots of 125-L capacity (0.48 m superior diameter, 0.8 m height and 0.35 m inferior diameter) were filled with top soil collected from the first 20-cm depth of a high fertility grassland (Duric Hapludand soil, CIREN 2003), and a 3-mm sieve was used for the subsequent homogenisation. Thus, the top 10 cm of pot soil had 5.7 pH (H$_2$O), 23 mg/kg Olsen P, 266 mg/kg exchangeable K and 45.5 mg/kg mineral N (NO$_3$ + NH$_4$). Pots were kept outside during the experiment.

Pasture establishment and irrigation

Each pot received a fertilisation equivalent of 45 kg/ha P, 60 kg/ha K$_2$O and 1000 kg/ha CaCO$_3$, which was mixed with the top 5 cm of soil. During January 2008 seeds of *L. perenne* cv. Impact were hand-sown in the pots in a square design (1.9 × 1.9 cm) to a depth of 1 cm and to an equivalent seed rate of 60 kg/ha. Thus, each mini-sward received 488 seeds, simulating the density of an established *L. perenne* pasture. Plant species other than *L. perenne* were manually eliminated. On 21 March, all the mini-swards were cut to a 5-cm height and the period of evaluation began.

The mini-swards were irrigated daily between sowing time and canopy closure; after that, irrigation was every second or third day. During the measurement period of the *L. perenne* growth dynamics (21 March–21 June), soil was irrigated with 30 mm of water when its tension, measured with a tensiometer (Irrometer model R, Irrometer Co., Riverside, CA, USA), reached 40 kPa.

Experimental design

The experiment was arrayed according to a completely randomised block design with a factorial arrangement of the treatments, three N levels and three defoliation frequencies, distributed in three blocks. The N levels were the equivalent of an autumnal application of 0, 25 or 75 kg N/ha, that was broadcast in monthly applications of 0, 8.3 or 25 kg N/ha. The defoliation frequency was based on leaf stage, defined as the time taken to full emergence of one new leaf (Donaghy *et al.* 2008), cutting and harvesting the mini-swards when the grass reached the 1.5-, 2.5- or 3.5-leaf stage.

Growth dynamics of L. perenne

The dynamics of *L. perenne* growth were evaluated during autumn, between 21 March and 21 June.

Following the defoliation that homogenised the mini-swards (21 March), three tillers per mini-sward were randomly selected and marked with coloured paperclips at their base. Every third day, the appearance of new leaves and daughter tillers was recorded on the marked tillers: lamina length was measured for fully expanded leaves from the tip of the lamina
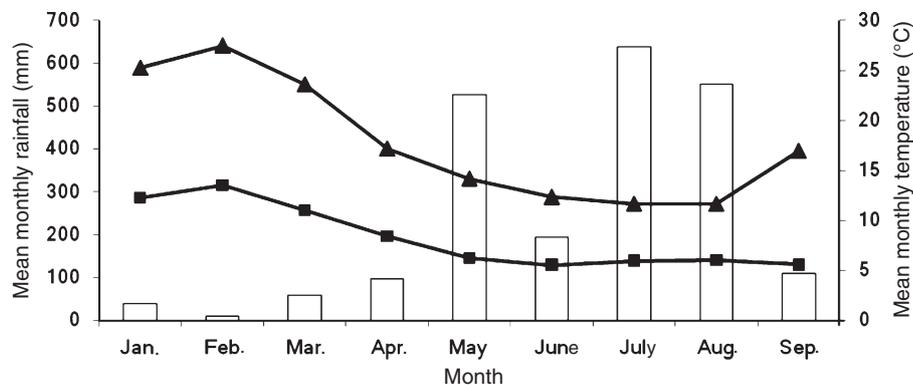

Fig. 1. Mean monthly maximum (▲) and minimum (■) temperature and monthly rainfall (□) for Valdivia, from January to September 2008.

to its ligule; and for growing leaves, lamina length was measured from the tip of the lamina to the ligule of the previously fully expanded leaf.

The calculated variables during the growth dynamics measurement period on marked tillers were:

$$\text{Leaf appearance rate (LAR; leaves/day)} = (n-1)/t \quad (1)$$

where n = number of appearing leaves; t = period of time between the appearance of the first and last leaf.

A new leaf was considered as 'appeared' when its tip was visible (Wilhelm and McMaster 1995).

Phyllochron, as defined by Klepper *et al.* (1982), was calculated as the inverse of LAR.

$$\text{Tiller appearance rate (TAR; tillers day}^{-1}\text{ tiller}^{-1}) = n/t \quad (2)$$

where n = number of tillers appearing after the first phyllochron until the end of the evaluation; t = period between the first phyllochron and the end of the evaluation.

Leaf elongation rate per tiller (LER):

$$\text{LER (mm/day)} = [((l_2 - l_1)/t_1) + ((l_3 - l_2)/t_2) + \cdots$$
$$+ ((l_n - l_{n-1})/t_{n-1})]/(n-1) \quad (3)$$

where l = leaf blade length at the nth measurement; t = time between two consecutive measurement events; n = number of leaf elongation measurements performed.

The overall accumulated number of appeared leaves (LAR) and tillers (TAR), and the LER between consecutive measurement dates were related to temperature. The slope of these regression curves showed the overall accumulated appearance of leaves and tillers, and also the overall LER. These analyses eliminated the variation induced by sources of variation, such as N level, defoliation frequency and block effect.

The temperature (minimum, maximum and average) was recorded on a daily basis at the meteorological station of the Isla Teja Campus, Universidad Austral de Chile.

Soil mineral N evaluation

When the evaluation of the growth dynamics concluded (21 June), a composed soil sample (five subsamples randomly selected) was taken from each mini-sward to a depth of 10 cm. Thus, each mini-sward generated an individual soil sample. These samples were analysed for mineral N content (N-NO$_3^-$ and N-NH$_4^+$) at the Soil Chemical Laboratory, Universidad Austral de Chile, using the Kjeldhal digestion methodology described by Sadzawka *et al.* (2006).

Accumulated herbage mass and herbage nutritive value

When the mini-swards reached the corresponding leaf stage, they were trimmed to a height of 5 cm and the collected foliage was oven-dried at 60°C for 48 h before determining the herbage mass. The accumulated herbage mass was calculated by adding together the herbage masses obtained from each mini-sward at each trimming event conducted between 21 March and 21 June.

The forage collected from each mini-sward at each trimming event was evaluated for CP (Bateman 1970), NDF (Van Soest *et al.* 1991), ADF (AOAC 1996), digestible organic matter on dry matter basis [DOMD; Tilley and Terry (1963)] and water-soluble carbohydrates in the forage [WSC$_F$; MAFF (1985)]. The analyses were performed at the Animal Nutrition Laboratory, Universidad Austral de Chile. Metabolisable energy (ME) was calculated from DOMD using the Garrido and Mann (1981) equation:

$$\text{ME (Mcal/kg DM)} = 0.279 + 0.0325 \times \text{DOMD} \quad (4)$$

Reserve of water-soluble carbohydrates in the stubble (WSC$_R$) in L. perenne

Following the dynamics of growth evaluation, the mini-swards were left to grow for another growth cycle until they reached their corresponding treatments (1.5-, 2.5- or 3.5-leaf stage). The mini-swards were then defoliated and the herbage between 0 and 5 cm was collected and analysed for WSC$_F$ at the Animal Nutrition Laboratory. Dead material attached to the pseudostems was removed and the pseudostems were then stored at 4°C. The herbage samples were oven-dried at 102°C for 2 h, then ground through a 0.5-mm sieve. Reserve water-soluble carbohydrates (WSC$_R$) were extracted using cold water in an orbital shaker for 1 h. The resulting extract was filtered, and a sample was then analysed using the anthrone method as described by MAFF (1985).

Statistical analysis

For all the studied variables, a mini-sward was the experimental unit. For growth dynamics measurements, individual tillers constituted the unit of observation and the three marked tillers per mini-sward were averaged. To compare between treatment means, ANOVA was applied for all the variables, and Tukey's honestly significant difference was used to separate treatment means.

Regression analysis between leaf elongation and mean daily temperature was performed. Based on the obtained equation from the regression analysis, the x-intercept (i.e. zero pasture growth) was used as the base temperature for the thermal time calculation, as growing degree days (GDD). Accumulated GDD were regressed against accumulated leaf and tiller number. The statistical analysis was performed using STATGRAPHICS Plus version 5.1 (StatPoint Technologies Inc., Warrenton, VA, USA).

Results

Soil mineral N and WSC$_R$

At the end of the growth dynamics evaluation, both N fertiliser supply and defoliation frequency had no significant effect ($P > 0.05$) on soil mineral N content (Table 1). However, they both had a significant effect on WSC$_R$ content, with an increase in the WSC$_R$ content of the pseudostems (assessed tissues) as defoliation frequency ($P \leq 0.01$) and N application ($P \leq 0.05$) decreased; there was no interaction between both sources of variation.

Accumulated herbage mass

The accumulated herbage mass did not significantly increase ($P > 0.05$) with N addition (Table 2). The defoliation frequency showed a significant effect ($P \leq 0.01$) over the accumulated

Table 1. Mineral nitrogen (mg/kg) in the soil at the end of the growth dynamics measurement period (21 June) and reserve water-soluble carbohydrates [WSC$_R$, (g/kg)] at completion of the first growth cycle after the last defoliation treatment (within measurement time) as affected by nitrogen application and defoliation frequency

*$P \leq 0.05$; **$P \leq 0.01$; n.s., not significant; h.s.d., honestly significant difference

Source of variation	Level	Dependent variables	
		Mineral nitrogen (mg/kg)	WSC$_R$ (g/kg)
Nitrogen (kg/ha)	0	13.77	109.88
	25	13.30	105.87
	75	12.13	98.97
	h.s.d.	n.s.	9.38*
Defoliation frequency (leaf stage)	1.5	12.60	86.93
	2.5	13.61	100.41
	3.5	12.99	127.11
	h.s.d.	n.s.	13.18**

herbage mass, with the mini-sward defoliated at the 3.5-leaf stage exhibiting a significantly higher herbage mass production (69.17 g) than the mini-sward defoliated at the 2.5- and 1.5-leaf stage (54.17 and 54.89 g, respectively). There was no significant difference between trimming the mini-swards at either the 1.5- or 2.5-leaf stage.

Growth dynamics of L. perenne

The overall LER did not vary ($P > 0.05$) when either the N addition or defoliation frequency increased (Table 2). Analysed monthly, at the end of autumn, *L. perenne* LER increased with N addition (Table 3).

There was a significant linear relationship ($P \leq 0.01$) between LER and the mean daily temperature (Fig. 2). *L. perenne* leaf elongation began when the mean daily temperature was above 4.9°C.

LAR, and therefore phyllochron, were not affected ($P > 0.05$) either by N application or by defoliation frequency (Table 2).

There was a significant linear relationship ($P \leq 0.01$) between the accumulated leaf number and GDD (Fig. 3). Thus, for each additional GDD, the total number of leaves increased by 0.0085.

TAR was not modified ($P > 0.05$) by N addition, but it was affected ($P \leq 0.05$) by the defoliation frequency (Table 2). Defoliated plants at the 2.5- and 3.5-leaf stage exhibited a significantly higher TAR (0.0285 and 0.0263 tiller/day, respectively), than plants defoliated at the 1.5-leaf stage (0.0144 tiller/day).

There was a significant linear relationship ($P \leq 0.01$) between the accumulated tiller number and GDD (Fig. 4), such that, for each additional GDD, the accumulated tiller number increased by 0.0027. Active tillering started when the thermal time was close to 350 accumulated GDD, generating on average 1.9 emerged tillers per marked tiller from that point until the end of the measurement period (Fig. 4).

Herbage nutritive value

The nutritive value of the forage was not altered by N supply ($P > 0.05$). The defoliation frequency significantly modified the herbage nutritive value (Table 4). Thus, the DOMD and ME contents were significantly higher ($P \leq 0.01$) in plants defoliated at the 1.5- and 2.5-leaf stage, in relation to plants defoliated at the 3.5-leaf stage. Plants defoliated at the 1.5- or 2.5-leaf stage did not differ significantly ($P > 0.05$) regarding DOMD and ME content. NDF and ADF content increased in an opposite direction to that observed for DOMD and ME.

CP content was significantly higher ($P \leq 0.01$) in plants defoliated at the 1.5- and 2.5-leaf stage than plants less frequently defoliated. No significant difference was found ($P > 0.05$) between plants defoliated at the 1.5- and 2.5-leaf stage.

WSC in the harvested herbage were lower ($P \leq 0.01$) in plants defoliated at the 1.5-leaf stage compared to plants defoliated at the 2.5- or 3.5-leaf stage.

Discussion

The results show that N had no effect on the analysed variables, with the exception of WSC$_R$. In the present trial, it is most likely that the *L. perenne* N requirements were fulfilled by the levels of available soil mineral N at the beginning of the study. Factors that probably enhanced the soil N mineralisation were soil preparation (Zhang *et al.* 2008) and the occurrence of high summer temperatures plus the irrigation administered to the plots during the establishment of the mini-swards, factors that

Table 2. Leaf appearance rate [LAR, (leaves/day)], phyllochron [PHYL, (days/leaf)], tiller appearance rate [TAR, (tillers/day)], leaf elongation rate [LER, (mm/day)], accumulated herbage mass production [HP, (g/mini-sward)] and pseudostem weight [PW, (g)] as affected by nitrogen supply and defoliation frequency

**$P \leq 0.01$; n.s., not significant; h.s.d., honestly significant difference

Source of variation	Level	Dependent variables					
		LAR[A] (leaves/day)	PHYL (days/leaf)	TAR[A] (tillers/day)	LER (mm/day)	HP (g/mini-sward)	PW[A] (g)
Nitrogen (kg/ha)	0	6.14	16.58	2.30	7.48	58.73	1.96
	25	5.73	17.95	2.05	7.66	57.03	1.76
	75	5.87	17.40	2.56	7.67	62.47	1.71
	h.s.d.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Defoliation frequency (leaf stage)	1.5	5.95	17.24	1.44	7.51	54.89	1.16
	2.5	5.80	17.50	2.85	8.11	54.17	1.32
	3.5	5.99	17.20	2.63	7.19	69.17	2.95
	h.s.d.	n.s.	n.s.	1.13**	n.s.	11.56**	0.43**

[A] $\times 10^{-2}$.

Table 3.   Leaf elongation rate [LER, (mm/day)] between nitrogen applications

*P ≤ 0.05; n.s., not significant; h.s.d., honestly significant difference

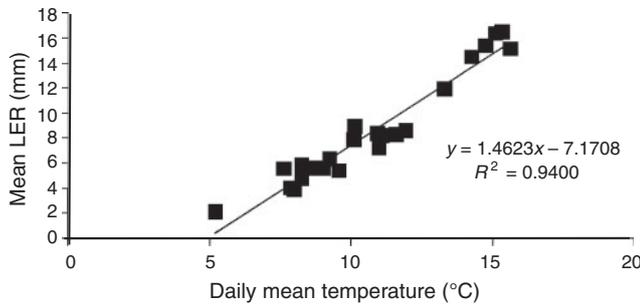| Source of variation	Level	LER (mm/day)		
		(21 March–20 April)	(21 April–31 May)	(1 June–21 June)
Nitrogen	0	12.70	6.98	4.22
(kg/ha)	25	12.33	6.76	5.06
	75	11.97	7.28	5.67
	h.s.d.	n.s.	n.s.	1.38*

Fig. 2. Regression curve and equation for daily mean temperature (°C) and mean leaf elongation rate (LER) (mm).

favour soil microorganism activity (Gill *et al.* 1995; Zhang *et al.* 2008). A high level of soil mineral N may maximise tiller density (Volenec and Nelson 1995) and herbage production (Hopkins *et al.* 1990). When *L. perenne* swards were fertilised to a dose of 450 or 900 kg N/ha, no differences were found in herbage mass production (Hopkins *et al.* 1990), suggesting a maximum limit of response to the available soil mineral N for *L. perenne* swards.

As winter approaches, due to the temperature diminishment, pasture growth rate is reduced (López *et al.* 2003), leading to a decrease in N uptake (Kemp *et al.* 2004). In addition, the vulnerability of N to be moved by water below the level where it can be reached by the root system of the sward increases (Gill *et al.* 1995). During the present study, the rainfall between the N applications was 73.5 mm (21 March to 20 April), 552.5 mm (21 April to 31 May) and 125.7 mm (1 June to 21 June). It is likely
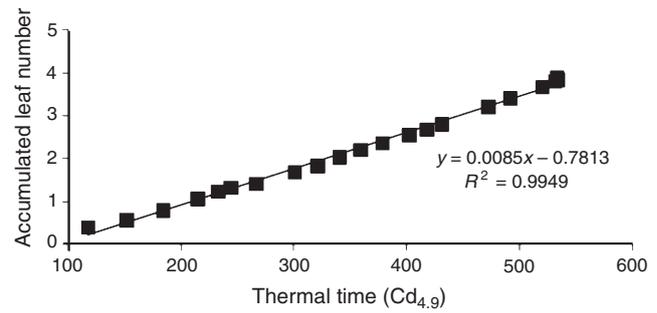

Fig. 3. Regression curve and equation for thermal time ($Cd_{4.9}$) and accumulated leaf number.
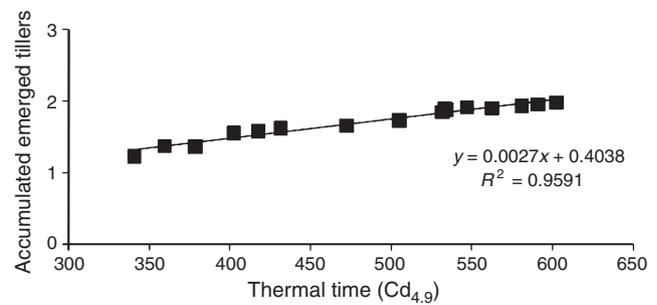

Fig. 4. Regression curve and equation for thermal time ($Cd_{4.9}$) and accumulated number of emerged tillers.

that a combination of pasture growth and N soil extraction, and the heavy rains that occurred between 15 and 23 May (469.5 mm) may have moved N vertically beyond the reach of the mini-swards' root system, lowering the pool of available N for plant uptake, thus allowing a response to the applied doses towards the end of the evaluated period.

Major differences were found to be induced by the defoliation frequency. It has previously been shown that N addition increases the LER (Ryle 1964; Kavanová *et al.* 2008), TAR (Ryle 1964; Harris *et al.* 1996; McKenzie 1998), herbage mass production (Hopkins *et al.* 1990; Schils 1997) and CP content of the forage (Belanger and McQueen 1998; Duru 2003).

Table 4.   Water-soluble carbohydrates on the harvested forage [WSC$_F$, (g/kg)], digestible organic matter on dry matter [DOMD, (%)], metabolisable energy [ME, (MJ/kg)], crude protein [CP, (%)], neutral detergent fibre [NDF, (%)] and acid detergent fibre [ADF, (%)] of a *Lolium perenne* sward as affected by nitrogen supply and defoliation frequency during autumn

**P ≤ 0.01; n.s., not significant; h.s.d., honestly significant difference

Source of variation	Level	Dependent variables					
		WSC$_F$ (g/kg)	DOMD (%)	ME (MJ/kg)	CP (%)	NDF (%)	ADF (%)
Nitrogen (kg/ha)	0	106.74	76.34	11.55	20.80	33.91	24.73
	25	103.06	76.51	11.59	21.27	34.4	25.01
	75	103.12	76.05	11.51	21.70	34.78	24.90
	h.s.d.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Defoliation frequency	1.5	88.12	76.89	11.63	23.30	32.83	23.77
(leaf stage)	2.5	109.73	77.11	11.67	23.11	33.41	24.09
	3.5	111.51	75.25	11.38	17.36	36.86	26.78
	h.s.d.	11.51**	1.09**	0.04**	1.46**	2.18**	0.87**

The variation in WSC_R observed in the present study is in agreement with earlier reports, i.e. their concentrations increased when the defoliation interval was longer (Fulkerson and Slack 1994; Fulkerson and Donaghy 2001) and when N addition was lower (Auda *et al*. 1966).

L. perenne *growth dynamics*

L. perenne growth dynamics were not affected by N application, and the defoliation frequency modified the tiller dynamics only. Donaghy and Fulkerson (1998) reported that the mean LER of *L. perenne* during the first 11 days of regrowth after defoliation was significantly reduced in plants cut at the 1-leaf stage in relation to plants defoliated at the 3-leaf stage, reporting also a linear relationship between LER and WSC_R status (expressed as mg/tiller) at defoliation over the same period of time. Other trials have shown that the WSC_R status of plants normally does not limit leaf elongation (Skinner and Nelson 1995). In this study, the LER was not affected by the defoliation frequency, and hence not by the WSC_R status. This is in agreement with the findings of Davies *et al*. (1989) for *L. perenne* and *L. multiflorum*. The differences between the results obtained in the present study and the ones reported by Donaghy and Fulkerson (1998) may arise from the periods of time considered, as the former takes into account the LER of the whole autumn season, whereas the latter considers the LER of the first 11 days after defoliation.

It has been shown that there is a close positive relationship between the TAR and the defoliation interval, such that while the defoliation interval of *L. perenne* plants increased (1, 2 and 4 weeks), more tillers per plant were initiated (Hume 1991). In the same way, when *L. perenne* plants were defoliated at the 3-leaf stage, they produced more tillers than plants defoliated at the 1-leaf stage (Donaghy and Fulkerson 1998). However, Turner *et al*. (2006*a*) showed no differences in the TAR when *L. perenne* plants were defoliated at the 2-, 3- or 4-leaf stage.

The variation in WSC_R has a similar pattern to the TAR regarding the defoliation interval, such that both TAR and WSC_R significantly diminished when the defoliation frequency increased from the 3.5-leaf stage to the 1.5-leaf stage. This relationship between the WSC_R and defoliation interval has previously been shown by Donaghy and Fulkerson (1998) and Turner *et al*. (2006*a*).

It appears that *L. perenne* has an internal hierarchy in terms of WSC_R accumulation and use. First, defoliated plants use the WSC_R to support tillering immediately after defoliation. Once the leaf area has recovered at the point when the balance between WSC generation from photosynthesis and WSC usage for tillering and plant development is positive, the individual plant starts accumulating WSC to be used after the next defoliation. In the present study, plants at the 2.5-leaf stage had reached the maximum TAR, so the TAR did not increase any further; however, WSC accumulation continued up until the 3.5-leaf stage. Priority for allocation of the WSC_R for regrowth has also been proposed by Donaghy and Fulkerson (1998), who found that reestablishment of the lost leaf area due to defoliation was the first concern for the defoliated plant, followed by resumption of root growth, and finally daughter tiller initiation coinciding with WSC_R replenishment. The lack of difference in the TAR between defoliation treatments at the 2.5-

and 3.5-leaf stages can be explained by the fact that defoliation was within the recommended interval (Turner *et al*. 2006*a*), indicating that reserves have a positive effect on tillering until a certain threshold is reached. From that point onwards, other factors (i.e. amount and quality of light) may limit tiller initiation.

Regarding the LAR, it has been reported that the defoliation frequency has no effect on it (Hume 1991; Donaghy and Fulkerson 1998), a finding in agreement with the results of the present study.

Accumulated herbage mass production and growth dynamics

Donaghy and Fulkerson (1998) reported that plants defoliated at the 3-leaf stage yielded a higher herbage mass production than plants defoliated at the 1-leaf stage after 27 days of regrowth. This difference was partly due to a 2-fold increase in the LER, partly due to an 82% increase in the number of tillers per plant, and partly due to an increase in tiller DM, expressed as mg/tiller, in plants defoliated less frequently. The results of the present study support these findings, mainly because accumulated herbage mass and tillering rate from plants defoliated at the 3.5-leaf stage were greater than those at the 1.5-leaf stage. Tiller mass was not assessed, but when the dry weight of pseudostems were compared, the individual weight of pseudostems from plants defoliated at the 3.5-leaf stage was significantly higher than the individual weight of pseudostems from plants defoliated at the 1.5- and 2.5-leaf stages, matching these results with those of the sward accumulated herbage mass. Therefore, the increase of the individual weight of pseudostems and the sward accumulated herbage mass when plants were defoliated at the 3.5-leaf stage, suggests that the weight of individual tillers also increased. Nevertheless, in our study LER did not differ between treatments.

An explanation for the lack of effect of tillering on herbage production between treatments cut at the 1.5- and 2.5-leaf stages can be found in the late start of active tillering (26 April), 37 days after measurements began. The late start in tiller appearance did not allow the tillers enough time to develop. The colder temperatures slowed their growth rates, suggesting that the tillers could have been very small at the harvesting dates, contributing in a non-significant way to the accumulated herbage mass production.

Growth dynamics and temperature

Leaf elongation was linearly related to the mean daily temperature. Peacock (1976) and Bos and Neuteboom (1998*b*) reported a linear relationship between the temperature and the LER for *L. perenne* and *Triticum aestivum*, respectively. Leaf elongation is the result of meristematic activity, and the increase in elongation due to temperature is a result of the dynamisation of the cellular cycles occurring in the meristematic tissues (Durand *et al*. 1999). In the present study leaf elongation, calculated as the *x*-axis intercept of the linear regression from the data, began at 4.9°C. This base temperature ($Cd_{4.9}$) was used for calculating the thermal time as GDD. The base temperature for leaf elongation obtained in the study was similar to that reported by Schapendonk *et al*. (1998), but higher than the reported by Peacock (1976) and Berone *et al*. (2007) for *L. perenne.* These differences may

arise from different calculation methods or different ways of treating data.

Relating leaf number to accumulated temperature, there was a linear relationship between the variables, suggesting a constant rate of leaf appearance during the measurement period and, therefore, a constant phyllochron. Similar findings have arisen from a series of reports: Klepper *et al.* (1982) and Bartholomew and Williams (2005). A trial by Acharan *et al.* (2010), using the same site where the present study was performed, reported that the individual phyllochron of *L. perenne* plants grown in a mixed *L. perenne*/*Trifolium repens* sward changed. From the middle of June to the end of August, the individual phyllochron decreased from 62 to 21 GDD. From the middle of September onwards, the phyllochron increased to 56.3 GDD and remained relatively static until the end of November when it rose to 91.6 GDD, presumably due to the start of the reproductive phase. For each of these sections, a single line describing the relation between thermal time and accumulated leaf number could be attained. Therefore, these results suggest that the linear relation determined in the present study would suffer breaks as the year progressed, resulting from season-induced changes in the plants' physiology.

The TAR was also related to temperature, as previously reported by Ryle (1964) and Bos and Neuteboom (1998*a*). However, none of these investigators related tiller number with thermal time. When we related these variables, a significant linear relationship was observed, similar to the one with leaves. This is to be expected due to the fact that tillers are dependent on leaf appearance because of the formation of tillering sites. This is in accordance with the findings reported by Bartholomew and Williams (2005).

Nutritive quality

The nutritive quality was unaffected by N application. However, the defoliation frequency had a significant effect over the studied variables. The ME content decreased when the defoliation interval was longer, with the DOMD showing a similar trend. The decrease in the reported values for the former variable is to be expected as digestibility declines with plant age at rates that are dependent on the change in cell wall carbohydrate and lignin concentrations in the plant tissue (Sinclair *et al.* 2006). A decrease in digestibility can be explained due to the presence of higher amounts of sclerenchyma and vascular tissue with an increasing number of leaves on a tiller (Ducrocq and Duru 1997).

The CP content decreased when the defoliation interval was longer. Other studies have reported a decline in the CP content as the plant maturity grew. Sinclair *et al.* (2006) observed a linear decay in the CP concentration for *L. perenne* as a function of regrowth duration, similar to that reported by Donaghy *et al.* (2008) for *Festuca arundinacea*, Turner *et al.* (2006*c*) for *Bromus willdenowii* and Rawnsley *et al.* (2001) for *Dactylis glomerata*. The relative decrease in CP concentrations may be in part due to an increase in fractions such as NDF and ADF and a small or null absolute increase in the N content of the plant.

The WSC in the harvested forage (expressed as WSC per plot) increased when the defoliation interval grew, complying with the findings of Fulkerson and Slack (1994), who reported

that the WSC present in leaf tissue increased linearly up to the 4-leaf stage in *L. perenne*.

When our nutritive quality data are contrasted with the nutritional requirements (NRC 2001) of a 600-kg dairy cow producing 20 L of milk with a fat content of 3.5% (2.167 kg CP/day, 167.4 MJ ME/day with a maximum consumption capacity of 18.2 kg DM/day), all three defoliation frequencies satisfy daily cow nutrient requirements. Assuming that intake corresponds to maximum consumption capacity, ME intake is exceeded by 26.4, 26.91 and 23.7%, and CP intake is exceeded by 95.7, 94.3 and 45.4%, for the 1.5-, 2.5- and 3.5-leaf stages, respectively. However, fibre recommendations are slightly below (1.5- and 2.5-leaf stages) or at the minimum limit (3.5-leaf stage), i.e. 35% NDF (Kolver and Muller 1998). Given the results, it can be seen that in an autumn *L. perenne* pasture, a better balance between CP, WSC and the fibrous fractions was achieved when plots were cut at the 3.5-leaf stage.

Conclusion

The defoliation frequency proved to have a significant effect on the growth dynamics, DM production and nutritive quality of a *L. perenne* sward during autumn. The differences in DM yield were due to changes in individual tiller weight, induced by differential defoliation treatments. The nutritive quality of the harvested forage was shown to be better balanced when plants were cut at the 3.5-leaf stage, due to the higher NDF and WSC_F, and lower CP concentrations. At the applied rates, N had no effect on the *L. perenne* growth dynamics.

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