Ecol Res
DOI 10.1007/s11284-011-0857-9

ORIGINAL ARTICLE

C. A. Busso · C. Gittins · G. F. Becker
L. Ghermandi

Tiller hierarchy and defoliation frequency determine bud viability in the grass *Poa ligularis*

Received: 15 March 2011 / Accepted: 20 May 2011

Abstract Bud viability after various defoliation frequency treatments was determined in the perennial bunchgrass *Poa ligularis* under arid field conditions from 2002 to 2005. Bud respiratory activity was examined on various stem base hierarchies using the tetrazolium test, as validated with the vital stain Evan's blue. The hypothesis of this work was that the total and viable axillary bud numbers on stem bases of all study stem base hierarchies are reduced as defoliation frequency increases. Interpretation of the results differed when they were expressed as a percentage rather than on a number per stem base basis. The total number of axillary buds per stem base was similar in all defoliation frequencies. When the results were expressed on a percentage basis, the order on stem bases having metabolically active buds was daughter tillers > stem bases with green tillers > stem bases without green tillers in all defoliation frequencies. The reverse order was found when considering dead buds. How the results are expressed thus deserves our attention when reporting results on bud viability in perennial grasses. An increased defoliation frequency increased the percentage of dead and dormant buds after the third or fourth defoliation of *P. ligularis* during the 1st study year. These percentages of bud viability, however, increased after the first defoliation during the 2nd study year. Bud viability was affected not only by the cumulative effects of defoliation but also by climatic variables throughout the seasons. However, our results show that *P. ligularis* can be defoliated up to twice a year without affecting bud viability, and thus its potential capacity for regrowth after defoliation.

Keywords Bud viability · Defoliation frequency · Perennial grass · *Poa ligularis*

C. A. Busso (✉)
Departamento de Agronomía,
Universidad Nacional del Sur, and CERZOS (CONICET),
San Andrés 800 (8000), Bahía Blanca, Argentina
E-mail: cebusso@criba.edu.ar

C. Gittins
INTA EEA Alto Valle, Ruta Nac. 22 km 1190 (8332),
Allen, Río Negro, Argentina

G. F. Becker
INTA EEA Bariloche, Paraje Villa Verde s/n (8400),
S. C. de Bariloche, Argentina

L. Ghermandi
Laboratorio ECOTONO, INIBIOMA (CONICET-UNCOMA),
Quintral 1250 (8400), S. C. de Bariloche, Argentina

Introduction

Grass follows a clonal growth pattern characterized by the sequential development of identical growth units (tillers) that derive from tiller apical meristems. New tillers are developed from axillary buds on parent tillers to repeat the process (Dahl 1995). These buds constitute a source of meristematic tissue and are critical for plant longevity. In the late 1980s, Busso et al. (1989) emphasized that longevity in two perennial grasses depended partially on the percentage of stem bases either with or without metabolically active axillary buds within each grass crown. Viable axillary buds are also critical for initiating plant growth (from stored total non-structural carbohydrates), thus contributing to tiller recruitment when no photosynthetic tissues remain in the plant as a result of a given disturbance (Busso et al. 1990). Defoliation can influence tiller recruitment by affecting (1) substrate availability for axillary bud growth, (2) the degree of inhibition of bud outgrowth, (3) the number of metabolically active axillary buds, and (4) the microclimatic conditions for tiller growth (Briske and Anderson 1992).

Determinations on axillary bud viability have been made on grass tillers coming from different locations within the tussock (e.g., center, periphery: Olson and Richards 1988; Busso et al. 1989). However, no studies have compared the number of viable axillary buds on previous-year stem bases of various hierarchies within the tussock: previous-year stem bases either with or without green tillers, or daughter tillers.

Published online: 21 June 2011

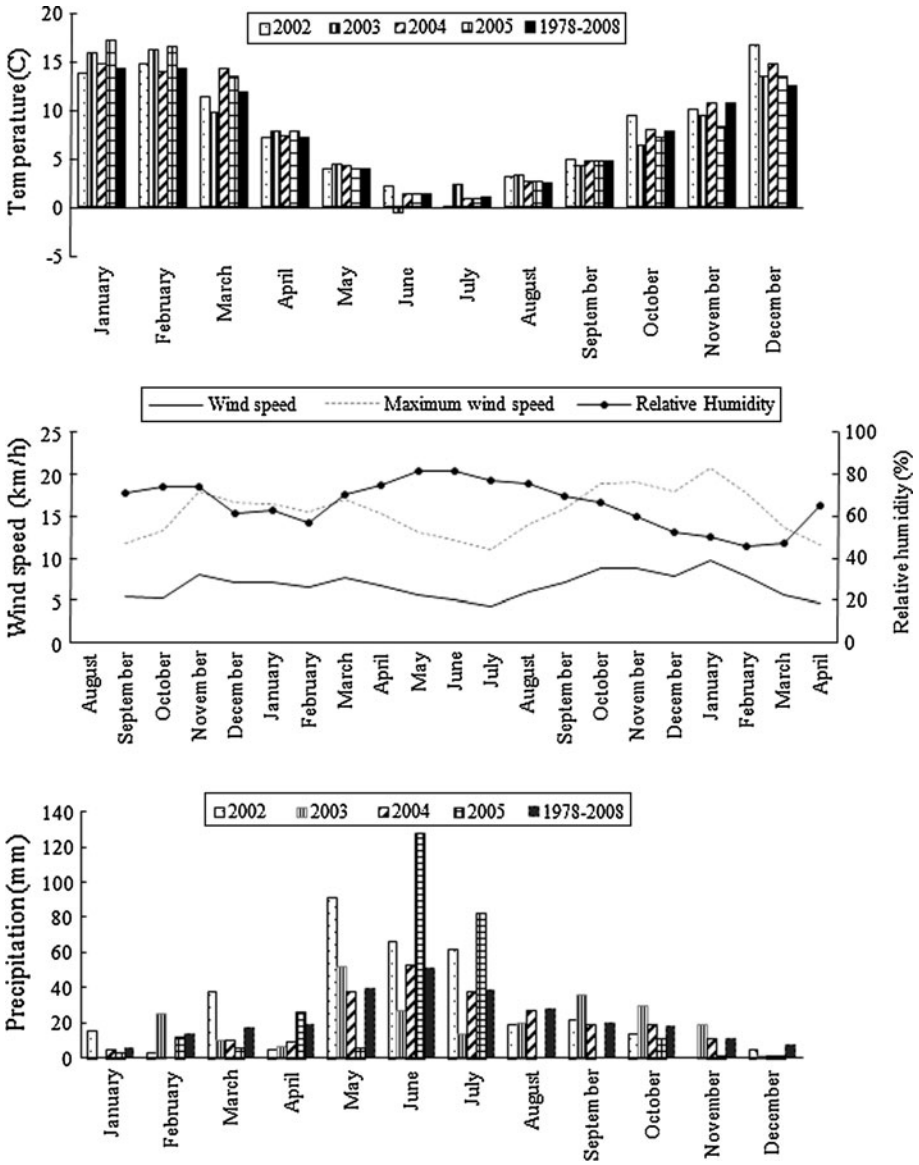

Fig. 1 Mean monthly or long-term (1978–2008) mean monthly temperatures, mean monthly maximum and minimum wind speeds, mean monthly relative humidities, and mean monthly or long-term (1978–2008) mean monthly precipitations during the *P. ligularis* study growing seasons of 2002–2003, 2003–2004 and 2004–2005. Data were obtained from a meteorological station located 3 km from the study site. Missing data during 2004 were due to technical constraints in the meteorological station

Increased defoliation frequency reduces axillary bud number in various perennial grasses (Mullahey et al. 1990, 1991). Plants of *Panicum virgatum* (Beaty and Powell 1976) and *Eragrostis trichodes* (Moser and Perry 1983) reduced their tiller number when defoliated frequently. Frequent defoliations determine an excessive aerial tissue loss, and a subsequent reduced photosynthesis (Newton and Hay 1996). This will in turn reduce the amount of carbon and energy available for bud and/or tiller recruitment (Wang et al. 2004). Severe defoliations have been shown to produce a loss of bud viability in several studies (Newton and Hay 1996; Wang et al. 2004). Busso et al. (1989) showed that severe, once-a-year defoliations under water stress reduced the number of metabolically active axillary buds to a minimum for tiller replacement in the grass *Agropyron spicatum*. However, the effect of various defoliation frequencies on the number of viable axillary buds has not yet been addressed on stem bases of various hierarchies within the grass crown.

This study was conducted on *Poa ligularis* in western-central Argentina, where the main economic activity is sheep production on natural vegetation (Ares et al. 1990). *Poa ligularis* is one of the major grazed, and often over-grazed, perennial grass species in this region (Defossé et al. 1990). This C_3, dioecious tussock grass either re-grows or is set from seeds during the fall, vegetates during winter, elongates internodes and flowers in early spring, sets seeds during this season and disperses them in the summer, when plants enter a dormant stage (Campanella and Bertiller 2008). The hypothesis underlying this work was that increases in defoliation frequency determine a reduction in the total (metabolically active + dormant + dead) and metabolically active numbers of axillary buds on stem bases of all study hierarchies. Our objective was to determine

the effects of various defoliation frequencies on the amount of viable, dead or dormant axillary buds on different hierarchies of *P. ligularis* stem bases during a 3-year-period in the field.

Materials and methods

Study site

The research was conducted at the Agropecuarian Experimental Station (EEA) that INTA Bariloche has in Pilcaniyeu (41°8′S, 71°19′W, Province of Rio Negro, 1,000 m a.s.l), 90 km east of San Carlos de Bariloche, Argentina, within the Phytogeographical Province of Patagonia (Stronati et al. 2011). Climatic information was provided by an automatic meteorological station located at the study site (Fig. 1). The ecological area includes a landscape of mountains and plateaus. Soils are from medium to wholly deep, of loam-sandy and silty textures. The exceptions are soils at the top of mountains, which have low depth and rocky exposures. Slopes range from 2 to 15% in the plateaus (Lores et al. 1983). A complete soil horizon description was reported by Lores et al. (1983). The climate is arid and cold, with 200–300 mm annual precipitation, occurring mostly during fall and winter. The scarce precipitation and its distribution during winter lead to a high summer water deficit (Paruelo et al. 2000). Strong winds from the west reduce thermic sensation by about 4.2°C. This effect is greater during the summer, which makes summers temperate to cold (Coronato 1993). Long-term (1978–1992) annual precipitation is 280 mm and the mean annual temperature is 10.7°C (Bustos and Rochi 1993). Mean annual temperatures are 15 and 2.1°C for the warmest (January) and coldest (July) months, respectively (Bustos and Rochi 1993). Vegetation corresponds to the Western District of the Patagonian Province (Stronati et al. 2011). Shrubby-gramineous steppes are dominant. Major shrub species are *Adesmia campestris*, *Mulinum spinosum*, *Senecio filaginoides* and *Berberis heterophylla*. Dominant grasses include *Poa ligularis*, *Stipa speciosa*, *Stipa humilis* and *Festuca argentina* (Stronati et al. 2011). Plant cover ranges from 40 to 60%. Grasses, shrubs and forbs are about 70, 25 and 5% of total plant cover, respectively (Lores et al. 1983).

Experimental procedures

Within a 4-year-exclosure, 144 plants (48 plants/growing cycle × 3 growing cycles) of *P. ligularis* were selected randomly and marked permanently. For each of the three study growing cycles, 48 plants (8 replicates/treatment × 6 treatments) were selected randomly out from the 144 plant pool; within each treatment ($n = 8$), plants were harvested destructively both before ($n = 4$) and after ($n = 4$) treatment application. For each defoliation frequency except the controls, plants were defoliated back to 5 cm stubble each time they grew up to 10 cm height. All these plants were defoliated leaving 5 cm stubble [stem length measured from the stem base (crown) that remains after a defoliation event] at the end of the 2001/2002 growing season. In this way, only growth produced from the start of the 2002/2003 growth cycle onward was considered in the study. All these 144 plants were exposed annually to one of six treatments: 0 (control), 1, 2, 3, 4 or 5 defoliations each study growth cycle. Defoliation dates are listed in Table 1. The cumulative effects of the study defoliation frequencies (treatments) on bud viability, as affected by various variables (e.g., sampling date; tiller hierarchy; tiller location within the tussock), were determined. Plants defoliated either four or five times each year could be exposed to only three defoliations during 2003–2004, since they did not regrow after the third defoliation (see Table 1). Harvesting dates after defoliation frequencies 1, 2, 3, 4 or 5 (Table 1) were, respectively, 14 August, 25 October, 26 November, 2 January and 15 March in 2002–2003; 26 September, 2 December and 6 January in 2003–2004, and 15 December and 25 February in

Table 1 Dates on which defoliation frequencies [DF; 0 = undefoliated (control); plants defoliated from 1 to 5 times each study year)] were imposed on plants of *Poa ligularis* from 2002 to 2005

DF	Defoliation date (day/month)														
	2002–2003					2003–2004					2004–2005				
0	Control (undefoliated plants)														
1	8 Aug					22 Aug					28 Oct				
2	8 Aug	28 Sep				22 Aug	23 Oct				28 Oct	16 Dec			
3	8 Aug	28 Sep	30 Oct			22 Aug	23 Oct	10 Dec			28 Oct	16 Dec	–		
4	8 Aug	28 Sep	30 Oct	5 Dec	4 Jan	22 Aug	23 Oct	10 Dec	–[a]	–	28 Oct	16 Dec	–	–	
5	8 Aug	28 Sep	30 Oct	5 Dec	4 Jan	22 Aug	23 Oct	10 Dec	–	–	28 Oct	16 Dec	–	–	–

[a]Absence of dates (–) on any study defoliation frequency and year indicates that the defoliation treatment could not be applied because regrowth did not reach 10 cm height. At each date of destructive stem base harvesting during 2002–2005, tillers were sampled before ($n = 4$) and after ($n = 4$) plants were either defoliated from 1 to 5 times or remained undefoliated. At each sampling date, three stem base hierarchies were sampled: previous-year stem bases either with or without tillers and current-year stem bases of daughter tillers. Within any study stem base, axillary buds were classified according to their degree of metabolic activity as either metabolically active or dormant or dead following Busso et al. (1989)

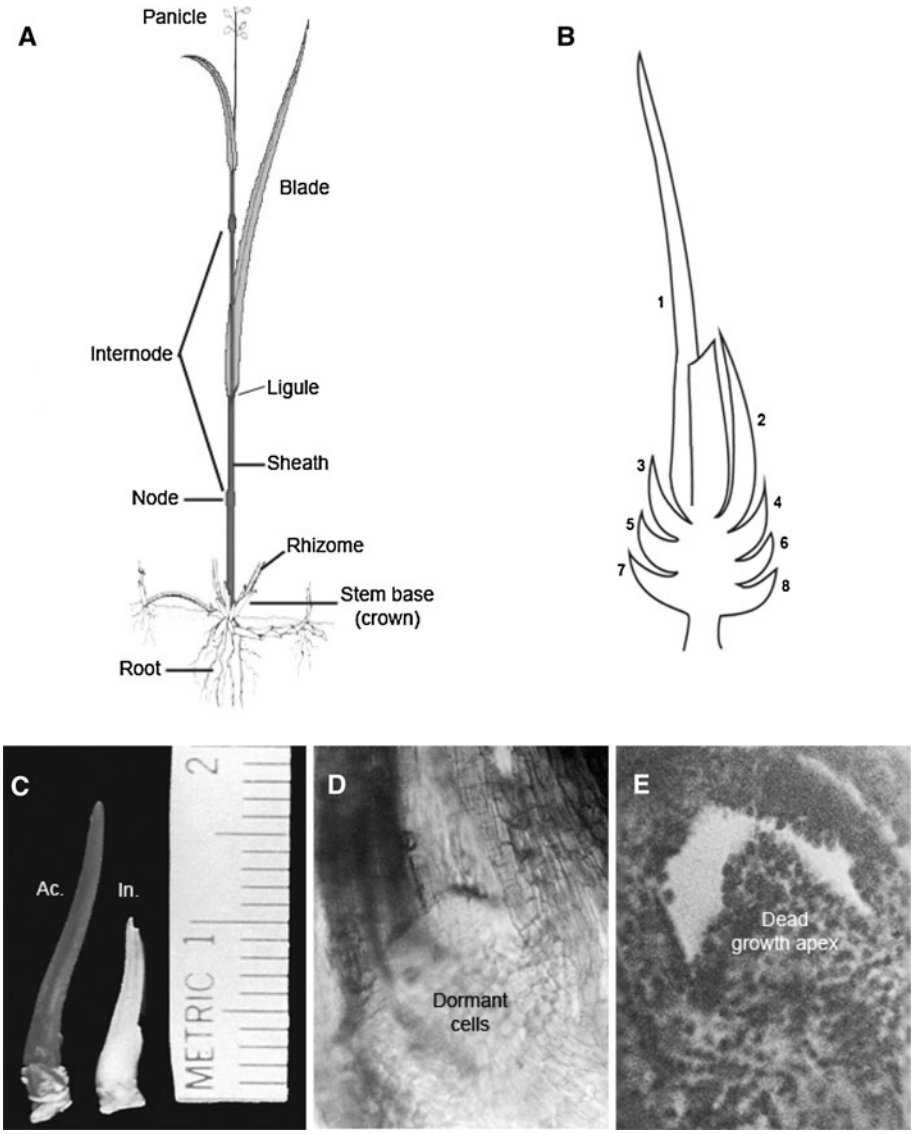

Fig. 2 Schematic representations of a tiller of *Poa ligularis* (**a**) and a typical axillary bud stem base (**b**); buds in the stem base are numbered *1–8* from uppermost to lowermost (modified from Mueller and Richards 1986). **c–e** Photographs of stem bases showing metabolically active [*Ac*. stained red with triphenil tetrazolium chloride (TTC)] and inactive (*In*. unstained with TTC) buds (**c**); TTC-unstained, dormant bud tissue after incubation in Evans's blue (**d**); and TTC-unstained, dead bud tissue after incubation in Evans's blue (**e**). Note the darkened nucleii within each cell

2004–2005. A schematic representation of the plant of *P. ligularis* and other research details are provided in Fig. 2 to help better understanding of the study.

In the laboratory, two previous-year stem bases with green tillers, one from the center and the other from the periphery, were selected on each destructively harvested plant; two daughter tillers with equal distribution were also chosen (see Becker et al. 1997). Two previous-year stem bases without green tillers, one from each study location within the plant, were also sampled from the destructively harvested plants during the 2003/2004 and 2004/2005 growing cycles. Observations of metabolic activity on axillary buds followed Busso et al. (1989). This allowed bud classification in three viability classes: metabolically active, dormant or dead. Briefly, previous-year stem base halves were incubated in colorless triphenil tetrazolium chloride [TTC, 0.6% (w/v)] at 30°C in darkness during 15 h. Bud apexes stained either red or pink were considered metabolically active (see Fig. 2). Change from colorless to either red or pink indicates the enzymatic reduction from TTC to insoluble red formazan. Buds unstained with TTC were tested using the vital stain Evan's Blue [0.25% (w/v)] that does not penetrate intact semi-permeable membranes. Thus, tissues that were unstained or dark blue-stained using the vital stain were considered dormant or dead, respectively (see Fig. 2).

The mean total number per stem base will refer to the sum of all metabolically active plus dormant plus dead buds in that stem base. Counts were made to obtain the numbers of these bud viability classes on each study stem base. The percentage of the total bud pool (number) representing metabolically active, dormant or dead buds was calculated.

Statistical analysis

The first part of the analysis compared the mean total number of buds per stem base, and the mean bud number per stem base within each viability class

(metabolically active, dormant, dead), with respect to treatment (i.e., defoliation frequencies). The second part of the analysis was the comparison of the bud percentage in the various viability classes with respect to the total study bud pool within any stem base.

Since there were no differences ($P > 0.05$) in the number of metabolically active, dormant or dead buds among the various previous-year stem base hierarchies or current-year daughter tillers, analyses were conducted within each stem base hierarchy. Data analysis was performed using the Statistica package (http://www.statsoft.com/).

Within each growing cycle, the total number of metabolically active, dormant or dead buds was analyzed using a two-way ANOVA: factors were the defoliation treatments (control versus defoliated) and the previous-year stem base hierarchies. Tukey's honestly significant difference (HSD) (Tukey 1953) was conducted whenever F tests were significant at $P < 0.05$ in any statistical analysis. During 2002–2003, the percentage of dormant buds was so low that it was excluded from the analysis.

Generalized linear models were used to determine the probability that buds in previous-year stem bases and daughter tillers were either metabolically active, dormant or dead. A logistic multinomial regression model was applied. This model describes the relationship between a categorical response variable and a pool of continuous and discrete explicative variables using the estimated parameters (β) from the maximum likelihood estimation (McFadden 1984; Agresti 1990). The matrix of the explicative variables (X) used in the analysis is exclusively of spatial nature, locating the response variable Y as a function of the independent variables X. When a bud viability level was not present [e.g., we had only two levels in the response variable (i.e., active and dead)], the binomial distribution and the logit link function were used in the analysis. Variables used included the defoliation treatment (two levels: control or defoliated), previous-year stem base location within the tussock (two levels: center or periphery) and sampling date. When the model has more than two levels in the response variable (multinomial distribution), estimation of the logit model demands omission of one of the levels from the selected pool. This is because there is a need to transform the levels of various dichotomous variables (dummy variables) in such a way that one of the levels could be taken as a reference. In this way, each level is entered into the model individually. Because the stage of metabolically active buds was present with the highest percentage in all stem base hierarchies, this stage was selected as the reference level. Estimated parameters for each of the various levels were measured in relation to this stage. Thereafter, variables were evaluated in three different combinations: (1) location (center, periphery) versus sampling date interaction; (2) location versus treatment (control, defoliated plants) interaction; and (3) treatment versus sampling date interaction.

Results

Numbers of metabolically active, dormant and dead buds per stem base were similar ($P > 0.05$) between defoliated and undefoliated plants. However, frequencies of dormant and dead buds were at times different ($P < 0.05$) between the various stem base hierarchies when the total study bud pool was considered in the analysis during either the 2002–2003 or 2003–2004 or 2004–2005 growing cycles.

Stem base hierarchy, total bud number and bud viability on a number per stem base unit basis

Total bud number per stem base was similar between defoliated and undefoliated plants, and among tiller hierarchies within each defoliation treatment, in all study defoliation frequencies during all three study periods (from 2002 to 2005, Table 2). During 2002–2003, the number of metabolically active axillary buds was similar ($P > 0.05$) between previous-year stem bases with green tillers and daughter tillers (Table 3). Previous-year stem bases with green tillers and daughter tillers also showed similar ($P > 0.05$) values for numbers of dead buds per stem base (Table 3).

In 2003–2004, the number of metabolically active axillary buds per stem base was similar ($P > 0.05$) in all tiller hierarchies for both defoliation treatments (Table 3). The number of dormant buds was similar ($P > 0.05$) on previous-year stem bases either with or without green tillers for undefoliated and defoliated tillers (Table 3). Previous-year stem bases either with or without green tillers showed a similar ($P > 0.05$) dead bud number on undefoliated and defoliated tillers. On daughter tillers, only one previous-year stem base showed dead buds on defoliated tillers, and there were no dead buds on undefoliated tillers.

The number of metabolically active axillary buds was similar ($P > 0.05$) on previous-year stems with green tillers and daughter tillers on undefoliated and defoliated tillers during 2004–2005 (Table 3). There was only one previous-year stem base without green tillers having metabolically active axillary buds. Finally, the number of dormant buds on previous-year stem bases with green tillers was similar on control and defoliated plants. Previous-year stem bases either with or without tillers showed a similar ($P > 0.05$) dead bud number on both defoliation treatments (Table 3). Undefoliated stem bases either with or without green tillers showed a similar ($P > 0.05$) dead bud number per stem base (Table 3); this result was similar to that found on previous-year stem bases either with or without green tillers of defoliated plants. The only exception was on daughter tiller stem bases where defoliation reduced ($P < 0.05$) the bud number per stem base after plants were defoliated twice (Table 3).

Table 2 Total (metabolically active + dormant + dead) number of axillary buds per stem base after the various DF treatments

df	2002–2003				2003–2004						2004–2005					
	With		Daughters		With		Without		Daughters		With		Without		Daughters	
	Con	Def	Con	Def	Con	Def	Con	Def	Con	Def	Con	Def	Con	Def	Con	Def
1	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.2 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	1.4 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	2.0 ± 0.3	2.0 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	2.5 ± 0.3	2.7 ± 0.3
2	2.0 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	2.4 ± 0.2	1.9 ± 0.2	2.2 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	2.0 ± 0.2	2.5 ± 0.2	2.7 ± 0.3	1.8 ± 0.3	1.5 ± 0.3	1.7 ± 0.3	2.8 ± 0.3*	2.0 ± 0.3*
3	2.3 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	2.6 ± 0.2	2.0 ± 0.2	2.6 ± 0.2	1.4 ± 0.2	2.4 ± 0.2	1.9 ± 0.2	2.3 ± 0.2						
4	2.4 ± 0.2	2.6 ± 0.2	2.2 ± 0.2	2.5 ± 0.2												
5	2.1 ± 0.2	1.88 ± 0.2	1.9 ± 0.2	2.1 ± 0.2												

Plants of *P. ligularis* were defoliated (*Def*) from 1 to 5 times or remained undefoliated (control: *Con*) during 2002–2005. At each sampling date, three stem base hierarchies were studied: stem bases either with (*With*) or without (*Without*) green tillers and daughter tillers (*Daughters*). During 2002–2003, stem bases without green tillers were not determined. Each value is the mean ± 1SE of $n = 6$–8; ±95% confidence intervals ranged from 0.7 to 3.4 during 2002–2005

* Values significantly different at $P < 0.05$

Table 3 Numbers of metabolically active (*Active*), dormant (*Dormant*) and dead (*Dead*) axillary buds within each DF on control (*Con*; undefoliated) or defoliated (*Def*) tillers that showed stem bases either with (*With*) or without (*Without*) green tillers and daughter (*Daughter*) tillers during the growing seasons from 2002/2003 to 2004/2005

df	Bud viability stage	2002–2003				2003–2004						2004–2005					
		With		Daughters		With		Without		Daughters		With		Without		Daughters	
		Con	Def	Con	Def	Con	Def	Con	Def	Con	Def	Con	Def	Con	Def	Con	Def
1	Active	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.2	1.3 ± 0.3	1.0 ± 0.5	1.6 ± 0.1	1.6 ± 0.2	1.7 ± 0.3	1.8 ± 0.3	0.0 ± 0.0	1.0 ± 0.8[b]	2.2 ± 0.3	1.8 ± 0.2
	Dormant	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.2	1.2 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Dead	1.0 ± 0.5	1.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.1	1.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.5	0.0 ± 0.0	1.7 ± 0.3	1.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
2	Active	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 0.1	1.5 ± 0.1	1.9 ± 0.3	1.8 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.3	2.1 ± 0.3
	Dormant	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.3	1.5 ± 0.3	1.2 ± 0.2	1.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.8	1.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Dead	1.0 ± 0.3	1.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.5	1.1 ± 0.1	1.2 ± 0.1	0.0 ± 0.0	1.0 ± 0.5[a]	0.0 ± 0.0	1.0 ± 0.5	2.3 ± 0.4	1.8 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
3	Active	1.3 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	1.8 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	0.0 ± 0.0	1.0 ± 0.5	1.5 ± 0.1	1.4 ± 0.1						
	Dormant	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.5	1.0 ± 0.3	1.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0						
	Dead	1.1 ± 0.2	1.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.3	1.0 ± 0.5	1.3 ± 0.1	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0						
4	Active	1.2 ± 0.2	1.6 ± 0.2	1.6 ± 0.1	1.7 ± 0.2												
	Dormant	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0												
	Dead	1.5 ± 0.2	1.9 ± 0.2	1.3 ± 0.3	1.8 ± 0.2												
5	Active	1.6 ± 0.2	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.1												
	Dormant	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0												
	Dead	1.3 ± 0.2	1.5 ± 0.2	0.0 ± 0.0	1.5 ± 0.5												

Each value is the mean ± 1SE of n equal to either 17–96 on 49% of samplings or $n = 2$–14 on 51% of samplings

[a]Data came from only one bud

[b]Only one stem base showed metabolically active buds

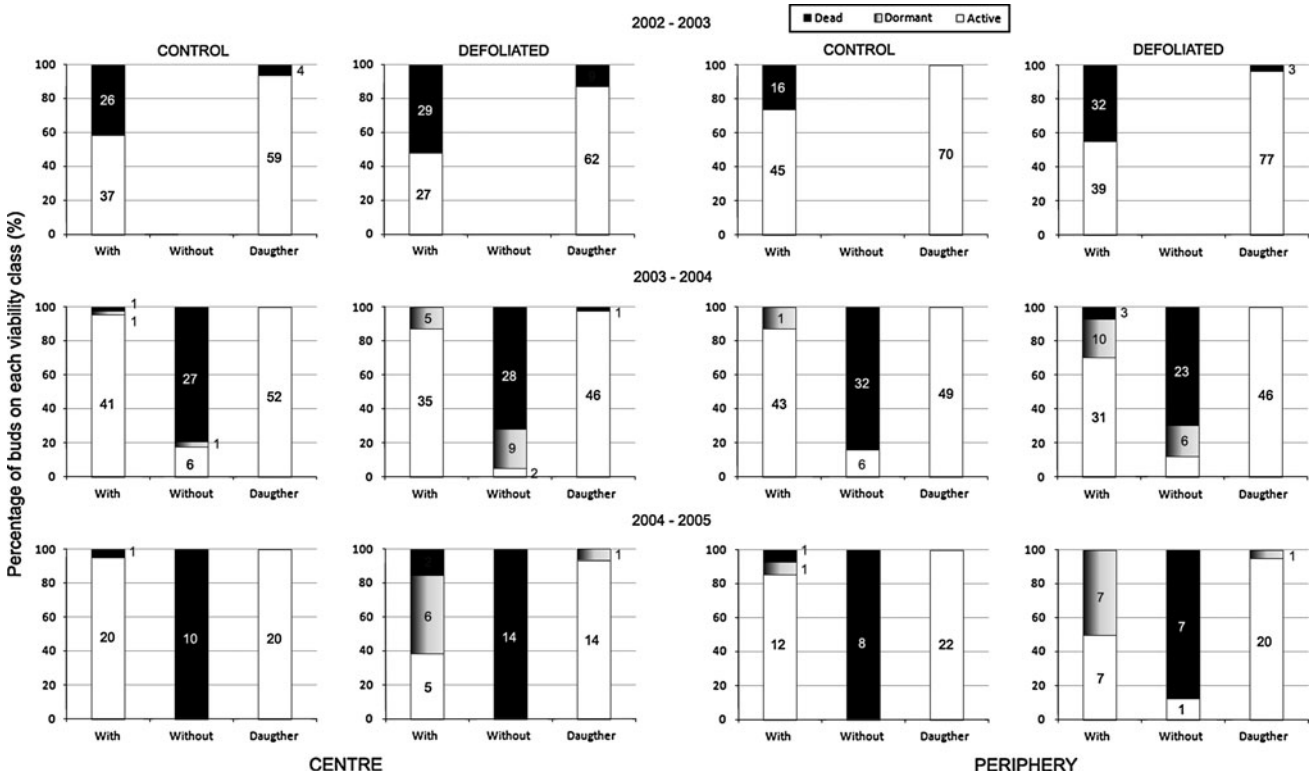

Fig. 3 Percentage of metabolically active, dormant or dead buds on stem bases either with or without green tillers, or daughter tillers, obtained from the plant (either centrally or peripherally) on control or defoliated plants of *P. ligularis* during the growing seasons of 2002–2003, 2003–2004 and 2004–2005. Buds coming from all defoliation frequencies were pooled within each stem base hierarchy and viability class; numbers either within or to the right of histograms are the numbers of observed buds. The percentage of buds on each viability class within each stem base hierarchy (*Y* axis) was calculated from the total bud pool (active + dormant + dead)

Tiller location, defoliation treatment, sampling date, and bud viability on a percentage unit basis

Percentage of metabolically active axillary buds were daughter tillers > previous-year stem bases with green tillers > previous-year stem bases without green tillers on stem bases from the plant center and periphery on undefoliated and defoliated plants during 2002–2003, 2003–2004 and 2004–2005 (Fig. 3). Previous-year stem bases without green tillers showed the greatest percentage of dead buds among all three stem base hierarchies (see Fig. 3). On the contrary, the greatest percentage of metabolically active buds was shown on daughter tillers, with intermediate percentage values on previous-year stem bases with green tillers (Fig. 3).

The interaction between tiller location within the tussock and sampling date was not significant ($P > 0.05$) in 2002–2003. Also, bud viability was similar ($P > 0.05$) on stem bases coming from the plant center and periphery. Percentage of dead buds was 34 ± 19% ($n = 119$) in the plant center, and 49 ± 18% ($n = 132$) in the plant periphery. The percentage of dead buds showed significant differences ($P < 0.05$) between sampling dates on stem bases with green tillers and daughter tillers (Table 4). It increased ($P < 0.05$) between July and November 2002, and kept increasing until reaching a maximum ($P < 0.05$) in February 2003 on stem bases with green tillers (Table 4). At this later time, the percentage of dead buds reached its minimum on daughter tillers ($P < 0.05$) (Table 4). The greatest percentage of dead buds appeared to be lower on daughter tillers than on stem bases having green tillers (Table 4).

Comparing the percentage of dead buds on previous-year stems with green tillers closely between undefoliated and defoliated plants during the 2002–2003 growth cycle, we see that as many undefoliated as defoliated plants had a high percentage of such buds (Table 5). Undefoliated plants increased ($P < 0.05$) the percentage of dead buds by 82% from July–September 2002 to 13 December of the same year. A similar situation was observed on defoliated plants that increased ($P < 0.05$) the percentage of dead buds from July–September 2002 to late November 2002–early January 2003 (Table 5). Defoliated plants increased ($P < 0.05$) the percentage of dead buds about three times in comparison to undefoliated controls after the third and fourth defoliations (Table 5). The greatest ($P < 0.05$) percentage of dead buds on stem bases of undefoliated daughter tillers was in mid-December 2002 (Table 5). The greatest percentage of dead buds on defoliated daughter tiller stem bases occurred after the fourth defoliation was conducted in mid-December 2002. A couple of weeks after that,

daughter tiller stem bases had increased ($P < 0.05$) the percentage of dead buds by 47% in comparison to undefoliated controls (Table 5).

During the 2003–2004 growing cycle, the percentage of dormant buds on stem bases with green tillers of defoliated plants had increased ($P < 0.05$) by 23 ± 5% after the third defoliation (Table 6). During that growing cycle, percentage of dormant buds on stem bases with green tillers did not differ ($P > 0.05$) among sampling dates; the only exception was before and after the first defoliation. Once again, the percentage of dormant buds on stem bases with green tillers was greater ($P < 0.05$) on defoliated than on undefoliated plants after the first and third defoliations (Table 6). Stem bases with green tillers showed a low percentage of dead buds that did not differ ($P > 0.05$) between sampling dates, on either undefoliated or defoliated plants (Table 6). Undefoliated and defoliated plants showed a similar ($P > 0.05$) percentage of dead buds on each sampling date during 2003–2004 (Table 6).

Previous-year stem bases without green tillers showed a relatively lower percentage of dormant than dead buds during 2003–2004 (Table 7). Percentage of dormant buds on this tiller hierarchy increased ($P < 0.05$) 2 months after the first defoliation, and again ($P < 0.05$) 2 months after the second defoliation (Table 7). Dead bud percentage increased ($P < 0.05$) substantially after the first defoliation, and this value was similar ($P > 0.05$) to that after the third defoliation, when most buds were dead (Table 7). These results were very different to those measured on previous-year stems with green tillers (Table 8). Stem bases located at the tussock center have a low percentage of dormant and dead buds (Table 8). The percentage of dormant buds on stem bases with green tillers at the plant periphery was increased ($P < 0.05$) after the first defoliation, and this percentage remained unchanged ($P > 0.05$) after plants were defoliated up to three times (Table 8). After the first defoliation, the percentage of dormant buds was 5 times greater on stem bases located at the plant periphery than at the plant center (Table 8).

Table 4 Percentage (%) of dead buds in previous-year stem bases with green tillers and daughter tillers on plants of *Poa ligularis* before defoliation (BD) and after defoliation (AD) during the sampling dates in 2002–2003 (1–5 indicates the number of times that plants were defoliated during such growing season)

Date	Percentage of dead buds (%)	
	Previous-year stem bases with green tillers	Daughter tillers
6 July 2002 (BD1)	10 ± 7 a	0
14 August 2002 (AD1)	10 ± 6 ab	0
24 September 2002 (BD2)	10 ± 7 a	0
25 October 2002 (AD2)	29 ± 5 abc	0
6 November 2002 (BD3)	38 ± 7 bc	0
26 November 2002 (AD3)	45 ± 7 cd	0
13 December 2002 (BD4)	69 ± 9 de	28 ± 6 a
2 January 2003 (AD4)	58 ± 10 d	26 ± 9 a
1 February 2003 (BD5)	85 ± 6 e	3 ± 1 b
15 March 2003 (AD5)	45 ± 5 c	0

Each value is the mean ± 1 SE of $n = 8$–20. Different letters within a column indicate significant differences ($P < 0.05$) between sampling dates

Discussion

Several studies on the effects of various disturbances on bud viability of perennial grasses have reported their results on an either bud number or percentage per stem base basis, but not both forms of expression (Busso et al. 1989, 1993; Becker et al. 1997; Flemmer et al. 2002). Other studies did report their findings using both expression forms, but did not indicate that interpretation of the results might change by expressing them in either form (Derner and Briske 1999). Our findings show that result interpretation can change if data on bud

Table 5 Percentage (%) of dead buds on previous-year stems with green tillers ($n = 10$–17) or daughter tillers ($n = 12$–15) before and after defoliation of plants of *P. ligularis* during the growing season of 2002–2003

Sampling date	Percentage of dead buds (%)			
	Previous-year stem bases with green tillers		Daughter tillers	
	Undefoliated tillers	Defoliated tillers	Undefoliated tillers	Defoliated tillers
6 July 2002 (BD1)	10 ± 5ab, a	10 ± 6a, a	0	0
14 August 2002 (AD1)	0a, a	18 ± 8a, a	0	0
24 September 2002 (BD2)	0a, a	21 ± 6a, a	0	0
25 October 2002 (AD2)	31 ± 6c, a	27 ± 7ab, a	0	0
6 November 2002 (BD3)	35 ± 5c, a	41 ± 5ab, a	0	0
26 November 2002 (AD3)	25 ± 7bc, a	70 ± 7bc, b	0	0
13 December 2002 (BD4)	82 ± 8d, a	60 ± 5bc, a	29 ± 6a, a	27 ± 6ab, a
2 January 2003 (AD4)	33 ± 7c, a	86 ± 10cd, b	0b, a	47 ± 5a, b
1 February 2003 (BD5)	77 ± 7d, a	93 ± 8d, a	0b, a	7 ± 4b, a
15 March 2003 (AD5)	55 ± 6cd, a	36 ± 6ab, a	0	0

Different letters to the left of the comma represent significant differences ($P < 0.05$) among sampling dates within each defoliation treatment. Different letters to the right of the comma represent significant differences ($P < 0.05$) between defoliation treatments within each stem base hierarchy. Each value is the mean ± 1 SE of $n = 10$–17

Table 6 Percentage (%) of dormant and dead buds on previous-year stem bases with green tillers of defoliated and undefoliated plants during the 2003–2004 growing season

Sampling date	Previous-year stem bases with green tillers			
	Percentage (%)			
	Dormant buds		Dead buds	
26 August 2003 (BD1)	0a, a	0a, a	0a, a	0a, a
26 September 2003 (AD1)	0a, a	5 ± 3c, b	0a, a	0a, a
22 October 2003 (BD2)	0a, a	13 ± 4bc, a	0a, a	13 ± 4a, a
2 December 2003 (AD2)	7 ± 3a, a	7 ± 4ab, a	0a, a	0a, a
16 December 2003 (BD3)	0a, a	12 ± 5ab, a	8 ± 3a, a	0a, a
6 January 2004 (AD3)	0a, a	23 ± 5bc, b	6 ± 3a, a	8 ± 3a, a

The percentage of dormant or dead buds on previous-year stem bases with green tillers was similar ($P > 0.05$) between defoliated versus undefoliated tillers. allowing pooling of data coming from defoliated and undefoliated tillers for statistical analysis. Different letters to the left of the comma indicate significant differences ($P < 0.05$) between sampling dates within each defoliation treatment, and those to the right of the comma indicate significant differences between undefoliated and defoliated plants. Each value is the mean ± 1 SE of $n = 11$–17. Data from defoliated and undefoliated tillers were pooled since there were no significant ($P < 0.05$) differences among them

Table 7 Percentage (%) of dormant and dead buds on previous-years stem bases without green tillers on plants of *P. ligularis* before and after defoliation during the sampling dates in 2003–2004

Sampling date	Previous-year stems without green tillers	
	Percentage (%)	
	Dormant buds	Dead buds
26 August 2003 (BD1)	0a	56 ± 6a
26 September 2003 (AD1)	8 ± 3bc	80 ± 7b
22 October 2003 (BD2)	5 ± 3b	91 ± 7b
2 December 2003 (AD2)	17 ± 6bc	65 ± 6ab
16 December 2003 (BD3)	28 ± 5c	69 ± 5b
6 January 2004 (AD3)	4 ± 2b	93 ± 8b

Different letters within each column indicate significant differences ($P < 0.05$) between sampling dates. Each value is the mean ± 1 SE of $n = 18$–27

viability in perennial grasses are expressed based on either a bud number per stem base on any given viability class or the percentage that the reported viability class represents of the total bud pool in that stem base. This may happen if data variability with respect to any given mean is high enough, which is often the case in bud viability studies (e.g., Derner and Briske 1999; Flemmer et al. 2002; see the range of the ±95% confidence intervals for the mean total bud pool per stem base at the various defoliation frequencies in this study: i.e., Table 2). Our long experience in studies of bud viability (Busso et al. 1989, 1993; Becker et al. 1997; Flemmer et al. 2002) and that of our colleagues (Peláez et al. 2001) reveals some constraints at the time of tiller manipulation that reduces the availability of the sampled stem bases. This is due to several factors that often affect study results in perennial grasses: (1) breaking of plant material (i.e., stem bases, buds) when peeling off the sheaths to expose the axillary buds; (2) failure to clearly observe the growth apex to determine if it is viable or not; (3) unsuccessful efforts when trying to determine if TTC-unstained tissues are unstained or not after using the vital stain Evan's Blue. These constraints mean that a given number of samples has to be discarded from the total sampling conducted. In turn, this contributes to increased data variability in the study parameter, and is the cause of the variable number of samples often reported for any given mean (e.g., Derner and Briske

Table 8 Percentage (%) of dormant and dead buds on previous-years stem bases with green tillers on plants of *P. ligularis* before and after defoliation during the sampling dates in 2003–2004

Sampling date	Previous-stem bases with green tillers			
	Percentage of dormant buds (%)		Percentage of dead buds (%)	
	Centre tillers	Periphery tillers	Centre tillers	Periphery tillers
26 August 2003 (BD1)	0 a	0a	0a	0a
26 September 2003 (AD1)	7 ± 4a	35 ± 11b	0a	0a
22 October 2003 (BD2)	0a	13 ± 7b	0a	13 ± 6a
02 December 2003 (AD2)	15 ± 8a	0a	0a	0a
16 December 2003 (BD3)	7 ± 4a	7 ± 5ab	7 ± 4a	0a
6 January 2004 (AD3)	13 ± 6a	7 ± 4ab	0a	14 ± 5a

Different letters within each column indicate significant differences ($P < 0.05$) between sampling dates. Each value is the mean ± 1 SE of $n = 10$–17

1999; Flemmer et al. 2002). Assuming a high enough data variability, let us work through the following example where no differences were found either in the mean total bud number (e.g., 2.7 for undefoliated plants; 1.7 for defoliated plants) or in the mean dormant number of buds per stem base (e.g., 1 for undefoliated plants; 0.7 for defoliated plants) on defoliated versus undefoliated plants of a given species. If these data for dormant buds are presented on a percentage basis, either 37% or 41.2% of the total bud pool was dormant on undefoliated or defoliated plants, respectively. In other words, results in this example were reversed if presented on a number or percentage per stem basis: relatively speaking, the mean dormant bud number was greater in undefoliated than in defoliated plants, while the mean percentage of dormant buds from the total bud pool was lower in undefoliated than in defoliated plants.

Another situation in which result interpretation on bud viability studies might change depending on how they are expressed, for example, is when the mean total number of buds per stem base decreases as the level of any disturbance increases. In this case, let us assume that the mean number of buds per stem base on any study viability class remains similar among disturbance levels. This will determine that the mean proportion (i.e., percentage) of buds per stem base of that viability class will increase as the level of the disturbance increases. For example, let us suppose that the mean total number of buds per stem base is six before and three after any given disturbance imposition; however, the mean number of dead buds per stem base is two both before and after imposition of that disturbance. Thus, although there is no difference in the mean number of dead buds among disturbance levels, the mean proportion of dead buds is 33.3% before and 66.7% after the disturbance. Overall, we suggest that, when reporting results on bud viability, both bud numbers and percentages on any given bud viability class should be reported. This is because both are necessary to a complete understanding of the effects of any disturbance on (1) the total bud number per stem base; (2) the bud number on any viability class within that stem base, and (3) the percentage that any viability class represents of the total bud pool in that stem base. In our study, when results were expressed on a percentage basis, the order of stem bases having metabolically active buds was daughter tillers > stem bases with green tillers > stem bases without green tillers. The reverse order was found when considering dead rather than metabolically active buds. These results, which differ from those expressed on a number per stem base basis (where no differences were found among stem base hierarchies), agree with those reported in perennial grasses by Busso et al. (1989) in *Pseudoroegneria spicata* and *Agropyron desertorum*, and Becker et al. (1997) in *Stipa tenuis* and *Piptochaetium napostaense*.

Even after 2 years of successive, severe and frequent defoliation, total bud number per stem base was maintained similar to undefoliated controls on plants of *P. ligularis* during 2002–2005. This result indicates that the potential capacity for photosynthetic surface area reestablishment was maintained in plants of this species after severe disturbance. However, daughter tillers showed a reduction in the number of buds per stem base after the second defoliation by February 2005. Busso et al. (1989) on *Agropyron spicatum*, Mullahey et al. (1990) on *Andropogon gerardii*, Mullahey et al. (1991) on *Calamovilfa longifolia*, Hendrickson and Berdahl (2002) on *Thinopyrum intermedium* and *Psathyrostachys juncea*, and Wang et al. (2004) on *Leymus chinensis* reported a reduction in the number of buds per stem base as a result of severe defoliations. Reductions in the number of buds per stem base may carry several undesirable consequences: (1) a lower ability to respond to resource pulses, (2) a lower recuperation rate of the tiller populations exposed to disturbances or stresses, (3) lower rangeland productivity and (4) lower regrowth production potential after herbivory (Dalgleish 2000). Anyhow, our findings demonstrate that *P. ligularis* may be defoliated twice a year at least during a 2-year period without reducing its potential capacity for producing regrowth.

The total bud number per stem base ranged from one to five. Similar values were found on stem bases of other perennial grasses like *Phalaris aquatica* (Cullen et al. 2005), *Bouteloua curtipendula* and *Hilaria belangeri* (Hendrickson and Briske 1997), *Trichachne californica* (Cable 1971), *Stipa tenuis* and *Piptochaetium napostaense* (Becker et al. 1997).

The number of metabolically active and dead buds was similar in all stem base hierarchies both in undefoliated plants and in plants defoliated up to five times per year during the 2-year study period. These results disagree with those of Busso et al. (1989) and Becker et al. (1997), who reported a greater number of metabolically active buds on stem bases having green tillers than on those without green tillers. The number of metabolically active and dormant, but viable, buds per stem base was greater than two in all tiller hierarchies and defoliation treatments. In a parallel study, Gittins (2011) demonstrated that from 0.2 ± 0.3 (plant periphery; plants defoliated three times; 2002–2003) to 2.5 ± 0.3 (plant periphery; control plants; 2002–2003) daughter tillers were produced per stem base in all stem base hierarchies from either the plant center or periphery, defoliation treatments and study years (2002–2005). These results indicate that the potential bud number per stem base available for regrowth was enough for tiller replacement even after a high frequency of defoliation (i.e., plants defoliated up to five times per year during a 2-year period).

Most buds on daughter tillers were metabolically active on both defoliation treatments after 1 and 2 years of successive, high frequency-defoliations. This result agrees with that of Busso et al. (1989), and indicate that a certain photosynthetic surface area appears necessary to maintain metabolically active buds on the same stem base. This suggestion is reinforced when we showed that (1) stem bases without green tillers did not have metabolically active buds at the beginning of the 3rd study

year. At this time, after 2 years of successive defoliations, numbers of dead buds per stem base were similar in all defoliation frequencies on stem bases without green tillers, and (2) stem bases with green tillers showed a dormancy five times greater at the periphery than at the plant center after the 2nd year (2003–2004) of successive defoliations. At this time, tiller height and total green leaf length were greater on tillers at the plant center than at the plant periphery (Gittins 2011).

A seasonal pattern of bud viability was observed in the three study growing seasons. At the beginning of the dry season during late spring, early summer (December–January: end of the growing season: Giorgetti et al. 2000), there was an increase in the percentage of dead and dormant buds per stem base in all stem base hierarchies; this has been shown in other grass and legume species (Newton and Hay 1992; Painter et al. 1993). This reduction in bud metabolic activity towards a dormant stage might have been associated with (1) defoliation treatment effects (Busso et al. 1989), (2) differences in light quality and quantity that reach the stem bases (Deregibus and Trlica 1990), (3) air temperature (Newton and Hay 1992) and water stress (Busso et al. 1989) increases, (4) tissue senescence or necrosis (Davies and Evans 1990) and/or (5) deterioration of the bud vascular bundles (Gifford and Evans 1981). Growth arrest and entrance to a dormant stage may secure survival in perennial grass species in semiarid environments, where summers present scarce or no precipitation, by protecting and maintaining meristem viability (Koller 1969). The percentage of dead buds increased with time in all stem base hierarchies and defoliation treatments. Some of these buds may have undergone senescence through time, similar to the results of Mueller and Richards (1986) on *Agropyron desertorum,* and of Chalmers and Schmidt (1979) on *Cynodon dactylon*.

An increased defoliation frequency increased the percentage of dead and dormant buds on previous-year stem bases with green tillers or daughter tillers after the third or fourth defoliation of *P. ligularis* during the 1st study year. These increases in the percentage of dead or dormant buds, however, occurred after the first defoliation during the 2nd study year; this was very likely the result of the cumulative effects of defoliation on bud viability. These results are similar to those of Busso et al. (1989), Mullahey et al. (1990), Hendrickson and Berdahl (2002) and Wang et al. (2004) on perennial grasses. We have to recognize, however, that the reductions in bud metabolic activity per stem base with increasing defoliation frequency should most likely not only be attributed to the defoliation treatments but also to precipitation decreases and temperature increases as the growing season progressed. This is because water and high temperature stresses decrease bud metabolic activity (Busso et al. 1989; Peláez et al. 2001). Other field studies of defoliation frequency effects on perennial grass growth attributed the reductions on growth parameters only to the increase in defoliation frequency, disregarding the simultaneous effects of water and high temperature stresses (e.g., Beaty and Powell 1976). Anyhow, our results show that *P. ligularis* can be defoliated twice a year without affecting its bud viability, and thus its potential capacity for recovery after defoliation.

Mean summer precipitation during 2002–2003 (35 mm) was similar to the long-term (1978–2008: 34.7 mm) average during that season, while that value was 57% and 40% lower than the long-term average during the growing seasons of 2003–2004 (14.8 mm) and 2004–2005 (21 mm), respectively. In addition, summers during 2003–2004 (mean = 14.3°C) and 2004–2005 (mean = 15.7°C) were 2.1 and 12.1% warmer than the mean monthly temperatures during the 2002–2003 warm season (14°C). These conditions, conducive to drier summers, may have contributed to the increased percentage of dormant buds on previous-year stem bases with green tillers during these years. Busso et al. (1989) in *Agropyron* species and Flemmer et al. (2002) in *Stipa tenuis* reported a reduction of bud metabolic activity as a result of water stress conditions.

Spring during 2002 was at least 39.5% drier (18.2 mm) than that in 2003 (49.8 mm) and 2004 (30.1 mm). Additionally, the mean temperature in that year (8.1°C) was 5.6% greater than the long-term average (1978–2008: 7.7°C), which may have increased evapotranspiration. These abiotic conditions most likely contributed to the greater dead bud frequencies on previous-year stem bases with green tillers and daughter tillers during 2002–2003 than in the other study years. Various authors have reported that dry and high temperature stress conditions were conducive to a greater percentage of dead and dormant buds in perennial grasses (Busso et al. 1989; Peláez et al. 2001; Flemmer et al. 2002).

After the fourth defoliation, stem bases with green tillers and daughter tillers showed an increased percentage of dead buds in comparison to controls during the early, dry summer in January 2003. From the third defoliation onwards, the percentage of dormant buds was greater on defoliated than on undefoliated stem bases with green tillers in January 2004; similar results were obtained in 2004–2005. A reduced bud metabolic activity as a result of an increased defoliation frequency was reported under water stress by Busso et al. (1989) in *Agropyron spicatum*, and under natural field conditions by Flemmer et al. (2002) in *Stipa clarazii*, *S. tenuis* and *S. gynerioides*. Flemmer et al. (2002) also showed that dead bud number was greater on defoliated than on undefoliated plants of those grass species. Jones and Davies (1988) and Newton and Hay (1996) also reported a loss of bud viability due to severe defoliations. Increased bud mortality as a result of increased defoliation frequency can contribute to reduce plant perenniality by reducing the viable bud bank. This is especially true of perennial grasses, where perenniality is determined mostly through vegetative reproduction (Briske and Richards 1995). In this case, and because of increased plant mortality, the proportion of empty surface area spaces could be increased in rangelands.

In turn, this could favor establishment of undesirable perennial grasses and woody plant species (Distel and Bóo 1996). We recognize, however, that those empty spaces could also be occupied by annual, desirable forbs like *Medicago minima* and *Erodium cicutarium* during wet years, which can produce an abundant, palatable forage production (Pelaéz et al. 1995). A change in species composition to undesirable perennial grasses and/or woody species in arid zones, with more lignified aerial tissues and greater C/N ratios, could lead to slowing down of nutrient cycles (Moretto and Distel 2003).

Activation of a dormant meristem bank after a disturbance is a mechanism through which some plants can tolerate herbivory (Lehtila and Larsson 2005). However, it has to be recognized that the greater the bud inhibition period, the lower the possibility that those buds will produce tillers (Mueller and Richards 1986). Given appropriate conditions, dormant buds (of low metabolic activity) could become active and produce green tillers. These dormant buds could thus contribute to plant perenniality and tussock expansion, together with the already existing metabolically active buds; this is specially true in dormant buds that are located at the plant periphery (see Olson and Richards 1988). If the number of green tillers per plant is increased as a result of the increased physiological activity of dormant buds, there will be subsequent increases in the (1) soil exploration by roots for water and nutrient uptake, and (2) the number of sites available for the potential colonization by fungi, and the resulting formation of arbuscular mycorrhizal associations (Briske and Richards 1995). Greater values for soil volumen exploration by the root system and arbuscular mycorrhizal colonization have been associated with an increased nutrient acquisition in the perennial grasses, and can contribute to their competitive ability (Tilman and Wedin 1991; Jackson and Caldwell 1996), mostly in poorly productive environments where competition for soil resources is intense (Jackson and Caldwell 1996). In a plant competitive situation, the probability of soil nutrient capturing increase by increasing rooting length and root association with arbuscular mycorrhizal fungi (Ryser and Lambers 1995).

The percentage of dormant and dead buds was similar, and often low, on stem bases with green tillers coming from the plant center and periphery. This implies that the potential regrowth capacity might be similar at both plant locations considering that dormant buds may regain physiological activity. These results differed most from those obtained on stem bases without green tillers that showed a high percentage of dead buds during 2003–2004. Daughter tillers, instead, showed the lowest dead bud frequencies per stem base when comparing the three study stem base hierarchies. They also showed a lower dead bud percentage at the plant periphery than at the plant center. This indicates a greater potential contribution of daughter tillers located at the plant periphery for tussock expansion. In addition, daughter tillers had only metabolically active and dormant buds, and exhibited an absence of dead buds, during a 6-month-observation period during the 1st study year. In the 2 subsequent study years, bud metabolic activity was equal to or greater than 99% on daughter tillers. Thus, the potential contribution to regrowth, on a tiller scale, would be greater from daughter tillers than from stem bases either with or without green tillers. On a plant scale, of course, the relative contribution of any stem base hierarchy to plant regrowth would be determined by the relative proportion of each stem base hierarchy within the tussock. The available growing period for each stem base hierarchy is another factor to consider when seeking determining its relative contribution to plant regrowth. Olson and Richards (1988), for example, reported a low contribution from daughter tillers to total plant dry matter production because of their limited growing period. Our results on the effects of various defoliation frequencies on perennial grass bud viability varied with the study tiller hierarchy within the plant. Thus, we suggest that studies on bud viability in perennial grasses should specify the study tiller hierarchy they are working with for an appropriate comprehension of their results.

This research on the effects of the frequency of defoliation on bud viability of perennial grasses showed that: (1) bud viability is reduced as defoliation frequency is increased in *P. ligularis*, (2) the study tiller hierarchy must be specified because results may vary accordingly, and (3) results must be reported in terms of both bud numbers and frequencies per stem base for appropriate result interpretation.

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