

## ***Lotus tenuis* SEEDLING ESTABLISHMENT AND BIOMASS PRODUCTION IN FLOODING PAMPA GRASSLANDS (BUENOS AIRES, ARGENTINA)**

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### ABSTRACT

Biomass and plant density of *Lotus tenuis* Waldst. & Kit. ex Willd. have been reported in decreasing in grasslands and pastures. Our objective was to determine if *L. tenuis* biomass and plant density can be increased in grassland through seed addition. Two separated experiments under cattle grazing exclusion were conducted in three paddocks of a Flooding Pampa grassland. The first experiment was from autumn 2004 to autumn 2006 and the second from autumn 2005 to autumn 2007. Different *L. tenuis* seed additions (0, 57, 229, 917 and 1833 seeds m<sup>-2</sup>) were broadcast into experimental plots. In the second experiment, besides seed additions there was a reseeded of approximately 900 seed m<sup>-2</sup> from seed rain produced by plants of grassland. Seed density explained the 81% and 19% of the variation in seedling density and *L. tenuis* biomass, respectively. Seedling emergence occurred mainly between autumn and early spring, while seedling mortality was mainly between late spring and early summer. *Lotus tenuis* adult plant density and biomass production increased with seed additions. Total biomass production in the plant community varied between 589.94 ± 26.89 and 1042.44 ± 54.39 g m<sup>-2</sup> yr<sup>-1</sup> and the differences were principally attributed to precipitations. *Lotus tenuis* biomass contribution was of approximately 10%. The results suggest that *L. tenuis* seedling and plant establishment and biomass production can be increased through seed addition and/or seed rain through grazing exclusion during reproductive period.

**Key words:** Legume, forage quality, population attributes, seedling mortality, seed addition.

### INTRODUCTION

Flooding Pampa grasslands (Buenos Aires, Argentina) have limited amounts of native forage legumes and in their biomass contribution respect to the other species of plant communities. In the Flooding Pampa grasslands *Lotus tenuis* Waldst. & Kit. ex Willd. is one of the most economically important exotic legumes. *Lotus tenuis* spread by seeds and its seedling establishment is facilitated by disturbance generated by fire, grazing and cattle dung (Sevilla *et al.*, 1996; Littera, 1997; Juan *et al.*, 2000; Vignolio and Fernández 2010). This species fixes N via symbiosis, benefits the growth of grass through soil N availability, increases the primary productivity and forage quality, is consumed by cattle and can grow

in soils where the low fertility and/or flooding conditions affect negatively the persistence of other forage legumes (Colabelli and Miñón, 1993; Colabelli and Viviani Rossi, 1997; Quinos *et al.*, 1998; Refi and Escuder, 1998; Vignolio *et al.*, 1999; Juan *et al.*, 2000).

Defoliation has been reported to lower *L. tenuis* plant number and biomass production (Miñón and Refi, 1993; Colabelli and Miñón, 1994; Acuña and Cuevas, 1999). *Lotus tenuis* seedling recruitment can be increased by controlling grazing (Sevilla *et al.*, 1996), but continuous and heavy grazing can negatively affect seed production and plant survival (Fernández *et al.*, 2008; Vignolio *et al.*, 2010). In different grasslands it has been documented that the persistence, recruitment of plant species and primary productivity can be limited by seed availability in the soil bank (Oersterheld and Sala, 1990; Tilman, 1997; Zobel *et al.*, 2000; Lenz and Facelli, 2005; Martin and Wilsey, 2006; Thomsen *et al.*, 2006). Under continuous grazing, the soil seed bank of the palatable species can be significantly reduced and affect the persistence of plant population (Sternberg *et al.*, 2003). Seed limitation can be minimized through seed additions (Oersterheld and Sala,

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1990; Martin and Wilsey, 2006) and/or through grasslands management that allows natural reseeding (Bologna *et al.*, 1996).

Our objective was to determinate if *L. tenuis* seedlings and plant establishment, and primary production in a Flooding Pampa grassland, can be increased through seed addition and natural reseeding. We measured these population plant attributes because they are key stages in the propagation and productivity of the species in the grasslands.

## MATERIALS AND METHODS

### Study area

The study was located in a grassland of the Flooding Pampa situated in San Marcos, Ayacucho (37°39' S, 58°29' W) Buenos Aires Province, Argentina. Grassland was used for extensive beef cattle breeding with a continuous stocking rate of 0.7 AU ha<sup>-1</sup>. The primary productivity is maximum during spring-summer and minimum in winter. The Flooding Pampa grasslands climate is temperate, subhumid, usually affected by floods in winter and spring and drought in summer. Annual precipitation average is approximately 950 mm.

### Experimental layout

Three paddocks of 35 x 35 m were selected in the grassland. Each paddock was wire fenced to exclude cattle grazing during the experimental period. Plant community of the paddock was composed principally by short monocots (grasses, sedges and rushes): *Agropyron* sp., *Carex* spp., *Cynodon dactylon* (L.) Pers., *Cyperus* spp., *Distichlis spicata* (L.) Greene, *Juncus* spp., *Paspalum dilatatum* Poir., *Paspalum vaginatum* Sw., *Sporobolus indicus* (L.) R. Br., *Stenotaphrum secundatum* (Walter) Kuntze, *Jarava plumosa* (Spreng.) S.W.L. Jacobs and J. Everett. Non-legume dicots: *Ambrosia tenuifolia* Spreng., *Aster scamatus* *Symphotrichum subulatum* (Michx.) G. L. Nesom, *Plantago lanceolata* L. and *Setaria parviflora* (Poir.) Kerguélen. Legume dicots: *Adesmia bicolor* (Poir.) DC., *Lotus tenuis* and *Trifolium repens* L. Soil test was done in each paddock (Table 1).

In each paddock, two separated experiments were conducted. The first experiment was from March 2004 to April 2006 on 15 plots and the second one from April 2005 to April 2007 on other 15 different plots (Table 2). Permanent 15 fixed plots (1.50 x 1.50 m) were arranged in each paddock in a completely randomized design, with three replications for each five seed additions. The distance among plots was 50 cm. *Lotus tenuis* seeds (cv. INTA Pampa) were used. Seeds were scarified with sand paper to break physical dormancy and inoculated with *Rhizobium loti* and germination percentage was determined. Seed number in conditions of germination was: 0 (control); 57; 229; 917; 1833 seeds m<sup>-2</sup> for 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> seed addition, respectively. Soil seed bank recorded in this same grassland, near of experimental paddock under continuous stocking was approximately 50 seeds m<sup>-2</sup> (Fernández *et al.*, 2008).

*Lotus tenuis* was sowed in early autumn because its recruitment is suitable due to warm climate and precipitations (Table 2). Due to slow rate of *L. tenuis* seedling growth, in both experiments, one day before of seed additions, plant communities of the paddocks were mechanically mowing at 4 cm height with bar of 0.50 m width. This disturbance to reduce plant competition, provide gaps and obtain uniform seedling establishment. Plant biomass was hand removed after mowing. On 23 March 2004 *L. tenuis* seeds were hand-sowed onto plots (depth of 0 mm) (Table 2).

### First experimental period

**Lotus tenuis seedling density.** Seedling density was recorded in each period during autumn, winter, spring and summer (Table 2). Plots were divided into nine subplots of 0.5 x 0.5 m each one, and seedling numbers were determined in sixteen 100 cm<sup>2</sup> quadrants placed at fixed locations (Figure 1). Seedling mortality was considered like reduction in seedling number from measurement to measurement.

**Biomass production.** Aerial biomass production of plant communities was determined through standing crop harvest. Biomass was hand harvested at 4 cm high in same

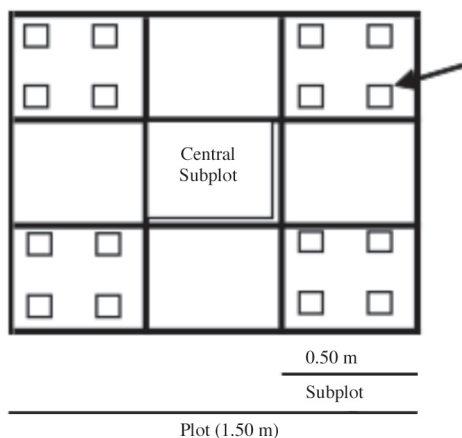
**Table 1. Soil test of the upper 15 cm (mean ± SE) analysis of pH, electrical conductivity (EC), phosphorus (P; Bray 1 method), organic matter (OM, Walkley and Black method) and interchange Na in the experimental paddocks of a Flooding Pampa grassland.**

Paddock	pH	EC	P	OM	Na
	soil:H <sub>2</sub> O, 1:2.5	mmohs cm <sup>-1</sup>	mg kg <sup>-1</sup>	%	cmol <sub>c</sub> 100 g <sup>-1</sup>
1	8.17 ± 0.56	2.02 ± 0.42	5.60 ± 0.38	5.15 ± 1.15	9.17 ± 1.51
2	9.20 ± 0.23	2.67 ± 0.54	6.77 ± 0.21	3.70 ± 0.75	10.70 ± 1.30
3	9.45 ± 0.20	2.17 ± 0.47	7.25 ± 0.60	4.05 ± 0.93	14.72 ± 1.87
Probability	0.083	0.62	0.06	0.56	0.08

**Table 2. Grassland operations and data collection during the first and the second experiment at Flooding Pampa.**

First experiment	Date	Second experiment	Date
Seed addition	23 March 2004		
SR 1	24 May 2004		
SR 2	1 July 2004		
SR 3	27 September 2004		
SR 4	22 December 2004		
BH 1	Seed rain 30 December 2004		
BH 2			
		Seed addition	19 April 2005
		SR 1	23 June 2005
		SR 2	WD
		SR 3	13 September 2005
		SR 4	17 November 2005
		BH 1	30 November 2005
BH 3	1 December 2005		
BH 4	25 April 2005		
		BH 2	11 April 2006
		BH 3	15 December 2006
		BH 4	13 April 2007

References: Brace indicates the period of *Lotus tenuis* seed rain during summer 2005 and increasing the soil seed bank during second experimental period; BH: biomass harvest; SR: seedling record; WD: without data.



**Figure 1. Experimental plot and central subplot. The arrow indicates one of the 16 quadrants of 100 cm<sup>2</sup> where *Lotus tenuis* seedling were recorded. Harvest of aerial biomass production of plant community and the number of *L. tenuis* adult plants were determined in the central subplot.**

central subplot (0.5 x 0.5 m) of each plot (Figure 1). Four harvests were realized between December 2004 and April 2006 (Table 2). The first harvest was 1 wk after the last *L. tenuis* seedling record (Table 2). Biomass was classified in four groups, *L. tenuis*, non-legumes dicots, legumes different to *L. tenuis*, and monocots, which were dried (60 °C for 72 h) and weighed afterwards. After each harvest

all the paddocks were mechanically cut at 4 cm height and the biomass was hand removed.

***Lotus tenuis* plant establishment.** Immediately after the last aerial biomass harvest of first and second experiment, 25 April 2006 and 13 April 2007, respectively, the number of *L. tenuis* adult plants was recorded in the same central subplot of each plot (Table 2, Figure 1).

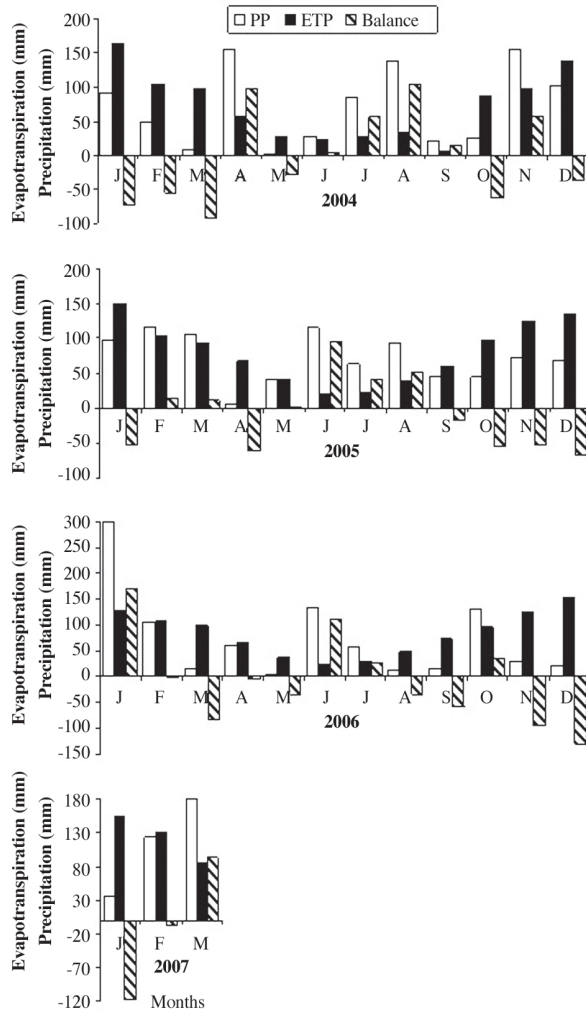
### Second experiment

Procedure of second experiment was done with the same methodology that in the first one. The plots were sowed on 19 April 2005 and the study finished on 13 April 2007 (Table 2). *Lotus tenuis* seed rain of the plants growing in the grassland was produced during reproductive season, between first and second harvest, 30 December 2004 to 31 March 2005 (indicated in Table 2 as seed rain). According to our experiments done in the same plant community, near of the paddocks, when the grazing was excluded on December at the end of reproductive period, the seed rain was approximately of 900 seed m<sup>-2</sup> (Fernández *et al.*, 2008).

### Climate data

Due to the impossibility of having a meteorological station in the paddocks, precipitation data was provided by the meteorological station in Ayacucho city, distant approximately 20 km from experimental

site. Evapotranspiration data (Penman-Monteith method) was proportioned by EEA-INTA Balcarce, distant at approximately 50 km from the paddocks. During the years 2004, 2005 and 2006 the accumulated precipitations were 931, 865 and 1076 mm, respectively. The evapotranspiration was higher than precipitation in 21 months, principally during spring and summer seasons (Figure 2).



**Figure 2. Precipitation (PP, mm), evapotranspiration (ETP, mm) and water balance recorded during the experimental period.**

### Statistical analyses

A randomized complete block design with three replicates and five seed additions as treatments were used in each paddock. Data were analyzed by ANOVA using SYSTAT procedures. Seedling density and biomass production were analyzed using a repeated-measures procedure.

Means were separated by the least significant difference test (LSD) at  $P \leq 0.05$ . The results are presented as mean  $\pm$  standard error.

## RESULTS

The biomass contribution of exotic legumes, *Trifolium repens*, *T. fragiferum* and the native *Adesmia bicolor* was not higher than  $2.15 \text{ g m}^{-2}$ , approximately 0.3% respect to the total biomass production. These legumes were not present in some plots, therefore they were not considered in the calculus.

### *Lotus tenuis* seedling and plant establishment

In both experiments, *L. tenuis* seedling density increased with seed addition. Seedling emergence occurred mainly in autumn and early spring and the mortality was mainly between late spring and early summer (Tables 3 and 4). Seedling number recorded in the first and second experiment varied with seed additions ( $P \leq 0.0001$  and  $P \leq 0.031$ ), date ( $P \leq 0.025$  and  $P \leq 0.042$ ) and interaction seed addition  $\times$  date ( $P \leq 0.0001$  and  $P \leq 0.0001$ ) was recorded, respectively. During both experiments *L. tenuis* clorotic seedlings were recorded.

Seedling density recorded in the last record at first period (22 December 2004) was related to seed addition:

$$\text{Seedling (N}^\circ \text{ m}^{-2}) = 0.0925 \times \text{Seed addition (N}^\circ \text{ m}^{-2}) + 3.856 \quad [1]$$

$$(r^2 = 0.802; P \leq 0.0001; n = 45)$$

and seed addition more 50 seed  $\text{m}^{-2}$  estimated in the soil seed bank:

$$\text{Seedling (N}^\circ \text{ m}^{-2}) = 0.0925 \times \text{Seed addition (N}^\circ \text{ m}^{-2}) - 0.766 \quad [2]$$

$$(r^2 = 0.802; P \leq 0.0001; n = 45)$$

Plant number established did not vary with seed additions and paddock. However, in the 4<sup>th</sup> and 5<sup>th</sup> seed addition plant number was higher ( $54.44 \pm 9.14 \text{ plants m}^{-2}$ ) than among 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> ones ( $31.66 \pm 4.70 \text{ plants m}^{-2}$ ). *Lotus tenuis* seedling density in control conditions (1<sup>st</sup> seed addition) of second experiment, increased approximately one hundred times (Table 4), respect to the same seed additions of first one (Table 3). This increment was attributed to seed rain. *Lotus tenuis* plant number established in the second experiment did not vary significantly with the seed addition and paddock, being the average  $21.42 \pm 17.46 \text{ plants m}^{-2}$ .

### Biomass production

Total dry biomass production by plant community did not vary significantly with *L. tenuis* seed addition. Total biomass production was lower in paddock 2 ( $692.06$

**Table 3. *Lotus tenuis* seedling density (mean ± SE) recorded in paddocks of a Flooding Pampa grassland during the first experiment. References: seed addition 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>.**

Date	1 <sup>st</sup> (Control)	Seed addition (N° m <sup>-2</sup> )			
		2 <sup>nd</sup> (57 m <sup>-2</sup> )	3 <sup>rd</sup> (229 m <sup>-2</sup> )	4 <sup>th</sup> (917 m <sup>-2</sup> )	5 <sup>th</sup> (1833 m <sup>-2</sup> )
Seedling (N° m <sup>-2</sup> )					
24 May 2004	2.46 ± 0.97aA	16.35 ± 3.08aAB	29.93 ± 2.84aA	144.13 ± 19.01bA	291.97 ± 38.61cBA
1 July 2004	0.69 ± 0.69aA	7.63 ± 2.50aA	31.64 ± 7.54aA	212.49 ± 29.35bB	388.88 ± 61.28cBC
27 Sept. 2004	4.85 ± 2.50aA	20.13 ± 4.26aB	56.24 ± 6.75aB	270.83 ± 25.19bB	459.71 ± 67.77cC
14 Dec. 2004	4.08 ± 1.96aA	7.77 ± 1.66aA	25.77 ± 5.04aA	89.44 ± 11.10bA	172.88 ± 21.85cA

Values followed by the same capital letter within columns and by the same small letter within file are not different according LSD test ( $P > 0.05$ ).

**Table 4. *Lotus tenuis* seedling density (mean ± SE) recorded in a paddock of a Flooding Pampa grassland during the second experiment. References: seed addition 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>.**

Date	1 <sup>st</sup> (Control)	Seed addition (N° m <sup>-2</sup> )			
		2 <sup>nd</sup> (57 m <sup>-2</sup> )	3 <sup>rd</sup> (229 m <sup>-2</sup> )	4 <sup>th</sup> (917 m <sup>-2</sup> )	5 <sup>th</sup> (1833 m <sup>-2</sup> )
Seedling (N° m <sup>-2</sup> )					
23 June 2005	146.52 ± 49.99abA	104.16 ± 19.00aA	143.74 ± 28.79abA	273.58 ± 38.56abA	557.63 ± 101.87cA
13 Sept. 2005	424.99 ± 111.79aB	327.77 ± 99.67aB	317.35 ± 74.70aB	379.85 ± 84.73aA	554.16 ± 76.27aA
17 Nov. 2005	368.05 ± 92.39aBA	272.91 ± 77.23aBA	278.47 ± 62.93aAB	365.27 ± 65.54aA	395.13 ± 48.81aA

Values followed by the same capital letter within columns and by the same small letter within file are not different according LSD test ( $P > 0.05$ ).

± 37.38 and 589.94 ± 26.89 g m<sup>-2</sup> yr<sup>-1</sup>) than in paddock 1 (995.96 ± 33.42 and 953.07 ± 55.80 g m<sup>-2</sup> yr<sup>-1</sup>) and 3 (1042.44 ± 54.39 and 743.77 ± 46.69 g m<sup>-2</sup> yr<sup>-1</sup>), by the first and second experiment, respectively. In average monocots, dicots non legume and *L. tenuis* biomass contribution was approximately 80, 10 and 10%, respectively.

In the first experiment, *L. tenuis* biomass production varied with the seed addition ( $P \leq 0.002$ ) and paddock ( $P \leq 0.004$ ) (Table 5). *Lotus tenuis* total biomass production by the 4<sup>th</sup> and 5<sup>th</sup> seed addition (109.51 ± 30.68 g m<sup>-2</sup>) was significantly higher ( $P \leq 0.026$ ) than the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> addition (42.73 ± 8.40 g m<sup>-2</sup>). *Lotus tenuis* total biomass production was related to seedling number recorded in the last record of (22 December 2004):

$$\text{Biomass (g m}^{-2}\text{)} = 0.445 \times \text{Seedling (N}^{\circ}\text{ m}^{-2}\text{)} + 42.987 \quad [3]$$

$(r^2 = 0.190; P \leq 0.003; n = 45)$

In the second experiment, *L. tenuis* total biomass production varied with date ( $P < 0.02$ ). Except in the last record, biomass production did not vary with seed addition, being in average 73.40 ± 9.68 g m<sup>-2</sup> (Table 6).

#### Climate data

During years 2004, 2005 and 2006 the accumulated precipitations were of 931, 865 and 1076 mm, respectively. During the experimental period 21 months were recorded with negative water balance. The more affected months corresponded to spring and summer seasons (Figure 2).-

**Table 5. *Lotus tenuis* biomass (mean ± SE) recorded in a paddock of a Flooding Pampa grassland during the first experimental period. References: seed addition 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>.**

Date	1 <sup>st</sup> (Control)	Seed addition (N° m <sup>-2</sup> )			
		2 <sup>nd</sup> (57 m <sup>-2</sup> )	3 <sup>rd</sup> (229 m <sup>-2</sup> )	4 <sup>th</sup> (917 m <sup>-2</sup> )	5 <sup>th</sup> (1833 m <sup>-2</sup> )
Biomass (g m <sup>-2</sup> )					
30 Dec. 2005	12.22 ± 4.55a	8.38 ± 3.71a	14.83 ± 3.27ab	11.91 ± 3.62a	30.85 ± 11.40b
31 May 2005	15.02 ± 5.63a	6.80 ± 2.52a	7.33 ± 2.10a	14.36 ± 5.40a	31.20 ± 9.26b
1 Dec. 2005	4.72 ± 1.53a	11.06 ± 4.54ab	11.11 ± 5.94ab	34.27 ± 16.42bc	40.75 ± 13.61c
25 April 2006	13.95 ± 7.69b	6.66 ± 2.04a	16.08 ± 4.82a	30.44 ± 13.08ab	25.24 ± 5.35ab

Values followed by the same small letter within file are not significantly different according LSD test ( $P > 0.05$ ).



**Table 6.** *Lotus tenuis* biomass (mean  $\pm$  SE) recorded in paddocks of a Flooding Pampa grassland during the second experimental period. References: seed addition 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup>.

Date	Seed addition (N <sup>o</sup> m <sup>-2</sup> )				
	1 <sup>st</sup> (Control)	2 <sup>nd</sup> (57 m <sup>-2</sup> )	3 <sup>rd</sup> (229 m <sup>-2</sup> )	4 <sup>th</sup> (917 m <sup>-2</sup> )	5 <sup>th</sup> (1833 m <sup>-2</sup> )
	Seedling (N <sup>o</sup> m <sup>-2</sup> )				
30 Nov. 2005	4.89 $\pm$ 1.37aA	5.77 $\pm$ 1.88aA	5.41 $\pm$ 2.21aA	6.02 $\pm$ 1.23aA	6.45 $\pm$ 1.31aA
11 April 2006	13.91 $\pm$ 4.24aAB	16.84 $\pm$ 7.22aAB	17.06 $\pm$ 5.07aA	19.28 $\pm$ 5.16aAB	24.00 $\pm$ 8.75aAB
15 Dec. 2006	19.83 $\pm$ 7.40aB	36.69 $\pm$ 15.25aB	20.44 $\pm$ 7.95aA	21.57 $\pm$ 6.15aAB	27.47 $\pm$ 8.97aAB
13 April 2007	9.52 $\pm$ 2.93aAB	24.49 $\pm$ 9.00abAB	19.55 $\pm$ 5.27abA	25.51 $\pm$ 10.61abB	42.21 $\pm$ 14.81bB

Values followed by the same capital letter within columns and by the same small letter within file are not significantly different according LSD test ( $P > 0.05$ ).

## DISCUSSION

In the first period seed addition explained the 81% and 19% of the variation in seedling density and *L. tenuis* biomass, respectively. *Lotus tenuis* response to seed addition could have been conditioned by its grazing history and climate variables, for example, precipitations. Under continuous grazing the removal of reproductive and vegetative organs of *L. tenuis* can negatively affect the seed production and consequently the soil seed bank size (Bologna *et al.*, 1996; Sternberg *et al.*, 2003; Fernández *et al.*, 2008). *Lotus tenuis* soil seed bank recorded in the same grassland, near of the paddocks, was estimated in approximately 50 seed m<sup>-2</sup> (Fernández *et al.*, 2008). Therefore, the increment of *L. tenuis* seedling with the seed additions could be in response to the low soil seed bank. In the second experiment, *L. tenuis* seed rain produced during the period 30 December 2004 to 31 March 2005 increased the soil seed bank and determining little differences in seedling density between control and 2<sup>nd</sup> and 3<sup>rd</sup> seed addition.

Favorable conditions for *L. tenuis* seedling emergence was in spring, as well as it was reported by Sevilla *et al.* (1996) in a pasture composed by *Festuca arundinacea* Schreb. *Lotus tenuis* seedling mortality was important in this experiment, being consistent with the reports about its low implantation efficiency, considered as seedling established respect to seed sowed. Implantation efficiency of *L. tenuis* in the Flooding Pampa grasslands and pastures were recorded between 9.5 and 0.1% (Miñón y Colabelli, 1993). *Lotus tenuis* seedling mortality by different environmental factors as fungal (Juan *et al.*, 2000), seed predation by rodents, failure of some seedling to penetrate the soil surface and summer water deficit (Colabelli and Miñón, 1993; Wilsey and Polley, 2003; Lenz and Facelli, 2005) and plant competition (Sevilla *et al.*, 1996) are some factors that can explain the decrease of seedling number in the experiments.

*Lotus tenuis* seedling started to die off as soon as plant communities began to regrow. *Lotus tenuis* seeds are

small (approximately 1 mg) and the seedling with poor vigour. Seedling recruitment and survival are dependent on open gaps or low plant competition (Colabelli and Miñón, 1993; Miñón and Colabelli, 1993; Sevilla *et al.*, 1996; Lateralra, 1997; Juan *et al.*, 2000). Low seedling density in early summer reflects the high mortality risks in this season. Water availability during summer in the Flooding Pampa grasslands, appears to be an important environmental factor controlling *L. tenuis* plant survival and biomass production (Colabelli and Miñón, 1993; Vignolio *et al.*, 2010). Oesterheld and León (1987) did not find differences in biomass production in pastures of 2, 5 and 13 yr old growing in the Flooding Pampa. The hypothesis proposed by these authors and others (Costa and García, 1997; Fernández Grecco, 2001) was that soil fertility and water would be the decisive factors determining the biomass production of the grasslands. This appreciation agrees with the results of Wilsey and Polley (2003) who added seeds of 20 species -perennial C<sub>4</sub> and C<sub>3</sub> grasses, legumes and forbs- in subhumid grassland of Texas, USA. In hot and dried summers, water availability was an important factor controlling seedling establishment and biomass production (Wilsey and Polley, 2003). Flooding Pampa grasslands, can remain exposed to temporary summer drought and available water can be an abiotic key element to the survival and biomass production of these grasslands (Wilsey and Polley, 2003; Lenz and Facelli, 2005; Thomsen *et al.*, 2006). The lower biomass production of the plant community (average of the three paddocks) in the second experiment (762 g m<sup>-2</sup> yr<sup>-1</sup>) respect to the first one (910 g m<sup>-2</sup> yr<sup>-1</sup>) can be attributed to the scarce precipitations during November and December 2006. Total biomass produced by plant community was not significantly increased through *L. tenuis* seed additions and seed rain. However, *L. tenuis* biomass contribution is important in the Flooding Pampa grasslands rich in grass species, poor in native legume and in soil N (Colabelli and Viviani Rossi, 1997; Costa and García, 1997; Fernández Grecco, 2001).

### Management implications

In the Flooding Pampa grasslands the growth of native legumes and their biomass production were insignificant respect to *Lotus tenuis*, which is an economically important species of these grasslands. Plant communities containing *L. tenuis* are generally heavily grazed, indicating a higher palatability and forage quality, but affecting negatively the seed production (Fernández *et al.*, 2008). Fluctuations in propagule availability can play an important role in the *L. tenuis* seedling recruitment and biomass production in the grasslands.

### CONCLUSIONS

Our results provide preliminary information about management of *L. tenuis* in these grasslands to reduce the declination of its plant stand and biomass production. Seedlings, plant establishment and biomass productivity can be increased through seed addition and/or natural reseeding. Natural reseeding can be reached by increasing the soil seed bank size through grazing exclusion during *L. tenuis* reproductive period.

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### RESUMEN

**Establecimiento de plántulas y producción de biomasa de *Lotus tenuis* en pastizales de la Pampa Deprimida (Buenos Aires, Argentina).** En pastizales y pasturas ha sido documentada la reducción de la densidad de plantas y de la biomasa de *Lotus tenuis* Waldst. & Kit. ex Willd. Nuestro objetivo fue determinar si su densidad de plantas y su producción de biomasa pueden ser incrementadas en un pastizal mediante la adición de semillas. Dos experimentos sin pastoreo fueron realizados en tres potreros de un pastizal de la Pampa Deprimida. El primer experimento fue realizado entre otoño 2004-otoño 2006 y el segundo entre otoño 2005-otoño 2007. Diferentes cantidades de semillas (0, 57, 229, 917 y 1833 semillas m<sup>2</sup>) fueron esparcidas a mano en las parcelas experimentales. En el segundo experimento, además de la adición de semillas, también se produjo la siembra de aproximadamente 900 semillas m<sup>2</sup>, por los aportes de las plantas de *L. tenuis* del pastizal. La densidad de semillas explicó el 81% y el 19% de la variación de la densidad de plántulas y la producción de biomasa de *L. tenuis*, respectivamente.

La emergencia de plántulas fue principalmente entre otoño-principios de primavera y la mortalidad entre primavera tardía-verano. La densidad de plantas adultas y la biomasa producida por *L. tenuis* se incrementaron con la adición de semillas. La biomasa total del pastizal varió entre 589,94 ± 26,89 y 1042,44 ± 54,39 g m<sup>-2</sup> año<sup>-1</sup> y las diferencias fueron atribuidas principalmente a las precipitaciones. La contribución de *L. tenuis* fue del 10%. Los resultados sugieren que el establecimiento de plántulas, de plantas y la producción de biomasa de *L. tenuis* pueden aumentar en el pastizal por la adición de semillas y/o por la lluvia de semillas mediante la exclusión del pastoreo durante el período reproductivo.

**Palabras clave:** leguminosa, calidad forrajera, atributos poblacionales, mortalidad de plántulas, adición de semillas.

### LITERATURE CITED

- Acuña, H.P., y G. Cuevas. 1999. Efecto de la altura y frecuencia de la defoliación, bajo corte y pastoreo, en el crecimiento y productividad de tres especies del género *Lotus* en suelos arcillosos. *Agricultura Técnica* 59:296-308.
- Bologna, J.J., J.S. Rowarth, T.J. Fraser, and G.D. Hill. 1996. Management of birdsfoot trefoil (*Lotus corniculatus* L.) pastures for productivity and persistence. *Proceedings of the Agronomy Society of New Zealand* 26:17-21.
- Colabelli, M.R., y D.P. Miñón. 1993. Métodos de intersembrado de *Lotus tenuis* y *Trifolium repens* en pastizales de la Pampa Deprimida bonaerense. *Revista Argentina Producción Animal* 13:225-233.
- Colabelli, M.R., y D.P. Miñón. 1994. Rendimiento y cambios botánicos de pasturas de *Lotus tenuis* puro y en mezcla bajo régimen de corte. *Agricultura Técnica* 54:39-45.
- Colabelli, M., y E.M. Viviani Rossi. 1997. Efecto de dos métodos de intersembrado de *Lotus tenuis* sobre su implantación y sobre la producción de forraje de pastizales del área ganadera del salado (Argentina). *Avances en Producción Animal* 22:123-128.
- Costa, J.L., y F.O. García. 1997. Respuesta de un pastizal natural a la fertilización con fósforo y nitrógeno en un Natracuol. *Revista de Investigaciones Agropecuarias* 28:31-39.
- Fernández Grecco, R. 2001. Efecto de la fertilización nitrogenada invernal sobre la acumulación de forraje de un pastizal natural de la Pampa Deprimida, Argentina. *Agricultura Técnica* 61:319-325.

- Fernández, O.N., O.R. Vignolio, y G.S. Cambareri. 2008. Persistencia de *Lotus tenuis* bajo pastoreo por vacunos: períodos críticos para la supervivencia y la reproducción. p. 34-35. In Ruiz, O. (ed.) IV Taller Interdisciplinario de *Lotus*: Aspectos Genéticos, Moleculares y Ecofisiológicos de *Lotus* spp. y sus Simbiontes, Chascomús. Noviembre 2008. IIB-INTECH, CONICET, UNSAM, Buenos Aires, Argentina.
- Juan, V.F., L. Monterroso, M.B. Sacido, and M.A. Cauhépé. 2000. Postburning legume seedling in the Flooding Pampas, Argentina. *Journal of Range Management* 53:300-304.
- Laterra, P. 1997. Post-burn recovery in the flooding Pampa: Impact of an invasive legume. *Journal of Range Management* 50:274-277.
- Lenz, T.I., and J.M. Facelli. 2005. The role of seed limitation and resource availability in the recruitment of native perennial grasses and exotics in a South Australian grassland. *Austral Ecology* 30:684-694.
- Martin, L.M., and B.J. Wilsey. 2006. Assessing grassland restoration success: relative roles of seed additions and native ungulate activities. *Journal of Applied Ecology* 43:1098-1109.
- Miñón, D.P., y M.R. Colabelli. 1993. Intersiembra de *Lotus tenuis* en tres comunidades nativas de la Pampa Deprimida. *Revista Argentina de Producción Animal* 13:133-140.
- Miñón, D.P., y R.O. Refi. 1993. Persistencia de pasturas de *Festuca arundinacea*, *Trifolium repens* y *Lotus tenuis* bajo pastoreo continuo. *Diálogo* 38. p. 95-102. Metodología de Evaluación de Pasturas.
- Oosterheld, M., y R.J. León. 1987. El envejecimiento de las pasturas implantadas: su efecto sobre la productividad primaria. *Turrialba* 37:29-35.
- Oosterheld, M., and O.E. Sala. 1990. Effects of grazing on seedling establishment: the role of seed and safe-site availability. *Journal of Vegetation Science* 1:353-358.
- Quinos, P.M., P. Insausti, and A. Soriano. 1998. Facilitative effect of *Lotus tenuis* and *Paspalum dilatatum* in a lowland grassland of Argentina. *Oecologia* 114:427-431.
- Refi, R.O., and C.J. Escuder. 1998. Nitrogen fixation by *Trifolium repens* and *Lotus tenuis*-based pastures in the Flooding Pampa, Argentina. *Agronomie* 18:285-297.
- Sevilla, G.H., O.N. Fernández, D.P. Miñón, and L. Montes. 1996. Emergence and seedling survival of *Lotus tenuis* in *Festuca arundinacea* pastures. *Journal of Range Management* 49:509-511.
- Sternberg, M., M. Gutman, A. Perevolotsky, and J. Kigel. 2003. Effects of grazing on soil seed bank dynamics: An approach with functional groups. *Journal of Vegetation Science* 14:375-386.
- Thomsen, M.A., C.M. D'Antonio, K.B. Suttle, and W.P. Sousa. 2006. Ecological resistance, seed density and their interactions determine patterns of invasion in a California coastal grassland. *Ecology Letters* 9:160-170.
- Tilman, D. 1997. Community invisibility, recruitment limitation, and grassland biodiversity. *Ecology* 78:81-92.
- Vignolio, O.R., G.S. Cambareri, y N.O. Maceira. 2010. *Lotus tenuis* (Fabaceae). Producción y manejo agronómico. *Revista Argentina de Producción Animal* 30:97-116.
- Vignolio, O.R., and O.R. Fernández. 2010. Cattle dung as vector of spreading seeds of exotic species in the Flooding Pampa grasslands (Buenos Aires, Argentina). *Annales Botanici Fennici* 47:14-22.
- Vignolio, O.R., O.N. Fernández, and N.O. Maceira. 1999. Flooding tolerance in five populations of *Lotus glaber* Mill. (Syn. *Lotus tenuis* Waldst. et Kit.). *Australian Journal of Agricultural Research* 50:555-559.
- Wilsey, B.J., and H.W. Polley. 2003. Effects of seed additions and grazing history on diversity and productivity of subhumid grasslands. *Ecology* 84:920-931.
- Zobel, M., M. Otsus, J. Liira, M. Moora, and T. Möls. 2000. Is small-scale species richness limited by seed availability or microsite availability? *Ecology* 81:3274-3282.