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Responses of *Lotus glaber* (Leguminosae) cv. Chajá to defoliation in reproductive stage

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Lotus glaber is a herbaceous forage legume, which became naturalized in the Flooding Pampa grasslands (Buenos Aires Province, Argentina). Experiments to evaluate defoliation effects on seed and shoot production were conducted in field conditions in Balcarce (Argentina). *Lotus glaber* plants were clipped (85% aerial biomass) at the beginning of the flowering period. Clipped plants commenced flowering again 21 days after being cut and compensated the vegetative biomass without affecting the reproduction. Clipped and control plants did not differ in seed number, pods and umbels per plant, mean seed weight and seed viability. *Lotus glaber* has indeterminate flowering, thus allowing to compensate vegetative and reproductive attributes under clipping conditions. *Lotus glaber* seed weight stability is important because this attribute is positively related to seedling vigor.

Key words: defoliation, *Lotus glaber*, seed and pods per plant, seed weight and viability

Introduction

Plant responses to defoliation depend on intensity and frequency of tissue removal, phenological stage, age, size, nutrient availability and reserves for growth (Fehr *et al*. 1981, Maschinski & Whitham 1989). *Lotus glaber* is a Mediterranean herbaceous forage legume naturalized in the Flooding Pampa grasslands (Buenos Aires Province, Argentina). *Lotus glaber* stem density and biomass production decreased as experimental period increased under grazing and mechanical defoliation conditions (Miñón & Refi 1993, Quadrelli *et al*. 1997, Acuña & Cuevas 1999). In *Lotus corniculatus* the seed production was correlated with the duration of the first flowering pulse (Ollerton & Lack 1998). Grazing can retard or suppress flowering and negatively affect seed production and seedling density (Olmos 2001).

The effects of defoliation on seed production in reproductive season were not reported in *L. glaber*.

The objective of this research was to evaluate the effects of defoliation at early flowering stage on vegetative and reproductive attributes of *L. glaber*.

Materials and methods

Study site and treatments

The study site was located at Unidad Integrada de Balcarce (F.C.A., U.N.M.P. — E.E.A., I.N.T.A., Balcarce, Argentina 37°47´S, 58°17´W). On 23

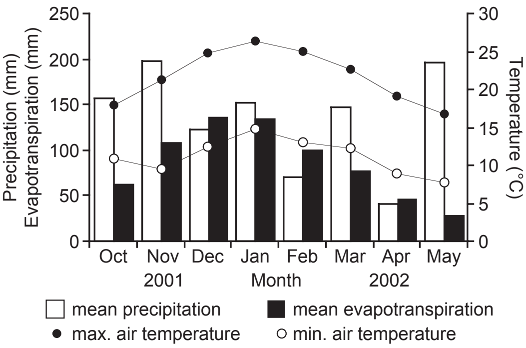

Fig. 1. Monthly mean precipitation, evapotranspiration and air temperatures during the field experiment.

July 2001, *L. glaber* cv. Chajá seeds were inoculated with *Rhizobium loti* (Quadrelli *et. al.* 1997) and they were sown one per pot in greenhouse conditions. On 15 October 2001, 45 *L. glaber* plants with mean dry weight (± S.E.) of 39.52 ± 3.28 mg pl^{-1} were transplanted to an experimental field. The soil was a typical Argiudol (pH (soil: H_2O, 1:2.5) 5.9, EC 0.3 mmhos, available P by the Bray 1 method 9.4 ppm, NO_3-N 6.3 ppm, total N 0.255%). Five plants were 75 cm apart in nine rows; the space between rows was also 75 cm. Additional plants were transplanted to reduce border effects. The experimental field was kept free of weeds by hand-removal, protected from herbivores and pollinated by honey bees. Climatic records were provided by the Unidad Integrada Balcarce (Fig. 1).

On 26 December 2001, 30 plants were selected at random. The plants had flowers (110.46 ± 12.72 umbels with flowers per plant), immature pods (33.53 ± 8.74 umbels with pods per plant) and prostrate growth. Defoliation was performed on 15 plants with hand scissors, leaving a radius of 6 cm of shoot from the center of the plant. The clipped biomass (CB) (± S.E.), being 85.15% (± 2.27%) of the total aerial biomass, was estimated on 6 extra plants. The standing biomass (SB) of clipped plants and CB were separated and dried (96 h, 60 °C), and used to estimate the total biomass per plant at the beginning of the treatments (TBB). The function used to estimate total biomass of clipped plants was:

$$\mathrm{TBB} = 1.1172 \times \mathrm{CB}$$
$$(n = 6;\ R^2 = 0.991;\ P < 0.0001).$$

The number of flowers and pods per umbel was recorded on 7 and 16 January, 1 and 15 February, 1, 21 and 26 March 2002. On each date, ten umbels with flowers and ten with pods were randomly selected in each plant. The pod to flower ratio, and the number of seeds per pod were determined on eleven dates: 3, 7, 9, 16, 18, 23, 25, 29 January 2002; 5, 13 February 2002; 4 March 2002. On each date, 3 umbels per plant with the same number of flowers and maturity stage were tagged with telephone wire rings, and mature pods were hand harvested. Seed mean weight and germination test were analyzed on pods originated from umbels which were tagged between 3 and 16 January. Seed germination was recorded on three randomly selected plants per treatment. Three samples of 50 seeds per plant were incubated in a germination room (22.21 ± 0.15 °C and 8 h light).

On 26 March 2002 the aerial biomass was harvested and dry weight was determined (96 h, 60 °C). Senescent leaves were lost and not included in the biomass production. The lost seed number (LSN) and their weight (LSW) during harvest and dehiscence were included in the calculus and were estimated for each plant as follows:

$$\mathrm{LSN} = \text{empty pod number} \times \text{mean number of seed per pod}$$
$$\mathrm{LSW} = \mathrm{LSN} \times \text{mean seed weight}$$

Statistical analysis

The effects of clipping treatments on vegetative and reproductive biomass and its attributes were determined with Student's *t*-test ($p < 0.05$). Seed germination percentages were arcsine square root transformed before analysis. The relation between number of umbels per plant and the number of seeds per plant was tested with regression analysis (Systat 1992).

Results

At the beginning of the experiment the total biomass (± S.E.) per plant was 16.62 ± 1.58 g plant^{-1} (n = 15). Clipped plants commenced flowering 21 days after being cut. By mid-January 2002, only 20% of clipped plants had pods (Fig. 2).

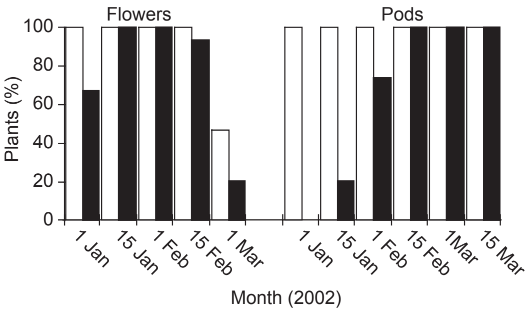

Fig. 2. Percentage of *Lotus glaber* plants with flowers and pods during the experimental period in control (white bars) and defoliation (black bars) treatments.

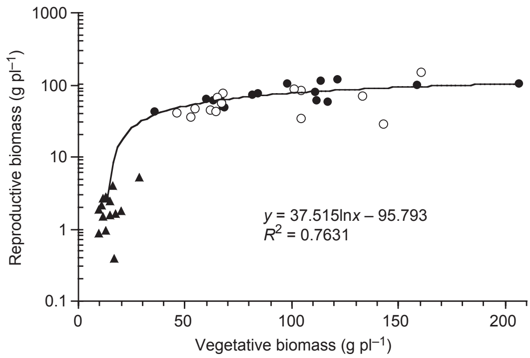

Fig. 3. Relationship between vegetative and reproductive biomass in *Lotus glaber*. ● = control; ○ = clipped plants; ▲ = biomass at the beginning of the experiment.

The reproductive variables were not significantly different between treatments (Table 1). Approximately 35% of the flowers did not produce pods in both treatments (Table 1).

Regression analysis showed that 94.50% of variation in seed number per plant was significantly explained by the number of umbels per plant (seed plant^{-1} = 34.18 × umbels plant^{-1}, $R^2 = 0.945$, $n = 30$, intercept = 0). The total aerial biomass per plant (vegetative plus reproductive biomass, without including the removed biomass) (± S.E.) was not significantly different between the control and defoliation treatments 178.55 ± 16.47 g plant^{-1} and 149.00 ± 15.19 g plant^{-1}, respectively. For both treatments, reproductive biomass per plant decreased with vegetative biomass lower than 50 g and did not differ among plants with vegetative biomass higher than that value (Fig. 3).

Discussion

Plants clipped at early reproductive stage delayed flowering. Plant stability of the reproductive and vegetative variables may be due to plasticity in different attributes. *Lotus glaber* plasticity of reproductive and vegetative attributes were reported

Table 1. Means (± S.E.) of reproductive components of *Lotus glaber* plants growing under different treatments. For a same variable, means with the same letter are not significantly different ($p < 0.05$) between treatments.

Variables	Treatments		Record date
	Control	Defoliation	
Flowers per umbel (*n* plant^{-1})	4.31 ± 0.08[a]	4.12 ± 0.08[a]	7 and 16 January 1 and 15 February 21 and 26 March
Pods/flower (%)	65.22 ± 2.56[a]	68.90 ± 1.63[a]	3–29 January 5 and 13 February 4 March
Pods per umbel (*n* plant^{-1})	3.40 ± 0.14[a]	3.04 ± 0.09[a]	7 and 16 January 1 and 15 February 21 and 26 March
Seeds per pod (*n* plant^{-1})	10.68 ± 0.63[a]	11.05 ± 0.86[a]	3–29 January 5 and 13 February 4 March
Seed weight × 1000 (mg plant^{-1})	1108.00 ± 30.55[a]	1096.84 ± 42.32[a]	3 and 16 January
Seed germination (%)	99.55 ± 0.44[a]	99.77 ± 0.22[a]	3 and 16 January
Umbels (*n* plant^{-1})	1684 ± 189[a]	1457 ± 147[a]	26 March
Seeds (*n* plant^{-1})	58551 ± 6254[a]	50345 ± 6700[a]	26 March
Pod (*n* plant^{-1})	5565 ± 570[a]	4450 ± 478[a]	26 March

for plants growing in different experimental conditions (Stofella *et al*. 1998, Kade *et al*. 2003). A delay in flowering and pod production caused by clipping was also recorded for other legumes (Collins & Aitken 1970, Lowther *et al*. 1992). Indeterminate flowering of *Lotus glaber* allows the plant to produce the same amount of seeds per plant without affecting mean seed weight. Clipping species with indeterminate flowering at their early reproductive stage does not substantially affect their growth or seed production if the growth is not limited by resources and climatic conditions (Paige & Whitham 1987).

The reproductive *L. glaber* biomass did not increase linearly with the vegetative biomass as it had been reported in other perennial herbaceous plants (Samson & Werk 1986). The reproductive biomass proportion decreased progressively as the vegetative biomass increased (Fig. 3). By the end of the reproductive season, climatic conditions are more limiting for flower, seed and pod production than for vegetative growth, resulting in a reduced reproductive biomass proportion (Vignolio *et al*. 2002).

The seed weight stability in *L. glaber* also agrees with previous studies in other species growing in grasslands under natural herbivory or mechanically cut (Paige & Whitham 1987). In *L. glaber* seed weight stability is important because this species has low seedling vigor (Hur *et al*. 1994) and seeds with lower weight would have negative demographic consequences on the legume. The number of flowers developed on each umbel exceeded the number of mature pods. This may be due to limitation in resources for pod development and/or the non-pollination of some flowers. Similar results were reported in *Lotus corniculatus* (Stephenson & Winsor 1986) and *Lotus pedunculatus* (Lowther *et al*. 1992). Stephenson and Winsor (1986) found abortion of flowers and pods in *Lotus corniculatus*. Aborted pods were those with fewer seed number or with seeds produced by self-pollination.

The vegetative growth and reproduction of *L. glaber* were not affected significantly when the plants were clipped in early reproductive season. In field conditions, the plants can be grazed with different frequency during the reproductive season. Further investigations must be carried out to know the effects of defoliation on *L. glaber* in interaction with the plant communities of grasslands.

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