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Effects of flooding at early summer on plant water relations of *Lotus tenuis*

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Introduction

Flooding is a natural disturbance of crescent importance affecting forage production in many grassland ecosystems. The anoxic environment associated with flooding induces a sequence of changes on plants including alterations of physiological processes related to the survival of plants under anaerobic conditions (Armstrong, 1979; Koslowski and Pallardy, 1984). At the physiological level, flooding could greatly affect plant water relations. In flood–sensitive species, closing of stomata, with or without leaf dehydration, and reduction of transpiration can occur in few hours (Bradford and Hsiao, 1982; Else *et al*., 1996); meanwhile in flood–tolerant species the same parameters (*e.g.* stomatal conductance, transpiration and leaf water potential) could be maintained for weeks (Insausti *et al*., 2001). Many studies have evaluated physiological differences in flooding tolerance between species. However, it is known that the same species could present different tolerance depending on the season of occurrence of the flooding event (Crawford, 2003). In this respect, studies looking for differences in flooding responses of the same species at different seasons are scarce.

The Flooding Pampa grasslands cover a wide area of the Buenos Aires province (Argentina) dedicated mainly to cattle breeding (Soriano, 1992). Flooding is one of the major stress factors affecting the structure and functioning of the plant communities located in the lowland topographical positions (Insausti *et al*., 1999). The most regular situation corresponds to large floods (1-2 months) from the end of winter to spring, while short floods (1-2 weeks) can occur with less frequency at the end of spring and the beginning of summer. *Lotus tenuis* Waldst. & Kit. (=*Lotus glaber Mill.)* possess a great potential as forage species for temperate areas due to their ability to withstand numerous and diverse environmental stresses, such as flooding and salinity (Vignolio *et al.*, 1999; Striker *et al.*, 2005; Teakle *et al.*, 2006). Previously, we studied the effects of 40 days of flooding at early spring on water relations of this species. The study showed that flooded plants maintained stomatal conductance and transpiration at similar rates than controls until 25 days of experiment, and later registered a slight decrease in such parameters without affecting biomass accumulation (Striker *et al*., 2005). However, monitoring plant water relations of *L. tenuis* under qualitatively more stressful conditions to plant growth, as summer flooding, has never been addressed. In this way, flooding at early summer is expected to be more injurious to plant growth due the higher temperature and evaporative demand for plant transpiration at such season (Vervuren *et al*., 1999; Van Eck *et al*., 2004). This work reports this issue.

Materials and methods

Plant material and study site

At early spring, adult plants of similar size of the legume *Lotus tenuis* were extracted in grassland soil blocks (= mesocosms, 0.3 × 0.3 × 0.25 m) from an extensive stand of lowland grassland located in the Department of Pila, Province of Buenos Aires, Argentina (36º 30´S, 58º 30´W). These grasslands corresponded to the plant community defined phytosociologically by *Piptochaetium montevidensis*, *Ambrosia tenuifolia*, *Eclipta bellidioides* and *Mentha pulegium* (*sensu* Burkart *et al*., 1990). This plant community is found in flat areas associated with typical Natraquoll soils (Soriano, 1992). After extraction, individuals were immediately put in plastic containers and placed interspersed in the experimental garden of the Faculty of Agronomy at the University of Buenos Aires.

After an acclimatization period of 2 months, two treatments were applied following a completely randomized design with five replicates: (1) control - watered daily to field capacity and allowed to drain freely, and (2) flooded - drilled holes of containers were coated, mesocosms were flooded and maintained at a level of 6 cm above soil surface by 15 days. A subsequent 30 days grown–period at drained conditions were allowed to evaluate plant recovery. During the experiment, oxygen diffusion rate (ODR) at control and flooded conditions was periodically recorded using a calomel electrode as reference and a platinum electrode inserted at a depth of 5 cm.

Water relations measurements

Stomatal conductance (g_s) and transpiration rate (E) were measured in young leaves (including three leaflets per leave) above water level in similar positions using a Li-1600M steady state porometer (Li-Cor Inc., Lincoln, NE, USA). Leaf water potential (Ψ_w) was recorded on the stem corresponding to the same leaves by a Schölander-type pressure chamber (Bio-Control, Buenos Aires, Argentina). At the same time, air temperature and relative humidity were monitored (Temperature and RH Probe HUMICAP H, Vaisala, Finland) and used to calculate the air vapor pressure deficit (VPD_{air}) as a characterization of the atmospheric evaporative demand. Measurements were performed approximately at midday on clear days (average PPFD of 1700 μmol m^{-2} s^{-1}). Each parameter was registered every three days within the flooding period and every five days within the recovery period.

Plant harvest was carried out at the end of experiment. Plant biomass was separated into shoot, crown and root. Harvested material was weighed after oven drying for 72 h at 70°C. All data sets were checked to satisfy ANOVA assumptions and analyzed by repeated measures analysis of variance (RMANOVA). The analyses were performed separately for the flooding period and the recovery phase. Contrasts between control and flooded plants on individual dates were performed with *a posteriori* Tukey tests. Biomass data was evaluated by Student's *t*-test. Results are presented as means of five replicates ± standard error.

Results and discussion

Flooding rapidly decreased oxygen diffusion rate from 60 ± 1 to 6 ± 1 × 10^{-8} g cm^{-2} min^{-1} in the first three days of treatment and near zero at day 15 indicating the anaerobic conditions imposed by the treatment (Figure 1).

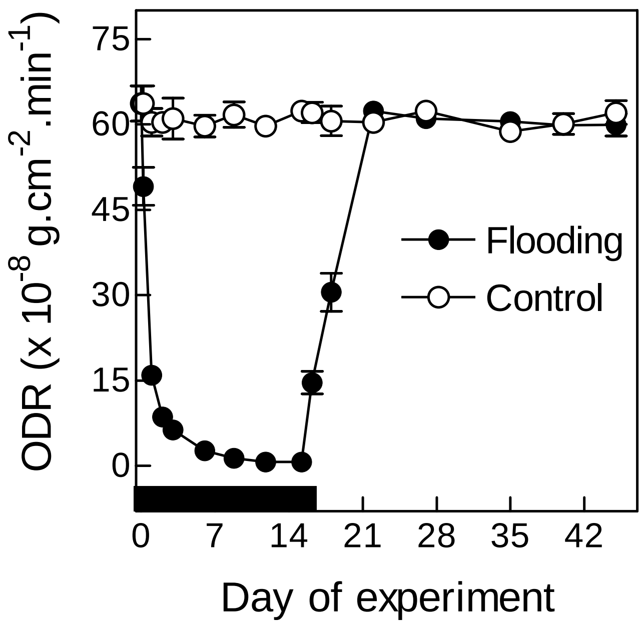

Figure 1. Oxygen diffusion rate (ODR) in control and flooded conditions. Horizontal closed bar indicate the flooding period of 15 days. Values are means ± SE of five replicates.

Repeated measures ANOVA indicated significant flooding (P< 0.01) and time effects (P< 0.001) on leaf water potential (Ψ_w), stomatal conductance (gs) and transpiration rate (E) of *Lotus tenuis*. The treatment-time interaction was significant (P< 0.001), showing that in general the negative effects of flooding increased over time (Figure 2). Interestingly, *a posteriori* mean-comparison tests revealed that negative effects of flooding, in these parameters at higher evaporative demand (Figure 2a), occurred after a week of experimental period. Decreases in stomatal conductance and transpiration rate by stomatal closing in response to flooding and anaerobic soil conditions were proposed as a mechanism to regulate water balance of plants and minimize leaf dehydration (Bradford and Hsiao, 1982; Ashraf, 2003). In this respect, the fact that flooded plants of *L. tenuis* showed a slight stabilization of the transpiration rate between the 6^{th} and 15^{th} day of flooding with progressive reductions of g_s is in line with this idea. However, the decrease in Ψ_w under flooding, occurred after g_s reductions, indicating that stomatal closure was not enough to prevent leaf dehydration at the higher atmospheric evaporative of summer (average value of VPD_{air} of 3.28 KPa). These responses differ with those occurred at early spring flooding with lower evaporative demands (average value of VPD_{air} of 1.82 KPa). At early spring, flooded plants of *L. tenuis* could regulate their water balance through stomata closure and could maintain Ψ_w with respect to controls, even after 40 days of flooding (Striker *et al*., 2005). In this sense, early summer flooding, an event of lower frequency in the Flooding Pampa grasslands, was clearly more injurious to plant performance of *L. tenuis* than winter-spring flooding of annual occurrence although did not provoked death of any plant.

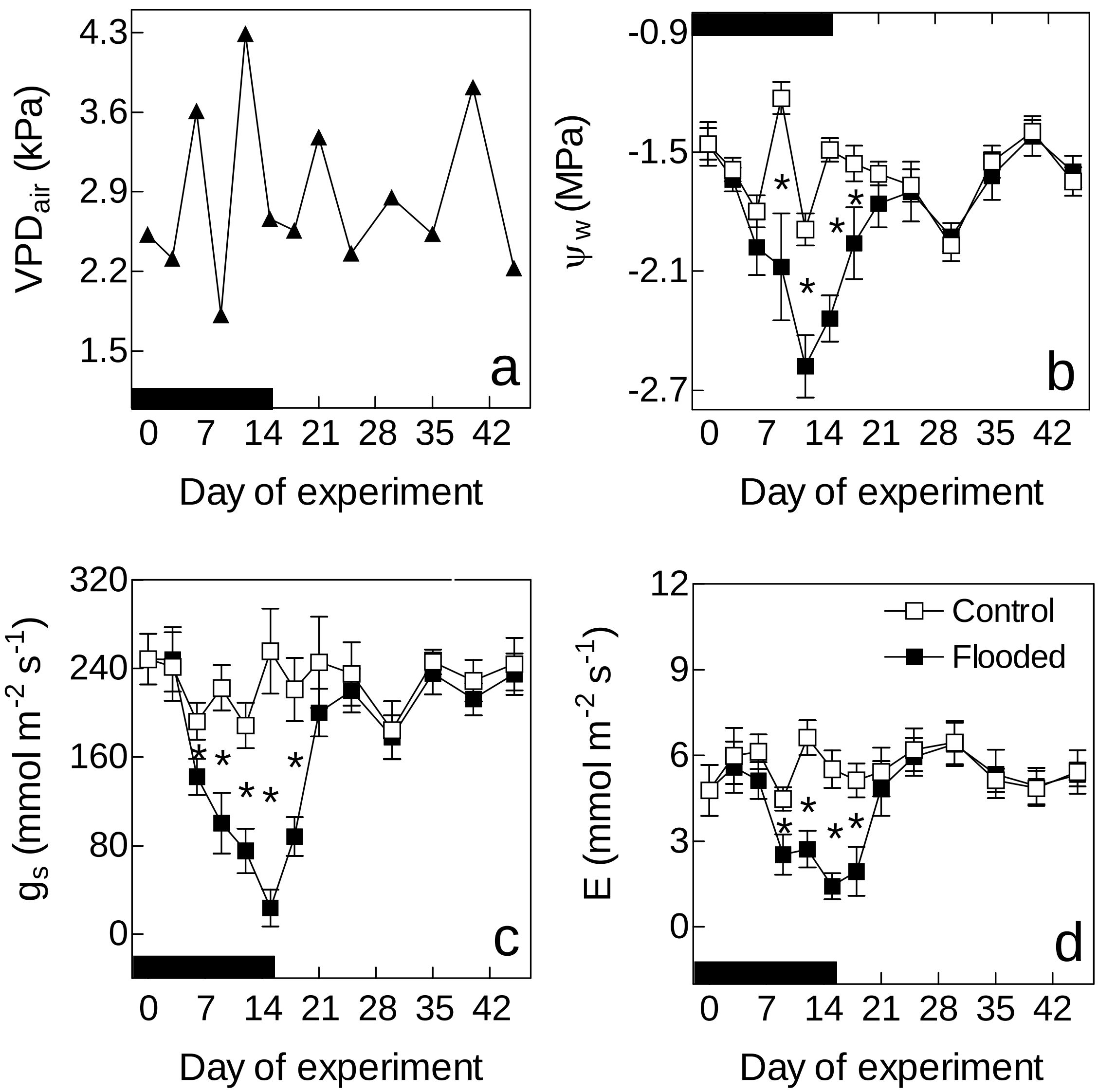

Figure 2. (a) Air vapour pressure deficit (VPD_{air}), (b) Leaf water potential (ψ_w), (c) stomatal conductance (g_s) and (e) transpiration rate (E) of *Lotus tenuis* plants grown under control (open squares) and flooded (closed squares) conditions. Horizontal closed bars indicate the duration of the experimental flooding (15 days). Asterisks indicate significant differences ($P< 0.05$) between treatments within date based on the Tukey test. Values are means ± SE of five replicates.

As expected, when flooding was discontinued all soil blocks recovered the values of oxygen diffusion rate similar to controls blocks in the first five days (Figure 1). In accordance with soil reoxygenation, during the recovery phase pre-flooded plants of *L. tenuis* recovered their water status (Ψ_w), and showed a stomatal behavior (gs) and transpiration rates (E) similar than control plants (RMANOVA, treatment effect: $P > 0{\cdot}05$; Figure 2). In spite of the higher capacity of *L. tenuis* to recover their physiological parameters when flooding was released, the plant growth was negatively affected by this stress as revealed a 46% lower total biomass of flooded plants at the end of the experiment in comparison to control plants (Table 1). In accordance, flooded plants of *L. tenuis* registered lower shoot, crown and root biomass (44%, 27% and 60%, respectively) in comparison to control plants ($P < 0.05$ for all cases; Table 1). The unique report available studying both plant water relations and biomass responses on a flood-tolerant legume crop *(Lupinus luteus*) is in agreement with our results (Davies *et al*., 2000). In this work, *Lupinus luteus* plants were flooded for 14 days and were capable to recover their stomatal conductance and leaf water potential after a week of growing under control conditions, but suffering reductions in shoot and root biomass on pre-flooded plants about 25 to 56% (Davies *et al*., 2000). In particular for *L. tenuis*, Vignolio *et al*. (1994) evaluated the effects of 17 days of summer flooding on biomass responses and found a reduction in biomass of 30% as consequence of a lower shoot and root mass in flooded plants.

Table 1. Plant biomass of *Lotus tenuis* subjected to control and flooding conditions. Flooding endured 15 days. Harvest was carried out after plants grown by 30 days at drained conditions for recovery.

Biomass (g plant^{-1})	Control	Flooded	*P-value*
Shoot	12.9 ± 1.9	7.2 ± 2.2	0.035
Crown	4.3 ± 0.4	3.1 ± 0.3	0.043
Root	8.2 ± 1.2	3.2 ± 0.9	0.01
Total	25.4 ± 1.9	13.5 ± 2.1	<0.001

Conclusion

Lotus tenuis was able to tolerate two weeks of flooding at higher evaporative demand, typical of early summer season, but showing an important reduction in plant growth. This contrast with the typical response of *L. tenuis* at early spring, in which plants can adjust their physiological behavior in a way that plant biomass accumulation is not affected by flooding. The study denotes the importance of seasonal variations of flooding events on plant performance of *L. tenuis*, and provides information that contributes to the knowledge of this forage legume and its utilization in agricultural areas subject to periodic flooding events.

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