

## Short-term responses to salinity of an invasive cordgrass

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### Abstract

Salinity is one of the main chemical factors in salt marshes. Studies focused on the analysis of salinity tolerance of salt marsh plants are very important, since they may help to relate their physiological tolerances with distribution limits in the field. *Spartina densiflora* is a South America cordgrass, which has started its invasion of the European coastline from the southwestern Iberian Peninsula. In this work, short-term responses in adult tussocks of *S. densiflora* from southwestern Spain are studied over a wide range of salinity in a greenhouse experiment. Our results point out that *S. densiflora* has a high tolerance to salinity, showing high growth and net photosynthesis rates from 0.5 to 20 ppt. *S. densiflora* showed at the lowest salinity (0.5 ppt) high levels of photoinhibition, compensated by higher levels of energy transmission between photosystems. Adaptative mechanisms, as those described previously, would allow it to live in fresh water environments. At the highest salinity (40 ppt), *S. densiflora* showed a high stress level, reflected in significant decreases in growth, net photosynthesis rate and photochemical efficiency of Photosystem II. These responses support *S. densiflora* invasion patterns in European estuaries, with low expansion rates along the coastline and faster colonization of brackish marshes and river banks.

**Abbreviations:** A – net photosynthesis rate; Chl – chlorophyll;  $F_0$  – basal fluorescence;  $F_p$  – peak of fluorescence;  $F_v$  – variable fluorescence;  $F_v/F_p$  – potential photochemical efficiency of PSII;  $G_s$  – stomatal conductivity rate; PPFD – photosynthetic photon flux density; PS II – Photosystem 2;  $T_{1/2}$  – half-time for transition from  $F_0$  to  $F_p$ ;  $\Psi_{leaf}$  – leaf water potential

### Introduction

Coastal ecosystems, such as salt marshes, are one of the areas most affected by the introduction of alien species. The genus *Spartina* counts different species that behave as salt marsh invaders all around the world. Their invasion patterns seem to depend on complex relationships between biological interactions with autochthonous species

and habitat physical conditions such as salinity (Kittelson and Boyd 1997; Hacker et al. 2001). Thus, salinity is one of the main chemical factors in salt marshes, determining vegetation distribution with respect to elevation (Banerjee 1993) and distance to the sea (Wilson et al. 1996), through species tolerance to ion concentration and modulation of the outcomes of interspecific interactions (Broome et al. 1995; Gough and

Grace 1998). In this context, works focused on the analysis of salinity tolerance of salt marsh invaders are very important since they may help to relate physiological tolerances with distribution limits in the field (Rozema et al. 1988).

*Spartina densiflora* is such a species, which is invading Europe (Castillo et al. 2000), northwest Africa (Fennane and Mathez 1988) and North America (Kittelson and Boyd 1997) from South American marshes (Mobberley 1956). In Europe, its invasion started in the Gulf of Cádiz (south-western Iberian Peninsula), where it has colonized at least eight estuaries and very contrasted habitats, from low to high marshes. However, studies on the tolerance of this species to environmental stress are very scarce (Kittelson and Boyd 1997; Nieva et al. 1999; Castillo et al. 2000).

The work described in this paper aimed to analyze short-term performance of *S. densiflora* from the southwestern Iberian Peninsula over a wide range of salinity. The specific objectives were to examine water potential, growth, leaf gas exchange and chlorophyll fluorescence in adult *S. densiflora* tussocks in five salinity treatments, from fresh water to hypersalinity, in a greenhouse experiment. We also sought to compare whether these measures of performance were in accordance with its actual distribution and discuss possible evolution of its invasion depending on its salinity tolerance. Our results may help to understand invasion mechanisms of cordgrasses.

Growth, leaf gas exchange and chlorophyll fluorescence have been identified as adequate tools to analyze stress due to salinity (Mishra et al. 1991; Belkhdja et al. 1994; Ewing et al. 1997).

## Materials and methods

### General methods

*Spartina densiflora* adult tussocks were collected, in June 1998, in Odiel river salt marshes (south-western Iberian Peninsula) from 'Punta del Sebo' low marsh. Plants were transplanted into clean sand culture in small plastic pots for propagation in a greenhouse (Ewing et al. 1995). For the experiment, plants (5–18 live stems per tussock) were repotted in expands perlite (Floreal. Agroperlita F-3) in 11 cm diameter pots (Trovadec plastics).

Plants were grown using five salinity treatments (0.5, 10, 15, 20 and 40 ppt), immersing the pots

2 cm in a saline/nutrient solution (20% modified Hoagland's solution). The salinity treatments were obtained by using Hoagland's Solution, and Hoagland's Solution plus sea salts (Instant Ocean) to give 10, 15, 20 and 40 ppt. The last treatment was obtained progressively, though the treatment of 20 ppt took 1 week. Concentrations of the full-strength nutrient solution were 1.02 ppt KNO<sub>3</sub>, 0.49 ppt Ca(NO<sub>3</sub>)<sub>2</sub>, 0.23 ppt NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 0.49 ppt MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.86 ppt H<sub>3</sub>BO<sub>3</sub>, 1.81 ppt MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.08 ppt CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.22 ppt ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.09 ppt H<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O, 0.6 ml FeSO<sub>4</sub>·7H<sub>2</sub>O 0.5% and 0.6 ml tartaric acid 0.4%. The solution was changed once a week. Five replicates of each treatment were used. The experiment was carried out for 29 days in September 2000, in a greenhouse with controlled temperatures between 21 and 25 °C; the photoperiod was extended to 15 h with the use of incandescent lights (Osram Vialox NAV-T (SON-T) 400 W) with a photosynthetically active photon flux density (PPFD) of 250 μE/m<sup>2</sup>s at canopy level.

The following measurements were made: leaf water potential, leaf expansion, leaf gas exchange and chlorophyll *a* fast kinetic fluorescence parameters.

### Leaf water potential

Leaf water potential ( $\Psi_{\text{leaf}}$ ) was measured by a Scholander bomb during the noon solar hour on adult leaves of each of four tillers (each from a different clump, chosen at random) in every treatment, at the end of the experiment.

### Leaf expansion

Leaf expansion was measured on five tillers (each from a different clump, chosen at random) in every treatment at the end of the experiment by placing a marker of inert sealant on the base of the youngest accessible leaf. The distance between the marker and the leaf base was measured at the beginning and the end of a 3-day period (Ewing et al. 1995).

### Gas exchange

Measurements of the net photosynthesis rate (A) and stomatal conductivity rate (G<sub>s</sub>) were carried out at the end of the experiment on the second

youngest leaf of each of three tillers (each from a different clump, chosen at random) in every treatment after 2 h of lighting with a PPFD of  $250 \mu\text{E}/\text{m}^2\text{s}$  at canopy level. Measurements were made using a portable infrared  $\text{CO}_2$  analyzer (ADC LCA-3, Analytical Development Co. Ltd, Hoddesdon, UK) in differential mode and in an open circuit; it was coupled to a Parkinson Leaf Chamber (ADC PLC-3N), using a radiant flux density of  $210 \mu\text{E}/\text{m}^2\text{s}$  (Long and Hällgren 1993).

### Chlorophyll fluorescence

Chlorophyll *a* fast kinetic fluorescence was measured on the second-youngest leaf of each of five tillers (each from a different clump, chosen at random) at every treatment, in the prevailing air temperature at PPFD of  $250 \mu\text{E}/\text{m}^2\text{s}$ . Chlorophyll fluorescence measurements were made with a portable non-modulated fluorimeter (Plant Stress Meter, PSM Mark II, Biomonitor S.C.I. AB, Umeå, Sweden) and white leaf enclosures (Biomonitor 1020) for dark adaptation. Details of the instrument are provided by Öquist and Wass (1988). Leaves were dark-adapted for 30 min before measurements of the fluorescence transient over 2 s and with an actinic stimulation at PPFD of  $400 \mu\text{E}/\text{m}^2\text{s}$ .

The initial fluorescence ( $F_0$ ) which depends on the size of the PSII chlorophyll antenna and on the functional integrity of PSII reaction centers (Krause and Weis 1991) was determined by the shutter aperture, which is fast enough to give satisfactory resolution. A flash of actinic light yielded a peak of fluorescence ( $F_p$ ) for this light level. The half-time for transition from  $F_0$  to  $F_p$  ( $T_{1/2}$ ) was determined, and it is related to reduction rate of  $Q_A$ ,  $Q_B$  and PQ; in fact, it has been used to determine the amount of functional PSII centers and the size of the PQ pool (Bolhár-Nordenkamp and Öquist 1993). The ratio of variable to peak fluorescence ( $F_v/F_p = (F_p - F_0)/F_p$ ) was used as a measure of the maximum photochemical yield of PSII; this ratio correlates with the number of functional PSII reaction centers and was used to quantify photoinhibition (Krivoshcheva et al. 1996).

### Data analysis

Analysis was carried out using 'Statistica' release 5.1 (Statsoft Inc.). Pearson correlation coefficients

were calculated between salinities and physiological variables. Physiological measurements were compared between treatments by a one-way analysis of variance. The least significant differences (LSD) between means were calculated only if the F-test was significant at the 0.05 level of probability. Data were tested for homogeneity of variance with the Levene test ( $P > 0.05$ ). Deviations were calculated as the standard error of the mean (SEM).

## Results

### Leaf water potential and leaf expansion

$\Psi_{\text{leaf}}$  was very highly negatively correlated with salinity concentration ( $r = -0.96$ ,  $P = 0.01$ ,  $n = 5$ ), oscillating between  $-0.52$  MPa at the lowest salinity (0.5 ppt) and  $-3.47$  MPa at the highest one (40 ppt) (Figure 1).

Leaf expansion was as well negatively correlated with salinity concentration ( $r = -0.92$ ,  $P = 0.03$ ,  $n = 5$ ). The highest leaf expansion was recorded at the lowest salinity ( $2.55 \pm 0.14$  cm/day), which was similar to those recorded at 10, 15 and 20 ppt and significantly higher than that

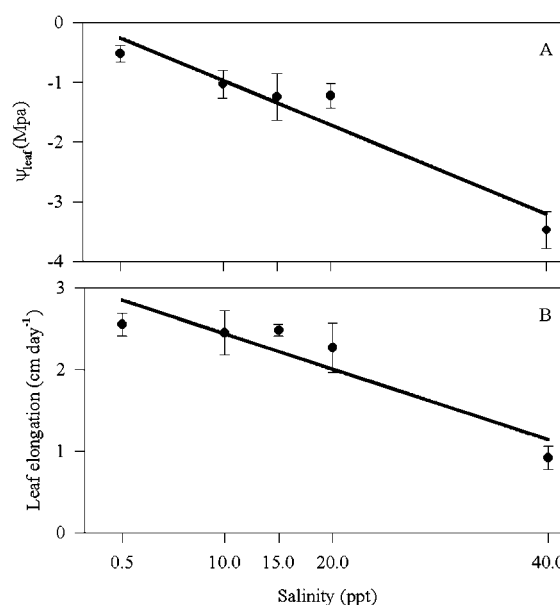


Figure 1. Leaf water potential ( $\Psi_{\text{leaf}}$ ) (A) and leaf elongation (B) in relation to salinity in *S. densiflora* from the southwestern Iberian Peninsula. Regression equations ( $n = 5$ ):  $\Psi_{\text{leaf}}$ ,  $y = -0.22 - 0.07x$ ; leaf elongation,  $y = 2.87 - 0.04x$ .

recorded at 40 ppt (LSD post-hoc test,  $P = 0.001$ ; ANOVA,  $F = 11.4$ ,  $P < 0.001$ ) (Figure 1).

#### Gas exchange

$G_s$  oscillated between  $95.6 \pm 16.0$  and  $148.6 \pm 21.3$   $\text{mmol/m}^2\text{s}$ , without significant differences between treatments ( $F = 1.53$ ,  $P < 0.266$ ) (Figure 2).

The net photosynthesis rate was negatively correlated with salinity ( $r = -0.98$ ,  $P = 0.01$ ,  $n = 5$ ), decreasing to half from fresh water ( $16.6 \pm 0.7$   $\mu\text{mol/m}^2\text{s}$ ) to hypersalinity ( $7.6 \pm 1.0$   $\mu\text{mol/m}^2\text{s}$ ) (Figure 2). On the other hand,  $A$  was positively correlated with  $\Psi_{\text{leaf}}$  ( $r^2 = 0.99$ ,  $P = 0.01$ ,  $n = 5$ ) and leaf expansion ( $r^2 = 0.96$ ,  $P = 0.01$ ,  $n = 5$ ).

#### Chlorophyll fluorescence

The ratio  $F_v/F_p$  showed a minimum at the lowest and the highest salinity concentrations (ANOVA,  $F = 3.9$ ,  $P < 0.017$ ).  $T_{1/2}$  was significantly higher

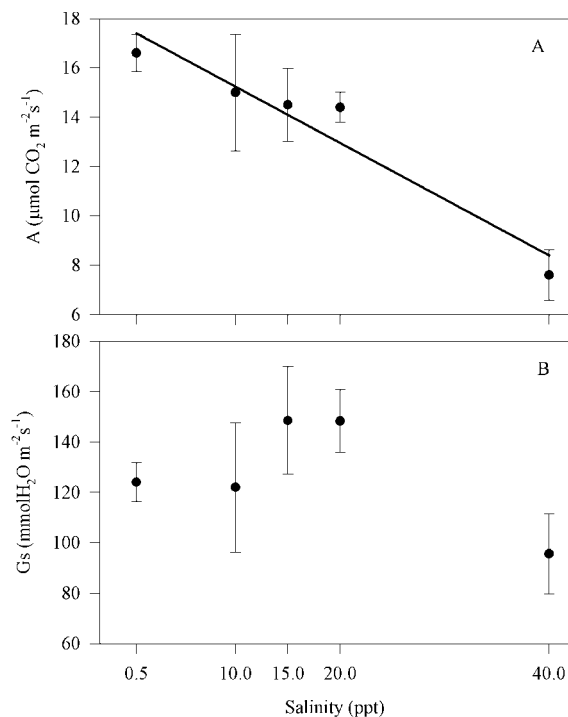


Figure 2. (A) Net photosynthesis rate ( $A$ ) and (B) stomatal conductance ( $G_s$ ) in relation to salinity in *S. densiflora* from SW Iberian Peninsula. Regression equation ( $n = 5$ ):  $A$ ,  $y = 17.45 - 0.22x$ .

at 0.5, 10 and 40 ppt than at 15 and 20 ppt (ANOVA,  $F = 5.8$ ;  $P < 0.003$ ), oscillating between 243 and 294 ms. The initial fluorescence ( $F_0$ ) did not vary significantly with salinity, oscillating around 0.05 in every treatment.  $F_p$  and  $F_v$  showed minimum values at the lowest and the highest salinities; however, these differences were not significant with respect to the other treatments (ANOVA,  $F = 2.1$ ,  $P < 0.114$ ;  $F = 2.3$ ,  $P < 0.097$ ; respectively) (Figure 3).

#### Discussion

This study shows that adult tussocks of the invasive cordgrass, *Spartina densiflora*, from the southwestern Iberian Peninsula show a high short-term tolerance to salinity with high values in growth and net photosynthesis rate from 0.5 to 20 ppt. These results are in agreement with *S. densiflora* distribution in invaded European estuaries, where it colonizes habitats with contrasted salinity regimens as low and brackish marshes and saltpans (Nieva et al. 2001). The high acclimation capacity of *S. densiflora* to salinity was also reflected in the regulation of its  $\Psi_{\text{leaf}}$ , behaving as an osmo regulator (Nieva et al. 1999).

*S. densiflora* showed a lower  $F_v/F_p$  ratio at the lowest salinity than at intermediate salinities, which resulted from a decrease in  $F_p$ . This photo inhibition in fresh water would be due to the dissipation processes of excessive energy, related to non-photochemical quenching such as thermal deactivation and xanthophyll cycle (Griffiths and Maxwell 1999), and/or a limitation in Chl synthesis. It has been described that NaCl increases the Chl content in coastal species (Beer et al. 1976). This photoinhibition would be compensated by adaptative biochemical mechanisms, such as higher levels of energy transmission between photosystems through maintenance of an abundant and active PQ pool – higher  $T_{1/2}$  values – (Fernández-Baco et al. 1998). These mechanisms would allow *S. densiflora* to keep high net photosynthesis and growth rates in fresh and brackish water environments.

At the highest salinity (40 ppt), a high stress level was recorded in *S. densiflora*, reflected in significant decreases in growth, net photosynthesis rate and photochemical efficiency of PS II. This decrease in  $A$  was high (around 50%

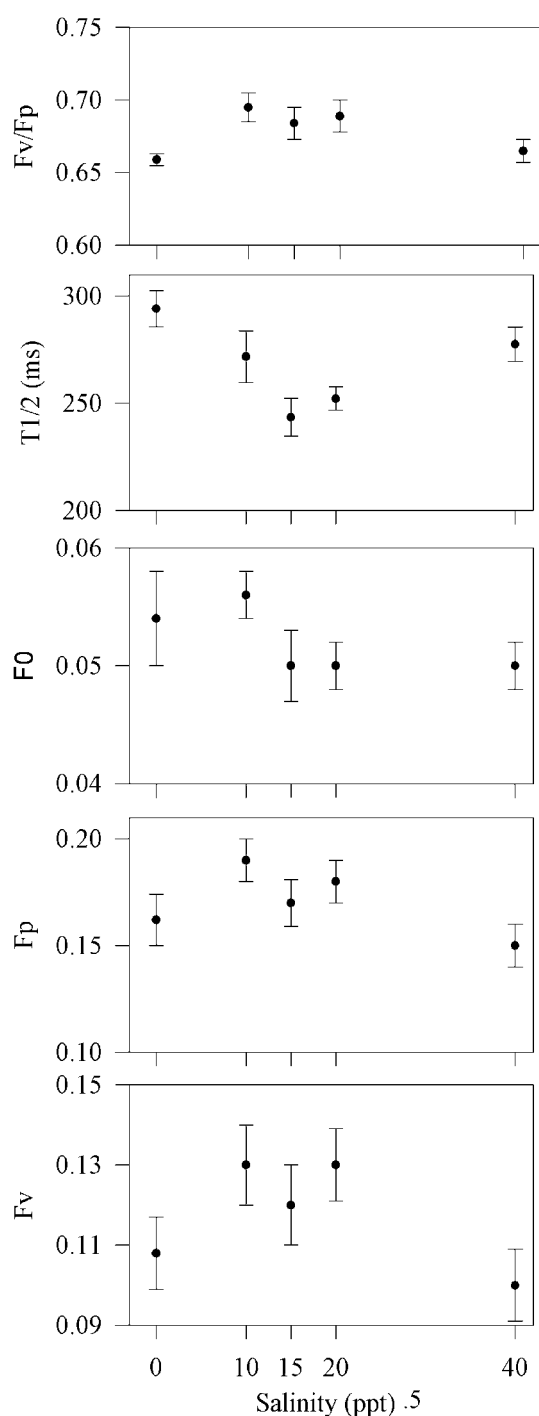


Figure 3. Potential photochemical efficiency of PSII ( $F_v/F_p$ ), half-time for transition from  $F_0$  to  $F_p$  ( $T_{1/2}$ ), initial fluorescence ( $F_0$ ), peak fluorescence ( $F_p$ ) and variable fluorescence ( $F_v$ ) in relation to salinity in *S. densiflora* from the southwestern Iberian Peninsula.

compared with fresh water treatment) as it used to be in  $C_4$  species (Drake 1989) and it was independent of  $G_s$  values. It has been described as the primary effect of salinity on metabolic events (Drake 1989) through an excess of ions in the plant tissue and/or an inhibition of nutrient ion uptake (Greenway and Munns 1980). These alterations interfere with metabolic functions such as protein synthesis, which determinates photoinhibition levels (Ohad et al. 1985), and they lead to biochemical changes that affect carboxylase activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Antolín and Sánchez-Díaz 1993). Salt stress was also reflected in a significant decrease of  $F_v/F_p$ . The nature of this photoinhibition seemed to be related with photoprotective mechanisms such as xanthophyll cycle, since a slight decrease in  $F_p$  and  $F_v$  was recorded. As well as in fresh water, *S. densiflora* photosynthetic apparatus showed adaptation mechanisms to salinity stress reflected in a significant increase of  $T_{1/2}$  at the highest salinity, suggesting high levels of energy transmission between photosystems (Fernández-Baco et al. 1998). This adaptation capacity would not be enough to compensate salt stress effects, since, as pointed out previously, other processes such as the Calvin cycle may also be involved.

Our results support *S. densiflora* invasion patterns in European estuaries, with low expansion rates near the sea, and an active colonization of brackish marshes and river banks, where it competes with native oligohaline species (Nieva 1996). Similar results were found by Kittelson and Boid (1997) working in adult *S. densiflora* tussocks in invaded Californian marshes. Nieva et al. (1999), working with invading *S. densiflora* in the Gulf of Cádiz, found an increase in  $A$  with a decrease in salinity, and Valiela et al. (1978) suggested that salinity might be limiting the growth of *Spartina alterniflora* wild populations. All these results suggest that *S. densiflora* invasion would be favored in brackish environments and by anthropogenic disturbances lowering salinity, such as fresh water emissions.

The elevated salinity tolerance of *S. densiflora* together with its high competitive ability – growing in very dense tussocks with a phalanx growth (Figueroa and Castellanos 1988) – would allow this neophyte to invade a wide range of habitats displacing native species, such as other invasive

weeds in salt marshes (Rea and Storrs 1999; Merriam and Feil 2002). Studies rooted to analyze competitive interactions of *S. densiflora* with native species in relation with environmental conditions are needed to clarify how far this alien species is affecting autochthonous communities.

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