Physiological comparison of alien Senecio inaequidens and S. pterophorus and native S. malacitanus: Implications for invasion

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Abstract

Drought is common in Mediterranean-type climates. Water stress can have serious physiological consequences for plant fitness. Here we analysed the response of two alien invasive species, Senecio inaequidens DC. and S. pterophorus DC., and one native non-invasive, Senecio malacitanus Huter, in terms of photosynthesis, water relations and growth. The proportional reduction in growth as a result of water stress was smaller in S. malacitanus, followed by S. inaequidens and finally S. pterophorus. Variations in relative growth rate were related to differences in unit leaf rates, which are strongly correlated with photosynthesis. At a similar level of leaf relative water content (RWC), photosynthesis in S. inaequidens and S. malacitanus did not differ, whereas it was lower in S. pterophorus. S. malacitanus started to show a reduction in RWC later than the other species. The hypothesis that alien invaders have greater physiological tolerance to drought than native non-invaders is not supported by our results since S. malacitanus showed a more adaptive response to drought than the aliens and was also the most resistant of the three species to water shortage. Differences in invasiveness would therefore be explained by a combination of traits, including establishment capacity, competitive capacity and drought resistance, among others.

Keywords: Alien invasiveness; Leaf traits; Mediterranean-type climate; Photosynthesis; Physiological performance; Global change

Introduction

Introduced plant species may become particularly competitive and invasive in new environments, thus threatening local biodiversity. However, the invasion success of invasive plants is determined by their ability to cope with new environmental constraints (see Alpert et al., 2000). In a Mediterranean-type climate, vegetation is often subject to periods of high temperature and irradiance and little or no precipitation during summer, thus producing severe drought stress in plants. Moreover, an increase in aridity has been predicted for Mediterranean ecosystems (Houghton et al., 2001). Plants subjected to water restriction respond through plasticity in several ecophysiological traits (reduced leaf area, stomatal closure, osmotic adjustment, etc.) to limit stress (Monneveux and Belhassen, 1996). In most plant species, water limitation leads to a decrease in photosynthetic rates as a result of stomatal closure and the
consequent reduction in CO2 availability (see review by Chaves et al., 2003). In conditions of mild water stress, stomatal closure is the main factor that limits photosynthesis, while with increasing drought non-stomatal limitation has also been reported (Flexas et al., 2002; Lawlor and Cornic, 2002; Peña-Rojas et al., 2004).

Invasive plants in the Mediterranean basin are also exposed to summer water shortages, and the effect of this environmental constraint on their physiological attributes is crucial to the success or failure of invasion. Although many studies demonstrated that invaders outperform natives when resources are readily available, they are not so successful when resources are scarce (Blicker et al., 2002; Burke and Grime, 1996; Gerlach and Rice, 2003; Kolb et al., 2002; Leger and Rice, 2003; Morris et al., 2002).

We are currently studying two alien species of Senecio (S. inaequidens and S. pterophorus, Asteraceae), which are invading Catalonia (NE Spain), in order to understand the life history traits that can explain their invasive capacities. Senecio inaequidens is widespread throughout Western Europe and during the last 20-years is expanding towards the south and east of continental Europe and S. pterophorus has recently been introduced in the north-eastern Iberian Peninsula and new scattered populations have been recorded in the south of Catalonia (Chamorro et al., 2006). Both species mainly colonise disturbed habitats but they are also invading natural habitats. Previous studies showed that emergence of both species in natural invaded habitats were strongly dependent on disturbance. Although these species are not competitive against local vegetation (Garcia-Serrano et al., 2004; Sans et al., 2004), recruitment during periods of abundant rainfall can increase their competitive ability and allows a successful invasion (Caño et al., 2007). Moreover, in optimal conditions both aliens can outcompete the native Senecio malacitanus, a species that forms sparse populations in temporal rivers (ramblas) of the south-eastern Iberian Peninsula and the Maghreb (Garcia-Serrano et al., 2005, 2007). Despite that S. inaequidens was also more competitive than S. malacitanus under moderate water stress (Garcia-Serrano et al., 2007), we still do not know whether the two aliens are able to resist the same water constraint as S. malacitanus.

Because the two alien species are expanding towards the south of the Iberian Peninsula, it is important to know whether they are adapted to resist drought stress as it occurs in the south of the Mediterranean region in order to prevent future invasions. For this reason, we subjected the two alien species to a severe water constraint in controlled conditions and we assessed their physiological response compared with that of the native congener, S. malacitanus. Studies comparing native and alien invasive plant performance provide insights about to what extent invasive plants may outperform co-occurring native species depending on growth conditions (see the review by Daehler, 2003). Particularly, comparison among phylogenetically related invasive and non-invasive species is useful to avoid confounding effect of phylogeny on the traits analysed (Mack, 1996; Radford and Cousens, 2000), especially when these species occupy similar habitats and have comparable growth patterns. The experiment was performed with potted plants in order to accurately control the soil water content and because for ethical reasons it is not possible to realise experiments directly in ecosystems where the two alien are not present. We used young plants because the flowering period is not synchronous among the three species (Garcia-Serrano et al., 2004; Sans et al., 2004). Moreover, as seedlings emerging in spring are subjected to drought stress after a few months of growth (Caño et al., 2007; Garcia-Serrano et al., 2004), they do not have the time to acclimatize through morphological or metabolic changes. Therefore, the short duration of the water stress treatment used in our study, provided physiological responses that are crucial to explain the survival of seedlings in the field. Furthermore, we also studied the relative growth rate (RGR, plant biomass increase over time per unit of plant mass) of the young plants because the susceptibility to environmental constraints (Pino et al., 1996; Solbrig and Solbrig, 1984) and the competitive capacity of seedlings (Marafion and Grubb, 1993) are related to seedling size, which depends on RGR. Thus, several authors have hypothesized that a high RGR favours plant invasion (Davis and Pelsor, 2001; Davis et al., 2000).

We addressed the following questions: (1) Do the three species differ in RGRs, leaf photosynthesis and water relations in response to water availability and drought stress? (2) Are the aliens more affected by water limitation than the native species? (3) Can these differences limit the expansion of the two aliens towards the arid regions of the South of Iberian Peninsula?

### Material and methods

#### Species studied

The three species are morphologically and ecologically similar: they have the same life form and they colonise wasteland and disturbed areas such as railroad and road margins, recently abandoned fields and seasonal rivers. However, they differ in population structure and distribution range.

*S. inaequidens* DC. and *S. pterophorus* DC. are two semi-lignose spreading shrubs native to South-Africa. They have woody stems, at least below, and they grow up to 80 and 150 cm, respectively. Leaves of *S. inaequidens* are linear (ca. 0.3 cm) and usually entire,
while those of *S. pterophorus* are wider (0.5–1.5 cm), sharply and coarsely serrate, densely grey-pubescent on lower side. *S. inaequidens* and *S. pterophorus* are native to Eastern Cape Province (South Africa) and their distribution ranges in South-Africa overlap. Both species colonise similar habitats. *S. inaequidens* mostly colonises rocky slopes, gravely river beds and grazed pastures and *S. pterophorus* forms scattered populations in forest margins, grasslands and fynbos. However, both species were introduced and expanded towards western places of Cape region, where they invade many ruderal habitats (Bond and Goldblatt, 1984).

*S. inaequidens* was accidentally introduced to Europe at the end of the 19th century (Ernst, 1998), and today is widespread across Western and Central Europe, where it forms dense populations in old fields and road margins. It also colonises heavily grazed grasslands. *S. pterophorus* was recently introduced to continental Europe, first recorded in 1995 near Barcelona (Pino et al., 2000). Its geographical distribution is increasing, as shown by a recent exhaustive survey performed in Catalonia (Chamorro et al., 2006). Although *S. pterophorus* forms very dense populations mainly in disturbed riverbeds, it is currently spreading rapidly into natural communities (Chamorro et al., 2006). Furthermore, *S. pterophorus* was introduced to South Australia about 1930 and spread to the south-eastern part of South Australia (Scott and Delfosse, 1992), where it tolerates a wide range of soil types and occupies areas that receive 500–1500 mm of rainfall per year (Walsh, 1999). In agricultural areas, *S. pterophorus* can cause heavy losses in productivity and in natural areas because it is a strong competitor, forming dense thickets that exclude native species (Parsons and Cuthbertson, 1992). *S. inaequidens* and *S. pterophorus* are classified as “novel, invasive colonisers” following Davis and Thompson’s (2000) categorisation scheme, although the distribution area of the former is much larger.

*S. malacitanus* Huter is morphologically similar to the alien studied species but it is native to southern Europe and northern Africa. It is a sparsely hairy or glabrescent dwarf shrub up to 50 cm, with leaves linear to linear-lanceolate ca 0.3 cm, entire or obscurely dentate, grey-green and slightly fleshy. *S. inaequidens* is a “successional coloniser” sensu Davis and Thompson (2000), which forms sparse populations in disturbed temporal rivers (“rambles”) and other dry, rocky and stony disturbed places nearby. Its geographical distribution is limited to the southeast of the Iberian Peninsula, the Balearic Islands and the Maghreb, in areas with semi-arid climates (under 350 mm rainfall/year, (INM, 2004)).

**Experimental design and growth conditions**

Seeds from the three species were collected from populations growing in the Iberian Peninsula. Seeds were harvested in 15 randomly selected individuals in each population. Those from *S. inaequidens* were collected from an early-abandoned field at Cantallops (Catalonia, near the Spanish–French border; Universal Transverse Mercator (UTM) zone 31 T, 0492E 4695N) while those from *S. pterophorus* were gathered from a population on the abandoned fields close to banks the Ripoll river (near Barcelona; UTM zone 31 T, 0425E 4602N). Seeds from *S. malacitanus* were from a population growing in a seasonal Mediterranean river at the Rambla del Rambutjar (near Sant Vicent de Raspeig, Alicante; UTM zone 30S, 0712E 4261N). In August 2003, we germinated the seeds and grew 30 plants of each species in a mixture of 25% perlite, 25% vermiculite and 50% of soil in pots with a diameter of 14 cm and height of 17.5 cm at the University of Barcelona experimental fields (Calcic Luvisol; FAO-UNESCO, 1988). The small diameter of the pot was selected to prevent excessive evaporation through the soil surface. Perlite and vermiculite were added to prevent substrate from compacting during the dry period. Plants were placed in an open space covered by a 10 × 10 m² transparent roof 5 m high but open to the south and north, in order to simulate natural open conditions whilst avoiding rainfall. Plants were randomly arranged in 9 × 10 position squares and irrigated every two or three days with a modified Hoagland nutritive solution (Hoagland and Arnon, 1938) at 50% dilution. They were left to grow for 2 months under unlimited water supply until reaching a size that would allow the measurement of relative water content (RWC), which implied leaf excision. After this period, we randomly selected eight to ten plants of each species and carefully divided them into roots, stems and leaves. Care was taken to gently wash all substrate from the roots without losing plant material. Dry weight was measured after drying material to constant weight at 60 °C in an oven-dryer. Leaf area was obtained after the harvest (before drying) by scanning all leaves from each plant on a flatbed scanner (Epson GT5000) and processing the images with ImagePC software (Scion Corporation, USA).

Sixteen of the remaining plants of each species were then used to perform two water treatments: eight randomly selected plants were assigned to the “control” treatment and were irrigated every one or two days until water drained through the holes in the bottom of the pots, while the other eight plants were assigned to the slowly imposed “water stress” treatment and were left without water for 12 days from 21 September (day 0). After this treatment, they were irrigated again and allowed to recover for two days. Substrate water content was monitored periodically by weighing pots. The dry weight of soil content from six additional pots was previously determined after drying at 60 °C in an air-forced oven (735.52 ± 14.9 g; mean ± SE throughout the
text) in order to calculate the ratio soil fresh weight/soil dry weight as a measure of water status. At the end of the experiment, on 3 October, all remaining plants were harvested and leaf area and dry weight of leaves, stems and roots was determined as described previously.

**Biomass allocation and growth rates**

Total Leaf Area (TLA), Specific Leaf Area (the leaf area per unit leaf biomass; SLA), Leaf Area Ratio (the leaf area per unit of plant biomass; LAR), Leaf Mass Ratio (the proportion of leaf biomass per unit of plant biomass; LMR), Unit Leaf Rates (increase in plant biomass over time per unit leaf area; ULR) and the Root to Shoot ratio (the ratio between belowground and aboveground biomass; RS) were determined for all harvested plants. Relative growth rate was calculated using a Fortran version of the program HP curves written by Hunt and Parsons (1974). RGR is defined as 
\[(\ln W_f-\ln W_0)/t,\]
where \(W_0\) is the dry biomass of the plants before the stress treatment, \(W_f\) is the dry weight of the plants at the end of the experiment and \(t\) is the number of days between the two harvests.

**Leaf water content**

On days 0, 2, 5, 8, 11 and 13, one or two young, fully expanded leaves from each plant were collected at pre-dawn (6–7 am), when plant water content is in equilibrium with soil water content. Leaves were excised, immediately weighed (pre-dawn weight; \(W_{PD}\)) and then allowed to hydrate to saturation with distilled water for 24 h at 4 °C in Petri dishes. They were then weighed again (saturated weight; \(W_s\)). Finally we measured their dry weight after drying them at 60 °C to constant weight (dry weight; \(W_D\)). Pre-dawn leaf relative water content (RWCPD) was then calculated as
\[\text{RWCPD} = (W_{PD}-W_D)/(W_s-W_D).\]

**Leaf gas exchange**

On days 0, 2, 5, 8, 11 and 13, we measured leaf gas exchange in one young, fully expanded leaf per plant. Experimental plants were grown in an open space simulating natural temperatures and photoperiod and avoiding rainfall (see Experimental design and growth conditions subsection). Punctual measurements were carried out under sunshine and in the morning to avoid any signs of midday stomatal closure and assure comparable air temperature and humidity conditions. Mean (± SE) ambient temperature (22.34 ± 0.36 °C) and humidity (77.23 ± 2.08%) were similar during the period of leaf gas exchange determination. Punctual measurements of net assimilation rate (\(A\)), leaf stomatal conductance (\(g_s\)) and intercellular CO₂ concentration (\(C_i\)) were performed with an open gas exchange system (LI-6400; Li-COR, Lincoln, Nebraska, USA) equipped with a LI-COR light source (LI-COR 6400-02B LED). This system allows the control of the environmental conditions during measurements, thereby permitting valid comparisons between measurements. Light intensity was set at 1300 μmol m⁻² s⁻¹ and CO₂ concentration at 350 μmol mol⁻¹. Relative humidity of the sample air was kept at 53.04 ± 0.02%, leaf temperature at 26.5 ± 0.004 °C, water mole fraction as 17 ± 1 mol H₂O mol⁻¹air⁻¹ and flow was fixed at 200 μmol s⁻¹. Results were expressed per leaf-projected area, obtained with a flatbed scanner and processed using imagePC analyser software. Instantaneous leaf water use efficiency (\(WUE_i\)) was calculated as \(A/g_s\).

**Statistical analysis**

Analysis of variance (ANOVA) was used to examine growth parameters, and significant effects and interactions were further tested with the least-squares means method. Data were log-transformed when required to achieve normality and homocedasticity of residuals.

Substrate water status, RWCPD and gas exchange measurements (\(A, g_s, C_i\)) were analysed with a repeated measures analysis of variance (ANOVAR) with two between-subject factors (species at three levels and water stress at two levels) and time as a within subject (repeated) factor. Degrees of freedom in the ANOVAR were corrected for sphericity with the Greenhouse-Geisser ε.

\(A, g_s, C_i\) were further compared between species and water treatments at similar RWC. RWCPD in the water stress treatment was 67.1 ± 8.7% for \(S. inaequidens\), 59.1 ± 5.9% for \(S. pterophorus\) and 65.6 ± 5.7% for \(S. malacitanus\) at days 8, 8 and 11, respectively (\(F_{2, 21} = 0.38, p = 0.69\)). Post-hoc multiple comparisons of means were performed with the least-squares means method.

All analyses were performed with the GLM procedure of SAS (1999).

**Results**

**Growth parameters**

RGRs differed significantly among species (\(F_{2, 42} = 24.7, p < 0.001\)) and water treatments (\(F_{1, 42} = 81.5, p < 0.001\)), and the interaction between these two factors was also significant due to the different pattern of response of each species to drought (\(F_{2, 42} = 6.95, p < 0.01\)). Under the control treatment, the native \(S. malacitanus\) and the alien \(S. inaequidens\) had the same RGR, whereas the alien \(S. pterophorus\) showed a lower rate (Table 1). However, initial biomass for RGR
Table 1. Mean (±SE) of growth parameters for the three Senecio species under control and water-stress conditions.

<table>
<thead>
<tr>
<th></th>
<th>RGR g g⁻¹ day⁻¹</th>
<th>ULR g m⁻² day⁻¹</th>
<th>TLA cm²</th>
<th>LAR m² g⁻¹ (× 10⁻³)</th>
<th>LMR g⁻¹ (× 10⁻³)</th>
<th>SLA m² g⁻¹</th>
<th>RS</th>
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</thead>
<tbody>
<tr>
<td><strong>S. inaequidens</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.081±0.006a</td>
<td>7.52±0.55a</td>
<td>80.00±7.66b</td>
<td>9.27±0.92ab</td>
<td>0.35±0.017b</td>
<td>26.05±1.44</td>
<td>0.62±0.06b</td>
</tr>
<tr>
<td>Stress</td>
<td>0.035±0.008c</td>
<td>3.12±0.50c</td>
<td>49.57±2.88c</td>
<td>9.99±1.50</td>
<td>0.37±0.030</td>
<td>26.45±1.67</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td><strong>S. pterophorus</strong></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.060±0.003b</td>
<td>4.81±0.26b</td>
<td>120.25±11.65a</td>
<td>10.45±0.47a</td>
<td>0.39±0.009a</td>
<td>26.89±1.28ns</td>
<td>0.80±0.05a</td>
</tr>
<tr>
<td>Stress</td>
<td>0.014±0.002d</td>
<td>1.16±0.18d</td>
<td>65.10±3.79bc</td>
<td>10.02±0.46</td>
<td>0.37±0.014</td>
<td>26.91±0.73</td>
<td>0.81±0.06</td>
</tr>
<tr>
<td><strong>S. malacitanus</strong></td>
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</tr>
<tr>
<td>Control</td>
<td>0.078±0.003a</td>
<td>7.43±0.41a</td>
<td>46.03±5.48cd</td>
<td>9.32±0.35b</td>
<td>0.33±0.011b</td>
<td>28.40±0.68</td>
<td>0.45±0.02c</td>
</tr>
<tr>
<td>Stress</td>
<td>0.063±0.004b</td>
<td>6.68±0.54a</td>
<td>31.06±1.86d</td>
<td>7.73±0.27</td>
<td>0.30±0.009</td>
<td>26.01±0.77</td>
<td>0.56±0.04</td>
</tr>
</tbody>
</table>

Different letters indicate significant differences between means. If only one letter is shown for both treatments within a species, there are no differences between treatments but there are between species. See text for abbreviations.

calculation was significantly different between species (S. inaequidens 0.45±0.09 SE; S. pterophorus 0.56±0.04 SE; S. malacitanus 0.18±0.02 SE; F₂, 2₅ = 24.6; p<0.001) because the growth of the two aliens during the first 2 months was greater than the native species. RGR decreased significantly under water deficit conditions for all species (Table 1), with the greatest drop detected in S. pterophorus (76.6% reduction), followed by S. inaequidens (56.8% reduction) and finally S. malacitanus (only a 19.2% reduction).

ULRs also differed significantly between species (F₂, 4₂ = 45.31, p<0.001) and water treatments (F₁, 4₂ = 70.45, p<0.001), and the interaction was also significant due to the differential effect of drought on this parameter in each species (F₂, 4₂ = 10.13, p<0.001). Under the control treatment, ULR did not differ between the native S. malacitanus and the alien S. inaequidens, whereas in S. pterophorus this rate was significantly less than in the other two species. However, the initial differences in biomass and leaf areas may explain the differences in ULR. Nevertheless, in the water stress treatment, S. malacitanus did not show a significant reduction in ULR, whereas this rate decreased by 58.5% in S. inaequidens and 78.9% in S. pterophorus (Table 1). TLA was significantly different between species (F₂, 4₂ = 35.2, p<0.001) and water treatments (F₁, 4₂ = 40.35, p<0.001); and again, the three species presented differential responses to water treatment, as shown by the significant interaction between these two factors (F₁, 4₂ = 4.92, p<0.05). There were no differences in TLA for S. malacitanus between treatments, whereas TLA decreased significantly in the other two species in response to water stress (Table 1).

Overall, LARs did not differ between treatments (F₁, 4₂ = 1.15, p = 0.29) nor was the interaction species * water treatment significant (F₂, 4₂ = 1.23, p = 0.30). However, significant differences were found between species for LAR (F₂, 4₂ = 3.26, p<0.05), with S. pterophorus showing the highest value, followed by S. inaequidens and S. malacitanus (Table 1). Differences between species were marginally significant at the beginning of the experiment (F₂, 2₅ = 3.28; p = 0.053) as a result of a significantly higher LAR in S. pterophorus.

LMR followed a similar pattern to that of LAR: there was no significant effect of water treatment (F₁, 4₂ = 1.17, p = 0.28), or species * water interaction (F₂, 4₂ = 0.96, p<0.39), though significant differences between species were detected (F₂, 4₂ = 9.90, p<0.001). S. pterophorus showed the highest LMR, followed by S. inaequidens and S. malacitanus (Table 1). However, differences in this parameter were not detected at the beginning of the experiment (F₂, 2₅ = 0.97; p = 0.39).

No significant differences between species (F₂, 4₂ = 0.58, p = 0.57) or water treatments (F₁, 4₂ = 0.47, p = 0.50) were found for SLA (Table 1) nor was there any interaction between the two factors (F₂, 4₂ = 89, p = 0.42).

The RS ratio was significantly different between species at the end of the experiment (F₂, 4₂ = 16.91, p<0.001), whereas no significant differences between water treatments (F₁, 4₂ = 2.59, p = 0.11) or in the interaction (F₂, 4₂ = 0.85, p = 0.43) were detected. S. pterophorus showed the highest RS ratio, followed by S. inaequidens and S. malacitanus. The RS ratio was not significantly different between species at the beginning of the experiment (F₂, 2₅ = 2.16; p = 0.13).

**Relative water content**

Pre-dawn leaf RWC (RWC₀) was strongly affected by water treatment, although the pattern differed between species (Table 2, Fig. 1a and b). Leaf RWC₀
in *S. malacitanus* was maintained at initial levels during drought for longer than in the two aliens, and did not reach the same level of reduction before the recovery period. The three species recovered their levels of leaf RWCPD after two days of irrigation.

### Leaf gas exchange

Repeated ANOVAs showed significant differences of net assimilation rate (*A*) among species and also for the time * species interaction throughout the experiment (Fig. 1c and d), even with comparable leaf RWCPD status (Fig. 2). These differences were mainly due to the low *A* of *S. pterophorus* compared to the other two species under both treatments. Water stress treatment greatly affected *A* in all species in a similar way (no species * water nor time * species * water interaction were significant; Table 2), although *S. malacitanus* was the last to show the effect of water stress on this parameter (Fig. 1d). *g* _s_ also varied significantly between species and treatments. *S. malacitanus* showed the highest *g* _s_ in the control treatment (Figs. 1e and 2), although *S. malacitanus* was the last to show the effect of water stress on this parameter (Fig. 1d). *g* _s_ decreased in *S. malacitanus* later than in the two aliens (Fig. 1f, Table 2). Under the same RWCPD conditions, no differences in *g* _s_ were found between the three species when subjected to water stress (Fig. 2).

*Ci* remained constant in the control treatment (Fig. 1g). In the drought treatment, the reduction in *g* _s_ at the beginning of the water stress period (Fig. 1f) was greater than the reduction in *A* (Fig. 1d), particularly for the two invasive species, thereby producing a decrease in *Ci* (Fig. 1h). Later, *g* _s_ decreased, whilst *A* continued to fall at a similar rate, producing an increase in *Ci*. *S. malacitanus* showed a slower reduction in *Ci*, while the two aliens showed a strong initial reduction and an increase in *Ci* after the 5th day of water stress (Fig. 1h). *Ci* values at the same RWCPD were not significantly different between water stress levels, but significant differences between species were detected as *Ci* was higher in *S. pterophorus* than in the other two species at all stress levels (Fig. 2).

### Discussion

#### Growth and drought stress

Under non-limiting water conditions, the RGRs of *S. inaequidens* and *S. malacitanus* were similar. In contrast, *S. pterophorus* grew at a slower rate. These results contrast with those from previous studies performed in a growth chamber in hydroponic culture with the same species, in which we found that *S. pterophorus* had the highest RGR, followed by *S. inaequidens* and *S. malacitanus* (Garcia-Serrano et al., 2005). Moreover, the RGRs in that study were one order of magnitude higher than in the present one.

The differences in the results can be explained by the fact that the experiment by Garcia-Serrano et al. (2005) was performed during the first month after germination,
during the exponential growth phase when the RGR is at a maximum (Poorter and Garnier, 1999). In contrast, in the present experiment, we analysed growth after this early exponential phase (2 months after germination). Therefore, our RGR values would be expected to be lower than the RGR$_{\text{max}}$ during the exponential phase. Moreover, in our experiment we found that the RGR during this phase (first 2 months) was the highest in $S$. pterophorus, indicating that this species reaches the saturated phase before the other two.

RGR can be broken down into the product of LAR*ULR, and LAR can be further split into LMR*SLA (Hunt, 1982). The differences in RGRs detected between species in our study were not due to differences in SLA, as commonly found in studies comparing the growth of several species (Poorter and Garnier, 1999; Poorter and Remkes, 1990). Instead, the lower RGR of $S$. pterophorus was due mainly to its lower ULR, while LAR and TLA were the highest of the three species.

Under drought, the species that showed the highest RGR was $S$. malacitanus, followed by $S$. inaequidens and finally $S$. pterophorus. In the latter two, the drop in RGR under water stress was due to a reduction in ULR, while the other parameters (LAR, LMR and SLA) remained unchanged. In $S$. malacitanus, neither the
decreases in ULR nor LAR were statistically significant, though the combination of these small decreases in these two factors caused a drop in RGR. However, this reduction was less marked than in the other two species, particularly that seen in S. pterophorus. In response to water stress, S. pterophorus showed a decrease in TLA as a mechanism to avoid desiccation by reduction of the transpirating surface. The higher decrease of the TLA in S. pterophorus compared to that of S. inaequidens and S. malacitanus may be related with their larger leaves (see Species studied subsection) and it is probably responsible for the drop in the RGR of this species.

**Leaf gas exchange**

S. inaequidens and S. malacitanus did not show differences in $A$, whereas S. pterophorus showed significantly lower rates. These differences in $A$ per ULA were compensated partially by a higher LAR and TLA in S. pterophorus. However, the results on $A$ and RGR are consistent: the species with the lowest A, S. pterophorus, showed the lowest RGR, caused by a lower ULR, which is positively related to $A$ (Poorter and Garnier, 1999). As ULR is the rate of biomass production per ULA, a higher ULR should be associated with greater carbon assimilation.

Water stress had a strong effect on leaf gas exchange in all three species as a result of stomatal closure. Stomatal closure, the earliest response to water deficit, reduces water loss through transpiration. However, the associated decrease in intercellular CO$_2$ concentration leads to a decrease in net $A$. In the three species, we observed a decrease in stomatal conductance before any detectable reduction in RWC. The capacity to prevent desiccation by means of stomatal closure and to maintain a constant and elevated RWC has been reported elsewhere (Gulı´as et al., 2002).

A parallel decrease in $A$ and $g_s$, together with the observation that $C_i$ did not increase overall during the study and even decreased during the first days after stomatal closure, indicate that stomatal closure made a greater contribution to limiting $A$ than metabolism, at least at the beginning of water stress (Lawlor and Cornic, 2002). These observations suggest conservative water use by all the three species, which closed stomata before the loss of too much water. Nevertheless, it should be noted that stomatal conductance started to decrease later in the native S. malacitanus than in the two aliens. This native might have the capacity to maintain high water content during intense drought, as shown throughout the study by its higher RWC$_{PD}$ with respect to the other species. Moreover, its instantaneous leaf water use efficiency ($WUE_i$), calculated as $A/g_s$ during drought was also higher than for the other species. The slightly fleshy leaves of S. malacitanus may favour the better adaptation response to drought and the higher resistance to water shortage.

**Physiology, drought stress and invasive capacity**

It has been proposed that when life history traits are shared by invasive and native plants (life form, reproductive potential, etc.), successful invading plants must either capture limited resources more efficiently than natives or use resources at times when they are unavailable to native species (Vitousek, 1986). Several studies support this hypothesis (Baruch and Goldstein, 1999; Baruch et al., 2000; McDowell, 2002; Pattison...
et al., 1998); however, our comparison of three congeneric species did not reveal a relation between $A$ and invasive capacity, as the species with the lowest $A$ per unit area was the alien *S. pterophorus*, whereas the alien *S. inaequidens* showed similar assimilation values to the native *S. malacitanus*. In a study comparing two species of *Bromus*, one invasive and one non-invasive, Garnier et al. (1989) did not find any difference in assimilation rates. Moreover, in our study the alien species were less resistant to drought than the native, therefore the hypothesis that aliens generally show greater adaptive response to environmental conditions is not supported. This observation is consistent with the findings of Brock and Galen (2005), who found that the native *Taraxacum ceratophorum* was more drought-tolerant than the invasive *T. officinale*. Castillo et al. (2000) also reported that the native *Spartina maritima* was more tolerant to tidal submergence than the invasive *S. densiflora*. In this regard, a better ecophysiological adaptation of native species to environmental conditions can be expected; however, it would not explain the invasive success of exotic species. Therefore, invasive capacity must depend on other aspects of physiology. For example, the RGR$_{\text{max}}$ during first month after emergence of the aliens *S. inaequidens* and *S. pterophorus* was higher than for the natives *S. malacitanus* and *S. jacobaea* (Garcia-Serrano et al., 2005). This higher rate may confer a superior competitive capacity to the aliens when there are no restrictions in the available resources (Garcia-Serrano et al., 2004; Sans et al., 2004). Other characteristics, such as early flowering period, may also be of relevance in explaining the differences reported in invasive capacity between aliens and native species (Sans et al., 2004).

The hypothesis that alien invaders are characterised by greater physiological tolerance than native non-invaders is not supported by our results since *S. malacitanus* showed greater adaptation to drought than the aliens and was the most resistant of the three species to this stress factor. Furthermore, RS ratio in *S. malacitanus* was the lowest, which resulted in higher soil water availability per transpiring area, thereby allowing a greater water spending strategy in these plants. In fact, with increasing drought, this species maintained the highest RWCs with the lowest decline in $y_o$, and thus a photosynthetic advantage with respect to the other species. Despite the large physiological tolerance of *S. malacitanus* to drought, this species usually forms scattered populations. Few aspects of its ecology or life history distinguish it from *S. inaequidens* and *S. pterophorus*, as reported in earlier studies. The lower RGR$_{\text{max}}$ of *S. malacitanus* (Garcia-Serrano et al., 2005), its later and shorter flowering period (Sans et al., 2004) and its low chemical defences against herbivores (Caño et al., 2009) might explain why it has not been successful as an invasive species. However, equally, a lack of opportunity may have prevented it from becoming an invader, and therefore measures should be taken regarding accidental introductions of this species into areas where it is still not present.

Our results from the analysis of photosynthetic response of aliens *S. inaequidens* and *S. pterophorus* and native *S. malacitanus* to drought, as well as previous studies in field conditions (Caño et al., 2007; Garcia-Serrano et al., 2004; Sans et al., 2004), provide relevant data to explain the current ecological status of each species and allow us to make hypotheses about their future expansion. In our study, *S. pterophorus* was the most affected by water stress, which is consistent with our results from another experiment where we analysed the effect of drought on interspecific competition between these species. *S. pterophorus* was outcompeted by the native under moderate water stress (Garcia-Serrano et al., 2007). The narrow physiological tolerance of *S. pterophorus* to water stress is consistent with the observation of dense and persistent population invasion by this species mainly in disturbed river beds and relatively moist grasslands, whereas it is rarely found in competitive environments or in dry places (Caño et al., 2007; Chamorro et al., 2006). Therefore, according to our results this species probably will not progress towards the arid ecosystems in the southern parts of the Iberian Peninsula and does not constitute a threat for the native species.

*S. inaequidens*, in contrast, is less sensitive to limited water supply and other environmental stress factors, and shows greater resistance to competition under moderate stress (Garcia-Serrano et al., 2004). Consequently, this species has the capacity to colonise a wide range of habitat types from lowlands to areas with altitudes over 2000 m, in areas with moderate water restrictions as well as in those with high availability of this resource. It is found in abandoned fields and wastelands, as well as along railroad and road margins but it is absent in non-disturbed habitats with high competition (Garcia-Serrano et al., 2004). In a previous experiment we showed that *S. inaequidens* outcompetes *S. malacitanus* under moderate drought (Garcia-Serrano et al., 2005). However, in the present experiment we have demonstrated that this species was not as resistant, in terms of carbon assimilation and growth, as *S. malacitanus* to the extreme drought conditions. Furthermore, the annual rainfall limit of *S. madagascariensis*, the putative diploid of *S. inaequidens* (Lafuma et al., 2003), in Australia is around 400 mm (Sindel et al., 1998). Therefore, if the species progresses towards the south, it will be present only in habitats with moderate water stress, such as the permanent river beds, but it is unlikely that it will colonise the dry habitats such as temporal river beds, the natural habitat of *S. malacitana*us. Nevertheless, a regular survey of the sparse populations in the south Catalanian range and its
manual eradication are necessary to prevent its advancement.

We found that physiological restrictions can limit the expansion of *S. inaequidens* and *S. pterophorus*. However, global-change components can alter species resource dynamics in ecosystems (Dukes and Mooney, 1999) and therefore they can affect the current distribution range of both species. Also, evolutionary changes derived from the colonisation of the new range can result into ecotype variations allowing the colonisation of more arid environments. In this sense, it has been recently found (Caño et al., 2008) that the Spanish introduced populations of *S. pterophorus* displayed higher ability to survive throughout summer drought than native South African populations. Because invasion dynamics can be influenced by the interaction of ecological and evolutionary processes (Lambrinos, 2004), future studies should explore the role of such interactions on the invasive ability of *S. inaequidens* and *S. pterophorus* in order to find more predictive and effective management strategies.

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