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Functional Ecology, Volume 10, Issue 2 (Apr., 1996), 265-274.

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Functional Ecology

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Response of the tussock grass *Stipa tenacissima* to watering in a semi-arid environment

F. I. PUGNAIRE, P. HAASE, L.D. INCOLL and S. C. CLARK

Department of Pure and Applied Biology, University of Leeds, Leeds LS2 9JT, UK

Summary

1. *Stipa tenacissima* is a perennial tussock grass of arid and semi-arid zones around the Mediterranean basin that occupies extensive areas in south-eastern Spain. The environment of this region is extreme, with low irregular rainfall, high temperature and high irradiance, so that *S. tenacissima* tussocks are subjected to a high degree of environmental stress, especially during the summer months.
2. The ability of *S. tenacissima* to use a pulse of water applied in midsummer was tested in a field experiment in which extension growth and physiological performance of leaves of watered and unwatered plants were measured.
3. In unwatered plants, when leaf extension had ceased, leaf water potential, relative water content, leaf conductance and net photosynthetic rate were low and minimum fluorescence was high.
4. Leaf conductance and net photosynthetic rate doubled, leaf extension resumed, minimum fluorescence (F_0) fell and photosynthetic efficiency of photosystem II (F_v/F_m) rose with watering. As the soil dried out again, values of these variables approached or equalled those of unwatered plants.
5. These changes in F_0 and F_v/F_m suggested that the photosynthetic apparatus had not been damaged permanently, i.e. that *S. tenacissima* has reversible photoprotective mechanisms.
6. We conclude that the decreased photosynthetic rate of unwatered plants in summer results from non-damaging photoinhibition and low leaf conductance. The latter is partly a consequence of the folding of leaves, which was inversely proportional to relative water content.
7. The arrested development of *S. tenacissima* leaves was apparently not due to summer dormancy because leaves responded opportunistically within days to an applied pulse of water.

Key-words: Fluorescence, leaf extension, Mediterranean species, photosynthesis, water stress

Functional Ecology (1996) **10**, 265–274

Introduction

Dry Mediterranean ecosystems are often dominated by evergreen species (Mooney & Dunn 1970), with low growth rates and high resistance to stress, that are able to use pulses of nutrients and water to grow and reproduce (Chapin 1980). In such environments of low fertility, the unpredictable availability of water (Evenary, Shanan & Tadmor 1982; Le Houerou 1984) may favour the evergreen habit as one way for plants to utilize sporadic rainfall.

The seasonal low availability of water in Mediterranean ecosystems strongly influences plant growth and morphology. It may also cause a low supply of mineral nutrients, both because mineralization is decreased and because movement of nutrients to the root system is inhibited by lack of water in the soil (Nye

& Tinker 1977). A consequence of this lack of water is low leaf conductance, which lowers the transpiration rate and decreases the photosynthetic rate and growth (Pugnaire, Serrano & Pardos 1993). A decreased transpiration rate reduces evaporative cooling and hence increases leaf temperatures to potentially lethal levels. Nevertheless, plants of arid environments, for example resurrection plants or those with crassulacean acid metabolism, can on the one hand endure environmental extremes while on the other restore physiological activity very rapidly in response to pulses of water (Lebkuecher & Eickmier 1993).

The high irradiances and elevated temperatures characteristic of Mediterranean environments can cause serious difficulties for growth and survival through thermal imbalance, photoinhibition and

damage to the photosynthetic system. In excess light, photoprotective processes divert excitation energy away from the photosystem II (PSII) reaction centres (Björkman 1987), thereby reducing photosynthetic efficiency. If the capacity of photoprotective processes is exceeded, PSII can be damaged, further reducing plant growth. Photoinhibitory processes are enhanced by the concurrence of other stresses, such as lack of water, high temperature and lack of nutrients.

Acclimation to high irradiance and photoinhibition is particularly influenced by the availability of nitrogen, because lack of nitrogen can slow the turnover of protein and thus the repair of damaged cell parts (Henley *et al.* 1991).

The driest regions of the Mediterranean basin support a vegetation characteristic of semi-arid environments because, in addition to the several months of summer drought typical of Mediterranean climates, total annual precipitation is low and irregularly distributed within and between years. Some areas in south-eastern Spain with less than 250 mm annual rainfall support communities in which scattered leguminous shrubs such as *Anthyllis cytisoides* L. and *Retama sphaerocarpa* (L.) Boiss., often mixed with the perennial tussock grass *Stipa tenacissima* L., predominate (Bellot 1978). With increasingly drier conditions, these communities give way to steppe dominated by *S. tenacissima*, with almost monospecific stands of *Artemisia barrelieri* Besser occurring in disturbed areas.

In the course of measuring seasonal demography and ecophysiology of *S. tenacissima*, we found a rapid and distinctive positive response of leaf growth to the availability of soil water following rainstorms. During the summer, when the soil was dry, there was no leaf extension and very low rates of photosynthesis. It was not known, however, whether the cessation of leaf growth was simply a response to lack of water or whether it was caused by summer dormancy. Some of the perennial grasses of Mediterranean regions are summer-dormant and, even when irrigated, only start to grow when temperatures fall and photoperiods decrease (Laude 1953; Ofir & Dorenfeld 1992). Other species do not become dormant in summer and grow whenever water is available (Laude 1953). In Mediterranean populations of *Dactylis glomerata*, some forms were able to respond to added water while others remained dormant (Knight 1960). In semi-arid south-eastern Spain occasional brief summer rainstorms sharply increase soil moisture but the effect is only of short duration because of the high temperatures and evaporation. In drought-resistant species lacking summer dormancy, the ability to use these short peaks of soil moisture provided by unpredictable summer rain could be advantageous.

In this paper we report the results of a field experiment that assessed the adaptation of a population of *S. tenacissima* to high levels of water stress and its effect, under conditions of high temperature and

irradiation, on photoinhibition and photosynthesis. The experiment was designed to test whether (1) *Stipa* is able to use short pulses of water for growth during summer by increasing gas exchange, and (2) *Stipa* has photoprotective mechanisms to reduce the harmful effects of high irradiance.

Field site and species

The experiment was conducted in the Rambla Honda near Tabernas, Almería Province, Spain (37° 08' N, 2° 22' W, 600–900 m altitude), a dry valley at the foot of the Sierra de Los Filabres range. The local climate is semi-arid with a mean annual precipitation of 242 mm, and a pronounced dry season from June to September in which there is no rain in most years. Mean annual temperature at Tabernas (490 m altitude, 9 km to the south) is 17.9 °C, with mean temperatures of 10.7 °C in January and 27.1 °C in August (R. Lázaro, personal communication).

Stipa tenacissima grassland covers the rocky slopes of the sides of the valley up to the ridges at 800–1000 m (Puigdefábregas *et al.* 1994). It is a long-living perennial grass that forms tussocks and spreads vegetatively. Individual tillers usually have several senesced or dead leaves and two to three green leaves, which last up to 1.5 years and are produced continuously (Sánchez 1995). The upper surface of the leaf is grooved and the stomata are at the bottom of the grooves (S. C. Clark, unpublished data). The leaf can fold along its long axis, completely concealing the grooved surface. Root-to-shoot ratio in *S. tenacissima* is very low (0.4; L. Gutiérrez, personal communication), and growth rate is extremely low.

Soil samples to a depth of 7 cm were collected under several tussocks within the experimental plot. The soil is a typic torrifluvent (Soil Conservation Service 1975), with a high proportion of sand (86%) and low in N and CO₃²⁻. Average pH was 7.3 ± 0.3 (1:2.5 soil in water), conductivity was 0.3 ± 0.1 mS and organic matter concentration (measured by loss of mass on ignition at 430 °C) was 1.7 ± 0.1% w/w.

Methods

Sixteen tussocks of similar size (about 1 m in diameter) and appearance were selected at the downslope margin of an almost pure stand of *S. tenacissima* at 670 m altitude. Eight plants were chosen at random as controls and the remaining eight were supplied with 6 litres of water per day for 4 consecutive days, with the objective of wetting the soil profile over the horizons containing the majority of roots. Water was applied to the soil surface at the centre of the tussock with a medical gravity drip-feed system running continuously. The quantity of applied water was equivalent to a rainfall of 31 mm over each tussock. There was no runoff. Gas exchange and other variables were measured at intervals of between 1 and 4 days from 7

to 26 July 1993, but not all variables were measured on each 'measurement' day.

Preliminary experiments had shown that photosynthetic rates reached a maximum early in the morning and decreased rapidly to negligible values after 2 h (F.I. Pugnaire & P. Haase, unpublished data). Therefore, all measurements were carried out twice daily at the time of maximum and minimum photosynthetic rates (06.00–07.00 and 11.00–12.00 GMT, respectively). Each measurement of gas exchange and chlorophyll fluorescence was replicated twice in each plant.

Gas exchange was measured with a portable infrared gas analyser and leaf chamber (LCA-3 and PLC-N; Analytical Development Company Ltd, Hoddesdon, UK) by enclosing three leaves simultaneously and orientating them normal to the solar beam. All measurements were made at irradiances saturating for photosynthesis. The portable analyser did not control temperature in the leaf chamber. In previous measurements of gas exchange of *S. tenacissima*, the leaf area enclosed in the leaf chamber had been determined destructively with an area meter (Mk2; Delta T Devices, Burwell, UK). As the present experiment extended over 19 days, repeated harvesting would have increasingly damaged the plants. Therefore, a standard mean area of 2.13 cm² derived from the data of the previous measurements, was used in all calculations of photosynthetic rate and leaf conductance. This may have resulted in slight overestimates of the photosynthetic rate and leaf conductance in samples with widely opened laminae.

Relative water content (RWC) was determined by the method of Barrs & Weatherley (1962), except that three 15-cm long segments of leaves from each plant were cut under deionized water, weighed and placed in a dark humid chamber at room temperature with their cut ends covered by saturated cotton wool, until fully rehydrated.

Leaf water potential was measured with a pressure chamber (SKPM 1400; Skye Instruments Ltd, Llandrindod Wells, UK) at dawn and solar noon. Soil water potential and temperature at a depth of 10 cm were measured with a soil hygrometer/psychrometer and a dew-point microvoltmeter (PCT-55 and HR-33T; Wescor Inc., Logan, UT).

Imprints of the cross-sections of cut leaf segments were taken on blocks of plasticine to determine leaf width and the angle between the two halves of the lamina. The dimensions of these imprints were subsequently measured under a dissecting microscope. The relationship between RWC and degree of leaf folding was determined by taking imprints and weighing segments of leaves ($n=6$) from watered plants ($n=3$), which were allowed to dry out on a laboratory bench at room temperature and low light after total rehydration. The same technique of imprinting was used for leaves in which chlorophyll *a* and *b* concentrations were determined following Arnon's method (1949) after extraction with 80% acetone.

Tissue water relations were measured by the pres-

sure–volume technique (Tyree & Hammel 1972; Koide *et al.* 1989) on droughted plants in June before the start of the experiment. Leaves of three plants were cut under deionized water, transferred to a dark humid chamber with deionized water, and kept at room temperature until fully rehydrated. The pressure chamber was used to follow the decrease in water potential (ψ) as the leaves were allowed to dehydrate on a laboratory bench at room temperature (Davis & Mooney 1986). From the plot of $1/\psi$ against RWC, the osmotic potential at full turgor (ψ_{sat}), the osmotic potential (ψ_{tip}) and relative water content (RWC_{tip}) at the turgor loss point, the bound water in the apoplast (R_a) and the bulk modulus of elasticity near saturation (ϵ) were calculated.

Chlorophyll fluorescence of watered and control plants was measured with a chlorophyll induction fluorometer (PEA; Hansatech, Kings Lynn, UK). Leaves were dark adapted for at least 15 min prior to measurement. Fluorescence yield of PSII with all primary electron acceptors fully oxidized, minimum fluorescence (F_0), as well as maximum fluorescence yield (F_m) after applying a pulse of saturating light, were recorded. Variable fluorescence (F_v) and photochemical efficiency of PSII reaction centres were calculated as the difference $F_m - F_0$ and as the F_v/F_m ratio, respectively. The *S. tenacissima* leaves were not wide enough to cover the whole aperture of the leaf clips. Although the ratio F_v/F_m is independent of area, the variables F_0 and F_v are not. Therefore data for F_0 were recalculated to allow for the actual proportion of the aperture covered by each leaf.

Extension growth of four juvenile, not fully extended, leaves per plant ($n=8$ plants per treatment) was monitored by measurements of leaf length on days 7, 8, 10, 14 and 21.

The environmental variables, air temperature, relative humidity, net radiation, and wind speed and direction, were recorded by an automatic weather station.

Total nitrogen (N) concentration was determined by an elemental analyser (Roboprep, Europa Scientific, Crewe, UK) in dried samples from a set of leaves ($n=8$) from watered and control plants on day 5.

Statistical analysis was performed by analysis of variance using the GLM procedure of the SAS statistical package (SAS 1989). Differences between treatments were compared by *t*-tests.

Results

Environmental conditions between 7 and 26 of July 1993 were typical for an average summer. Mean daily temperature from 7 to 26 of July was $23.8 \pm 0.5^\circ\text{C}$, with a mean maximum of 30.4°C and a mean minimum of 17.8°C (Fig. 1). Total rainfall from 1 January to 26 July 1993 was 179 mm (compared with a 5-year average of 164 mm). Rainfall was negligible after 10 May (2 mm) so water stress was developing as temperature and radiation increased. Mean maximum

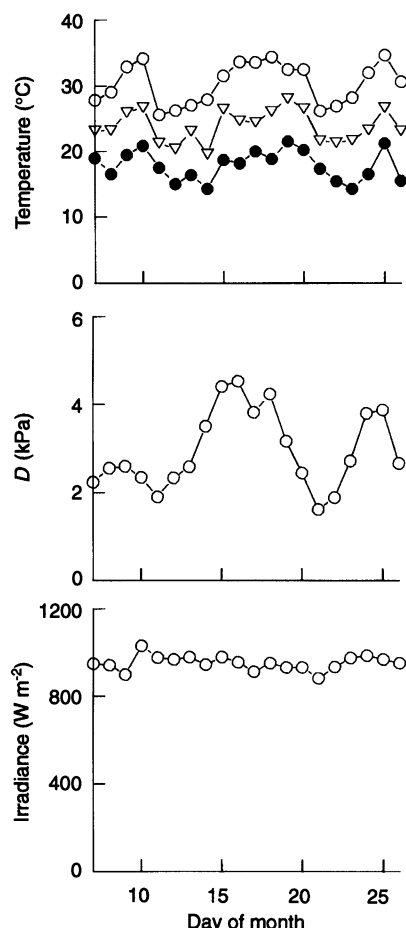


Fig. 1. Mean (▽), maximum (○) and minimum (●) values of temperature, and daytime mean of atmospheric saturation water vapour deficit (D) and solar irradiance, at Rambla Honda, Almería, from 7 to 26 July 1993.

irradiance was $932 \pm 21 \text{ W m}^{-2}$ (Fig. 1) and mean maximum photon irradiance was $\sim 1700 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The mean daytime atmospheric saturation water vapour deficit (D) was $2.26 \pm 0.06 \text{ kPa}$, but its range during the period of the experiment was $1.62\text{--}4.58 \text{ kPa}$ (Fig. 1). For the periods when gas exchange was measured, mean vapour pressure deficits were $2.32 \pm 0.19 \text{ kPa}$ for the early morning period (range $0.88\text{--}4.06 \text{ kPa}$) and $3.08 \pm 0.23 \text{ kPa}$ for the midday period (range $1.62\text{--}4.39 \text{ kPa}$).

EFFECT OF WATERING

Watering increased both dawn soil (ψ_{soil}) and leaf water potential (ψ_l) within 24 h (Fig. 2) and ψ at dawn continued to rise until the highest values ($\psi_{\text{soil}} = -0.6 \pm 0.5 \text{ MPa}$ and $\psi_l = -1.9 \pm 0.1 \text{ MPa}$) were measured 2 days after watering ceased. These data show that watering was effective to at least 10 cm depth, the position of the soil moisture sensor. Thereafter, dawn ψ fell in both as the soil dried, with ψ_l approaching the value for unwatered plants ~ 3 weeks later. The soil and leaf water potentials in unwatered tussocks at

dawn remained constant throughout at $-3.7 \pm 0.1 \text{ MPa}$ and $-4.2 \pm 0.2 \text{ MPa}$, respectively. (On 2 July, the gravimetric moisture content of dry soil at 15–20 cm depth in the Rambla Honda was $1.01 \pm 0.14\%$; J. Puigdefábregas, personal communication).

The diurnal change in ψ_{soil} between dawn and midday was similar at around -0.4 MPa under watered and unwatered tussocks.

Mean soil temperature under unwatered plants ($30.7 \pm 0.2^\circ\text{C}$) was significantly higher ($P < 0.0001$) than under watered plants ($29.5 \pm 0.1^\circ\text{C}$). Soil temperature increased by 0.6°C between early morning and solar noon in wet soil and by 2°C under unwatered plants.

In watered plants, early morning relative water content (RWC) increased until day 6 and always remained well above that of unwatered plants (Fig. 3). Maximum early morning (0.92) and midday RWCs values (0.94) were much higher than those of unwatered plants (0.84 and 0.82, respectively). The angle at $\text{RWC} = 1.0$ varied considerably between leaves from 120° to 50° (mean $= 90^\circ$), as did the RWC at 0° when leaves were closed (0.5 to 0.9, mean $= 0.73$) (Fig. 4).

GAS EXCHANGE

At $1300 \mu\text{mol m}^{-2} \text{ s}^{-1}$, mean photon irradiance was well above the light saturating point (F.I. Pugnaire, unpublished data) for *S. tenacissima* during the early morning measurements; it was about $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at midday. Gas exchange was very sensitive to the

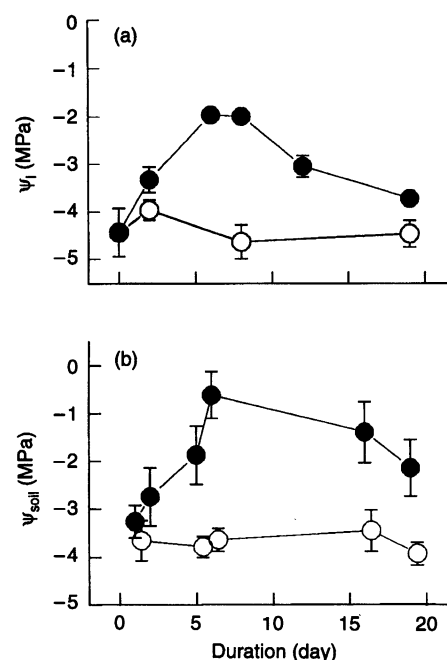


Fig. 2. Temporal variation of leaf (a) and soil (b) water potentials (ψ) at dawn in watered (●) and unwatered (○) tussocks of *S. tenacissima* in response to watering between 7 (day 0) and 11 July ($n = 8$; mean \pm SE; error bars are shown when larger than the symbol).

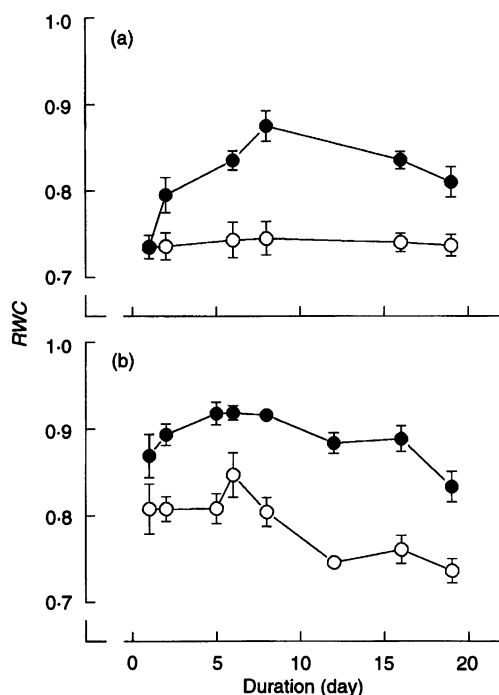


Fig. 3. Temporal variation of midday (a) and early morning (b) relative water content (RWC) of watered (●) and unwatered (○) tussocks of *S. tenacissima* in response to watering between 7 (day 0) and 11 July ($n=8$; mean \pm 1 SE; error bars are shown when larger than the symbol).

availability of water. Early morning leaf conductance (g_l) in unwatered plants remained low and constant (at a mean of $0.07 \pm 0.01 \text{ mol m}^{-2} \text{ s}^{-1}$ until day 16; Fig. 5a). Leaf conductance in watered plants increased quickly to a maximum value, double that of unwatered plants, 1 day after watering ceased, remained almost double until day 16, and then fell to almost equal that of control plants on day 19 (Fig. 5a).

The pattern of response of photosynthetic rate was similar, remaining low and changing little with time in unwatered plants. In watered plants, the maximum early morning value ($10.92 \pm 0.68 \mu\text{mol m}^{-2} \text{ s}^{-1}$) on day 5 was double that of unwatered plants, it remained almost double for the duration of the experiment (Fig. 5).

Although the mean depression of photosynthetic rate (ΔA) and leaf conductance (Δg_l) between early morning and midday over the whole experiment were very different for watered and unwatered plants, the proportional changes were similar (Table 1).

GROWTH

The measured change of length of leaves of unwatered plants over the period of the experiment, $+0.6 \pm 2.5 \text{ mm}$, was not statistically significant, while leaf length in watered plants increased by $21.5 \pm 2.5 \text{ mm}$. The extension rate increased to a maximum the day after watering ceased (Fig. 6), and then fell to almost equal that of unwatered plants after ~15 days.

FLUORESCENCE

Minimum fluorescence (F_0) of leaves on unwatered plants decreased slightly over the period of the experiment (Fig. 7). In watered plants, F_0 decreased significantly within 5 days and remained low until ψ_{soil} dropped below -1.5 MPa after 16 days, when it rose to equal that of unwatered plants again. The fluorescence ratio F_v/F_m , decreased significantly in unwatered plants over the first 16 days of the experiment, rising slightly over the last 3 days. The ratio had increased significantly in watered plants 1 day after watering ceased, and remained high until day 16 when it then fell to equal that of unwatered plants.

Although there was no significant change in mean F_0 between early morning and midday (Table 1), there were significant diurnal decreases in mean F_v/F_m in unwatered but not in watered plants.

CHEMICAL ANALYSIS AND WATER RELATIONS

There were no differences in nitrogen concentration between leaves of watered and unwatered plants after 4 days of growth. The nitrogen concentration was low ($6.4 \pm 0.3 \text{ mg g}^{-1}$ dry mass), but because the specific leaf area (SLA) was low ($0.28 \text{ dm}^2 \text{ g}^{-1}$) the nitrogen pool (2.28 g m^{-2}) and total chlorophyll content ($3.98 \pm 0.32 \text{ g m}^{-2}$) were high. The chlorophyll *a/b* ratio was 1.31.

Tissue water relations as derived from pressure/volume curves showed a high bound water in the apoplasm, a low bulk modulus of elasticity for a semi-arid species, and a low saturated/dry mass ratio (Table 2).

Discussion

Leaf extension of grasses is very sensitive to changes in leaf water potential (Boyer 1970; van Volkenburgh & Boyer 1985; Toft, McNaughton & Georgiadis 1987), even in species adapted to drought in semi-arid

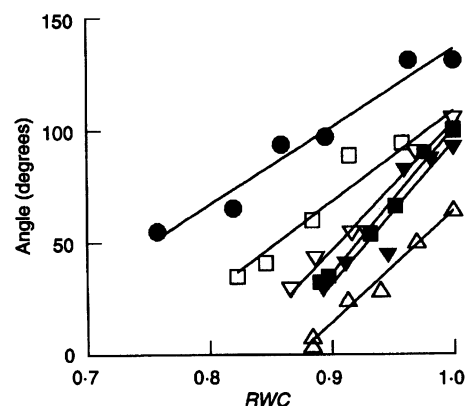


Fig. 4. Relationship of the angle between the two halves of the lamina and the RWC of excised *S. tenacissima* leaves ($n=6$; each leaf is represented by a different symbol and lines are regressions fitted to the data for each leaf) measured during dehydration at room temperature.

environments (Distel & Fernández 1988; Busso & Richards 1993). Evidence from this work at our study site at Rambla Honda shows that water is an important limiting factor for growth of *S. tenacissima*, a perennial evergreen species that apparently remains physiologically active the whole year round. The resumption of leaf growth following watering suggests that it is not summer dormant and hence that it should grow whenever water is available. In our experiment, leaf extension depended strongly on the availability of water, showing that short-term growth of *S. lenacissima* is dependent on the current water status of soil and plant; in arid ecosystems, however, long-term growth is limited by nutrient supply, especially of

nitrogen (Gutierrez & Whitford 1987). In species from semi-arid zones, ecologically similar to *S. tenacissima*, Busso & Richards (1993) described a decrease of 70% in daily extension rates when water-stressed and a decrease in extension rate as the leaf water potential decreased. In *S. tenacissima* there was no extension growth when ψ_{soil} was lower than about -3 MPa and ψ_l lower than about -4 MPa. When plants were watered, leaves extended at rates that appeared to be related directly to ψ_{soil} and ψ_l (compare Figs 2 and 6). The highest rates were measured when soil was wettest, i.e. when ψ_{soil} was higher than -1 MPa.

Both the diurnal decrease of carbon fixation and the low maximum photosynthetic rate (A_{max}) of unwatered *S. tenacissima* plants in natural conditions (Table 1) are typical responses of plants adapted to low-resource environments. In infertile habitats, A is often low to match the rate of supply of resources (Farquhar & Sharkey 1982; Chapin 1991; Collatz *et al.* 1991), because a high A_{max} would deplete the limiting resource and produce severe imbalance among carbon- and inorganic-nutrient-containing metabolites (Chapin 1991). The measured photosynthetic rates for both watered and unwatered plants were within the range for species from infertile habitats when under water stress (Larcher 1983; Lawrence 1987; Kyparissis & Manetas 1993). The photosynthetic rate was lower than might be expected for a leaf with a nitrogen pool of 2.28 g m^{-2} , perhaps because, in xeromorphic plants, there is a greater internal diffusive resistance that decreases the concentration of CO_2 at the carboxylation sites (Parkhurst *et al.* 1968). In addition, stomata in *S. tenacissima* are placed in deep grooves that are enclosed as the leaf folds, thereby increasing resistance of CO_2 diffusion. There was also an increase in gas exchange efficiency (A/g_{CO_2} , Farquhar, O'Leary & Berry 1982) with watering (Table 1), which shows an increase in water-use efficiency due to the removal of a limitation for CO_2 fixation (Schulze & Hall 1982). The photosynthetic rate may also have been low because more nitrogen is required for func-

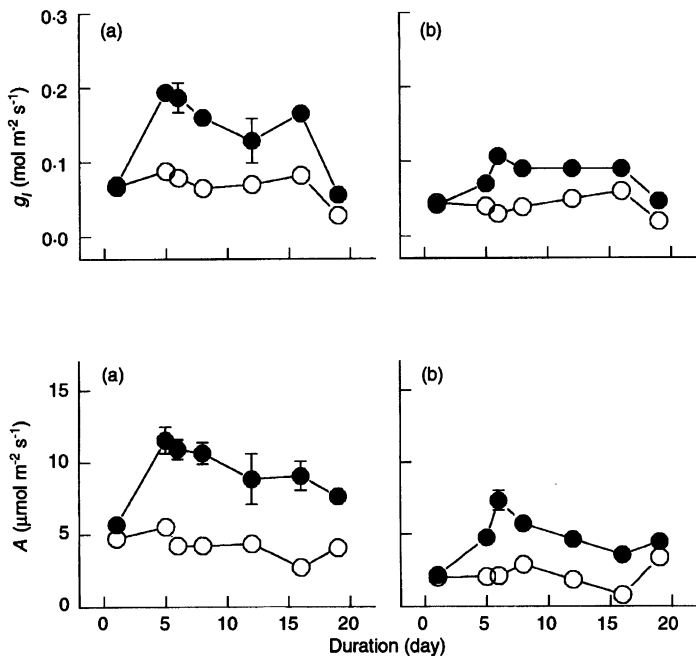


Fig. 5. Temporal variation of leaf conductance to water vapour (g_l) and photosynthetic rate (A) of watered (●) and unwatered (○) tussocks of *S. tenacissima* in the early morning (a) and at midday (b) in response to watering between 7 (day 0) and 11 July ($n=8$; mean \pm 1 SE; error bars are shown when larger than the symbol).

Table 1. Average values (\pm 1 SE) of relative water content (RWC), photosynthetic rate (A), leaf conductance to water vapour (g_l), minimum fluorescence (F_0) and efficiency of PSII (F_v/F_m) in watered and unwatered tussocks of *S. tenacissima* in the early morning and at midday between 6 and 27 July, except for intrinsic gas exchange efficiency (A/g_{CO_2} , where g_{CO_2} is leaf conductance to CO_2) which is for the fourth day of watering. ΔA and Δg_l are the absolute, and in parentheses proportional, changes in each variable between early morning and midday measurements. Significant differences (a) between treatments and (b) between early morning and midday values within treatments are indicated at $P<0.05$, $P<0.01$ and $P<0.001$ by *, ** and ***, respectively; NS = not significant

Quantity	Watered				Unwatered		
	Morning	(b)	Midday	(a)	Morning	(b)	Midday
RWC	0.88 \pm 0.01	***	0.85 \pm 0.01	***	0.78 \pm 0.01	***	0.75 \pm 0.01
A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	8.83 \pm 0.37	***	4.75 \pm 0.73	***	4.12 \pm 0.16	***	1.85 \pm 0.22
ΔA	-4.08 (-46%)				-2.27 (-55%)		
g_l ($\text{mol m}^{-2} \text{s}^{-1}$)	0.13 \pm 0.01	***	0.08 \pm 0.01	***	0.07 \pm 0.01	***	0.05 \pm 0.01
Δg_l	-0.05 (-39%)				-0.021 (-31%)		
A/g_{CO_2} ($\mu\text{mol mol}^{-1}$)	135 \pm 22	***	54 \pm 9	***	22 \pm 3	***	4 \pm 1
F_0	5.02 \pm 0.98	NS	6.54 \pm 1.10	*	7.29 \pm 0.19	NS	7.50 \pm 0.48
F_v/F_m	0.69 \pm 0.02	NS	0.69 \pm 0.01	***	0.64 \pm 0.01	**	0.57 \pm 0.01

tions other than photosynthesis in sclerophyllous leaves (Field & Mooney 1986).

Another consequence of watering was that RWC of *S. tenacissima* rose and, because of the positive relation between RWC and the angle formed by the two halves of the leaf (Fig. 4), leaves unfolded. As RWC changed diurnally (Table 1) the degree of folding also changed diurnally. Leaf folding has been described in tropical trees (Chiarello, Field & Mooney 1987), Mediterranean sclerophyllous plants (Kyparissis & Manetas 1993) and several other species (Begg 1980), and has the effect of increasing water-use efficiency and avoiding severe water losses. In those species, leaf folding was a function of turgor pressure. The water potential of unwatered plants in our experiment indicated that the leaves had always been near or below the ψ_1 at the turgor loss point (-3.75 MPa), and suggests that leaves must regain turgor at night, thereby causing the leaves to open and enabling them to assimilate CO_2 for a short period early in the morning, when RWC is above 0.87. This is the RWC at the turgor loss point obtained from pressure/volume curves (Table 2), which also suggests that leaves of unwatered plants would be at or below zero turgor most of the time, and of watered plants some of the time (Fig. 3). A was correlated positively with RWC ($r=0.85$, $P<0.0001$) and thereby to the leaf's angle of aperture, so that a reduction in RWC would fold the leaf and reduce assimilation of CO_2 and evaporation of H_2O . By combining data on RWC, gas exchange and leaf folding from several leaves, we calculated that a decrease of 10% in RWC on average closed the leaf by 37% and reduced the photosynthetic rate by 18%.

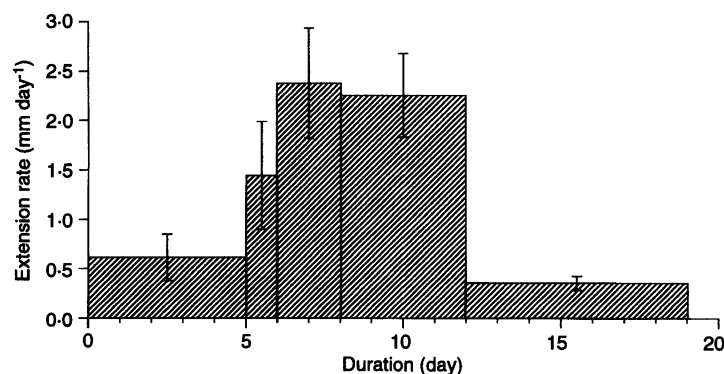


Fig. 6. Temporal variation in rate of extension of young leaves of *S. tenacissima* tussocks in response to watering between 7 (day 0) and 11 July ($n=8$; mean ± 1 SE). Leaves of unwatered tussocks did not change in length.

Table 2. The tissue water relations of *S. tenacissima* from Rambla Honda, Almería (Spain) in June 1993 as determined from pressure–volume curves (mean ± 1 SE; $n=3$)

Osmotic potential at saturation, ψ_{sat}	-1.95 ± 0.03 MPa
Water potential at the turgor loss point, ψ_{tlp}	-3.75 ± 0.35 MPa
Relative water content at the turgor loss point, RWC_{tlp}	0.87 ± 0.01
Bound water in the apoplast, R_a	$59.3 \pm 0.6\%$
Bulk modulus of elasticity near saturation, ϵ	9.86 ± 0.89 MPa
Saturated/dry mass ratio, m_s/m_d	1.53 ± 0.04

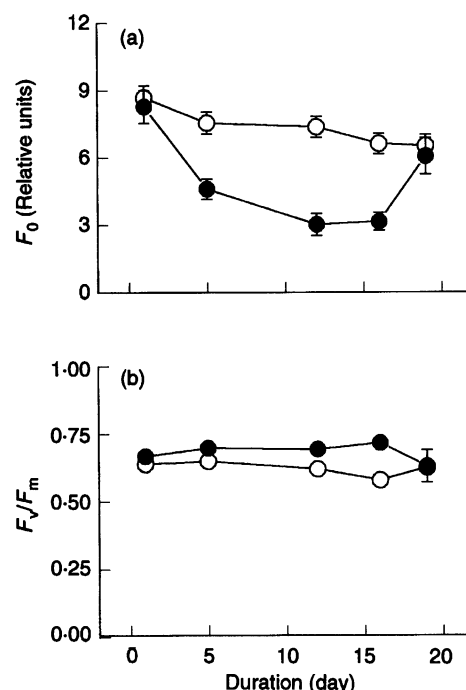


Fig. 7. Temporal variation in (a) minimum (F_0) fluorescence and (b) photochemical efficiency of PSII (F_v/F_m) in leaves of watered (●) and unwatered (○) *S. tenacissima* tussocks in response to watering between 7 (day 0) and 11 July ($n=8$; mean ± 1 SE; error bars are shown when larger than the symbol).

The osmotic potential at saturation of *S. tenacissima* is within the range for desert plants (Larcher 1983) and is similar to other ecologically similar species from arid zones, such as *Agropyron desertorum* and *Pseudoroegneria spicata* (Busso & Richards 1993). The relatively high bulk modulus of elasticity (Table 2) predicts a rapid fall of ψ_1 with a small decrease in RWC, a characteristic of plants from arid environments enabling them to avoid loss of water. F. Domingo, A. J. Brenner, S. C. Clark, C. Aguilera, L. Gutiérrez & L. D. Incoll (unpublished data) have also shown such a relationship of ψ_1 to RWC for *S. tenacissima*.

The saturated/dry mass of *S. tenacissima* is extremely low compared with mesophytic plants (Table 2), suggesting either a low proportion of living tissue in the leaf or a high proportion of thick-walled living cells. In *S. tenacissima* the former applies as its leaf has a high proportion of sclerenchymatous fibres (up to 45% w/w; Bolam 1965, cited in Hills 1988). The low saturated/dry mass ratio of the leaf probably accounts for the low concentration of nitrogen in leaves. On watering, leaves maintained the same concentration of nitrogen, which suggests that as the leaves grew, they mobilized nitrogen from storage pools elsewhere in the plant or from older senescing leaves. As nitrogen storage in this nutrient-limited environment is unlikely, we assume that the latter is more probable.

Stipa tenacissima responds to loss of turgor by folding its leaves. This does not, however, involve any substantial departure from their characteristically erect habit. This kind of canopy confers some advantage in dry, open habitats, as steeply orientated leaves intercept less radiation than horizontal leaves around solar noon (Werk & Ehleringer 1984; Ryel, Beyschlag & Caldwell 1993) and potential damage by excess radiation is reduced (Powles 1984; Demmig & Björkman 1987; Ehleringer & Forseth 1989; Krause & Weis 1991).

The increase in the fixation of CO_2 in watered plants was concurrent with a decrease in minimum fluorescence, F_0 (Fig. 7). Generally, F_0 decreases as a consequence of a decrease in photoprotection or an increase in CO_2 fixation. According to Genty, Briantais & Da Silva (1987) water stress does not affect F_0 . Therefore the rapid decrease of F_0 with watering suggests that this decrease is a consequence of increased photosynthetic rate.

On the other hand, the photochemical efficiency of PSII (F_v/F_m) increased in watered plants (Fig. 7) but was far from the optimal value of 0.83. According to Baker *et al.* (1994) most vegetation in direct sunlight undergoes 'mild' diurnal photoinhibition. Although F_v/F_m fell diurnally in unwatered plants (Table 1), it did not in watered plants, suggesting that unstressed *S. tenacissima* was able to cope with high light without the need to down-regulate photosynthesis by photoinhibition. Therefore excitation energy must be dissipated by other means despite the fact that photosynthetic rate and leaf conductance fell by 46% and 39%, respectively, on average between early morning and midday (Table 1). Energy could be dissipated as a consequence of leaf folding, which will decrease the area of leaf intercepting radiation. Furthermore, on folding, the leaf assumes a circular cross-section; it is likely that above a particular angle of incidence, light will be completely reflected. These two factors would combine to reduce the amount of excess light entering the leaf.

The near constant F_v/F_m from day to day in unwatered plants (Fig. 7) showed that their diurnal increase in photoinhibition was reversible and did not cause permanent damage (Baker *et al.* 1994). Over the 20 days of the experiment there was no significant change in F_v/F_m of unwatered plants (compare days 1 and 19, Fig. 7). The maximum seasonal value of F_v/F_m measured so far for *S. tenacissima* is 0.76 (A. J. Brenner, F. Domingo, S. C. Clark, C. Aguilera, L. Gutiérrez & L. D. Incoll, unpublished data) in late spring, suggesting that, with values as low as 0.57 in midsummer, there was a progressive inactivation of PSII reaction centres. The rise in F_v/F_m in watered plants suggests that this inactivation was at least partly reversible. Damage to PSII has been observed in droughted plants under high light (Björkman & Powles 1984) but evidence for an effect of water stress on photochemical efficiency is equivocal (Jones

et al. 1990; Epron & Dreyer 1993; Cornic 1994). Thus, although our data show that diurnal photoinhibition in *S. tenacissima* leaves is fully reversible and seasonal photoinhibition is partly reversible, this reversibility cannot be ascribed solely to properties of the photochemical system.

Although Almería Province is perceived as having 3 or more months of summer drought, variability of rainfall is high. Thus a 26-year data set for rainfall at Tabernas gives coefficients of variation for June of 198%, July 215% and August 193% (R. Lázaro, personal communication), so despite mean monthly rainfalls of 9.8, 2.1 and 2.3 mm, respectively, heavy falls do occasionally occur during this period. Our experiment has shown the response to applied water equivalent to a substantial rainfall in summer. The variability of rainfall at other times of the year, although lower, is over 100% for all months except November, February and April, and extended periods occur when the soil can be as dry as in summer. Although *S. tenacissima* leaves respond opportunistically to pulses of water at such times, the characteristics of the responses are not known and need investigation, as temperatures may be suboptimal for photosynthesis and growth, the parameters of tissue water relations may be different, a given sized pulse may take longer to evaporate, and the pulse may be applied to an already damp soil.

CONCLUSIONS

In unwatered plants of *S. tenacissima*, leaf water potential, relative water content, leaf conductance and photosynthetic rate were low and minimum fluorescence was high and leaves, which were closed, stopped growing. After watering, g_l and A doubled, F_v/F_m , ψ_l and RWC rose, F_0 fell and leaves, which opened in response to rising RWC, resumed growth. We conclude that a decreased A of leaves of droughted plants is a consequence of low leaf conductance and non-damaging photoinhibition and that the cessation of leaf growth was not due to summer dormancy.

The set of morphological and physiological traits present in *S. tenacissima* shows that it is well adapted to semi-arid conditions. Its evergreen habit and lack of summer dormancy allows it, like other species from semi-arid zones (Distel & Fernández 1988), to respond rapidly to an unpredictable climate and to use a sporadic water supply efficiently. Although in dryland ecosystems of temperate regions a substantial proportion of the total carbon gain occurs during winter months (65% of total; Emmingham & Waring 1977; Waring & Franklin 1979) when water is not limiting, evergreenness enables opportunistic species to exploit sporadic rain storms during summer drought or during the frequent periods when rainfall is scarce at other times of year. The conservative use of water may be an advantage during drought, but when

water is available photosynthesis is enhanced by high conductance (DeLucia & Heckathorn 1989; Evans & Black 1993). Furthermore, the presence of effective mechanisms of protection against high light and low availability of water contribute to the tolerance of *S. tenacissima* to stress and to the adaptation of this species to semi-arid conditions.

Acknowledgements

This research was carried out as part of the MEDALUS II (Mediterranean Desertification and Land Use) research project. MEDALUS II was funded by the EC under its Environment Programme, contract number EV5V CT92-0164, and the support is gratefully acknowledged. We thank CSIC and especially Dr J. Puigdefábregas for allowing us to use facilities of the Estación Experimental de Zonas Áridas and unpublished data from its meteorological station in the Rambla Honda. We thank A. J. Brenner for useful comments on an earlier draft of the manuscript and M. A. Domene, J. París and D. Herman for technical assistance.

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Received 10 January 1995; revised 25 September 1995;
accepted 13 October 1995