



## Ecophysiological significance of chlorophyll loss and reduced photochemical efficiency under extreme aridity in *Stipa tenacissima* L.

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### Abstract

*Stipa tenacissima* L., a perennial tussock grass widely found in semi-arid environments of the Iberian Peninsula and North Africa, is subjected to multiple stresses during the extreme summer conditions of south-east Spain. We characterised the photoprotective mechanisms of *S. tenacissima* during the transition from spring to summer and autumn. *S. tenacissima* experienced a marked water deficit ( $\Psi_{pd} < -8.4$  MPa) and the complete suppression of CO<sub>2</sub> assimilation in August, associated with a 72% reduction of maximal photochemical efficiency of PSII ( $F_v/F_m$ ). These reduced  $F_v/F_m$  values were related to the pre-dawn maintenance of high levels of epoxidized forms of xanthophyll-cycle pigments (DPS<sub>pd</sub>, ca. 42% higher than spring values), and with a 60% reduction in the concentration of total chlorophyll (Chl *a* + *b*). These changes were associated with a low capacity of dissipation of the excitation energy non-radiatively (measured as NPQ). Leaves showed a complete recovery of  $F_v/F_m$  and xanthophyll and chlorophyll concentrations after the autumn rainfall, which reached levels similar to that of spring. This poikilohydric-type response of *S. tenacissima* to stress allows for a greater tolerance of water shortage, high temperature and high light intensity, which are typical in these semi-arid environments and accounts for its distinctive opportunistic growth.

### Introduction

Seasonal stress determines the performance of plants in xeric Mediterranean grasslands (Madon and Medail, 1997). Perennial grasses such as *Agrostis castellana* and *Poa bulbosa* exhibit photoprotective mechanisms that ameliorate the combined effects of chilling temperatures and drought in winter (Figueroa et al., 1997). In summer, however, structural and physiological mechanisms seem to be insufficient to prevent the temporal suppression of photosynthetic activity in dry Mediterranean environments (Valladares and

Pugnaire, 1999). Resistance to summer drought in Mediterranean perennial grasses must rely on a combination of morphological and physiological traits including: (a) a deep root system for a greater water uptake at depth (Qian et al., 1997; Volaire et al., 1998); (b) the ability to reduce leaf transpiration rates (Maroco et al., 1997) or transpirational surface, either through leaf abscission (Werner et al., 1999) or by anatomical features that enable reversible leaf curling (Abernethy et al., 1998; Pugnaire et al., 1996); (c) high use efficiency of resource pulses (Pugnaire and Haase, 1996; Volaire et al., 1998); (d) large pool sizes of osmoprotectants (Abernethy et al., 1998; Ghasempour et al., 1998; Volaire et al., 1998); and/or (e)

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a high dehydration tolerance (Volaire et al., 1998). Drought avoiders would base their drought resistance mainly on *a* and *b*, retarding water loss and/or increasing water absorption, while the *c–e* traits would prevail in drought tolerators (sensu Bewley, 1979). Thus, perennial grasses that thrive in semiarid environments but lack a deep and extensive rooting system are more likely to be drought-tolerant rather than drought-avoiding species.

*Drought tolerance*, however, is an ambiguous term, which refers to species with different strategies and contrasted degrees of drought endurance. In Mediterranean woody species drought tolerators are characterised by maintaining a significant stomatal conductance and rate of carbon assimilation even at low leaf water potentials ( $< -2.0$  MPa; Epron et al., 1993), showing only a slight decrease in photochemical efficiency ( $F_v/F_m$ ) in summer, compared to that caused by winter photoinhibition (Karavatas and Manetas, 1999; Martínez-Ferri, 1999; Martínez-Ferri et al., 2000). Prolonged drought prevents restoration of regular physiological activity. Very few vascular plants tolerate severe dehydration of their vegetative tissues (Scott, 2000). These drought-tolerant species, known as resurrection plants, are functionally poikilohydric, cease photosynthetic activity when dehydrated, and either keep their photochemical apparatus (homoiochlorophylly) or lose a large fraction of their photosynthetic pigments (poikilochlorophylly) (Bewley, 1979; Tuba et al., 1996, 1997; and references therein). Unlike other drought tolerators, poikilohydric species exhibit a marked down-regulation of PSII activity accompanied by a differential non-photochemical dissipation of energy during drying and in the dehydrated state (DiBlasi et al., 1998).

The tussock-forming grass *Stipa tenacissima* is a stress-tolerant species that spread its biogeographical range from its primary origin in eastern arid steppes into extensive semiarid areas at the western end of the Mediterranean Basin (Gamarra and Montouto, 1999). Its current extension in south-east Spain, where annual rainfall is below 250 mm, and its colonising ability are likely due to traits that constitute a syndrome well suited to deal with extreme environmental stress. Its particular canopy, which minimises light interception and photoinhibition (Valladares and Pugnaire, 1999), and its ability to control water loss by reducing leaf area and leaf exposure to high light when water is limiting (Pugnaire et al., 1996), are key elements of its success. In this paper we try to characterise the mechanisms of drought resistance in *S. tenacissima*

under field conditions. We monitored water relations, gas exchange, chlorophyll fluorescence, and pigment concentrations of *S. tenacissima* tussocks in south-east Spain, tracking the same type of leaves from spring to autumn. We expected that severe water deficit during summer would force leaf dehydration, and consequently, we hypothesised that the drought resistance of this shallow-rooted species should be based on an ability of cells to recover from very low water contents.

## Materials and methods

### *Field site and species*

The field site is located in the northern foothills of the Sierra Alhamilla range near Tabernas, Almería Province, Spain ( $37^{\circ} 08' N$ ,  $2^{\circ} 22' W$ , 450 m elevation). The climate is semiarid with a mean annual precipitation of 242 mm and a pronounced dry season from June to September during which there is no rain in most years. Site and climate characteristics have been described elsewhere (Pugnaire and Haase, 1996; Pugnaire et al., 1996). Our plot was on a mainly flat area, gently oriented to the West with a slope of about  $15^{\circ}$ . Soil developed on a schist and quartz bedrock, showing a brown B horizon under an ocric A horizon. They are not calcareous, although the degree of saturation of the cambial complex is above 50% between 20 and 50 cm in depth. Soils can be classified as eutric cambisols CMe (FAO, 1998).

Frequently, low-land areas are steppe-like habitats dominated by *S. tenacissima* L., a long-living perennial grass that forms tussocks and spreads both by seeds and vegetatively. It has long and narrow leaves arranged in tillers, and each individual tiller usually has several senescent or dead leaves and two to three green leaves (Haase et al., 1999). Root-to-shoot ratio in *S. tenacissima* is very low and the plant arrests growth during the summer, when water deficit reaches a maximum. However, the arrested development of *S. tenacissima* leaves is not due to summer dormancy because leaves respond opportunistically within days to pulses of water (Pugnaire et al., 1996).

### *Experimental design*

Twelve *S. tenacissima* tussocks of similar size (about 1 m in dia.) and age were selected at the field site, and marked for the seasonal tracking of physiological measurements in May, August and November

1999. During each measurement period, microclimatic data of air temperature (T), photosynthetic photon flux density (PPFD), and relative humidity (RH) were recorded every 2 min with a combination of cross-calibrated temperature sensors (thermistor; Grant Instruments Ltd., Cambridge, U.K.), quantum sensors (SKP210; Skye Instruments Ltd., Powys, U.K.), and relative humidity probes (HMP 35A; Vaisala Oyj, Helsinki, Finland), respectively, connected to a Squirrel 1200 data logger (Grant Instruments, U.K.). Atmospheric saturation vapour pressure deficit ( $D_a$ ) was calculated from air temperature and air humidity data.

Soil water availability was measured by gravimetry. Six samples were taken with a borer under the tussocks on each sampling date; cores were placed in sealed plastic bags, taken to the laboratory and weighed, then dried at 110 °C for 48 h; volumetric soil moisture was obtained by difference in mass.

Relative water content (RWC) was determined for six plants by the method of Barrs and Weatherley (1962); about 1 g of 15-cm long segments of leaves from each plant were cut, weighed and placed in a dark humid chamber at room temperature with their cut ends covered by water saturated cotton wool, until reaching full rehydration, usually in about 24 h. Plant water potentials ( $\Psi$ ) were determined on freshly cut leaves at dawn ( $\Psi_{pd}$ ) and mid-day ( $\Psi_{md}$ ) at the sampling dates using a pressure chamber (SKPM 1400, Skye Instruments Ltd., Llandrindod Wells, U.K.) as described in Scholander et al. (1965).

Diurnal patterns of gas exchange were measured on 21 May, 28 August and 24 November 1999. Photosynthetic rate was measured at full sunlight on approximately ten mature leaves per tussock because of their small area, with a portable infrared gas analyser and leaf chamber (models LCA-4 and PLC-4C, Analytical Development Co., Hoddesdon, U.K.), equipped with microquantum sensors and thermocouples which monitored incident PPFD and cuvette and leaf temperature simultaneously to gas exchange measurements. Temperature inside the cuvette never exceeded the external air temperature by more than 2 °C during each measurement. Net photosynthetic rate ( $A$ ), stomatal conductance to water vapour ( $g_s$ ) and intercellular CO<sub>2</sub> concentration ( $C_i$ ) were calculated according to the model of von Caemmerer and Farquhar (1981). Both  $A$  and  $g_s$  were expressed on a projected leaf area basis, which was measured with an automatic image analyser (SigmaScan 2.0, Jandel Scientific Co., San Rafael, Canada). The area and dry mass of the leaf lamina in the leaf chamber were later recorded and

data were used for calculations of specific leaf area (SLA).

*In vivo* chlorophyll *a* fluorescence signals of the lamina of approximately ten attached mature leaves per tussock were monitored from pre-dawn to dusk on the same dates with a portable fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany) equipped with a leaf-clip holder to monitor incident PPFD (PPFD<sub>leaf</sub>) and leaf temperature ( $T_{leaf}$ ). The so-called saturation pulse method was used for determinations of all the fluorescence parameters (Schreiber et al., 1994). Briefly, leaf samples were exposed to a weak modulated measuring beam to assess the initial minimal fluorescence in the dark adapted state ( $F_0$ ) or the steady-state fluoresce in the light ( $F_t$ ). Then, a saturation flash of light (12 000  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) was given for 0.8 s to assess the maximal fluorescence levels, either in the dark, when PSII centres are closed ( $F_m$ ), or under light conditions ( $F'_m$ ).

Measurements of  $F_m$  and  $F_0$  were taken at pre-dawn to calculate maximal photochemical efficiency of PSII ( $F_v/F_m = [F_m - F_0]/F_m$ ). These  $F_m$  values were also used to calculate diurnal non-photochemical quenching of fluorescence (NPQ= $[F_m - F'_m]/[F'_m]$ ; Bilger and Björkman, 1990). Daily variation in the relative quantum yield of PSII photochemistry ( $\Delta F/F'_m = [F'_m - F_t]/F'_m$ ) was calculated according to Genty et al. (1989).

Two leaves from each *S. tenacissima* tussock were taken before dawn and at noon and immediately stored in liquid nitrogen until extraction of pigments (Martínez-Ferri et al., 2000). Leaf samples (100 mg) were extracted in 5 ml cool acetone in the presence of sodium ascorbate. After filtering, 30  $\mu\text{L}$  of the extract were injected into a Spherisorb ODS2 (Waters Corp., Milford CT, U.S.A.) reverse-phase steel column (25 cm, 5  $\mu\text{m}$  particle dia.). Chlorophylls and carotenoids were separated by HPLC (Waters, U.S.A.) equipped with a Waters 996 photodiode array detector (Waters, U.S.A.) as described in Val et al. (1994). Solvents for HPLC analysis (LabScan Ltd., Dublin, Ireland) were degassed before use by bubbling helium. For peak identification and quantification, pure commercial standards (VKI, Hørsholm, Denmark) were used. Leaf concentrations of violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) as well as the total pool (VAZ) were expressed in relation to total chlorophyll ( $\text{Chl}_{a+b}$ ) and on a dry weigh basis. The de-epoxidation state of the xanthophyll cycle pigments (DPS) was calculated as the ratio of antheraxanthin

and zeaxanthin to the total xanthophyll cycle pool as described by Adams et al. (1995).

Data were analysed with the STATISTICA 4.5 program (StatSoft Inc., Tulsa OK, U.S.A.). Differences in the diurnal course of gas exchange variables, chlorophyll fluorescence-based variables,  $PPFD_{\text{leaf}}$ ,  $T_{\text{leaf}}$  and pigment composition throughout the seasons were assessed by a repeated measurements analysis of variance (ANOVAR). In this model 'time of the day' and 'sampling date' were considered as within subjects repeated measurements factors. Where differences were detected, a least-significant difference test (LSD) was used for all pairwise comparisons. Assumptions of normality and homoscedasticity were tested using the Kolmogorov–Smirnov test and Cochran's C test, respectively. In the event of heteroscedasticity, variables were either log-transformed or analysed with non-parametric Kruskal–Wallis test.

## Results

### *Weather conditions*

All sampling dates were clear, sunny days in which maximum photon flux density ( $PPFD_{\text{max}}$ ) was reached little after mid-day along with maximal temperatures ( $T_{\text{max}}$ ) and atmospheric water vapour pressure deficits ( $D_a$ ) (Table 1). Although  $PPFD_{\text{max}}$  was higher during spring, August was the hottest date, with air reaching 44 °C, temperatures associated with the highest values of vapour pressure deficit (Table 1). A total of 81 mm of rain fell during our measurement period between May and November, irregularly distributed. There was no rain between May and July, and scattered storms left less than 10 mm in August and September, but the substantial rainfall did occur in October (26 mm) and November (above 43 mm). Leaf growth ceased at the on-set of the drought season, just after our May measurements, and resumed in November, right after the first Autumn rains.

### *Water content and specific leaf mass*

*S. tenacissima* showed marked seasonal changes in water status, showing a minimum of water content in the summer. In August, plants experienced a marked water deficit, with low values of leaf water potential ( $\Psi < -8.54$  MPa, below the range of the apparatus) and RWC (Table 2). These values indicate severe water shortage in summer. Pre-dawn  $\Psi$  values were significantly higher in November than in May ( $P < 0.001$ ),

reflecting that autumn rainfall made water availability higher in autumn than in spring. There were, however, no significant differences in the leaf relative water content (RWC) between the two sampling dates, suggesting that adequate osmotic adjustment has occurred in May. In both spring and autumn, plants suffered a significant mid-day decrease in  $\Psi$  of ca. 35 and 130%, respectively ( $P < 0.01$ ; Table 2). The lower  $\Psi_{\text{md}}$  in May translated into a lower but significant mid-day decrease in RWC ( $P < 0.05$ ). Specific leaf area (SLA) was significantly higher in November than in the two other months ( $P < 0.01$ ; Table 2).

### *Gas exchange*

Carbon assimilation in August was completely suppressed; plants were mainly respiring ( $A = -1.6 \pm 1.3 \mu\text{mol m}^{-2} \text{day}^{-1}$ ) and stomatal conductance was close to 0  $\text{mmol m}^{-2} \text{s}^{-1}$  (Figure 1a and b). Plants showed a maximum value of  $\text{CO}_2$ -assimilation rate in November, reaching 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at mid-day (Figure 1a). These values of  $\text{CO}_2$  fixation were associated with the highest values of  $g_s$  registered in all the sampling dates. Daily integrated assimilation rates in November were twice those registered in May ( $42 \pm 7.6 \mu\text{mol m}^{-2} \text{day}^{-1}$  and  $21 \pm 3.6 \mu\text{mol m}^{-2} \text{day}^{-1}$ , respectively;  $P < 0.001$ ).

### *Chlorophyll fluorescence*

Pre-dawn values of photochemical efficiency of photosystem II ( $F_v/F_m$ ) were below the optimal range (ca. 0.83 according to Maxwell and Johnson, 2000) in all the sampling dates. Plants showed similar pre-dawn values of  $F_v/F_m$  in May and November ( $0.75 \pm 0.01$  and  $0.77 \pm 0.01$ , respectively; Figure 1c), but experienced a significant drop of  $F_v/F_m$  in August, down to  $0.21 \pm 0.04$  ( $P < 0.001$ ). All plants showed a progressive and significant decrease in the photochemical efficiency of PSII ( $P < 0.001$ ) from pre-dawn to mid-day, when  $\Delta F/F'_m$  values reached a minimum in May and November, while in August, values did not vary significantly throughout the day, remaining always very low (Figure 1c). These minimum values were higher in November than in May and August ( $P < 0.05$ ). Diurnal variations in  $\Delta F/F'_m$  was negatively related to diurnal changes in NPQ in May, August and November ( $r^2 = 0.90$ ,  $r^2 = 0.75$  and  $r^2 = 0.96$ , respectively;  $P < 0.001$ ) (Figure 1d).

Table 1. Mean ( $\pm$ standard error), absolute maximum and minimum values of air temperature (T), photon flux density (PPFD), relative humidity (RH) and air water vapour pressure deficit (VPD) at the foot of the Sierra Alhamilla range (Tabernas, Almería, Spain) on three sampling dates of 1999

	May	August	November
T (°C)			
Mean	20.0 $\pm$ 0.30	29.1 $\pm$ 0.32	5.9 $\pm$ 0.23
Max	32.9	44.3	19.5
Min	9.4	18.7	-3.0
PPFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )			
Mean	755 $\pm$ 31	601 $\pm$ 26	326 $\pm$ 19
Max	2105	1905	1435
RH (%)			
Mean	36 $\pm$ 0.7	42 $\pm$ 0.9	65 $\pm$ 1.0
Max	66	80	99
Min	13	9	32
VPD (kPa)			
Mean	1.8 $\pm$ 0.05	3.2 $\pm$ 0.10	0.49 $\pm$ 0.02
Max	4.3	8.7	1.4
Min	0.41	0.46	0.01

Table 2. Mean values ( $\pm$ standard error) of pre-dawn volumetric soil water content ( $\theta$ ), water potential ( $\Psi$ ), relative water content (RWC), and specific leaf area (SLA), and total chlorophyll concentration ( $\text{Chl}_{a+b}$ ) of leaves of *S. tenacissima* on three sampling dates. Different letter within a variable indicates significant differences at  $P < 0.05$  (ANOVA and LSD)

	May	August	November	N
$\theta$ (%)	3.1 $\pm$ 0.68 <sup>a</sup>	0.8 $\pm$ 0.30 <sup>b</sup>	8.7 $\pm$ 1.2 <sup>c</sup>	6
$\Psi$ (MPa)				
Pre-dawn	-3.1 $\pm$ 0.22 <sup>a</sup>	< -8.5 <sup>b*</sup>	-1.0 $\pm$ 0.07 <sup>c</sup>	12
Mid-day	-4.2 $\pm$ 0.27 <sup>d</sup>	< -8.5 <sup>b*</sup>	-2.3 $\pm$ 0.29 <sup>f</sup>	6
RWC ( $\text{g g}^{-1}$ )				
Pre-dawn	0.86 $\pm$ 0.08 <sup>a</sup>	0.50 $\pm$ 0.02	0.87 $\pm$ 0.02 <sup>a</sup>	12
Mid-day	0.81 $\pm$ 0.01 <sup>b</sup>	-	0.86 $\pm$ 0.04 <sup>a</sup>	6
SLA ( $\text{m}^2 \text{kg}^{-1}$ )	1.45 $\pm$ 0.01 <sup>a</sup>	1.35 $\pm$ 0.02 <sup>a</sup>	1.82 $\pm$ 0.02 <sup>b</sup>	6
$\text{Chl}_{a+b}$				
( $\text{mg g}^{-1}$ )	0.690 $\pm$ 0.034 <sup>a</sup>	0.298 $\pm$ 0.013 <sup>b</sup>	0.842 $\pm$ 0.021 <sup>c</sup>	36
( $\text{mmol m}^{-2}$ )	542 $\pm$ 30 <sup>a</sup>	251 $\pm$ 14 <sup>b</sup>	511 $\pm$ 16 <sup>a</sup>	36

\*Measurements out of the range of the apparatus.

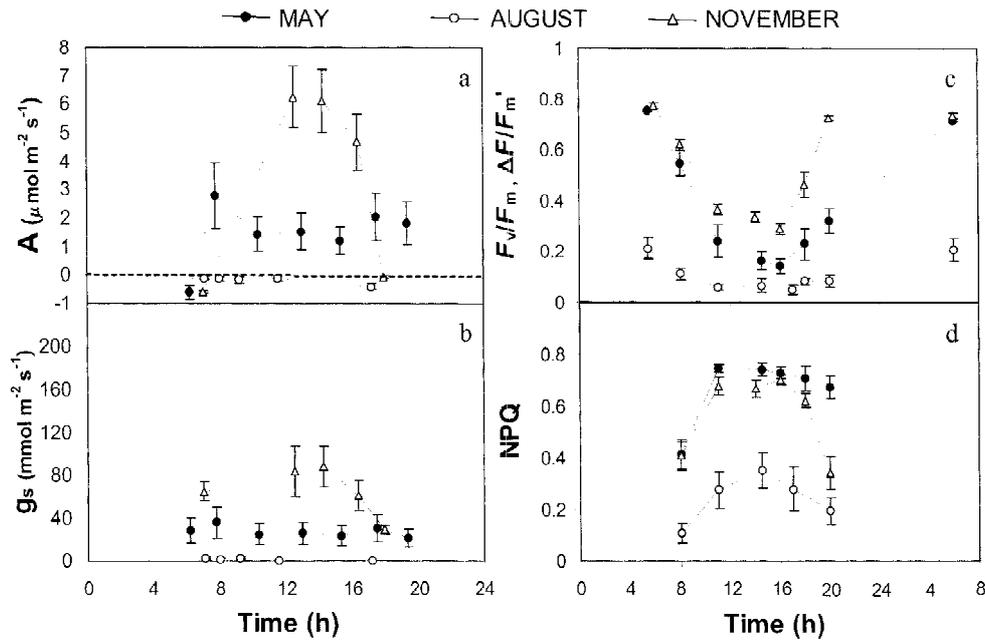


Figure 1. Diurnal variation of (a) photosynthetic rate,  $A$ ; (b) leaf conductance to water vapour,  $g_s$ ; (c) photochemical efficiency of PSII ( $F_v/F_m$  or  $\Delta F/F_m'$ ); and (d) non-photochemical quenching of chlorophyll fluorescence (NPQ) on the three sampling dates. Each point represents mean  $\pm$  standard error ( $n=6$ ).

#### Pigment concentrations and diurnal variation

The total amount of chlorophyll ( $\text{Chl}_{a+b}$ ) expressed on a dry weight and on a leaf area bases was significantly ( $P < 0.001$ ) reduced in August, compared with the values registered in May (57 and 54% reduction, respectively) and November (65 and 51% reduction, respectively, Table 2) on the same type of leaves. No significant diurnal variation in the quantity of  $\text{Chl}_{a+b}$  was found in any of the sampling dates. The amount of xanthophylls (VAZ) expressed either on a leaf area (Figure 2a) or on a dry weight bases was also significantly lower in August than in May and November ( $P < 0.001$ ). However, when xanthophylls were expressed per chlorophyll unit (VAZ), that tendency was reversed: the amount of VAZ on a total chlorophyll basis was twice as high in August as in May and November ( $P < 0.05$ ; Figure 2b). A daily depletion of the VAZ pool was observed in August and November but not in May ( $P < 0.05$ ; Figure 2b). Pre-dawn de-epoxidation state of xanthophylls ( $\text{DPS}_{\text{pd}}$ ) ranged from  $0.21 \pm 0.03$  to  $0.85 \pm 0.08$ , reaching the highest  $\text{DPS}_{\text{pd}}$  values in August ( $P < 0.001$ ). The significantly higher  $\text{DPS}_{\text{pd}}$  values in August were related to a higher amount of zeaxanthin, either expressed on plant dry weight basis or on a total chlorophyll basis. All plants

experienced a marked and significant ( $P < 0.001$ ) diurnal increase in DPS reaching maximum values at mid-day, except in August, when the degree of photoconversion of epoxidized forms remained unchanged throughout the day (Figure 2c).

#### Discussion

The response of *S. tenacissima* to summer drought is typical for poikilohydric plants, characterised by a decrease in chlorophyll concentration and in  $F_v/F_m$ , permanently high zeaxanthin levels, which allows a harmless dissipation of energy, and measurable respiration rates at extremely low water potentials (Casper et al., 1993; Kappen and Valladares, 1999). The decrease in leaf chlorophyll which is common during the summer in the Mediterranean Basin, is observed in species as functionally different as semi-deciduous shrubs (Kyparissis et al., 1995), broad-leaved sclerophyllic trees (Kyparissis et al., 2000) and pines (Elvira et al., 1998). The loss of up to 50% of leaf chlorophyll does not lead to leaf senescence in these species, but rather constitutes a reversible process that allows a decrease in light harvesting and reduces risks of over-excitation and photodamage. This reduction of the antenna size allows a sustained functionality of

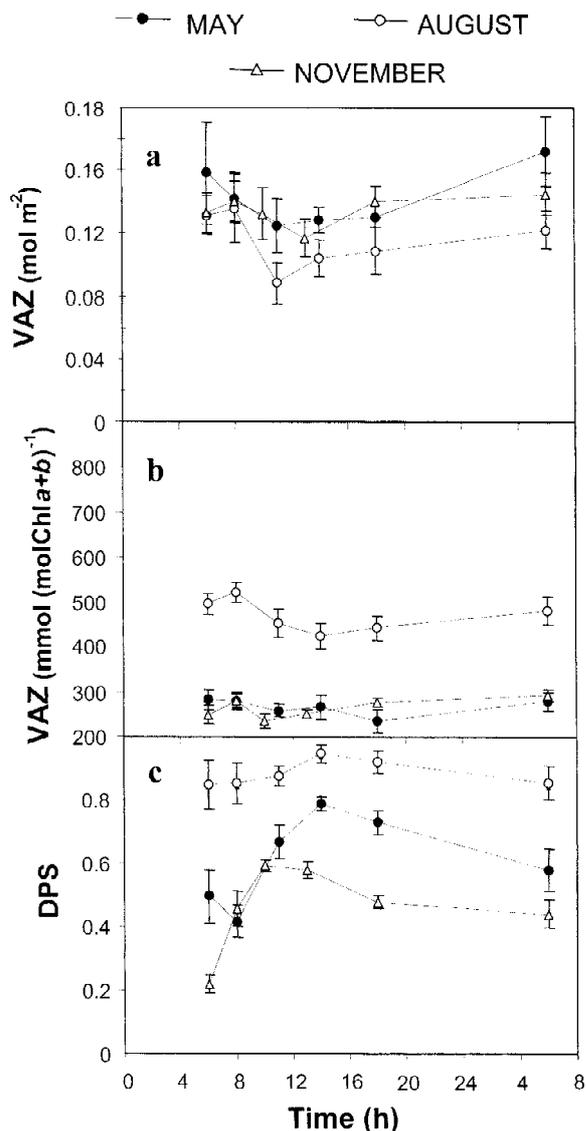


Figure 2. Diurnal variation of the (a) pool of xanthophyll-cycle pigments (VAZ) on a leaf area basis, (b) pool of xanthophyll-cycle pigments on a total chlorophyll basis and (c) de-epoxidation index (DPS) on the three sampling dates. Each data point represents mean  $\pm$  standard error ( $n=6$ ).

PSII throughout the dry season, as indicated by summer  $F_v/F_m$  values no lower than 90% of the annual maximal values. Exceptional reductions of chlorophyll content of up to 75% in *Lavandula stoechas* or 85% in *Rosmarinus officinalis* (Munné-Bosh and Alegre, 2000) never led to decreases over 20% of annual maximal  $F_v/F_m$  values. Our results show that *S. tenacissima* belongs to the group of species that reversibly lose a large proportion of their chlorophyll

in summer; but, contrary to other species, the maximum efficiency of PSII during drought decreased to an unprecedented 30% of previous  $F_v/F_m$  values, accompanied by a complete suppression of net carbon gain.

Diurnal rise in NPQ suggests that this dramatic decrease in  $F_v/F_m$  is not attributable to a photo-destruction of PSII. In high light, NPQ consists of two components, the high energy state quenching (qE) and the photoinhibitory quenching (qI; Horton and Ruban, 1994). A NPQ increase caused by qE suggests the persistence of functional PSII complexes (Richter et al., 1999), whilst if due to qI indicates reversible inactivation but not disorganisation of PSII reaction centres (Long et al., 1994). The low  $F_v/F_m$  in August must be at least partly caused by the large proportion of zeaxanthin that remained at night indicated by the high  $DPS_{pd}$  values. This phenomenon has also been observed in *Pinus sylvestris* exposed to high light intensities during dry periods (Ottander et al., 1995), and in Mediterranean species both during winter (García-Plazaola et al., 1999; Martínez-Ferri, 1999) and summer (Martínez-Ferri et al., 2000). This zeaxanthin pool would provide additional photoprotection early in the day by shortening the inductive phase of the harmless, non-photochemical energy dissipation (Ruban and Horton, 1999). However, the NPQ values in August were lower than those registered in May and November. This likely reflects that NPQ measures changes in heat dissipation relative to the pre-dawn state. Since the reference point in August was more quenched than in May and November, any increase in heat dissipation would have appeared as a smaller increase in quenching (cf. Maxwell and Johnson, 2000).

Unlike drought evaders, with life cycles restricted to wet periods, or drought avoiders, that prevent water potential decline by tight stomatal closure, esparto grass is a drought tolerator that withstands water shortage. Its tolerance is even superior to that of other well-known drought-tolerant species such as *Juniperus phoenicea*, or sclerophyllic species of *Quercus* (Martínez-Ferri et al., 2000). Our results indicate that esparto is able to withstand a severe dehydration of its leaves, decreasing pre-dawn relative water content below 40% and leaf water potentials below  $-8.5$  MPa during the summer months. The apparent coupling of leaf water content and soil moisture, along with the fact that the same leaves that dehydrated in summer turned green after the first autumn rains, confirm

that esparto grass fits the definition of poikilohydric species (Bewley, 1979; Gaff, 1989).

The poikilohydric nature of this species, along with the high capacity for mobilising nitrogen of other species in the same genus (Carrera et al., 2000) would explain the ability of *S. tenacissima* to use – and take advantage of – pulses of resources in semi-arid conditions (Pugnaire and Haase, 1996). Species with this strategy often show a trade-off between dehydration endurance and investment in structures for water uptake, transport and storage (Kappen and Valladares, 1999). The architecture of *S. tenacissima* helps to delay dehydration as other species do, such as the grass-like, poikilochlorophyllic *Xerophyta humilis* (Farrant et al., 1999). In addition, the reduction in the ratio of exposed leaf area to leaf mass during the dehydration process in *S. tenacissima* by leaf folding (Pugnaire et al., 1996) contributes to save water and to avoid photodamage during the dehydrating period (Farrant et al., 1999). The high degree of self-shading observed in *S. tenacissima*, which represents losses of up to 55% of potential CO<sub>2</sub> gain (Valladares and Pugnaire, 1999) is an additional contribution to a reduction of damage by radiation.

It is not clear whether *S. tenacissima* is a homeo- or poikilochlorophyllic species. Leaf chlorophyll concentration during summer was 67% lower than in autumn, a decrease similar to that observed for typical homeochlorophyllic species of *Ramonda* (70%, Markovska et al., 1994, Drazic et al., 1999) but also close to the values of typical poikilochlorophyllic species such as *Xerophyta scabrida* or *Borya nitida* (75–78%, Csintalan et al., 1998; Hetherington and Smillie, 1982). However, the values reached by *S. tenacissima* (ca. 0.3 mg g<sup>-1</sup> DW) are extremely low compared with other poikilohydric species in the Poaceae (>2 mg g<sup>-1</sup> DW, Quartacci et al., 1997), lower than those found in *Ramonda* (>2 mg g<sup>-1</sup> FW, Drazic et al., 1999), and even lower than in *X. scabrida*, which exhibited a chlorophyll loss of 85% when dehydrated (to ca. 0.5 mg g<sup>-1</sup> DW, Tuba et al., 1998). The low leaf chlorophyll concentration in *S. tenacissima* rendered leaves brown, making it very close to poikilochlorophyllic species.

The poikilohydric and poikilochlorophyllic habits seem to represent an adaptive advantage in species from open habitats (Smirnoff, 1993) such as perennial tussock grasses in arid and semi-arid ecosystems (Aguilar and Sala, 1999). Both habits explain the extreme drought resistance of *S. tenacissima*, its successful use of pulsed resources (Pugnaire and Haase,

1996) and its success as a colonising species in Mediterranean semiarid environments.

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