Diurnal and seasonal variations in the photosynthetic performance and water relations of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*

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Diurnal and seasonal fluctuations in the photosynthetic performance and water relations of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum* were monitored throughout two consecutive years at Garraf Natural Park in north-east Spain. Leaf gas exchange rates, chlorophyll fluorescence and shoot water potentials were measured once each season. Leaf nitrogen and carbon concentrations, leaf $\delta^{13}$C and $\delta^{15}$N and specific leaf area (SLA) were also measured once a year (August) on well developed mature leaves. *Globularia alypum* experienced seasonal fluctuations in their water potential, with the lowest values recorded in summer, whereas *E. multiflora* did not show significant differences in water potential among seasons. Moreover, lower water potentials were found in *G. alypum* than in *E. multiflora* throughout the entire study, suggesting that the latter behaved as a drought-avoiding species, whereas the former tolerated lower water potentials. In both species, maximum leaf gas exchange rates were observed in autumn and secondarily in spring; in contrast, photosynthetic and transpiration rates reached absolute minima in summer. The stronger fluctuations in water potential and leaf gas exchange rates found in *G. alypum* compared to *E. multiflora*, suggest that *G. alypum* is, sensu Levitt (1980), a water spender, whereas *E. multiflora* is a water conservative. This hypothesis is further supported by a higher integrated water-use efficiency (higher $\delta^{13}$C values) and a higher degree of sclerophyllity (lower SLA) in *E. multiflora* in comparison with *G. alypum*. *Globularia alypum* showed higher leaf gas exchange rates and higher predawn potential photochemical efficiency ($F_v/F_m$) than *E. multiflora* during most of the study. In spring and autumn, predawn $F_v/F_m$ values were within the optimal range, whereas chronic photoinhibition in summer and winter was detected in both species. However, whereas both species could maintain positive photosynthetic rates in winter, frequent negative values were found in summer, suggesting higher levels of stress during the drought period. These results together with the high correlations that were found between the net photosynthetic rates and several parameters of water availability (accumulated rainfall, soil moisture or midday water potential) provided further evidence of the key role of water availability in the regulation of the photosynthetic rates in these Mediterranean species. Warmer and drier conditions in future decades, as a consequence of climate change, may alter the present, slight competitive advantage of *G. alypum* and the fitness of both shrub species within semi-arid Mediterranean environments.

**Introduction**

The survival of a plant in a given environment depends largely upon its ability to photosynthesize at an adequate rate in order to grow faster than its competitors and balance its water loss to supply (Wuenscher and Kozlowski 1971). The rates of these two gas-exchange processes thus may play a major role in determining the outcome of competition among species and hence the species composition of plant communities (Wuenscher and Kozlowski 1971). In general, two contrasting water use strategies can be found in plants: prodigal and

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*Abbreviations* – A, net photosynthetic rate; E, transpiration rate; ETR, apparent electron transport rate; $F_0$, minimum dark-adapted fluorescence; $F_{m}$, maximum dark-adapted fluorescence; SLA, specific leaf area; WUE, water use efficiency.
conservative (Passioura 1982). The prodigal (or water-spending) strategy is related to high stomatal conductance associated with high water losses, but also high carbon gain and thus growth (Heilmeier et al. 2002). A usual way to assess the potential competitive ability of plants and to get information on specific adaptations to the prevailing climate has been the study of diurnal and seasonal fluctuations in photosynthetic performance and water relations. In particular, many studies on Mediterranean species show seasonal adjustments in these parameters (e.g. Harley et al. 1987, Nunes et al. 1992, Oliveira et al. 1992, Tretiach 1993, Grammatikopoulos et al. 1995, García-Plazaola et al. 1997, Karavatas and Manetas 1999), as a consequence of the marked seasonality of the Mediterranean climate.

Mediterranean summer is characterized by low precipitation and high temperature, high irradiance and high water vapour pressure deficit (Di Castri 1973). Although the photosynthetic apparatus is largely unaffected by water limitations (e.g. Genty et al. 1987, Havaux 1992), the combination of high light and high temperature can be particularly inhibitory to photosystem II (PSII) activity (Gamon and Pearcy 1990, Chaves et al. 1992, Havaux 1992). Indeed, many studies have described reductions in photochemical efficiency (e.g. Damesin and Rambal 1995, Valladares and Pearcy 1997, Matos et al. 1998) and low photosynthetic rates during summer drought due to stomatal control of water loss (e.g. Harley et al. 1987, Tenhunen et al. 1990, Damesin and Rambal 1995, Valladares and Pearcy 1997, Matos et al. 1998). However, co-occurring species often differ in their tolerance to drought (Duhme and Hinckley 1992, Castell et al. 1994). This variability is at least partly associated with differences in stomatal responses to dry conditions. Two drought adaptation strategies have been proposed: drought avoidance and drought tolerance (Levitt 1980). Drought avoidance is generally found in species with high stomatal sensitivity to drought. In these species, the high degree of stomatal control enables them to maintain high plant water potentials for extended periods of drought (Levitt 1980). In contrast, drought-tolerating species exhibit reduced stomatal sensitivity and, thus, simultaneous decreases in stomatal conductance and leaf water potential (e.g. Levitt 1980, Picon et al. 1996).

Although summer drought is generally considered the primary constraint to productivity and distribution of the Mediterranean vegetation (Di Castri 1973, Larcher 2000), some authors have suggested that winter cold stress also plays an important role in the development and distribution of Mediterranean evergreen species (Mitrakos 1980, Tretiach 1993). During Mediterranean winters, long rainless and cloudless periods can coincide with the coldest days (Kyparissi et al. 2000). Therefore, during such periods, photosynthesis may be limited directly by temperature or indirectly via hydraulic limitations on stomatal conductance (Oliveira et al. 1992, Grammatikopoulos et al. 1995). Indeed, low photosynthetic rates during winter (reviewed by Larcher 2000) and reductions of the efficiency of PSII under low temperatures (García-Plazaola et al. 1999, Karavatas and Manetas 1999, Larcher 2000, Oliveira and Peñuelas 2001) have also been reported.

Global change effects on Mediterranean climate are likely to produce warmer and drier conditions and more frequent and stronger droughts (IPCC 2001) with consequent effects on vegetation. Since co-occurring Mediterranean species have often different climatic constraints, each species will likely respond differently to the climate change. To predict how climate change may affect species distribution or dominance and as a consequence, community structure and ecosystem functioning, it is essential to have a broad knowledge of which climatic factors are constraining physiological activity of each species and how these constraints are manifested temporally. Therefore, the aims of this study were: (1) to determine whether two co-occurring Mediterranean shrub species, Erica multiflora and Globularia alypum, show different photosynthetic performance and/or different strategies of water use in response to the diurnal and seasonal variability in water availability and temperature; and (2) to identify the periods of environmental stress for these two species and the main abiotic factors causing such stresses. To approach these aims, we measured leaf water potentials, leaf gas exchange rates, chlorophyll fluorescence, specific leaf area (SLA), leaf nitrogen and carbon concentrations and leaf δ13C (to study integrated plant water use) and δ15N (to study plant N sources) throughout two consecutive years.

Materials and methods

Site and species description

The study was carried out in a dry shrubland (Rosmarino-Ericion) next to the village of Olivella in Garraf Natural Park, Barcelona, north-east Spain (41°18′N, 1°49′E) at 210 m above sea level and on a south-southeast slope (13°). The climate is typically Mediterranean, characterized by spring and autumn rainfalls, with cool winters and hot, dry summers. The soil is a petrocalcic calcixerpts (SSS 1998), thin (12–37 cm) and with a loamy texture and abundant calcareous nodules.

Erica multiflora L. and Globularia alypum L. are evergreen, scrophylous shrubs that typically occur in basic soils of the western Mediterranean Basin. They are two dominant shrubs of the studied shrubland. Both species resprout from underground organs after above-ground biomass removal. Vegetative growth occurs twice a year: in spring (from March to June) and autumn (from September to November). Flowering starts in August to September.

Environmental data

Precipitation was registered at the study site with a standard rain gauge. Soil moisture was measured every 1–2 weeks through the study period using time domain
of a measured shoot. Water-use efficiency (WUE), measured using ImagePC (v. 9 for Windows, Scion Co., Frederick, MD, USA), on nine fixed sampling points distributed within the study site. Air temperatures during the sampling period were obtained from the nearest weather station (El Rascler, Begues), at a distance of 8 km from the study site. Air temperatures in the leaf chamber (PLC4B; ADC Inc., Hoddesdon, Hertfordshire, England) and photosynthetic photon flux density (PPFD) at the moment of the leaf gas exchange measurements were monitored with a portable open-flow gas exchange system (ADC4; ADC Inc.).

**Shoot water potential**

Water potentials of apical shoots were measured seasonally with a Scholander-type pressure bomb (PMS Instruments, Corvallis, OR, USA). On each sampling date, shoots of three plants (one shoot per plant) of *E. multiflora* and *G. alypum* were measured at predawn (0230–0430 h in spring and summer and 0430–0630 h in autumn and winter, solar time) and midday (1100–13.00 h, solar time).

**Leaf gas exchange rates and chlorophyll fluorescence**

We measured leaf gas exchange rates and chlorophyll (Chl) fluorescence during 4–6 consecutive days in spring (May), summer (August), autumn (November) and winter (February) throughout 2 years (spring 1999 – spring 2001). We also measured Chl fluorescence in February 1999. Measurements were taken on one sunny shoot per plant, grown always in the spring of the current year. Each season, leaf gas exchange rates were measured on six to ten plants of *E. multiflora* and *G. alypum* in the morning (from 30 min after sunrise to 1130 h, solar time, at the latest) and in the afternoon (from midday to 1630 h, solar time, at the latest). Net photosynthetic rates (*A*), transpiration rates (*E*), and stomatal conductances were measured with a portable open-flow gas exchange system (ADC4; ADC Inc.). Branch tips with several leaves were introduced into the chamber of the system. All results are expressed on area basis, which was measured using ImagePC (v. 9 for Windows, Scion Co., Frederick, MD, USA) from photocopies of all the leaves of a measured shoot. Water-use efficiency (WUE), defined as mmol of net CO₂ uptake per mol of H₂O transpired, was calculated by dividing instantaneous values of *A* by *E*.

Components of Chl fluorescence were quantified with a portable modulated fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). After a dark-adaptation period of at least 30 min, we obtained minimum and maximum dark-adapted fluorescence (F₀, Fₘ) and F₀/Fₘ, where F₀ = Fₘ – F₀, F₀/Fₘ has been used as a measure of the potential (or maximum) photochemical efficiency of PSII. Measurements were performed on nine to 15 plants of *E. multiflora* and *G. alypum* at predawn and midday. The actual photochemical efficiency of PSII in the light-adapted state was estimated as: Φ(pxII) = ∆F/Fₘ = (Fₘ’ – F)/Fₘ’, where *F* is the steady-state fluorescence yield under the given environmental conditions, and Fₘ’ is the maximum level of fluorescence obtained during a saturating flash of light (when all the PSII traps are closed) under the same environmental conditions. From this index, we calculated the apparent electron transport rate (ETR) as:

$$ETR = \Delta F/F_m \times PPFD \times 0.84 \times 0.5$$

where PPFD was the photosynthetic photon flux density incident on the leaf, 0.84 was the coefficient of absorption of the leaves, and 0.5 was the fraction of electron required to the absorption of one quanta, as two photosystems are involved. We measured the ETR on nine to twelve plants of *E. multiflora* and *G. alypum* at morning (0700–1000 h, solar time) and midday (1100–1400 h, solar time).

**Isotope and elemental analyses**

Carbon (C) and nitrogen (N) leaf concentrations and foliar δ¹³C and δ¹⁵N were determined on leaves from the current year collected in August of 1999 and 2000. Each year, we sampled nine plants of *E. multiflora* and *G. alypum*. All analyses were carried out in an elemental analyser Carlo Erba EA1108 (Milan, Italy) coupled to a Delta C isotope ratio mass spectrometer with a CONFLO II interface (Thermo Finnigan MAT, Bremen, Germany). The calibrations were performed using interspersed international isotopic standards of carbon and nitrogen (IAEA, Vienna, Austria). The elemental analysis calibration was performed using atropine (ThermoQuest, Milan, Italy) as standard. The accuracy of the measurements was ± 0.15‰ for δ¹³C and ± 0.3‰ for δ¹⁵N.

**Specific leaf area**

We calculated the SLA of the leaves of the same shoots in which we measured leaf gas exchange rates in summer (August). After measuring the total leaf area of the shoots (see above), we dried all the leaves in an oven at 70°C to constant weight. We calculated the SLA as the ratio of the area of the leaves divided by their dry weight (cm² mg⁻¹). Shoots were collected at the end of the sampling day.

**Statistical analyses**

We performed ANOVAS within each year, season and round to study the differences between the two species. Since multiple tests were performed with the same variables, significance level (*P* = 0.05) was always adjusted for the number of statistical tests using a sequential Bonferroni correction to prevent group-wide type errors. To investigate the differences among seasons, we performed ANOVAS for each species within each year and round.
round, pooling the data from different years and/or rounds when the interactions were not significant. We used PPFD as a covariable in the ANOVAs whenever we analysed variables that were likely to be sensitive to instantaneous light levels. Pre-treatment measurements of the Chl fluorescence parameters (February 1999) were not considered in the analyses.

Reduced major axis regressions were used to investigate the relationships among the studied variables and the different parameters related to water availability (accumulated rainfall, soil moisture and shoot water potential). Finally, to analyse the differences between the two species in the leaf nitrogen concentration, the C/N ratio or the stable isotopes composition, we performed ANOVAs with year and species as fixed factors.

Results

Environmental data

The mean annual air temperature was 13.8°C in both 1999 and 2000. The maximum mean monthly air temperature occurred in August both years, whereas the minimum mean temperatures were reached in February in 1999 and in January in 2000 (Fig. 1). Total precipitation was 420.5 mm in 1999 and 489 mm in 2000. Maximum monthly rainfall occurred in October in 1999 and in December in 2000. The driest month was February in 1999 and July in 2000 (Fig. 1).

The mean annual soil moisture (v/v) was 21% in 1999 and 23% in 2000. Maximum soil moisture occurred at the end of autumn and beginning of winter, whereas minimum soil moisture was registered in summer (Fig. 1).

Shoot water potential

Globularia alypum had lower water potentials than E. multiflora (Fig. 2), although seasonally the differences between the two species were only significant in summer 2000 at predawn ($P = 0.001$) and midday ($P = 0.004$) and in autumn 2000 ($P = 0.002$) and winter 2000–01 ($P = 0.000$) at predawn. Midday values were significantly lower than predawn values for both species ($P < 0.001$).

Leaf gas exchange rates

In the morning, G. alypum showed five times higher leaf net photosynthetic rates ($A$) than E. multiflora in spring 1999 and two times higher in autumn 2000 ($P < 0.001$ in both cases) (Fig. 3). In the afternoon, net photosynthetic rates of G. alypum were higher (a mean of 2.5 times higher) than those of E. multiflora through all the study period (except in summer of both years), although in spring and winter of the second year differences were
not significant. Differences between morning and afternoon net photosynthetic rates of *E. multiflora* and *G. alypum* were almost significant (*P* = 0.08). The highest net photosynthetic rates of the two species occurred in autumn, except for *G. alypum* in the first year, when autumn and spring values were similar. The lowest values of *A* for both species and both years were in summer, although, in the second year, photosynthetic rates of *G. alypum* in summer were not statistically different from spring values.

In spring and autumn, maximum net photosynthetic rates of *G. alypum* were higher than maximum rates of *E. multiflora* for the same stomatal conductance (Fig. 4). In winter, maximum leaf net photosynthetic rates in relation to stomatal conductances were also higher for *G. alypum* plants, although the differences between the two species were smaller than in spring and autumn. In summer, both species showed similar ranges of leaf net photosynthetic rates and stomatal conductances.

In spring and autumn, leaf transpiration rates of *G. alypum* were 2.4 times higher than those of *E. multiflora* in the morning and 1.7 times higher in the afternoon (*P* < 0.01), except in autumn 2000 (Fig. 5). In winter and summer, differences were significant only in summer 2000 (*P* < 0.01). In both species, transpiration rates were lower in the afternoon than in the morning (*P* = 0.001) in all the seasons and years. In the first year, leaf transpiration rates of *E. multiflora* (morning and afternoon) and *G. alypum* in the afternoon did not differ significantly among seasons. However, in the first year in the morning, leaf transpiration rates of *G. alypum* were lower in winter and summer than in spring and autumn, whereas in the second year, morning transpiration rates of *E. multiflora* and *G. alypum* were higher in spring than in the other seasons (*P* < 0.001).

*Globularia alypum* and *E. multiflora* showed similar WUE (*A*/*E*) values throughout the study period, except in autumn 2000 (*P* < 0.001 in the morning and in the afternoon) and in winter 2000 in the morning (*P* = 0.004) (Fig. 5). There were no differences between morning and afternoon values of WUE in any of the two study species. *Erica multiflora* and *G. alypum* showed the lowest WUE values in summer, except in the second year in the morning, when there were no significant differences between spring and summer values.

**Relationship between leaf net photosynthetic rates and temperature or water availability**

When scatter plots are used to compare leaf net photosynthetic rates (morning and afternoon) versus temperature, several seasonal trends are highlighted (Fig. 6). There is a broad temperature optimum. *Erica multiflora* and *G. alypum* usually showed their maximum net photosynthetic rates in autumn and their minimum rates in summer. Despite temperatures while measuring in winter being very similar to those of autumn, net photosynthetic rates in winter were lower than in autumn. Negative
values of net photosynthetic rates were recorded in 
_E. multiflora_ and _G. alypum_ mainly in summer, although
some negative values were also observed in winter and
spring. Values were more negative for _G. alypum_ than for
_E. multiflora_.

The afternoon net photosynthetic rates of _G. alypum_
and _E. multiflora_ were significantly correlated with the
accumulated rainfall over the 3 months prior to the mea-
surements, the soil moisture and the midday water
potential (Fig. 7). Morning net photosynthetic rates
showed weaker correlations with the studied hydrologic
parameters in the case of _G. alypum_, whereas the correla-
tions were similar for _E. multiflora_.

**Chlorophyll fluorescence**

At predawn, potential photochemical efficiencies of
PSII (_Fv/Fm_) of _G. alypum_ plants were higher than for
_E. multiflora_ in all seasons, except in winter (1999, 2000
and 2001) and in autumn 2000 (Fig. 8). At midday, both
species showed similar values of _Fv/Fm_, except in winter
2000, when _Fv/Fm_ values of _E. multiflora_ were higher
than those of _G. alypum_ (_P = 0.001_). Midday values of
_Fv/Fm_ were significantly lower than predawn values
for both species in all the seasons (_P < 0.001_). Spring
and autumn values of _Fv/Fm_ were higher than winter
and summer values in both species, at predawn and
midday.

Apparent electron transport rates (ETR) of _E. multiflora_
plants at midday were usually higher than those for
_G. alypum_, although differences were statistically signifi-
cant only in summer (_P = 0.007_) and autumn (_P = 0.004_)
of the second year (Fig. 9). Midday ETR values of
_E. multiflora_ and _G. alypum_ were higher in autumn and
spring than in winter and summer.
Specific leaf area and foliar N concentration

Mature leaves of *G. alypum* had higher SLA values (4.33 \( \times 10^{-2} \pm 3.6 \times 10^{-4} \text{ cm}^2 \text{ mg}^{-1} \)) than mature leaves of *E. multiflora* (3.57 \( \times 10^{-2} \pm 2.6 \times 10^{-4} \text{ cm}^2 \text{ mg}^{-1} \)). The leaf nitrogen concentration was higher in *G. alypum* than in *E. multiflora* in both years (\( P = 0.004 \)) (Fig. 10). Leaf nitrogen concentration in 2000 was slightly higher than in 1999 for both species (\( P = 0.051 \)). *Globularia alypum* showed a lower C/N ratio than *E. multiflora* in both years (\( P < 0.001 \)) (Fig. 10). On the other hand, the C/N ratio was lower in both species in 2000 than in 1999 (\( P = 0.03 \)).

Leaf stable isotopes

In both years, lower \( \delta^{13} \text{C} \) values were found in *G. alypum* than in *E. multiflora* (\( P < 0.001 \)) (Fig. 11). There were no significant differences between years in the \( \delta^{13} \text{C} \) values of both species. There were no significant differences between the two species or the two years in the leaf \( \delta^{15} \text{N} \) values (Fig. 11).

Discussion

Leaf gas exchange rates, as well as shoot water potential values, of *G. alypum* and *E. multiflora* were within the range reported for other Mediterranean shrubs that co-occur with them, such as *Pistacia lentiscus* or *Quercus coccifera* (Rhizopoulou et al. 1991, Llusia` and Peñuelas...
Globularia alypum shrubs sustained more severe water deficits (lower shoot water potentials) than did *E. multiflora* throughout the study period (Fig. 2). Moreover, *G. alypum* plants experienced seasonal fluctuations in their water potential, with the lowest values recorded in summer, whereas *E. multiflora* plants did not show significant differences in shoot water potential among seasons (Fig. 2). As a consequence, differences in water potentials between the two species were specially marked in summer.

Seasonal adjustments in leaf gas exchange rates were observed in *G. alypum* and *E. multiflora*. Photosynthetic and transpiration rates of both species reached absolute minima in the summer (with frequent negative values of photosynthesis), whereas they showed high photosynthetic and transpiration rates during the periods with high water availability (autumn and spring) (Figs 3 and 5). Similar results have been reported for Mediterranean vegetation (e.g. Nunes et al. 1992, Castell et al. 1994, Damesin and Rambal 1995, Grammatikopoulos et al. 1995).

The marked reductions in gas exchange rates in summer in the absence of changes in shoot water potential indicates that *E. multiflora* behaved as a drought-avoiding species. In contrast, *G. alypum* is a more drought-tolerating species. The advantage of tolerating lower plant water potentials is that a strong gradient of water potential between the soil and the plant is produced, thus helping to maintain the flow of water into the plant (Dunn et al. 1976). However, decreases in plant water potential increase the risk of cavitation (Tyree and Sperry 1989), which can be responsible for the death of leaves and twigs during the summer (Correia and Catarino 1994).

The higher net photosynthetic (*A*) and transpiration (*E*) rates of *G. alypum* in comparison with *E. multiflora* throughout the year (except in summer) was usually related to higher stomatal conductances (Figs 3 and 5). However, *G. alypum* had higher values of *A* than *E. multiflora* for a same stomatal conductance (Fig. 4). This could be related to a greater photosynthetic capacity in *G. alypum*, which agrees with the higher leaf nitrogen concentration that we found in this species (Fig. 10). A positive correlation between leaf nitrogen and rates of net photosynthesis has been demonstrated in many studies (e.g. Field and Mooney 1986, Evans 1989), since the photosynthetic apparatus and, specifically, Rubisco is a major sink for nitrogen in leaves (Field and Mooney 1986, Evans 1989).

It has been proposed that stomatal closure in part of the day in arid ecosystems would avoid dehydration and therefore major tissue damage (Pereira and Chaves 1995). Accordingly, we found that transpiration rates of the two study species were lower in the afternoon than in
the morning throughout the study period (Fig. 5). A higher leaf-to-air vapour pressure difference in the afternoon than in the morning (data not shown) may cause the afternoon depression in transpiration rates (Beadle et al. 1985). It has also been suggested that afternoon depression of leaf gas exchange rates may be associated with long periods of high photon flux density (Correia et al. 1990).

Relationship between net photosynthetic rates and temperature

In our study, *E. multiflora* and *G. alypum* showed a broad range of temperature optima for net photosynthesis (Fig. 6), which is similar to the range reported for a number of Californian and Chilean shrubs (15–30°C, Oechel 1980) or for sclerophyllous shrubs of dry regions after compilation data from numerous authors (20–35°C, Larcher 1995).

Our results are in agreement with the general idea that the range of temperatures more favourable to the carbon uptake coincides with the temperature limits during the period of growth in the Mediterranean region (Chaves et al. 1992). Traditionally this period has been identified as spring. However, the two studied species present two growing periods, one in spring and the other in autumn, and for both species the range of temperatures most favourable to CO₂ assimilation coincides with the latter. C climatic conditions in autumn (higher rainfall and soil moisture, lower temperatures and thus lower vapour pressure deficit) allow higher net photosynthetic rates and for both species the range of temperatures most favourable to CO₂ assimilation coincides with the latter. Climatic conditions in autumn (higher rainfall and soil moisture, lower temperatures and thus lower vapour pressure deficit) allow higher net photosynthetic rates as in spring. Moreover, the highest *A* values in autumn coincide with the flowering and fruiting period. Although temperatures on sunny sampling days of winter were also within the optimum temperature range, net photosynthetic rates were not as high as in autumn, probably due to the lower water availability and/or the suboptimal temperature events in winter.

Relationship between net photosynthetic rates and water availability

Several authors have pointed out situations in which there is a lack of correlation between changes in leaf water potential and leaf gas exchange rates (Beadle et al. 1985, Reich and Hinckley 1989, Tenhunen et al. 1990, Matos et al. 1998, Giorno et al. 1999) and, thus, it has been suggested that stomata may respond to soil or root water status rather than to leaf water status (Reich and Hinckley 1989, Giorno et al. 1999). However, we found significant correlations between afternoon net photosynthetic rates and shoot water potentials at midday in both species (Fig. 7), although such correlations may be reflecting the significant correlations that we also found between shoot water potential at midday and soil moisture (see Fig. 7). Afternoon net photosynthetic rates were also highly correlated with the soil moisture and with the accumulated rainfall over the 3 months prior to the measurements in both species (Fig. 7), suggesting that water availability plays an important role in the regulation of net photosynthetic rates in the two studied species.

Water-use efficiency

Different authors have suggested that efficient use of water is a conservative ecophysiological ‘strategy’ that can be detrimental in a competitive water-limited environment (DeLucia and Heckathorn 1989, Read and Farquhar 1991). Accordingly, we found that the lowest instantaneous WUE (*A/E*) values were in summer (and spring in the second year) for both species (Fig. 5), in agreement with other studies on Mediterranean species that reported decreases in WUE as water deficit increased (e.g. Gratani et al. 2002, Moriana et al. 2000). It has been suggested that sometimes when stomata close, the ratio *A/E* falls sharply since net photosynthetic rates are reduced nearly to zero, but transpiration (mostly cuticular) continued because of the high evaporative demand (Larcher 1995). Our results contrast with those reported by a number of authors indicating that as water become less available, WUE increases in woody plants (e.g. Toft et al. 1989, Korol et al. 1999). Differences in the intensity of water stress may explain such contrasting results. Indeed, Chaves and Rodrigues (1987) reported that in *Vitis vinifera* the highest WUE was observed with mild water deficiency whereas, as stress increased, WUE fell.

Theory predicts that values of leaf δ¹³C are positively related, via the ratio between the concentration of CO₂ in the leaf and in the air, to the integrated WUE over the growing season (WUE_i) (Farquhar et al. 1989). *Erica-multiflora* leaves showed higher δ¹³C than *G. alypum*, which corresponds to a higher WUE_i (Fig. 11). However, instantaneous values of WUE measured in spring did not agree with this result, since we did not find differences between the two species (Fig. 5). Other studies have reported discrepancies between gas exchange and isotopic data (e.g. Vitousek et al. 1990, Gutierrez and Meinzer 1994, Picon et al. 1996). Hence, scaling from instantaneous values of WUE may not reflect the integrated leaf gas exchange activity over the growth period (Gutiérrez and Meinzer 1994).

Higher WUE_i in *E. multiflora* suggests that *E. multiflora* is more water conservative than *G. alypum*; the lower transpiration rates of *E. multiflora* in comparison with those of *G. alypum* support this. Assuming plant water competition, the more prodigal use of water of *G. alypum* in spring (lower WUE_i) would be advantageous because it allows *G. alypum* to use the water before the other species extract it and, as a consequence, to have higher photosynthetic rates. Accordingly, different studies have reported that plants with low WUE tended to have higher net photosynthetic rates under high soil moisture conditions than plants with high WUE (Ehleringer and Cooper 1988, Kolb and Davis 1994). However, these studies also showed that, when soil water
is limiting (e.g. summer drought), plants with low WUE are more susceptible to decreases in growth rate, water stress-induced embolism, shoot dieback and mortality (Ehleringer and Cooper 1988, Kolb and Davis 1994).

Finally, higher WUE in *E. multiflora* than in *G. alypum*, coincides with a lower SLA of *E. multiflora* leaves; that is, *E. multiflora* leaves are more sclerophyllous than *G. alypum* leaves. Several authors have found increases in this, with previous studies on Mediterranean vegetation that 0.62 ± 0.02 for *G. alypum* leaves. Several authors have found increases in δ¹³C and, thus, in the WUE, as the level of sclerophyllly increased (Vitousek et al. 1990, Groom and Lamont 1997). On the other hand, higher leaf N concentration and net photosynthetic rates in *G. alypum* coincides with higher SLA, in agreement with previous studies demonstrating a positive correlation between these variables (Reich et al. 1997). SLA reflects a fundamental trade-off in plant functioning between a rapid production of biomass (high SLA) and an efficient conservation of nutrients (low SLA) (Garnier et al. 2001).

### Photosynthetic efficiency

In our study, maximum predawn \( F_v/F_m \) values were within the optimal range reported by Björkman and Demmig (1987) and they occurred in spring of both years and in autumn of the first year, suggesting relatively low stress during these seasons (Fig. 8). Conversely, predawn values of \( F_v/F_m \) in summer and winter every year and in autumn 2000 were lower than 0.75, with a minimum of 0.57 ± 0.02 for *E. multiflora* and 0.62 ± 0.02 for *G. alypum* for winter 1999, in agreement with previous studies on Mediterranean vegetation that also reported lower \( F_v/F_m \) values during these seasons (e.g. García-Plazaola et al. 1997, Karavatas and Manetas 1999, Larcher 2000, Oliveira and Peñuelas 2001). Our results, thus, suggest the presence of chronic photoinhibition during summer and winter in the two study species, as no recovery of photochemical efficiency after night-time was observed. Persistent reductions in PSII efficiency are attributed predominantly to the retention of de-epoxidized components of the xanthophyll cycle (zeaxanthin and antheraxanthin) and their sustained engagement for energy dissipation activity, which may help to protect PSII from over-excitation and photodamage (e.g. Adams and Demmig-Adams 1995, García-Plazaola et al. 1999, Kyparissis et al. 2000). Nevertheless, sustained reductions of \( F_v/F_m \) values may also result from accumulation of non-functional PSII reaction centres and partial photoactivation of PSII (Niyogi 1999). In winter, the relatively high assimilation rates of both species, favours the hypothesis that photoinhibition was due to photoprotection rather than photodamage. In contrast, the observed decreases of \( F_v/F_m \) in summer could be accompanied by extremely low photosynthetic rates (sometimes even negative ones, Fig. 3), suggesting possible damage to the photosynthetic apparatus.

Higher net photosynthetic rates in *G. alypum* than in *E. multiflora* in spring and autumn would create a higher photochemical sink for the electrons contributing to protect the photosynthetic apparatus against the accumulation of excessive excitation energy. This is in accordance with the higher potential photochemical efficiency of *G. alypum* in comparison with *E. multiflora* plants at predawn during most of the study period (except in autumn 2000 and winter). Midday \( F_v/F_m \) values for *E. multiflora* were suboptimal (lower than 0.75) throughout the study period, except in November 1999, whereas values for *G. alypum* were lower than 0.75 in winter every year and in summer and autumn of 2000.

In C₃ plants, PSII activity is mainly partitioned between photosynthesis and photorespiration (Krall and Edwards 1992). However, despite the higher photochemical sink of *G. alypum*, we found similar or even lower apparent ETR in *G. alypum* than in *E. multiflora* (Fig. 9). These contradictory results may simple arise from the fact that photosynthetic rates are expressed by the total leaf area of the measured shoot, whereas the ETR values are expressed by the illuminated leaf area (which at least for *E. multiflora* never coincides with the total leaf area of the shoot). Alternatively, these results may indicate higher photorespiration rates in *E. multiflora*. Available data do not allow us to resolve this question.

The results of our study suggest that, under the present climate conditions, *G. alypum* is able to take a greater advantage of periods with high water availability than *E. multiflora*, showing a higher carbon gain and a better photosynthetic performance. However, the predicted warmer and drier conditions in future decades (IPCC 2001) will probably reduce such differences, lowering the present competitive advantage of *G. alypum*. Moreover, taking into account that both species, but in particular *E. multiflora*, showed suboptimal values of \( F_v/F_m \) at midday during most of the year, the drier and warmer conditions predicted for future decades may further decrease the photosynthetic performance of these two Mediterranean shrubs.

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