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Seasonal changes in photochemical efficiency in leaves of *Halimium halimifolium*, a mediterranean semideciduous shrub

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Abstract

The relationship between soil water availability, physiological responses [leaf chlorophyll (Chl) fluorescence, leaf water potential (Ψ), and stomatal conductance (g_s)] and plant stress was studied in *Halimium halimifolium* (L.) Willk, *Cistaceae*, in three sites with contrasted water regimes (Monte Blanco - MB, Monte Intermedio - MI, and Monte Negro - MN) of Doñana National Park (SW of Spain) along the day, in September (summer period) and December (winter period) 1994. In winter, differences among the areas were not significant, with Ψ, g_s, and photochemical efficiency values of -1.5 MPa, 0.200 cm s^{-1}, and 0.70, respectively. In summer, however, high declines of g_s (0.014 cm s^{-1} in MI and 0.021 cm s^{-1} in MB), photochemical efficiency (0.65 F_v/F_p in MB and MI sites) and Ψ (-3.76 in MI and -3.04 MPa in MB) were recorded. Winter-summer differences were minimum in well-watered sites (MN) and maximum in MI. The Ψ and photosystem 2 (PS2) fluorescence were correlated and showed similar seasonal patterns in all three areas.

Additional key words: chlorophyll fluorescence; drought; leaf water potential; photosystem 2; plant stress; soil water availability; stomatal conductance.

Introduction

Plants of Mediterranean type climate are subjected to water and heat stress during the summer. These stresses are generally associated with clear days and intense solar radiation (Valladares and Pearcy 1997).

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Abreviations: Chl = chlorophyll; F_v/F_p = photochemical efficiency; g_s = stomatal conductance; Monte Blanco = MB; Monte Intermedio = MI; Monte Negro = MN; PS2 = photosystem 2; Ψ = leaf water potential.

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The use of chlorophyll (Chl) fluorescence induction in plant leaves *in situ* is a good non-intrusive method for monitoring photosynthetic events and judging the physiological state of the plant (Baker 1991, Long *et al.* 1994, Gil Martínez 1995, Mohammed *et al.* 1995). The maximum photochemical efficiency (F_v/F_p) is highly correlated with the quantum yield of photosynthetic O_2 evolution (Krause and Weis 1991, Gil Martínez 1995, Schreiber *et al.* 1995). It is very sensitive to a variety of environmental stress-inducing factors: high irradiance, extreme temperatures, water scarcity, salinity, pollution, *etc.* (Mishra *et al.* 1991, Öquist and Huner 1991, Pastenes and Horton 1996, Fernández-Baco *et al.* 1998). A reduction in the photochemical efficiency of PS2 is called photoinhibition (Krause and Weis 1991, Govindjee 1995). Two main classes of photoinhibition can be distinguished on the basis of the relaxation time: (*a*) dynamic photoinhibition, or photosynthetic down regulation, a readily reversible process that provides photoprotection to PS2 that can be observed during the day, with a marked reduction in photochemical efficiency, in CO_2 uptake and with changes in content of xanthophyll pigments at midday (Schindler and Lichtenthaler 1996, Fernández-Baco *et al.* 1998), (*b*) chronic photoinhibition, a slow depression of photosynthesis associated with photodamage (Osmond 1994). This effect has been detected through seasonal changes in Chl fluorescence in trees and grassland species (Figueroa *et al.* 1997, Fernández-Baco *et al.* 1998).

Stomatal closure is a protective mechanism that reduces water losses during periods of limited water availability, but it also reduces photosynthetic activities by decreasing intercellular concentrations of CO_2 (Demmig-Adams *et al.* 1989, Epron *et al.* 1992). Another consequence is the production of a non-assimilatory electron flow which needs a protective mechanism (Wu *et al.* 1991, Schreiber *et al.* 1995).

Under the same climate and soil characteristics, different types of scrub vegetation coexist in Doñana National Park, with a spatial distribution determined only by water availability (González Bernáldez *et al.* 1975, García Novo 1997). *H. halimifolium* is the only shrub species which is present with significant cover in the different communities of the park, presenting diverse morphological and physiological characteristics depending of the area.

The aim of this study was to examine changes in the photochemical efficiency of PS2 in the two most contrasted seasons of the Mediterranean climate (summer and winter) in three sites of Doñana National Park with different soil water availability, by examining the diurnal changes in individual Chl fluorescence parameters in plants where Ψ and g_s cycles were known in detail (García Novo *et al.* 1996, Díaz Barradas *et al.* 1999).

Materials and methods

Doñana National Park is located in SW Spain facing the Atlantic Ocean. It has a Mediterranean type climate with some oceanic influence. Monthly average temperature is 9.3 °C in winter and 23.9 °C in summer. Annual rainfall lies around 600 mm (80 % concentrated between October and March). Summer drought is severe, with no precipitation during July and August. The Park, with an extension of 77 620 ha, includes three main ecological units: marsh, mobile dunes, and stabilised sands (García Novo 1979). The topography of the stabilised sands shows a series of arched ridges (old dunes) separated by flat depressions. The sands are dominated by a Mediterranean scrub, that has been ecologically studied by Allier *et al.* (1974), González Bernáldez *et al.* (1975a,b), García Novo (1977), Ramírez Díaz and Torres Martínez (1977), Díaz Barradas and García Novo (1990); the studies were summarised by García Novo and Merino (1993, 1997).

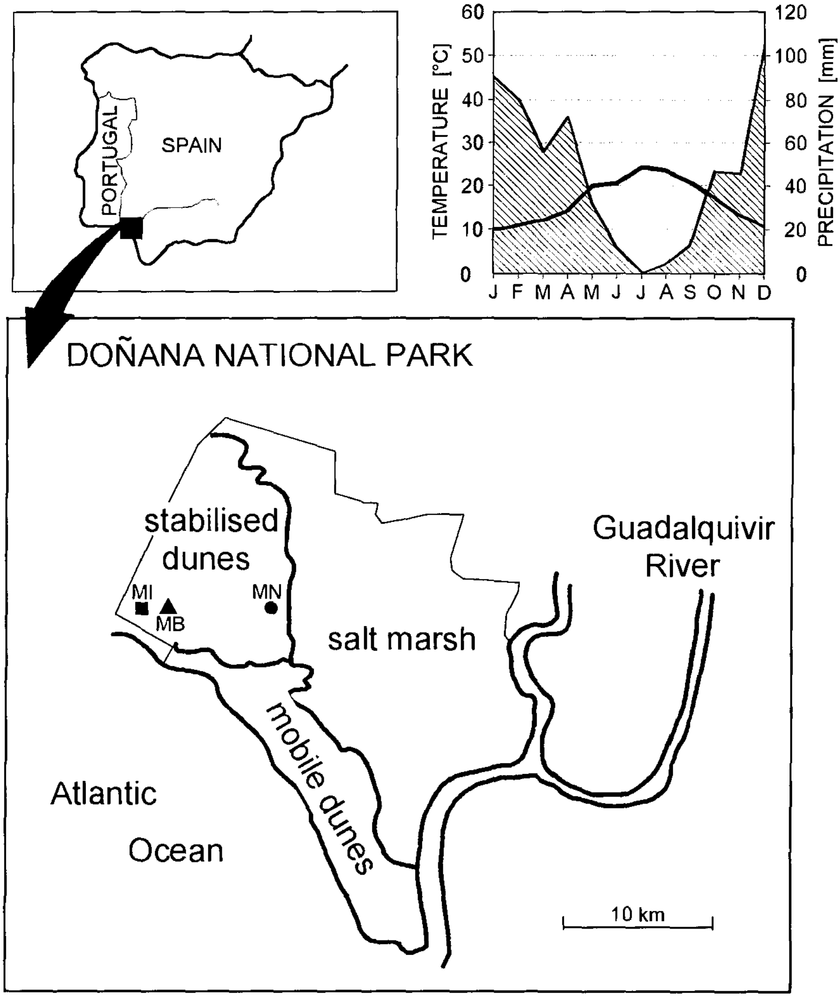

Fig. 1. Location of Doñana National Park in the SW of Spain with location of the sample sites within the Park. Ombrothermal diagram of the area is also shown.

Three main types of scrub are found in the stabilised sands, depending of the depth of the water table (see Table 1): Monte Blanco (MB) is located on top of the dune ridges. The vegetation is dominated by *Juniperus phoenicea, Halimium commutatum, Rosmarinus officinalis,* and *Cistus libanotis*. Monte Negro (MN) is located in the depressions. The vegetation is dominated by *Erica scoparia, Erica ciliaris, Ulex minor, Calluna vulgaris,* and *Cistus salvifolius*. Monte Intermedio (MI) is located on the slopes of the dune ridges, the vegetation is dominated by *H. halimifolium* and *Ulex australis*. Experimental sites were chosen in these three localities (Fig. 1). All three study sites shared a similar temperature, relative air humidity, irradiance, and sandy soil. The main differentiating feature was water availability, that was determined by the depth of the water table. Measurements were carried out in the months of September (when the accumulated summer stress showed the most marked consequences) and December (winter period).

Table 1. Water table depth (in winter and in summer) and scrub standing crop dry mass (average ± standard errors), for 10-12-year-old communities. Values from Garcia Novo (1979), Merino and Martín Vicente (1981), and Merino *et al.* (1990).

Type of scrub	Water table Summer depth	Winter depth	Standing crop [g m^{-2}]
Monte Blanco	2 to 3 m	over 3 m	500±250
Monte Intermedio	1 to 2 m	1.5 to 3.0 m	1300±350
Monte Negro	0 to 0.5 m	0.5 to 1.5 m	2200±500

H. halimifolium, a *Cistaceae* species with pubescent leaves and large yellow flowers, was chosen for this study. The high ecological plasticity of this species (Díaz Barradas *et al.* 1999) allows it to colonise different areas of the stabilised sands as well as the mobile dune system. The morphological features of *H. halimifolium* at the stabilised sands of Doñana National Park were compiled in Specht (1988), and the seasonal changes of canopy structure of this species at the mobile dune system by Díaz Barradas and García Novo (1990).

The soil water status of *H. halimifolium* in the study sites was the following: in MN, ground water was available always in summer, in MI only in wet summers, and in MB water availability depended on rainfall supply, because plant roots never reached the phreatic table and had to use the water of soil profile (Martínez García and Rodríguez 1988, Martinez *et al.* 1998). Summer descent of water table level did not affect plants growing in MB similarly as it affected individuals of MI.

Ψ was determined at predawn, midday, and late evening in each site in a set of seventeen randomly selected individuals. These moments of the day were chosen as they were those when the greatest contrasts occurred, with maximum variations. Plants were marked, so that the same plants could be measured every time. Measurements were made monthly throughout 1994 (García Novo *et al.* 1996, Díaz Barradas *et al.* 1999) with a pressure chamber (Scholander *et al.* 1965) using terminal shoots. g_s was measured at midday using a portable porometer *MK3* (*Delta-Device*). Three fully expanded leaves were measured at each chosen plant.

Chl fluorescence kinetics of intact leaves was measured at the prevailing air temperature using a portable non-modulated fluorimeter (Plant Stress Meter, *PSM*

Mark II, Biomonitor S.C.I. AB, Umeå, Sweden) and a white light-weight clamp cuvette (*Biomonitor 1020*) for dark adaptation (Öquist and Wass 1988). Leaves, on the same twenty marked individuals, were dark pre-treated for 20 min before measurement. The time used for dark adaptation of the leaves was sufficient to allow complete relaxation of the fast relaxing component of total non-photochemical quenching, mainly due to non-radiactive energy dissipation process (Herppich and Peckmann 1997). An actinic irradiance of 400 µmol m^{-2} s^{-1} was used to reduce all the electron acceptors of PS2 at the P-peak of the fluorescence induction. In each sampling site five randomly chosen leaves were measured in each marked plant.

Initial fluorescence (F_0) was determined when all PS2 reaction centres were open and the rate of photochemistry was not limited. F_0 depends on the size of the PS2 Chl antenna and on the functional integrity of PS2 reaction centres (Krause and Weis 1991). Superimposing a flash of actinic radiation yielded a peak of Chl fluorescence (F_p) dependent on the level of actinic stimulation. Variable Chl fluorescence (F_v) was determined as the change in fluorescence emission between the two defined states, F_0 and F_p. The half-time for transition from F_0 to F_m ($T_{1/2}$) was also determined. This fluorescence rise is related to the reduction rate of the first electron acceptors, Q_A, Q_B, and PQ, and has been used to determine the size of the PQ pool (Krause and Weis 1991). The ratio of variable to peak fluorescence [$F_v/F_p = (F_p - F_0)/F_p$] was used as a measure of photochemical efficiency of PS2; this ratio correlates with the number of functional PS2 reaction centres (Öquist *et al.* 1992) and is used to quantify photoinhibition (Osmond 1994, Krivosheeva *et al.* 1996).

Leaf Chl content values were determined by means of a *Minolta SPAD 500* chlorophyll meter, which had previously been calibrated with *H. halimifolium* leaves harvested in the same study sites. Each sample was put in a plastic bag, kept in darkness, and transported to the laboratory at an approximate temperature of 4 °C. Chl was extracted from each sample with 80 % acetone and determined spectrophotometrically following Lichtenthaler (1987). Values are the means of 20 leaves per marked plant.

Environmental variables were computed with each recording. These were air and soil temperature (with an electronic sensor connected to a *LI-1000* data logger), relative humidity (with the porometre), and midday PFD (Photosynthetic Flux Density) with a quantum sensor (model *Li-190 SB Li-Cor*) connected to a *Li-1000* data logger.

Changes of water table depth were computed in MN and MI sites by means of electric probe that was introduced into PVC tubes of 6 cm diameter, placed in the centre of each site between 1.5 and 5.0 m depth to record groundwater levels. Rainfall was measured with a Hellman's pluviometer with 200 cm^2 of collecting surface, located in the area.

A two way ANOVA ($n = 51 \times 2 = 102$) was used to detect significant differences among response patterns of plants growing in MN, MI, and MB, using Ψ, g_s, and the different PS2 fluorescence measurements (F_v/F_p, F_0, $T_{1/2}$). Means were compared using the Tukey test. Paired *t*-test was used to test differences between sites and solar times during the diurnal cycle. Ψ, g_s, and photochemical efficiency of PS2 were compared by means of correlation tests.

Results

Fig. 2 shows the variation of environmental variables throughout the year 1994: the absence of rainfall in summer was accompanied by a strong increase of temperature, which caused a pronounced water deficit in the vegetation. PFD, air temperature, and air and soil relative humidities were similar in the three areas. Water was always available to vegetation at MN, but never at MB.

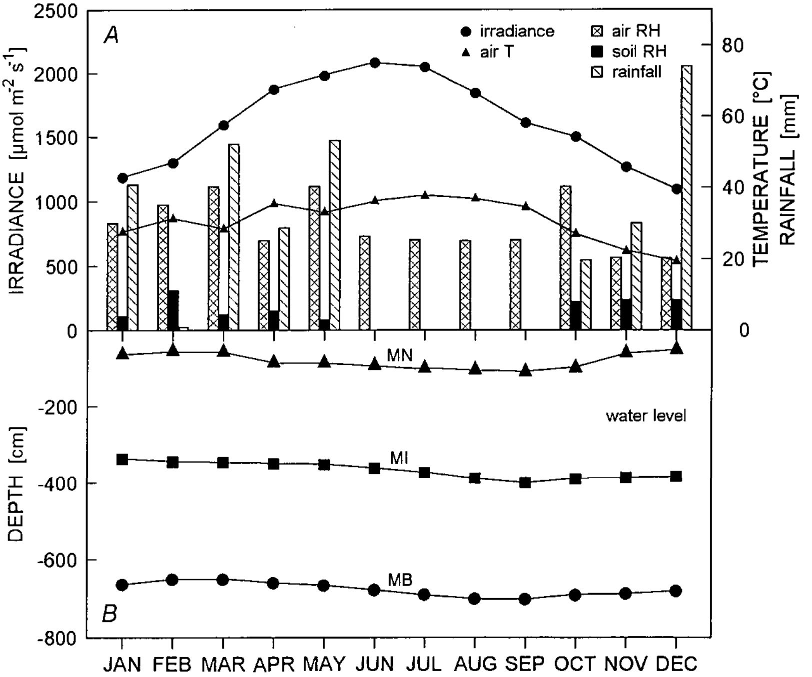

Fig. 2. *A*: Temporal changes of the environmental variables: irradiance, air temperature (T) [°C], air and soil relative humidity (RH) [%]. *B*: Water table level throughout 1994 in the three sites, Monte Blanco (MB), Monte Intermedio (MI), and Monte Negro (MN).

During summer, differences in Ψ were significant among the three populations (Table 2). The lowest Ψ values were found in MI and the highest in MN stands (Fig. 3). A similar pattern was observed in midday g_s, MI plants showed the minimum values, while MN showed the highest ones (Fig. 4).

Significant differences (Table 2) appeared between predawn mean values of photochemical efficiency at MN (0.84) with respect to the other two populations (0.75) (Fig. 5). At midday, suboptimal values were found in the three populations (between 0.71 in MN, and 0.65 in MB and MI). Recovery after midday decrease of photochemical efficiency was almost complete some hours after irradiance decreased in MN, but this did not occur in MI or MB. Leaf Chl content in summer was lowest in MB and highest in MN (Table 3).

Table 2. Two way ANOVA results.

		dF	F	P
Photochemical efficiency	Site	2	4.165	0.001
	Month	1	3.025	0.001
	Site×month	2	3.535	0.001
Resistance	Site	2	23.123	0.000
	Month	1	82.021	0.000
	Site×month	2	15.642	0.000
Leaf water potential	Site	2	7.656	0.000
	Month	1	28.202	0.000
	Site×month	2	4.511	0.000

Table 3. Chlorophyll content of *Halimium halimifolium* leaf in the three study sites.

	Summer period [g kg^{-1}]	[g m^{-2}]	Winter period [g kg^{-1}]	[g m^{-2}]
Monte Blanco	0.78±0.08	2.03	1.95±0.59	3.8
Monte Intermedio	0.90±0.15	2.34	2.00±0.62	4.3
Monte Negro	1.10±0.11	2.86	2.05±0.68	3.8

During winter, water availability in all three areas did not show significant differences, neither in Chl fluorescence, nor in Ψ and g_s. Only in MB it was possible to detect a slight decrease in midday photochemical efficiency of PS2 and a slower nocturnal recovery. Winter leaf Chl content was similar in the three populations, with an average value of 2.00±0.62 g kg^{-1}.

$T_{1/2}$ values in MN showed little variation throughout the day in both periods, while in MB and MI the variations were larger. In MI this variable underwent a slight increase during the day in summer and important daily fluctuations during winter. Variations in MB were smaller, especially in winter, when they were minimum (Fig. 5).

Basal Chl fluorescence (F_0) values remained almost constant in the three study areas in both periods. Only a slight increase was recorded in MN, as well as a decrease in MB, both at midday during the summer. In the winter, MN showed a progressive decrease throughout the day (Fig. 6).

When comparing summer with winter results of the photochemical efficiency of PS2, the lowest daily values were found at midday in both seasons. In predawn and dusk, the winter values were higher in MI and MB but the opposite occurred in MN. In MN, dusk and predawn photochemical efficiency values were lower than in summer, while midday photochemical efficiency showed very little variation (Figs. 3 to 5). Seasonal contrasts in Ψ were highly significant, with less negative values in winter for the three areas at predawn, midday, and dusk. The g_s values were lower and similar in winter, and higher and significantly different in summer.

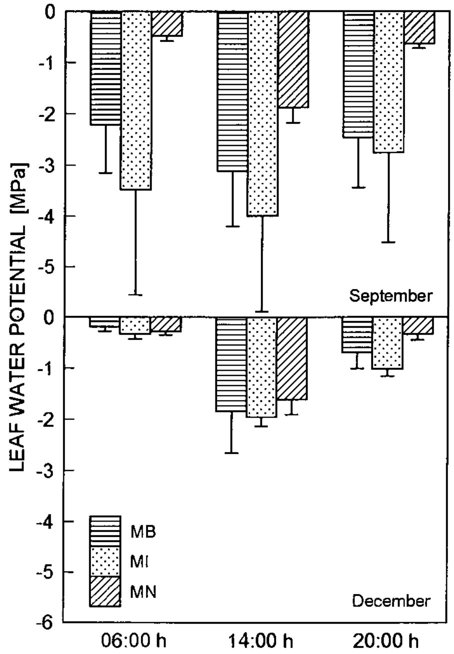

Fig. 3. Summer and winter leaf water potential [MPa] during day in the three sample areas Monte Blanco (MB), Monte Intermedio (MI), and Monte Negro (MN).

There was a correlation (Fig. 7) between photochemical efficiency and Ψ (individual values of photochemical efficiency in the three sites at predawn and midday and the logarithm of Ψ), with $p<0.001$ and $r^2 = 0.63$. The photochemical efficiency of PS2 decreased linearly together with Ψ. The correlation between g_s and PS2 fluorescence was not significant.

Discussion

H. halimifolium is a semi-deciduous shrub species that has developed different strategies to maximize its carbon gain and to avoid damage due to water stress and excessive irradiance in the different areas of Doñana National Park.

In both seasons a marked and reversible decrease in photochemical efficiency of PS2 occurred at midday. At the end of summer, the depression seemed to be related to the combination of high irradiance and water scarcity. The winter decrease of photochemical efficiency of PS2 could be related with low temperature in sunny days, when photosynthetically active radiation was fairly high. For similar results in Mediterranean grasslands species see Figueroa *et al.* (1997) and Fernández Baco *et al.* (1998).

The minimum F_v/F_p values recorded in September (0.65) were higher than those recorded for other *Cistaceae* (Werner *et al.* 1998) or for other scrub species

(Valladares and Pearcy 1997) in summer in Mediterranean climate, what supports the possible existence of efficient protecting mechanisms in this species. MN plants, not water deprived in summer, showed the lowest F_v/F_p values in winter in the three recordings. Hence the main stressing factor in clear winter days is probably cold in combination with high irradiance (1 660 μmol·m^{-2} s^{-1}).

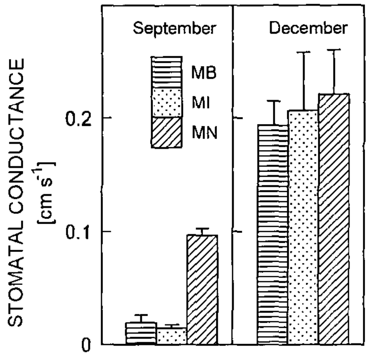

Fig. 4. Summer and winter midday stomatal conductance [cm s^{-1}] in the three sample areas Monte Blanco (MB), Monte Intermedio (MI), and Monte Negro (MN).

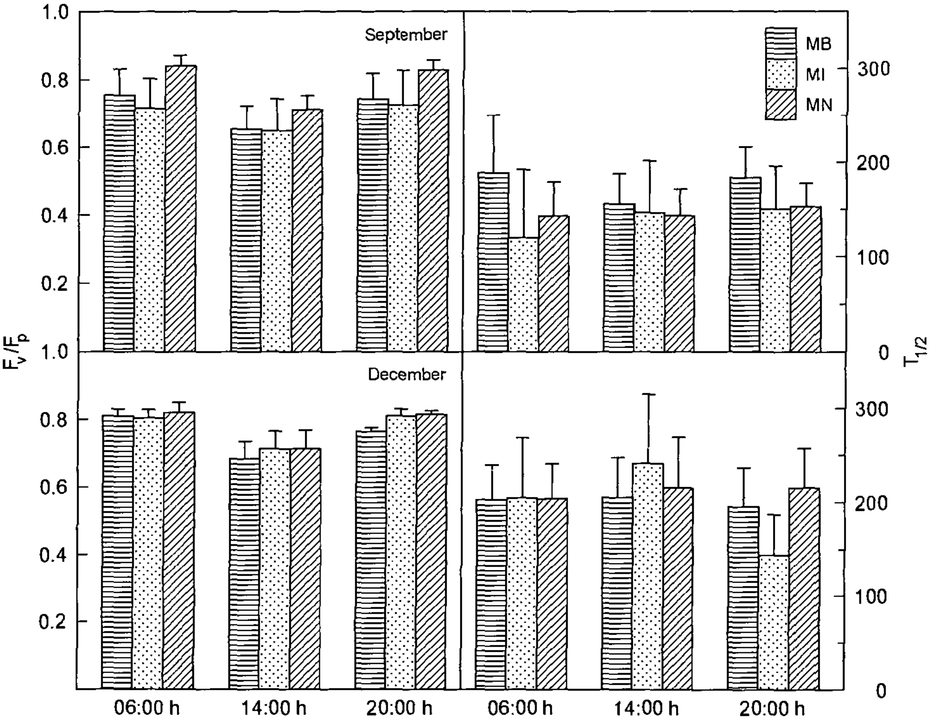

Fig. 5. Summer and winter photochemical efficiency of PS2 (F_v/F_p) and $T_{1/2}$ daily cycles in the three sample areas Monte Blanco (MB), Monte Intermedio (MI), and Monte Negro (MN).

The analysis of other Chl fluorescence typical parameters (F_0, F_v, F_p, and $T_{1/2}$) revealed that under presumably greater environmental summer stress (September), the evidence of photoinhibition reflects the existence of different photoprotection mechanisms to avoid damage of PS2 reaction centres.

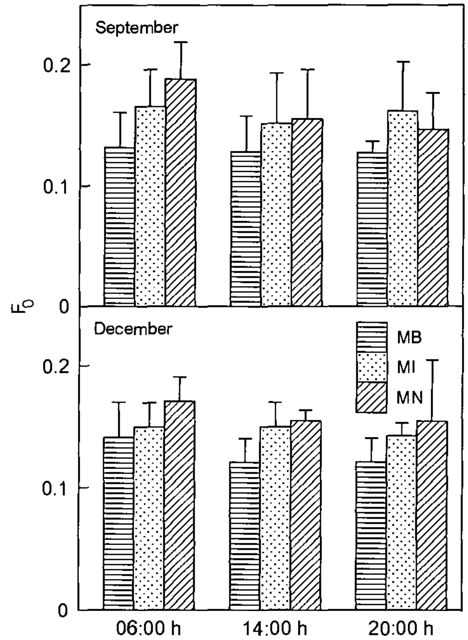

Fig. 6. Summer and winter initial Chl fluorescence (F_0) daily cycles in the three sample areas Monte Blanco (MB), Monte Intermedio (MI), and Monte Negro (MN).

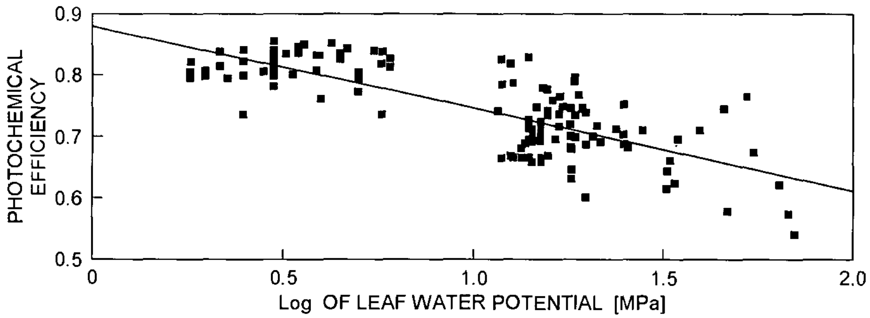

Fig. 7. Linear regression between photochemical efficiency of PS2 (F_v/F_p) and leaf water potential (individual values of photochemical efficiency in the three sites at predawn and midday and the logarithm of leaf water potential absolute values).

In all three populations studied, midday drop of photochemical efficiency of PS2 was mainly due to lower F_p values. The F_v decrease suggests the occurrence of photoinhibition, as caused by closure of reaction centres (Schreiber and Bilger 1987, 1993). The small variations that F_0 undergoes throughout one daily cycle, even its decrease in some cases (at midday in MB individuals) reveal that no structural damages occur at the level of PS2, and that thermic dissipation of absorbed radiant energy is increasing (Krause 1988, Bolhàr-Nordenkampf *et al.* 1991). This determines a difference among the three populations studied, showing greater energy dissipation effectiveness in MB than in the other two areas. This suggests that the studied species possess highly efficient protection mechanisms, which function even in the most stressing conditions. These mechanisms are both internal (thermal deactivation and a high deactivation rate by photochemical ways) and external, reducing the amount of radiation intercepted by the leaves. An important strategy to avoid excessive damaging irradiance in *H. halimifolium* is the structural regulation of radiant energy interception: changes in leaf pubescence, which increase leaf reflectance (Díaz Barradas and García Novo 1988), leaf dimorphism, and leaf angle, mainly vertical in summer (Díaz Barradas 1988).

The high photochemical deactivation rate is deduced by the almost constant values of $T_{1/2}$ throughout the daily cycle, what shows that energy transmission between photosystems is not interrupted or limited even under highest irradiance (Fig. 5). In the same sense, the xanthophyll cycle (in case of excessive excitation energy, violaxanthin is de-epoxidized to zeaxanthin *via* antheraxanthin) can play an important role in the dissipation of the excess of excitation energy (Demmig-Adams and Adams 1992, Ball *et al.* 1995, Demmig-Adams *et al.* 1995).

At the end of summer, leaf Chl content and PS2 photochemical efficiency were highest in MN, with lower values in MB and MI; in winter, however, all three sites showed similar values (Díaz Barradas 1988). Rhizopolou *et al.* (1991) found that young expanding leaves of four evergreen Mediterranean sclerophyll species exhibited the highest Chl content; the minimum values being reached at the end of the drought period. The decrease in Chl content in summer may be an adaptive mechanism for the avoidance of damage to the photosynthetic apparatus because of excess photons. Chl content per leaf area showed a similar pattern in September with the highest value in MN, but in winter different values were measured in each site, due to the largest width of MI leaves (Díaz Barradas *et al.* 1999) the highest Chl content was registered in this site.

Ψ of MN individuals underwent small variation along the year, while in the MB and MI individuals a drastic decrease occurred in summer, with a minimum value in September. Winter values in MB and MI were similar to the MN ones (Garcia Novo *et al.* 1996, Díaz Barradas *et al.* 1999).

The g_s values suggest that stomata close at midday so as to avoid water losses, mainly in MI and MB. Stomatal closure overrode the decrease in F_v/F_p, impeding gas exchange far more than would have occurred due to reduction at the PS2 activity alone (Valladares and Pearcy 1997). Strong limitations of net CO_2 assimilation, especially during periods of high irradiance, may promote an imbalance between photochemical activity at PS2 level and damage of PS2 reaction centre (Powles 1984, Kaiser 1987, Epron *et al.* 1992, Werner *et al.* 1999).

The significant mathematical correlation between F_v/F_p and Ψ suggests that under similar irradiance, air and soil temperatures, and air humidity in all three sites, the differences found in F_V/F_P daily cycles in *H. halimifolium* were mainly due to water availability. Although some authors doubt about PS2 sensitivity to water shortage (Havaux 1992, Jefferies 1994) because of the higher stability that thylakoid membranes acquire under these circumstances, a decrease of Ψ has in many cases been related to a drop in photochemical efficiency of PS2 (Demmig-Adams *et al.* 1989, Epron *et al.* 1992). Our results even suggest that the detected decrease of photochemical efficiency in individuals of *H. halimifolium* was related rather to water scarcity than to seasonal fluctuations of irradiance and temperature.

Our study points out the efficiency of the different protective mechanisms of *H. halimifolium* against the varied stresses of the Mediterranean climate, both internal (photoinhibition due to reaction centres closure, thermal deactivation), and external (reflectance and leaf angle, growth and structure), against excessive irradiance and temperature, and also a large capacity of regulation at different water availability, with internal (stomatal opening and water potential control) and external (structure, leaf area index, sclerophyll index, root system) mechanisms (Zunzunegui 1997, Díaz Barradas *et al.* 1999). *H. halimifolium* has more protective mechanisms than other *Cistaceae* species under the same Mediterranean climate. All these features allow *H. halimifolium* to adapt to the different fluctuating environments of Doñana National Park, combining different strategies in dependence on the area.

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