PHOTOSYNTHETICA 42 (3): 399-407, 2004

Seasonal differences in photochemical efficiency and chlorophyll and carotenoid contents in six Mediterranean shrub species under field conditions

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Abstract

The effects of summer and winter stress on the chlorophyll and carotenoid contents and photosystem 2 efficiency were examined in six Mediterranean scrub species. These six species belong to two different plant functional types: drought semi-deciduous (*Halimium halimifolium* L., *Rosmarinus officinalis* L., *Erica scoparia* L.) and evergreen sclerophylls (*Juniperus phoenicea* L., *Pistacia lentiscus* L., *Myrtus communis* L.). Two sites with different water availability were chosen. In the xerophytic site, despite they belong to two different functional types, *R. officinalis* and *J. phoenicea* showed a similar response. These were the most affected species in summer. *H. halimifolium* showed optimal values of F_v/F_m and non-significant seasonal changes in xanthophyll content. In the mesic site, *E. scoparia* and *M. communis* were apparently the most affected species by winter climatic conditions. *P. lentiscus* presented a pattern similar to *H. halimifolium*, except for elevated F_0 values. In all the studied species, lutein plus zeaxanthin content was negatively correlated with F_v/F_m in summer and with leaf water potential, thus indicating that the thermal dissipation of energy was a general pattern for all species. Under stress, plant response is more species-specific than dependent on its functional type.

Additional key words: carotenoids; chlorophyll fluorescence; photoinhibition; photosystem 2; sclerophyllous plants; semi-deciduous plants; water stress; xanthophyll cycle.

Introduction

It is assumed that even the most rapidly growing plants with the highest rates of photosynthesis do not utilise more than about half of the photons that their leaves absorb during the hours of peak irradiance in sun-exposed habitats (Björkman and Demmig-Adams 1994, Demmig-Adams and Adams 1996). This is particularly important in areas with Mediterranean climate, where high irradiance is combined with absence of precipitation and high temperatures in summer or moderate low temperatures in winter.

Under summer clima, stomata closure is a strategy to reduce water loss. This behaviour, found in many species (Lange *et al.* 1987, Demmig-Adams *et al.* 1989, Sobrado 1990, Epron *et al.* 1992), contributes to a reduction of carbon assimilation during the warmest period of the day.

This CO_2 uptake limitation contributes to an absence of a sink for the assimilated energy (Valladares and Pearcy 1997), and may cause chloroplasts to be subjected to an excess of energy resulting in the down-regulation of photosynthesis or in photoinhibition (Demmig-Adams and Adams 1996). In winter, low temperatures reduce the capacity of photosynthetic systems to utilise incident radiation, leading also to a photoinhibition process (Jung and Steffen 1997).

Photoinhibition is evident through the reduction in the quantum yield of photosystem 2 (PS2) and a decrease in variable chlorophyll (Chl) *a* fluorescence (Demmig and Björkman 1987). The decrease of efficiency of PS2

Received 15 June 2004, *accepted* 12 July 2004.

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Abbreviations: β-Car, β-carotene; Car, carotenoids; Chl, chlorophyll; DM, dry mass; F_0, initial fluorescence yield of dark-adapted leaves; F_m, maximal fluorescence yield of dark-adapted leaves; F_v/F_m, photochemical efficiency of photosystem 2; L+Z, lutein+zeaxanthin; NPQ, non-photochemical quenching of Chl fluorescence; PPFD, photosynthetic photon flux density; PS, photosystem; V, violaxanthin; Φ_{PS2}, quantum yield of photosystem 2.

Acknowledgements: The present work was supported by a project of the Fundación Ramón Areces (Spain). XII Concurso Nacional.

photochemistry under stress may reflect not only the inhibition of PS2 function, but also an increase in the dissipation of thermal energy (Demmig-Adams and Adams 1992a). The latter is often considered as a photoprotective mechanism.

The ability to dissipate such excess of excitation energy *via* non-photochemical quenching (NPQ) occurs together with an increase in the concentration of zeaxanthin in several species (Demmig *et al.* 1988, Björkman and Demmig-Adams 1994). This relationship strongly suggests that this xanthophyll plays a central role in the non-radiative energy dissipation process (Demmig *et al.* 1987, Bilger and Björkman 1990). Under excess of photons, violaxanthin (V) is de-epoxidised rapidly *via* the intermediate antheraxanthin to zeaxanthin. Under low irradiances or in the dark, zeaxanthin is epoxidised back to V, completing the so-called xanthophyll cycle which has been extensively studied by Gilmore and Yamamoto (1993), Demmig-Adams and Adams (1996), and Gilmore (1997). The xanthophyll cycle is a flexible process that responds not only to irradiation (diurnal fluctuation, sunflecks, sunshade), but also to a combination of other environmental factors, including temperature, water deficit, and nutrient availability (Demmig-Adams *et al.* 1996).

The Mediterranean climate, with its characteristic seasonality concerning the parameters most influencing plant growth (water, irradiation, temperature), provides an useful opportunity to study the role of carotenoids (Car) in the adaptation of plants to their environment. Several authors have studied the role of the xanthophyll cycle in Mediterranean woody species, *e.g.* Demmig-Adams *et al.* (1989) in *Arbutus unedo*, Faria *et al.* (1996) in four tree species, García-Plazaola *et al.* (1997) in *Quercus*, Kyparissis *et al.* (2000) in eight scrub species, and Munné-Bosch and Alegre (2000a) in *Lavandula stoechas.*

Under a Mediterranean climate, two different syndromes or plant functional types can be distinguished in shrub species: evergreen sclerophylls and drought semi-deciduous. They differ in their physiological and structural adaptations to cope with the stress factors (Gill and Mahall 1986, Werner *et al.* 1999, 2001). From an evolutionary point of view, sclerophylls from the Mediterranean basin have a tertiary tropical origin, against semi-deciduous, which have a quaternary Mediterranean origin (Herrera 1984, 1992, Verdú 2000).

The aims of this work were to assess the seasonal patterns in two critical periods of the year on six Mediterranean shrub species (three semi-deciduous and three sclerophylls) and to check whether species of the two syndromes exhibited a common response. The concentration of Car and chlorophyll (Chl) pigments, photochemical efficiency, non-photochemical quenching, and leaf water potential were measured with this purpose. We hypothesised that species of tropical origin (sclerophylls from Mediterranean basin) would be more sensitive to winter photoinhibition than semi-deciduous plants. Besides, the species from the dry area would need to be able to withstand the combination of summer stressing factors independently of the functional type they belong to.

Materials and methods

Study area: Doñana National Park, which is located in SW of Spain, facing the Atlantic Ocean, has a Mediterranean type climate with some oceanic influence. Monthly average temperature is 9.3 ºC in winter and 23.9 ºC in summer. Annual rainfall lies around 600 mm, but can range between 300 and 1 000 mm, depending on the year. Summer drought is severe, with no precipitation during July and August. On the stabilised sands, a large water table depth gradient underlies in association with the general topography from higher dunes to the marsh. Small-scale gradients of water table depth occurring in association with the dune topography were described by Allier *et al.* (1974) and Muñoz Reinoso (2001). Temporal dynamics in relation to rainfall fluctuation were analysed by Zunzunegui *et al.* (1998). Different types of scrub communities were identified on the stabilised sands in response to the general water table level gradient (García Novo 1997, García Novo and Merino 1997). Hygrophytic community, in the depressions, is dominated by *Erica scoparia* L., *Erica ciliaris* L., *Myrtus communis* L., *Pistacia lentiscus* L., *Ulex minor* L. Roth, and *Cistus salvifolius* L. Xerophytic community, on the top of the dunes, is dominated by *Juniperus phoenicea* L., *Halimium commutatum* L., *Rosmarinus officinalis* L., *Cistus libanotis* L., and *Stauracanthus genistoides* L. Intermediate community, on the dune slopes consists of *Halimium halimifolium* L. and *Ulex australis* L. The study was completed in Acebuche de Matalascañas, where the depth of the water table allowed us to readily identify the different scrub communities (Fig. 1).

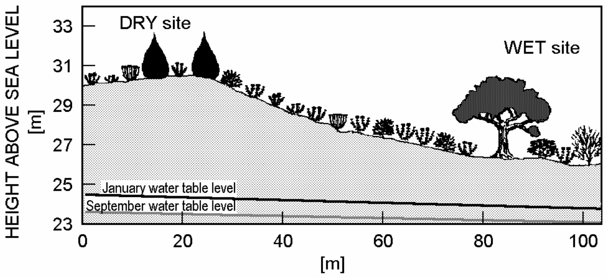

Fig. 1. Temporal variation of ground water table depth throughout the study period and approximate location of main species in the studied area. Pointed crown *Juniperus phoenicea*, round crown *Quercus suber*, other crowns are different scrub species.

Plants: Six species were selected for the study, in the xerophytic area: *H. halimifolium*, *J. phoenicea*, and *R. officinalis*, and in the hygrophytic area *H. halimifo-*

lium, P. lentiscus, *M. communis*, and *E. scoparia*. One population of each species was studied, except for *H. halimifolium* where two populations, one in the wet site and one in the dry site, were studied. Five plants of each population were randomly marked for the study. Field sampling was carried out when the most contrasting climatic conditions occurred, in February 1999 (low temperature and high irradiance) and in September 1999 (high irradiance, high temperature, and water deficit). According to Herrera (1984, 1992), the distribution of the seven selected populations into the two plant functional types is shown in Table 1.

Table 1. Distribution of the selected species into the two plant functional types (PFTs) in the studied areas.

Area	PFT I (semi-deciduous)	PFT II (sclerophyllous)
Xerophytic	*R. officinalis* *H. halimifolium*	*J. phoenicea*
Hygrophytic	*E. scoparia* *H. halimifolium*	*P. lentiscus* *M. communis*

Environment: Photosynthetic active photon flux density (PPFD) was recorded in the field with a quantum sensor (model *Li-190 SB*, *LI-COR*), connected to a data logger *Li-1000*, at solar midday on each sampling date. Data for annual precipitation and daily maximum and minimum temperatures were obtained from the Meteorological Station of Doñana National Park. Water table depth was measured by means of an electric portable probe introduced into 1.5 to 6.0 m deep PVC piezometers that were placed for this purpose in the centre of the sampling plots (Serrano and Toja 1995).

Chl fluorescence kinetics of leaves were determined by means of the pulse-amplitude modulation technique using a portable fluorometer (mini-PAM, *Walz*, *Effeltrich*, Germany) *in situ* on five south facing attached leaves per plant at midday. Fluorescence was excited by a pulse of modulated red radiation from a light-emitting-diode (LED, type *H-3000 Stanley*) connected to a fibre optics.

The optimal photochemical efficiency of photosystem 2 (PS2) was determined from the ratio of variable to maximal fluorescence, *i.e.* $F_v/F_m = (F_m - F_0)/F_m$, where F_0 and F_m are initial and maximal fluorescence of dark-adapted leaves during 20 min, a period sufficient to allow complete re-oxidation of the PS2 reaction centres. F_0 was determined with a modulated measuring radiation from a LED at a frequency of 600 Hz. F_m was obtained with a brief saturating pulse.

The effective quantum yield (Φ_{PS2}), or photochemical efficiency of PS2 in a light adapted state, was estimated on light-adapted leaves with a distance clip according to Genty *et al.* (1989) as $\Phi_{PS2} = (F'_m - F)/F'_m$ (F'_m = maximal and F = steady-state fluorescence under actinic irradiation). The leaf was fixed in such a way that the measuring fibre optics and the clip did not shade onto the leaf surface at the moment of the measurement. The fibre optics was adjusted to an angle of 60º from the leaf surface while measuring the fluorescence yield in the light-adapted state.

The non-photochemical quenching of Chl fluorescence (NPQ) was estimated from Chl fluorescence data according to Schreiber *et al.* (1995) as $NPQ = F_m - F'_m/F'_m$ in the same leaves which previously had been measured in the dark-adapted state.

Pigment determination: For all pigment analyses, leaves were collected around midday, immediately frozen in liquid nitrogen, and stored at –24 ºC until analysis.

For Car analysis, samples were first ground in a mortar in liquid nitrogen, followed by grinding in cold 85 % acetone. Extracts were analysed using the high performance liquid chromatography (HPLC) described by Mínguez-Mosquera *et al.* (1992). Samples were centrifuged to discard particulate residues prior to injection into the liquid chromatograph. The separation was performed on a *Nova-Pak C18* column (3.9×150.0 mm, 4 µm particle size, 6 nm pore size) containing dimethyl octadecyl silyl bonded to amorphous silica, protected with a guard cartridge (4 µm particle size, sealed with 2 µm filters). The pigments were eluted at a rate of 16.7 $mm^3\ s^{-1}$, and were detected by measuring the absorbance at 360–700 nm, using a programmable photodiode-array detector (*Waters 991*). Standards of β-carotene were provided by *Hoffmann-La Roche*, Switzerland. Standards of V were from Dr. L. Lubián, Instituto de Ciencias Marinas, CSIC, Cadiz, Spain. Lutein and β-carotene (β-Car) were supplied by *Sigma* (St. Louis, MO, USA). As juniper's leaf area was difficult to measure, the pigment contents were expressed on a Chl basis. Lutein and zeaxanthin under these conditions were eluted together, because their respective absorbances are very close and our column was not able to separate the two pigments.

Chl and total Car were extracted from fully expanded leaves with 100 % acetone, under low irradiance, and quantified spectrophotometrically by measuring the absorbancy at 661.4, 644.8, and 470.0 nm according to Lichtenthaler (1987). Pigment analysis was performed on 3 different samples for each population in each season.

Water status: Leaf samples were collected, stored at 4 ºC, and transferred to laboratory, where fresh mass was measured. They were then dried at 80 ºC for 24 h for dry mass (DM) determination. Leaf water potential at midday was determined in the field using a pressure chamber of the Scholander type (Scholander *et al.* 1965). The five plants marked in each population were measured.

Statistics: The results were subjected to an analysis of variance by site and by populations (two-way ANOVA). To detect significant differences among population response to winter and summer conditions, the Tukey's test was used. Means were compared with the Duncan test. The analyses were carried out using the statistical SPSS computer program. Regressions and correlations were calculated between F_v/F_m, leaf water potential, and L+Z.

Results

Environmental factors: The seasonal pattern is characteristic of a Mediterranean type climate, with no rainfall and elevated temperatures during the summer, and relatively wet and cold winter (Fig. 2). PPFD is very high in summer, reaching a maximum midday value of 1 700 µmol m^{-2} s^{-1} during the measurement day (2 000 µmol m^{-2} s^{-1} in June). PPFD was also high during winter clear days, reaching 1 300 µmol m^{-2} s^{-1} (these clear days are usually also the coldest days of the year with temperatures close to 0 °C at dawn).

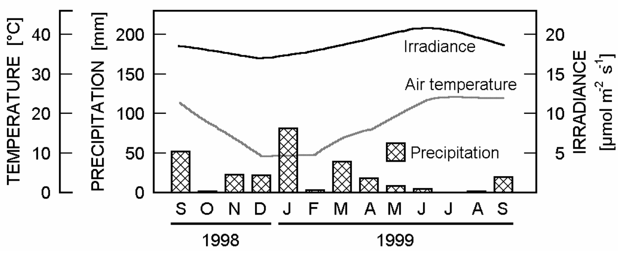

Fig. 2. Climatic diagram of the area (average temperature, monthly rainfall, and PPFD) during the study period.

Chl fluorescence: The maximum efficiency of PS2 photochemistry (F_v/F_m) varied from one species to another, even though they shared the same site (Fig. 3). At the dry area, *R. officinalis* and *J. phoenicea* showed significant ($p<0.001$) decreases in summer, while *H. halimifolium* presented optimal values in both seasons. At the wet area, the species exhibited different patterns: while *H. halimifolium* presented optimal values in both seasons, in *P. lentiscus* F_v/F_m decreased in summer, and in *E. scoparia* and *M. communis* the ratio decreased in winter. *M. communis* was the only species which showed a very low photochemical efficiency in winter ($p<0.4$).

The winter values of F_0 were close to 200 in all species; only *P. lentiscus* had higher values, close to 400. In summer, all semi-deciduous species and *J. phoenicea* showed a decrease of F_0 ($p<0.001$), whilst it increased in *M. communis* and *P. lentiscus* (Fig. 3).

The quantum yield of PS2 of light-adapted leaves showed variation within the same population due to differences in PPFD among leaves (Fig. 3). In the dry area, there was a significant decrease in summer ($p<0.001$) for all species, with *R. officinalis* leaves having the lowest values ($p<0.1$). In the wet area, *H. halimifolium, E. scoparia*, and *P. lentiscus* showed a more or less pronounced summer decrease, while *M. communis* presented the opposite pattern, with a decrease in winter.

The thermal dissipation of energy, which was measured as NPQ, showed considerable variation within the same species due to differences in PPFD and in leaf position among individuals (Fig. 3). In all populations, except in *H. halimifolium* from the hygrophytic area, energy dissipation increased in summer ($p<0.05$), coupled with the maximum irradiance.

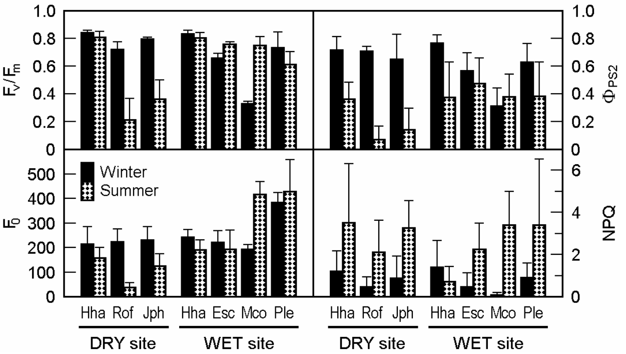

Fig. 3. Mean values ± SD of the maximum photochemical efficiency of photosystem 2, PS2 (F_v/F_m) of dark-adapted leaves, initial fluorescence (F_0) of dark-adapted leaves, effective quantum yield (Φ_{PS2}), or photochemical efficiency of PS2 in a light-adapted state and non-photochemical quenching (NPQ) of Chl fluorescence in winter and summer for the seven studied populations (*H. halimifolium*: Hha; *J. phoenicea*: Jph; *R. officinalis*: Rof; *P. lentiscus*: Ple; *M. communis*: Mco; *E. scoparia:* Esc).

Chl content was significantly different among populations ($p<0.001$), even if they shared the same site (Fig. 4). In the dry site, Chl contents decreased in the three populations during summer, *H. halimifolium* showing the highest values and *J. phoenicea* the lowest ones in winter. In the wet site, also *H. halimifolium* and *P. lentiscus* decreased their Chl contents in summer, however, *E. scoparia* and *M. communis* showed the opposite pattern, increasing their Chl contents in summer. *E. scoparia* exhibited the lowest Chl content in winter and *H. halimifolium* the highest one. The population of *H. halimifolium* from the dry site contained significantly less Chl in summer ($p<0.01$) than the population of the same species from the wet site. Chl *a/b* ratio decreased in summer in *R. officinalis*, *J. phoenicea*, and *H. halimifolium* from the wet site and in *E. scoparia*, while in the other populations it remained more or less constant (Fig. 4).

Car/Chl: Despite the seasonal variations in pigment contents, the Car/Chl ratio remained in the same range for most of the studied species (Fig. 4). Only *R. officinalis* leaves showed a significant increase ($p<0.001$) of this ratio in summer.

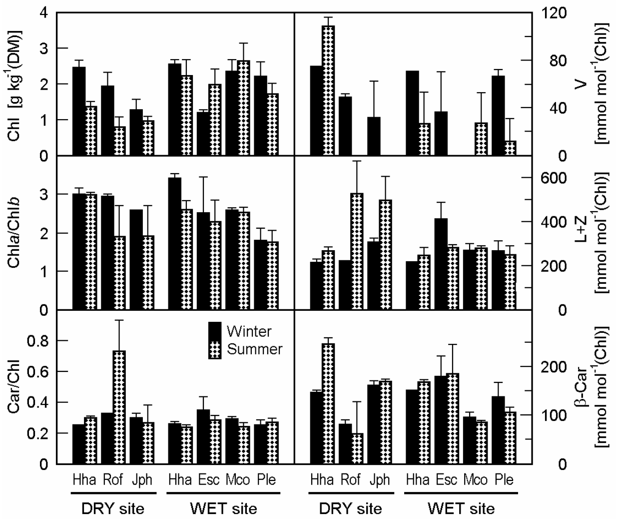

Fig. 4. Mean values ± SD of chlorophyll (Chl) content, Chl *a/b* ratio, carotenoids (Car)/Chl ratio, and of L+Z, V, and β-Car expressed on a Chl basis during winter and summer for the seven studied populations (*H. halimifolium*: Hha; *J. phoenicea*: Jph; *R. officinalis*: Rof; *P. lentiscus*: Ple; *M. communis*: Mco; *E. scoparia*: Esc).

Leaf water potential: Midday water potentials indicated that the six shrub populations were severely water stressed in September. However, this descent was more pronounced in two species of the dry area, *R. officinalis* and *J. phoenicea*, each one belonging to a different functional group (Fig. 5). Plants from the wet site showed higher leaf water potential independently of the plant functional type.

Car and xanthophylls: In summer, in *R. officinalis* and *J. phoenicea* the L+Z content increased significantly, while it decreased in *E. scoparia*. V could not be detected in these species. In winter, *E. scoparia* presented the highest values of L+Z and β-Car, while *H. halimifolium* from both sites and *P. lentiscus* showed the highest values of V (Fig. 4). Significant negative correlations ($p<0.01$) were found between F_v/F_m and the L+Z content in summer, while Car/Chl ratio was positively correlated with L+Z in both seasons (Fig. 6).

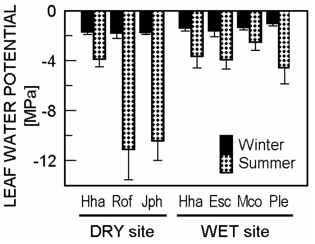

Fig. 5. Mean values ± SD of the midday water potential during winter and summer for the seven studied populations (*H. halimifolium*: Hha; *J. phoenicea*: Jph; *R. officinalis*: Rof; *P. lentiscus*: Ple; *M. communis*: Mco; *E. scoparia*: Esc).

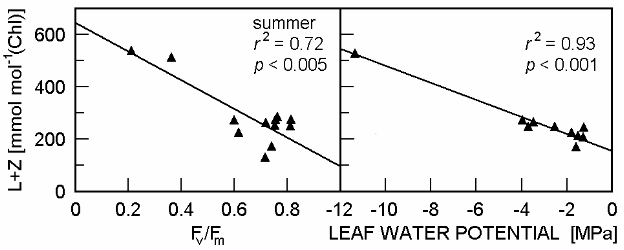

Fig. 6. Relationship of the maximum photochemical efficiency of photosystem 2 (F_v/F_m) and leaf water potential with the lutein+zeaxanthin (L+Z) content for the seven studied plant populations.

A negative correlation was found between L+Z and midday leaf water potential ($p<0.001$) in both seasons for the populations from the dry site and the semi-deciduous ones. No correlation was found for sclerophyllous populations from the wet site (Fig. 6).

Discussion

The severe conditions of Mediterranean summer affected both types of plant communities, although in the dry site, on the top of the dunes, their effects were enhanced because of the low field capacity of the sandy soil and the distance to the water table.

One semi-deciduous and one sclerophyll species, *R. officinalis* and *J. phoenicea,* apparently were the most affected by the severe environmental conditions of Mediterranean summer. These species exhibited the lowest values of F_v/F_m and Φ_{PS2}, and the highest L+Z content, while V could not be detected. Munné-Bosch and Alegre (2000b) describes this effect when a combination of high temperature, elevated radiation, and water deficit occurs. Our results suggest that all the components of the xanthophyll cycle were in a de-epoxidised state, which is associated with the harmless dissipation of excess excitation energy in the photochemical system. This was supported by the summer decrease of F_0 and the increase of NPQ values, evidencing a thermal dissipation of the absorbed energy (Gilmore and Yamamoto 1991, 1993, Demmig-Adams and Adams 1992b, Griffiths and Maxwell 1999). Kyparissis *et al.* (2000) found that in semi-deciduous species under Mediterranean summer conditions, 90 % of the xanthophyll cycle components had been converted to zeaxanthin and antheraxanthin at midday and total xanthophyll pool was maximal during summer.

During summer, *R. officinalis* showed a significant reduction in Chl content, similar to the results of Munné-Bosch and Alegre (2000b) in the same species. The reduction in Chl content in *J. phoenicea* was not significant. The decrease in Chl content is closely correlated to water deficit, elevated temperature, and excess excitation energy (Powles 1984, Hendry *et al.* 1987, Núñez-Olivera *et al.* 1996). This decrease may be caused by a degradation of Chl by photo-oxidation, and can also be regarded as a protective adaptive mechanism in stressed plants (Maslova and Popova 1993, Kyparisis *et al.* 1995, 2000). The lesser Chl content brings together a reduction of the amount of photons intercepted by leaves and also a higher capacity to dissipate excess excitation energy per PPFD intercepted (Munné-Bosch and Alegre 2000c).

The decrease in Chl *a/b* ratio recorded in *R. officinalis* and *J. phoenicea* in summer is expected under stress conditions (Núñez-Olivera *et al.* 1996, Faria *et al.* 1998), as this ratio usually increases in active tissues (Margalef 1982). This is a common pattern in Mediterranean species in summer (Núñez-Olivera *et al.* 1996, Faria *et al.* 1998). Only *R. officinalis* showed a significant increase in the Car/Chl ratio. This supports the major role that Car play in the energy dissipation process and the PS2 protection in this species under Mediterranean summer stress.

In winter, when soil water is available, photochemical efficiency and quantum yield showed optimal values and Chl content increased. A decrease in L+Z together with an increase in V was observed, independently of lower temperatures combined with high irradiance.

Although *J. phoenicea* is a sclerophyll, its response to seasonal environmental conditions in the same xerophytic site was similar to *R. officinalis,* a semi-deciduous plant. In contrast, the semi-deciduous species *H. halimifolium* was the only species able to grow in the two study sites. In both cases it showed a different seasonal pattern than *R. officinalis.* The maximum photochemical efficiency was close to optimal values at midday during both seasons, due to its structural regulation of radiant energy interception (Zunzunegui *et al.* 1999). This is achieved through changes in leaf pubescence (which increase leaf reflectance), in leaf dimorphism, and in leaf angle, mainly vertical in summer (Díaz Barradas and García Novo 1988).

The observed decrease in Φ_{PS2} at midday was completely reversible in *H. halimifolium* within few minutes of dark adaptation, which indicates that the photoinhibition observed in this species was due to photoprotective processes, as Munné-Bosch and Alegre (2000a) described for *L. stoechas*. The summer decrease of F_0 and an increase in NPQ (for the populations of the xerophytic area) suggest that no structural damages occurred at the level of PS2 and that thermal dissipation of energy took place.

In *H. halimifolium* the L+Z increase was smaller than in *R. officinalis* and *J. phoenicea*, and significant amounts of V could be detected. Probably because of the above-mentioned reasons, structural adaptations and leaf changes contribute to reduce the amount of absorbed energy.

Chl content decreased in summer for both populations, as described by Díaz Barradas *et al.* (1999), but with a significant higher decrease for the population of the xerophytic area, which can also be a photoprotective mechanism to maintain optimal photochemical efficiencies all over the year (Maslova and Popova 1993, Kyparissis *et al.* 1995, 2000). These slight seasonal differences in physiological variables measured in both populations (xerophytic and hygrophytic) suggest a plastic response of this species to climatic conditions (Díaz Barradas *et al.* 1999).

In the hygrophytic area, the studied species (*P. lentiscus*, *M. communis*, *H. halimifolium*, and *E. scoparia*) do not usually suffer water stress during summer because their roots can reach the ground water table (Merino *et al.* 1976, 1995, Zunzunegui *et al.* 2000). However, they did show remarkable species-specific variations in their response to seasonal conditions independently of the functional type.

P. lentiscus presented a similar seasonal pattern as *H. halimifolium*, showing the highest F_0 values in both seasons, thus suggesting a mechanism of energy dissipation *via* fluorescence. The same pattern occurred in *M. communis* in summer.

A winter depression of F_v/F_m was found in *E. scoparia* and especially in *M. communis*, together with a decrease in Chl content. This depression was also found by Karavatas and Manetas (1999) in Mediterranean sclerophyllous species and also in a variety of species during cold winter days (Adams and Demmig-Adams 1994, 1995, Adams *et al.* 1994). Several authors have suggested that Mediterranean evergreen sclerophylls are particularly sensitive to mildly low temperatures (Kyparissis *et al.* 2000). Based on bio-geographical criteria, Mitrakos (1980) proposed that mildly cold Mediterranean winters may limit the growth and distribution of evergreen sclerophylls and Verdú (2000) suggested a tropical origin for these species.

L+Z content in these species did not present significant seasonal changes as compared with species from the xerophytic area. Only *E. scoparia* showed a winter maximum which was correlated with a decrease in F_v/F_m. Hurry and Huner (1992), Koroleva *et al.* (1994), Brüggemann and Koroleva (1995), and Thiele *et al.* (1996) showed for vascular plants that acclimation to low temperature and excess-photon stress are associated with a strong increase in the pool size of xanthophyll cycle pigments, while acclimation to excess irradiance is associated to an increase in the contents of zeaxanthin. Several authors proposed that the flexibility of the conversion state of the xanthophyll cycle in tracking temperature changes is of photoprotective significance (Kyparissis et al. 2000).

The negative correlation between L+Z and F_v/F_m (Fig. 6) found in summer and the absence of significant correlation in winter may indicate that thermal dissipation of energy is a general pattern for all the species, as found in other Mediterranean species (Faria *et al.* 1998, Munné-

Bosch and Alegre 2000a). The correlation observed between L+Z (Fig. 6) and midday leaf water potential in semi-deciduous species indicated that L+Z was induced by both high irradiance and water deficit. The decrease in these pigments observed in winter, when water deficit does not occur but high irradiance does, confirms the importance of both environmental conditions to induce L+Z formation.

In summary, no general pattern in response to summer and winter Mediterranean climatic conditions was found to occur equally in both functional types. In the xerophytic site the species resist (*J. phoenicea*, *R. officinalis*) or tolerate (*H. halimifolium*) the interaction of drought, elevated irradiance, and high temperature in summer with recovery in winter. In the hygrophytic site, where water is available all the year long, a species-specific pattern was found in response to summer and winter climatic conditions. As already suggested by Kyparissis *et al.* (2000), our present results support the hypothesis that Mediterranean winter may also be stressful for some evergreen sclerophyllous plants and more intensely for the sclerophylls than for the semi-deciduous species (Oliveira and Peñuelas 2000).

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