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Drought tolerance in the Mediterranean species *Quercus coccifera*, *Quercus ilex*, *Pinus halepensis,* and *Juniperus phoenicea*

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Abstract

We investigated the strategies of four co-occurring evergreen woody species *Quercus ilex*, *Quercus coccifera*, *Pinus halepensis*, and *Juniperus phoenicea* to cope with Mediterranean field conditions. For that purpose, stem water potential, gas exchange, chlorophyll (Chl) fluorescence, and Chl and carotenoid (Car) contents were examined. We recognized two stress periods along the year, winter with low precipitation and low temperatures that led to chronic photoinhibition, and summer, when drought coincided with high radiation, leading to an increase of dynamic photoinhibition and a decrease of pigment content. Summer photoprotection was related to non-photochemical energy dissipation, electron flow to alternative sinks other than photosynthesis, decrease of Chl content, and proportional increase of Car content. Water potential of trees with deep vertical roots (*Q. coccifera*, *Q. ilex*, and *P. halepensis*) mainly depended on precipitation, whereas water potential of trees with shallow roots (*J. phoenicea*) depended not only on precipitation but also on ambient temperature.

Additional key words: carotenoids; chlorophyll fluorescence; field conditions; net photosynthetic rate; photosystem 2; precipitation; quantum yield; stomatal conductance; transpiration; water potential.

Introduction

The Mediterranean climate is characterized by a hot and dry summer and by a cold winter that limit production, growth, development, and distribution of plants in Mediterranean ecosystems. There is strong evidence that summer mild water stress *per se* does not affect the photosynthetic apparatus (Havaux 1992, Cornic 2000), but it is often accompanied by other stressful factors such as high irradiance or temperature that limit the photosynthetic capacity of plants because of stomatal control of water loss and, particularly, can inhibit photosystem 2 (PS2) activity (Öquist *et al.* 1992, Valladares and Pearcy 1997, Martínez-Ferri *et al.* 2000). Low winter temperatures also reduce photosynthetic activity and they are even worse than high temperatures for the same irradiance (Corcuera *et al.* 2005). Under these limiting conditions, the energy absorbed by the leaf can exceed that required for photosynthesis, leading to the over-excitation of the photosynthetic apparatus and promoting photoinhibition. Photoinhibition is a protective mechanism that prevents photochemical damage. Dynamic photoinhibition relies on fast reversible mechanisms (Adams *et al.* 1999), whereas chronic photoinhibition is associated with the maintenance of slow reversible energy dissipating mechanisms, repair processes, or with permanent damage to the photochemical apparatus (Osmond and Grace 1995).

Mediterranean field studies have shown a generalized decrease of chlorophyll (Chl) content under Mediterranean summer drought stress suggesting that Chl loss is not necessarily a symptom of unsuccessful adaptation to photoinhibitory and photo-oxidative conditions but could have a regulatory function (Kyparissis *et al.* 1995).

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Abbreviations: Car – carotenoid; DM – dry mass; DMSO – dimethyl sulphoxide; *E* – transpiration rate; F_0, F_m, and F_v – minimal, maximal, and variable chlorophyll fluorescence of dark-adapted leaves, respectively; F_0', F_m', and F_v' – minimal, maximal, and variable chlorophyll fluorescence of irradiated leaves, respectively; FM – fresh mass; g_s − stomatal conductance; P_N – net photosynthetic rate; PPFD − photosynthetic photon flux density; PS2 – photosystem 2; q_N – non-photochemical quenching; q_P – photochemical quenching; SLA – specific leaf area; Φ_{PS2} – quantum yield of linear electron transport; Ψ_{ws} – stem water potential.

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Mediterranean field conditions also affect carotenoid (Car) contents that show qualitative and quantitative variations (Kyparissis *et al.* 1995, Martínez-Ferri *et al.* 2000, Llorens *et al.* 2002). Cars, apart from functioning as accessory light-harvesting pigments, play a significant role in the photo-protection against photo-oxidative damage through the xanthophyll cycle (Verhoeven *et al.* 1996, Adams *et al.* 1999).

In this general context, we have undertaken a comparative study to investigate the strategies followed by four Mediterranean evergreen woody species to cope with extreme field conditions. The pioneer *Pinus halepensis* (Mill.), which behaves as a drought-avoiding species, was compared with a drought-tolerant gymnosperm species, *Juniperus phoenicea* (L.), and with the late-successional drought-tolerant angiosperms, *Quercus ilex* ssp. *ballota* (Desf.) Samp. and *Quercus coccifera* (L.) (Martínez-Ferri *et al.* 2000). Our aim was to quantify differences in important physiological parameters related to drought and temperature tolerance, by means of stem water potential, gas exchange, Chl fluorescence, and pigment contents that were followed during a day in every season throughout a two-year period. Our objectives were: (*1*) to identify the major adverse season in a Mediterranean ecosystem, (*2*) to determine the photo-protective mechanisms operating in Mediterranean evergreen woody species along seasons, and (*3*) to establish whether water potential is influenced by different climatic factors among species.

Materials and methods

Field site and plants: The study was established under open field conditions on a south-facing calcareous rocky slope (60 %) located at Cañada de Verich (40º52'N 00º07'W, Teruel, east Spain) at 750 m above sea level. This area presents low soil depth and water storing capacity as well as limited plant growth. The climate at this site is typically Mediterranean with cold winters, cool, wet springs and autumns, and hot, dry summers. During the experimental period, summer drought occurred from July to September, when monthly rainfall was nearly 30 mm and maximum daily temperatures were over 35 ºC.

The study was carried out with two conifers, *Pinus halepensis* and *Juniperus phoenicea*, and two evergreen *Quercus* species, *Q. coccifera* and *Q. ilex*. None of the trees was taller than 2 m, indicating that tree growth is restricted in this area. Three representative trees or shrubs of each species were selected as study specimens for measurements and sampling. The trees selected for the study were representative of the vegetation presented in the area. Besides, trees were very homogeneous all presenting a limited growth (1−2 m tall). All determinations and samplings were made on sunlit exposed leaves located at regular intervals around the outer crown. Individual parameters were measured seasonally from July 2001 to May 2002. The days on which measurements and sampling were done may be considered as representatives of winter, spring, summer, and autumn.

Stem water potential was measured with a pressure chamber (*Skye Instruments*, U.K.) as described by Scholander *et al.* (1965). Four twigs per plant of three trees per species were cut, bagged in plastic, and transferred promptly to a pressure chamber.

Gas exchange measurements were done in four fully expanded current-year leaves or twigs of three plants per species with a portable, open-flow gas exchange system (*ADC Bioscientific*, U.K.) equipped with an IRGA-porometer connected to a conifer cylindrical chamber *PLC4C* (*ADC Bioscientific*, U.K.). Measured variables were net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E). Trees were randomly measured within 90 min time avoiding that photosynthetic photon flux density (PPFD) changes could influence results. Air and leaf temperatures in the chambers were controlled by a Peltier unit and were maintained within 1−2 ºC of ambient air. All results were expressed on a leaf area basis, which was determined with a portable leaf area meter *AM100* (*ADC Bioscientific*, U.K.). Projected leaf areas of twigs of *J. phoenicea* and needles of *P. halepensis* were corrected multiplying by π/2 (Cregg 1992).

Chl fluorescence emission was measured seasonally from July 2000 to May 2002 with a *PAM-2000* portable fluorometer (*H. Walz*, Effeltrich, Germany) in six, current-year, fully expanded leaves (or twigs of *J. phoenicea*) of three plants per species. Fluorescence measurements were done in the same leaves or leaves of the same twig used in gas exchange measurements and both measurements were done immediately one after the other. The same leaves were used for measurements at predawn and midday. In the early morning, the minimum fluorescence yield F_0 of leaves adapted to darkness throughout the night was determined under weak red modulated radiation. The leaf was held in the leaf clip holder of the fluorometer at a standard distance from the optic fibre probe and a far-red (735 nm) pulse was sent to fully oxidize the electron transport chain. The maximum fluorescence yield F_m of the dark-adapted leaves was reached by exposing photosystems to saturating pulse (0.8 s; 12 000 µmol m^{-2} s^{-1}) of "white light". Predawn values of maximal (F_m) and minimal fluorescence (F_0) were used to calculate maximal photochemical efficiency of PS2 (F_v/F_m). The F_m values were also used to calculate midday non-photochemical quenching [$q_N = (F_m - F_m')/(F_m - F_0')$; Buschmann 1995]. Seasonal variation in midday quantum yield of linear electron transport (Φ_{PS2}), midday photochemical quenching (q_P), and midday photochemical efficiency of the open reaction centres of

PS2 (F_v'/F_m') were calculated according to Genty *et al.* (1989). For quenching analysis, minimal fluorescence yield of a pre-irradiated sample (F_0') was assessed in leaves darkened immediately after every saturation pulse and subsequently exposed to far-red radiation for 5.5 s.

Pigment analyses: Samples were collected generally at predawn, at noon, and by nightfall, every season, from May 2000 to May 2002. Leaves of different cohorts (1999, 2000, and 2001) were analysed separately in *Q. coccifera*, *Q. ilex*, and *P. halepensis*. Samples were wrapped in aluminium foil, quick-frozen in liquid nitrogen, and stored at –80 ºC until determination in the laboratory. Specific leaf area (SLA) was determined from leaf dry mass (DM) and leaf area (LA) measurements and this, together with FM/DM ratio (fresh mass/dry mass), used to express pigment content on a leaf area basis. Leaf dry mass (DM) was quantified after drying part of the sample in an oven at 70 ºC until constant mass. Chls and Cars were extracted from 10 mg of leaf material using 2.5 cm^3 dimethyl sulphoxide (DMSO) saturated with $CaCO_3$ (Barnes *et al.* 1992). Tubes containing the reaction mixture were incubated for 40 min at 65 ºC in the dark (shaking at regular intervals) and then extracts allowed to cool to ambient temperature before diluting 1 : 1 with fresh DMSO. Absorbance of extracts was then read spectrophotometrically (*Uvikon 922*, *Kontron*, Switzerland) at 480.0, 665.1, and 649.1 nm using 1-cm^3 quartz-glass cuvettes calibrated against a blank containing DMSO saturated with $CaCO_3$. The concentrations of Chl *a* and *b* as well as total Cars were calculated using equations of Wellburn (1994).

Statistical analysis: Significant differences between mean values obtained from 12−24 samples of three different tree specimens in four woody species was determined by a repeated measures' analysis of variance (ANOVAR) using the *JMP 5.0* software package (SAS Institute, USA). In this model, species was the between-groups factor and tree was nested under species. Within subjects, sampling date was considered as a repeated measure factor. Assumptions of normality and homoscedasticity were tested by Kolmogorov-Smirnov and Levene tests, respectively. Significant differences between means were determined with the least significant difference test (LSD) calculated at the 5 % level. Regressions were used to investigate the relationships between the studied variables.

Results

Stem water potential: Contrary to expectation, *Quercus* species and *P. halepensis* showed their lowest stem water potential (Ψ_{ws}) in winter (Fig. 1). Only *J. phoenicea* exhibited the lowest Ψ_{ws} in summer. The smallest Ψ_{ws} values were close to −1.5 MPa in *P. halepensis*, −2.5 MPa in *Quercus* species, and −3.5 MPa in *J. phoenicea*. Midday water potential (Ψ_{wsmd}) was significantly lower than predawn water potential (Ψ_{wspd}) for all species ($p<0.05$) except for *P. halepensis*. The biggest daily reduction of Ψ_{ws} between predawn and midday was recorded in summer in *Q. coccifera*, *Q. ilex*, and *J. phoenicea* and in spring in *P. halepensis*. Among species, the less negative Ψ_{ws} was measured in *P. halepensis* ($p<0.01$), whereas the lowest Ψ_{ws} was reached by *J. phoenicea*, especially in summer ($p<0.05$).

A positive correlation between predawn Ψ_{ws} and accumulated rainfall in the previous month (Fig. 2) suggests that the low Ψ_{ws} observed during winter is due to the low rainfall occurred in this season. It can also be observed from the regression lines that *P. halepensis* leads to maximum Ψ_{ws} with approximately 50 mm rainfall, whereas *Quercus* species and *J. phoenicea* increased their Ψ_{ws} with increasing precipitation. When precipitation was low, the lowest Ψ_{ws} corresponded to *J. phoenicea* and the highest to *P. halepensis*. These differences among species disappeared at high precipitations.

Gas exchange: g_s (Fig. 3*A,D*) was maximal in the morning in all species, lowering significantly at midday, this decrease being season-dependent with highs in spring and summer ($p<0.01$). *J. phoenicea* showed significant lower g_s than the other species ($p<0.01$), except in summer when g_s was similar to that of *P. halepensis*.

Maximum P_N (Fig. 3*B,E*) was recorded in spring and summer in *Quercus* species, in spring in *J. phoenicea*, and in winter in *P. halepensis*. A decrease of P_N at midday was found in the warmer seasons, in spring and especially in summer ($p<0.01$) when a 50 % decrease of P_N was observed in all species, in agreement with the highest reduction in g_s. The decrease of g_s, previously observed in *P. halepensis* in summer, involved a large decrease in P_N, particularly at midday. *J. phoenicea* exhibited the lowest P_N as a consequence of its low g_s ($p<0.05$).

E (Fig. 3*C,F*) was highest at midday during the warmer seasons of spring and summer in all species ($p<0.05$), with the exception of *P. halepensis* in summer because of stomatal closure. *P. halepensis* was the only species that did not achieve an increase of leaf *E* at midday in summer.

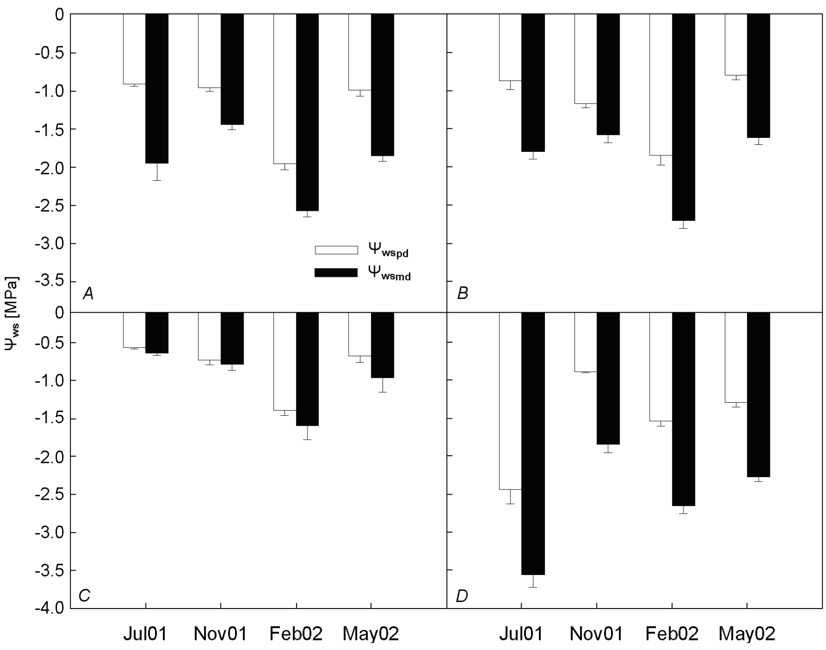

Fig. 1. Changes in stem water potential [MPa] measured at predawn (Ψ_{wspd}) and midday (Ψ_{wsmd}) in (*A*) *Q. coccifera*, (*B*) *Q. ilex*, (*C*) *P. halepensis*, and (*D*) *J. phoenicea* plants growing under Mediterranean field conditions from July 2001 to May 2002. Means ± standard error ($n = 12$).

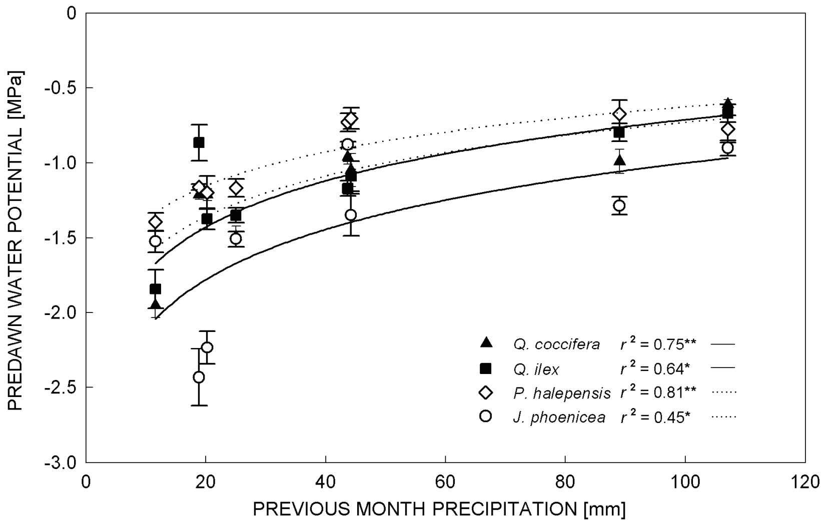

Fig. 2. Relationship between accumulated precipitation over the previous month and predawn stem water potential in *Q. coccifera*, *Q. ilex*, *P. halepensis*, and *J. phoenicea* plants growing under Mediterranean field conditions. Means ± standard error ($n = 12$). Asterisks indicate significant correlations: $^{**}p<0.01$, $^{*}p<0.05$.

Chl fluorescence: The maximum photochemical efficiency of PS2 (F_v/F_m, Fig. 4*A*) took place in summer and the lowest in winter in all species and was significantly higher in *P. halepensis* (0.78–0.82) than in other species in all seasons (0.70–0.79, $p<0.05$). Midday photochemical efficiency of the open reaction centres of the PS2 (F_v'/F_m', Fig. 4*C*) was significantly lower than predawn F_v/F_m in all species and seasons ($p<0.001$), and again, *P. halepensis* showed higher F_v'/F_m' and midday quantum yield of linear electron transport (Φ_{PS2}, Fig. 4*B*) than those of other species ($p<0.05$). Both F_v'/F_m' and Φ_{PS2} were higher in autumn and winter than in summer in all species. Differences between species in midday Φ_{PS2} were not due to differences in photochemical quenching (q_P, Fig. 4*E*). Nevertheless, midday non-photochemical quenching (q_N) was correlated with q_P, F_v'/F_m', and Φ_{PS2}, indicating that the photochemical efficiency decrease observed at midday was explained by a proportional increase of q_N (data not shown). Fig. 4 also shows that the midday diminution in Φ_{PS2} was due to a simultaneous reduction in q_P and F_v'/F_m', associated with an increase in q_N.

The ratio Φ_{PS2}/P_N (Fig. 5) is used as an indicator of the increase of electron transport to acceptors other than CO_2 when photosynthesis is inhibited by stomatal closure (Flexas *et al.* 2002). This ratio sharply increased in conifers when g_s was minimum, but much less in *Quercus* species, mainly due to the lower g_s values reached by conifers. Among *Quercus* species, *Q. coccifera* showed a higher Φ_{PS2}/P_N than *Q. ilex* when g_s was low.

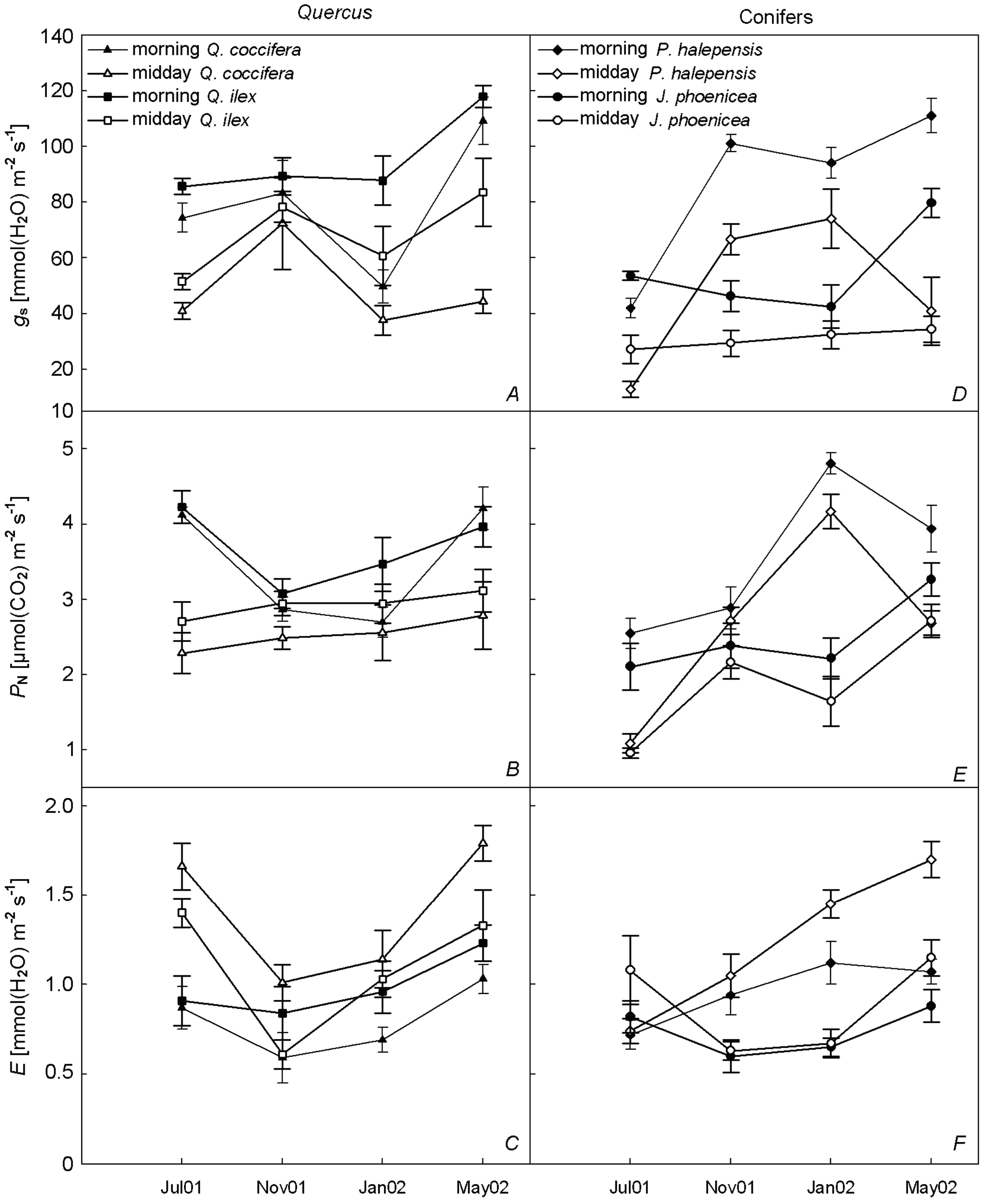

Fig. 3. Changes in (*A*, *D*) stomatal conductance (g_s), (*B, E*) net photosynthetic rate (P_N), and (*C, F*) leaf transpiration rate (*E*) measured at mid-morning and midday in current-year leaves of *Q. coccifera*, *Q. ilex*, *P. halepensis*, and *J. phoenicea* plants growing under Mediterranean field conditions from July 2001 to May 2002. Means ± standard error ($n = 12$).

Pigment content exhibited a seasonal pattern (Fig. 6), but no significant diurnal changes were found in any species (data not shown). Leaf Chl (*a*+*b*) and Car contents and Chl *a*/*b* were lower in spring and summer and higher in autumn and winter for all species ($p<0.01$). However, Car/Chl ratio did not show any consistent trend. *Q. ilex* presented the highest Chl and Car contents followed by *Q. coccifera* and *J. phoenicea*, whereas *P. halepensis* exhibited the lowest pigment contents ($p<0.01$). However *J. phoenicea* showed lower Chl *a*/*b* (Fig. 6*C,G*, $p<0.001$) and *P. halepensis* had lower Car/Chl ratio than the other species (Fig. 6*D,H*, $p<0.05$).

q_N was also positively correlated with the Car/Chl ratio (Fig. 7). The different slopes of the regression lines between *Quercus* and conifers suggest that, while high variations in the Car/Chl ratio corresponded to small changes in q_N in *Quercus* species, small variations of Car/Chl ratio caused higher changes in q_N in conifer species.

Discussion

Ψ_{ws} of *Q. coccifera*, *Q. ilex*, *P. halepensis*, and *J. phoenicea* was within the range reported for other Mediterranean species (Sala and Tenhunen 1996, Faria *et al.* 1998, Werner *et al.* 1999, Martínez-Ferri *et al.* 2000, Llorens *et al.* 2003). However, Ψ_{ws} was not as negative as previously reported under severe drought in the same species (Sala and Tenhunen 1996, Castillo *et al.* 2002) despite our plants have grown on a rocky outcrop with low soil availability. Contrary to our expectation, both *Quercus* species and *P. halepensis* attained their minimum Ψ_{ws} in winter. The positive correlation observed between Ψ_{ws} and the previous month precipitation (Fig. 2) indicates that the low Ψ_{ws} measured in winter reflected the limited water availability caused by the scarce rain taking place in this season. The chronic photoinhibition that also occurred in winter was not as marked as previously reported in our four Mediterranean evergreen species by Martínez-Ferri *et al.* (2004), and was similar to that obtained in *Q. ilex* by García-Plazaola *et al.* (2003) and Corcuera *et al.* (2005). Low winter F_v/F_m values have been related to changes in the pigment bed associated to the presence of zeaxanthin and antheraxanthin in the PS2 antenna, attributed to the inhibitory effect of chilling temperatures on the enzymatic conversion of zeaxanthin and antheraxanthin to violaxanthin (Adams and Demmig-Adams 1994). However, we have found low q_N values in winter in contradiction with previous reports of non-photochemical energy dissipation at low temperature (Gilmore and Björkman 1995). Martínez-Ferri *et al.* (2004) suggested that the larger quantity of violaxanthin+antheraxanthin+zeaxanthin (VAZ) observed in winter could confer greater stability on the membranes, hence, winter photo-protection would be associated with photophysical and structural aspects. The higher PS2 photochemical efficiency observed in *P. halepensis* is consistent with its lower VAZ/Chl ratio (Martínez-Ferri *et al.* 2004), lower q_N, and higher P_N during winter (Figs. 3 and 4).

Nevertheless, the low P_N and g_s measured seasonally in all species, as compared to values obtained by other authors in the same species (Sala and Tenhunen 1994, Peñuelas *et al.* 1998, Gratani *et al.* 2000, Larcher 2000), demonstrate that our plants were subjected to a continuous stress. Comparably, and accordingly to Flexas and Medrano (2002), g_s (Fig. 3*A*,*D*) together with Ψ_{ws} (Fig. 1) indicated that our plants were exposed to a severe or very severe drought.

The highest dynamic photoinhibition coincided with summer drought and was completely reversed in *P. halepensis*, indicating that photoinhibition was due to photoprotective processes, as corroborated by the increase of q_N (Adams and Demmig-Adams 1994, Munné-Bosch and Alegre 2000). However, photoinhibition was not completely reversed in both oaks and *J. phoenicea* and, although thermal dissipation of energy took place, predawn F_v/F_m did not return to optimum values. Martínez-Ferri *et al.* (2000) suggested that predawn de-epoxidated state of the xanthophylls results in a slight nocturnal quenching that persists throughout summer months, leading to a reduction in F_v/F_m. In spite of the drought-avoidance strategy of *P. halepensis* that limits P_N, this species showed higher photochemical efficiency

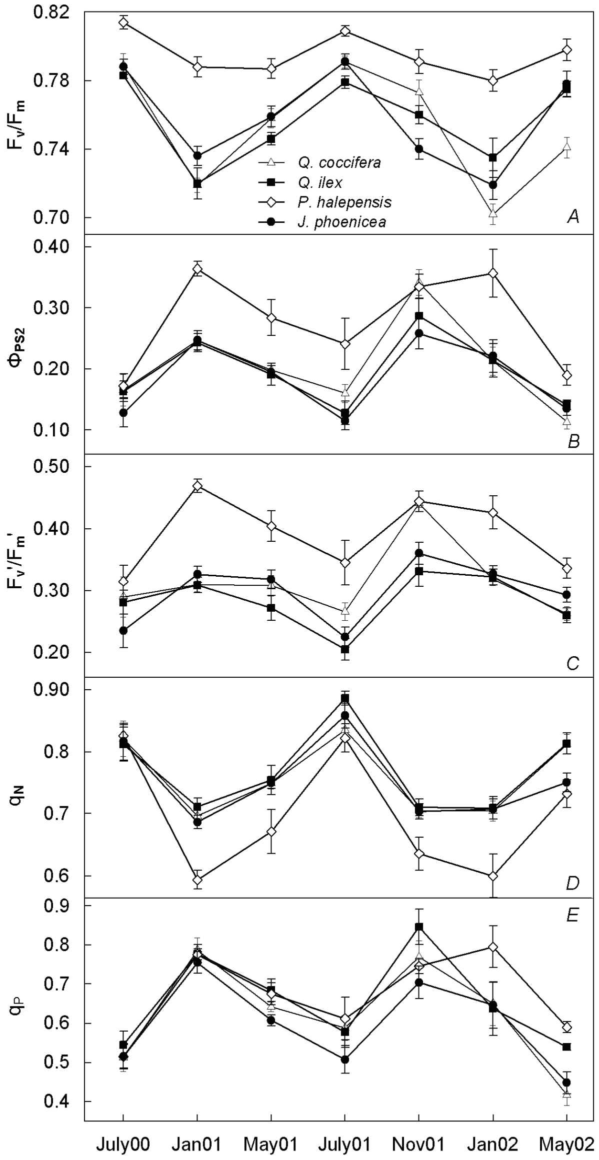

Fig. 4. Changes in (*A*) predawn maximum photochemical efficiency of PS2 (F_v/F_m), (*B*) midday quantum yield of linear electron transport (Φ_{PS2}), (*C*) midday photochemical efficiency of the open reaction centres of PS2 (F_v'/F_m'), (*D*) midday non-photochemical quenching of fluorescence (q_N), and (*E*) midday photochemical quenching of fluorescence (q_P) in current-year leaves of *Q. coccifera*, *Q. ilex*, *P. halepensis*, and *J. phoenicea* plants growing under Mediterranean field conditions from July 2000 to May 2002. Means ± standard error ($n = 18$).

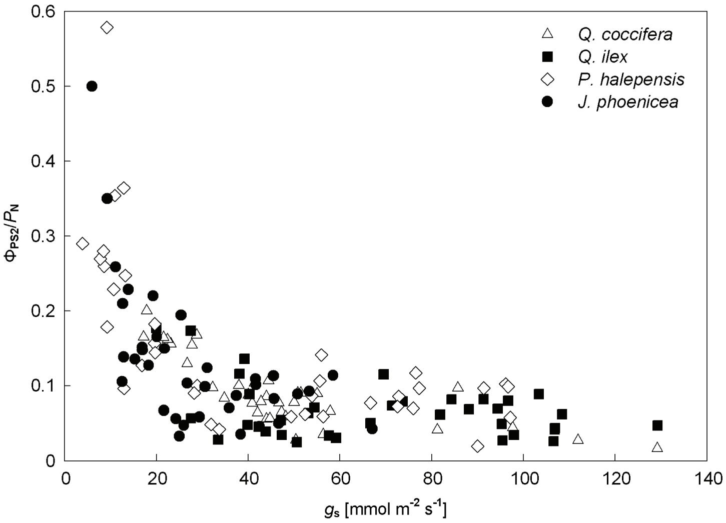

Fig. 5. Relationship between stomatal conductance (g_s) and the ratio of linear electron transport to CO_2 assimilation (Φ_{PS2}/P_N) in current-year leaves of *Q. coccifera*, *Q. ilex*, *P. halepensis*, and *J. phoenicea* plants growing under Mediterranean field conditions. Means ± standard error (*n* = 12).

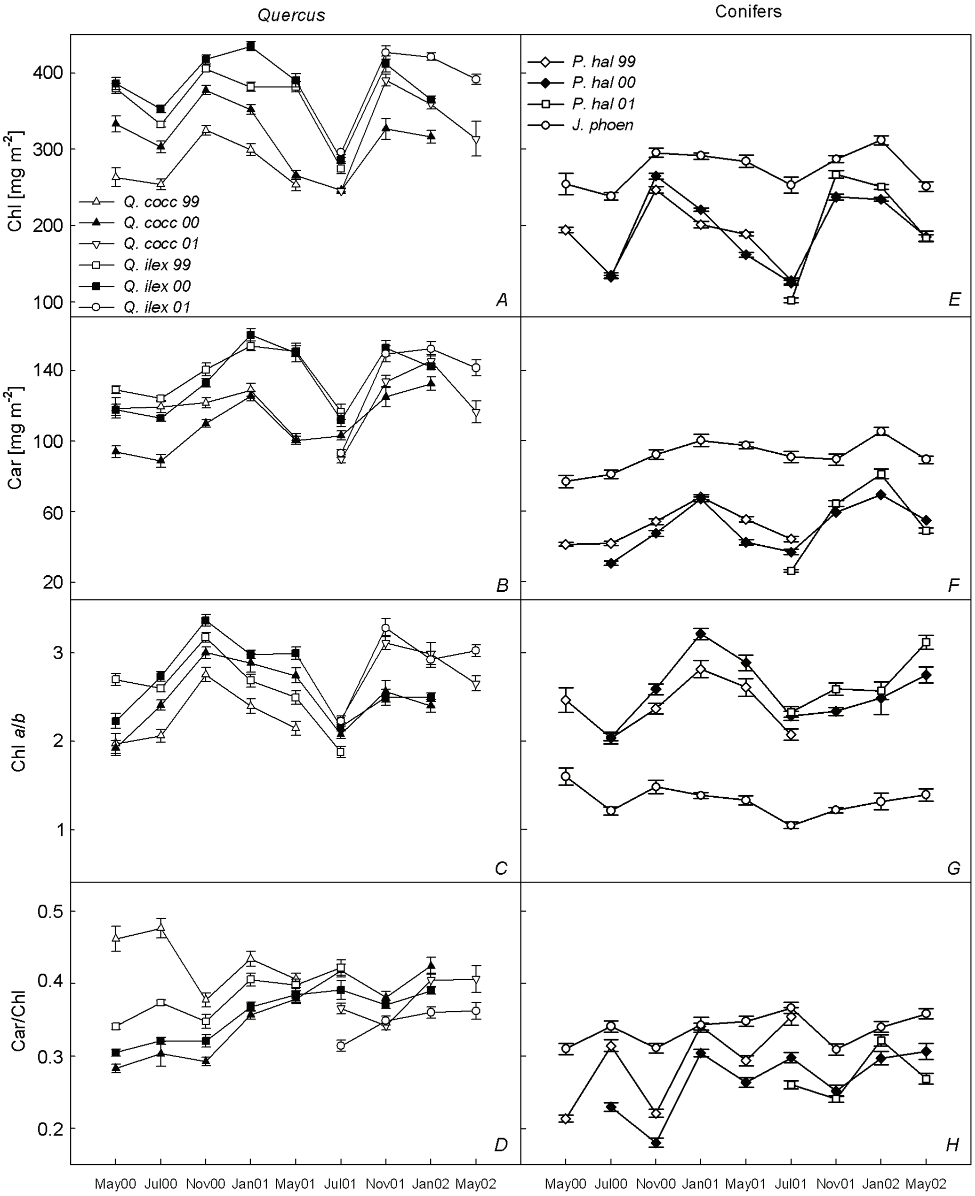

Fig. 6. Ratio of changes in (*A, E*) total chlorophyll (Chl) content, (*B, F*) total carotenoid (Car) content, (*C, G*) Chl *a/b* ratio, and (*D, H*) Car/Chl ratio in leaves of *Q. coccifera*, *Q. ilex*, and *P. halepensis* (1999, 2000, and 2001 cohorts) and *J. phoenicea* plants growing under Mediterranean field conditions from July 2000 to May 2002. Means ± standard error (*n* = 108).

and, contrary to what we could expect, lower midday photoinhibition than other species, even in summer (Fig. 4*C*). Thus, photorespiration and/or the Mehler reaction would have great importance as electron sinks under Mediterranean field conditions in conifers as the electron transport rate, not directly dissipated through photosynthesis, increased when g_s decreased (Φ_{PS2}/P_N, Fig. 5). The decrease of Chl content observed in spring and summer (Fig. 6*A,E*) may be caused by photo-oxidation of Chl, what can be regarded as a protective adaptive mechanism in stressed plants (Anderson *et al.* 1992, Munné-Bosch and Alegre 2000). The absence of senescence traits during the Chl content decline and the subsequent recovery in autumn suggests that summer Chl loss was an adaptive mechanism. Chl loss under excess sunlight reduces leaf photon absorption capacity, thus preventing over-excitation of photosystems (Anderson *et al.* 1992) and it also increases the capacity to dissipate excess excitation energy per intercepted PPFD (Munné-Bosch and Alegre 2000). Total Car content also decreased at the same time as Chl content did (Fig. 6*B,F*), but their decline was less pronounced than Chl, indicating a conservation of photo-protective capacity. The increase in Car/Chl in summer suggests an increase in β-carotene, VAZ, and lutein, the pigments associated with high irradiance protection (Kyparissis *et al.* 1995, Bungard *et al.* 1997, Llorens *et al.* 2002).

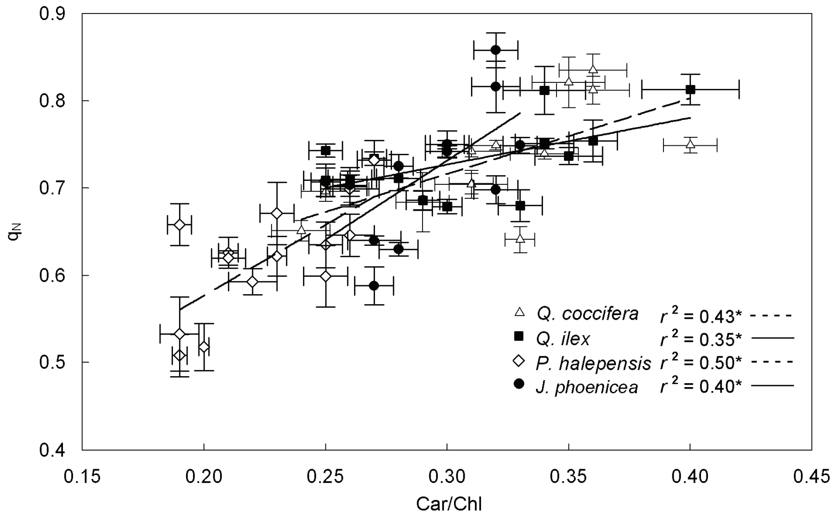

Fig. 7. Relationship between carotenoid/chlorophyll (Car/Chl) ratio and non-photochemical quenching of fluorescence (q_N) in *Q. coccifera*, *Q. ilex*, *P. halepensis*, and *J. phoenicea* plants growing under Mediterranean field conditions. Means ± standard error (*n* = 12). Asterisks indicate significant correlations: $^{**}p<0.01$, $^{*}p<0.05$.

The relationship between Ψ_{ws} and precipitation (Fig. 2) showed a threshold monthly rainfall of about 40 mm enough to restore maximum Ψ_{ws} in all species. *J. phoenicea* was the only species that reached the lowest Ψ in summer. Moreover, all species including *J. phoenicea* showed a positive correlation between predawn Ψ_{ws} and the previous month precipitation. As *J. phoenicea* presents shallow roots (Castillo *et al.* 2002) its Ψ_{ws} would be influenced not only by precipitation ($r^2 = 0.45$), but also by water evaporation from the superficial layers of the soil. Hence, the higher summer temperature led to higher water evaporation from the soil and, as consequence, to lower predawn Ψ_{ws} of *J. phoenicea* in this season. At the same time, predawn Ψ_{ws} of *Q. coccifera*, *Q. ilex*, and *P. halepensis* depended more on precipitation that on temperature as indicates the fact that the lowest predawn Ψ_{ws} was measured in winter. This dependence of Ψ_{ws} on previous month precipitation is an evidence of the deep roots of *Q. coccifera*, *Q. ilex*, and *P. halepensis* that are able to reach water stored in depth that is related to the rainfall recharge and not influenced by ambient temperature. *P. halepensis* showed higher Ψ_{ws} than the other species, what could be related to deeper roots and higher access to water. These suggest that plants of this genus maintain a more successful relationship between water loss and water supply (Picon *et al.* 1996).

In summary, the ability of Mediterranean species to counteract with these adverse situations is mainly related to their capacity to exploit water, to avoid water loss, and to counteract with excess energy absorbed during the most adverse periods. Plant development, growth, and eco-physiological parameters indicated extreme stress. We recognized two stress periods along the year, winter with low precipitation and low temperatures that led to a chronic photoinhibition, and summer, when drought coincided with high irradiation, leading to an increase of dynamic photoinhibition and a decrease of pigment contents. Summer photoprotection was related to non-photochemical energy dissipation, electron flow to alternative sinks other than photosynthesis, decrease of Chl content and proportional increase of Car content, most probably those associated with protection from high irradiance. Ψ_{ws} of trees with deep vertical roots (*Q. coccifera*, *Q. ilex*, and *P. halepensis*) mainly depended on precipitation, whereas Ψ_{ws} of trees with shallow roots (*J. phoenicea*) depended not only on precipitation but also probably on temperature as this increases water evaporation from the surface of the soil.

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