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Winter photoinhibition in the field involves different processes in four co-occurring Mediterranean tree species

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Summary Photoinhibition was examined in four co-occurring Mediterranean evergreen tree species during two consecutive winters. In response to low temperatures and saturating light, *Juniperus phoenicea* L., *Pinus halepensis* Mill., *Quercus coccifera* L. and *Q. ilex* ssp. *ballota* (Desf.) Samp. exhibited marked chronic photoinhibition, indicated by low predawn maximal photochemical efficiency of photosystem II (PSII) (F_v/F_m). Low F_v/F_m values were correlated with high concentrations of xanthophyll cycle components (VAZ) and with the maintenance of high concentrations of zeaxanthin overnight (DPS_{pd}). In all species, however, chronic photoinhibition was enhanced as the winter progressed in the absence of changes in DPS_{pd}, suggesting cumulative damage toward the end of winter.

Photoinhibition differed among species: *P. halepensis* always displayed significantly higher F_v/F_m values; and *Q. coccifera* had the lowest F_v/F_m values, showing a high sensitivity to the combination of high light and low temperatures. Differences among species were not fully explained by differences in the xanthophyll pool or its de-epoxidation state. Chronic photoinhibition overlapped with a dynamic photoinhibition as shown by the low values of photochemical efficiency of the open reaction centers of PSII at midday. Winter photoprotective strategies differed among species and may involve photoprotective mechanisms in addition to those associated with xanthophylls. The observed species-specific differences matched results obtained for the same species in summer; however, comparison of the two seasons suggests that the higher VAZ concentration observed in winter has an additional structural photoprotective role.

Keywords: Juniperus, photoprotection, Pinus, Quercus, xanthophyll cycle.

Introduction

Evergreen plants in extra-tropical regions are subjected to a combination of chilling temperatures and high solar irradiances during clear days in winter and early spring. Impairment of photosynthesis by low temperatures reduces the irradiance at which light becomes saturating. Plants exposed to saturating light commonly experience temporary or sustained photoinhibition (Berry and Björkman 1980, Öquist et al. 1993, Krause 1994, Schnettger et al. 1994, Demmig-Adams and Adams 1996, Neuner et al. 1999). Dynamic photoinhibition relies on fast reversible mechanisms (Demmig-Adams and Adams 1992, Long et al. 1994), 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 1994). Sustained photoprotection may confer an advantage to evergreen species inhabiting boreal (Ottander et al. 1995, Ivanov et al. 2001) and temperate regions (Groom et al. 1991, Adams and Demmig-Adams 1994) in which winter temperatures are mostly below the threshold for positive carbon balance. Evergreen plants are capable of down-regulating the photosynthetic process by several slowly reversible photoprotective mechanisms (Huner et al. 1993, Anderson and Aro 1994, Adams and Demmig-Adams 1994, 1995, Ottander et al. 1995). In this context, photoinhibition may be considered a protective mechanism because it prevents photochemical damage. However, in environments with mild winters, mostly clear days and a marked diurnal oscillation of temperatures, the maintenance of long-lasting photoprotective mechanisms could adversely affect the photosynthetic exploitation of relatively favorable winter conditions, which can be crucial in Mediterranean-type ecosystems to achieve a positive annual carbon balance (García-Plazaola et al. 1999*a*, 1999*b*, Joffre et al. 1999).

Despite the Mediterranean winter's mildness, sustained decreases in the maximal photochemical efficiency of photosystem II (PSII) (F_v/F_m) in Mediterranean evergreen species during winter have been reported (García-Plazaola et al. 1999*a*, 1999*b*, Karavatas and Manetas 1999, Kyparissis et al. 2000, Oliveira and Peñuelas 2000). Decreases in F_v/F_m during winter are associated with either damage to the photosynthetic apparatus (García-Plazaola et al. 1997) or a down-regulation process, such as an increase in thermal energy dissipation related to high concentrations of zeaxanthin and antheraxan-

thin overnight (García-Plazaola et al. 1999*a*, Kyparissis et al. 2000). Mediterranean evergreen species may differ in their susceptibility to photoinhibition depending on the photoprotective mechanisms involved in the photoinhibitory process. In addition, phenomorphological adaptations, such as leaf angle changes, pubescence, leaf reflectance, dimorphism or curling, protect some species from excess light energy (Oliveira and Peñuelas 2000, Werner et al. 2002). Martínez-Ferri et al. (2000) showed that photoprotective capacity differed qualitatively and quantitatively among co-occurring Mediterranean evergreen species under summer conditions and they classified species as photoinhibition-avoiding or photoinhibition-tolerant on the basis of their susceptibility to dynamic photoinhibition.

We examined the physiological behavior of four co-occurring Mediterranean evergreen tree species, *Juniperus phoenicea* L, *Pinus halepensis* Mill., *Quercus coccifera* L. and *Q. ilex* ssp. *ballota* (Desf.) Samp. during winter. Our specific objectives were: (1) to identify the photoprotective mechanisms operating in Mediterranean evergreen tree species during winter; (2) to determine the role of the xanthophyll cycle pool in the development of non-photochemical quenching; and (3) to test whether the differences in photoprotective capacity previously observed among species during summer are maintained in winter.

Materials and methods

Field site and plant material

The study was carried out on a south-facing, calcareous rocky slope (27°) located in Teruel, Spain (40°52′ N, 00°07′ W; central-east Spain) at 750 m a.s.l. The area is characterized by a Mediterranean-type climate, with dry and warm summers and cold winters. Mean annual precipitation is 382 mm (1960–1994), occurring mainly during autumn and winter. Mean maximum and minimum temperatures are 32 and –0.6 °C, respectively, reaching a maximum of 42 °C and a minimum of –10 °C. *Juniperus phoenicea*, *Pinus halepensis*, *Quercus coccifera* and *Quercus ilex* ssp. *ballota* co-occur scattered on this site and show evidence of restricted growth (Martínez-Ferri et al. 2000). Three trees per species were tagged at the site for measurements. Sampling was conducted on selected cloudless days in December 1997 and February 1998 (winter 1997–1998; three days per month), and in December 1998 and February 1999 (winter 1998–1999; two days per month). All measurements were made on current-year leaves located at regular intervals around the outer-crown surface. During the experimental period, air temperature (T), photosynthetic photon flux (PPF) and relative humidity (RH) were recorded every 2 min throughout the day with a combination of cross-calibrated temperature sensors (thermistor, Grant Instruments, Cambridgeshire, U.K.), quantum sensors (SKP210, Skye Instruments, Powys, U.K.) and relative humidity probes (HMP 35A, Vaisala, Helsinki, Finland), respectively, connected to a Squirrel 1200 data logger (Grant Instruments). Available data for daily maximum and minimum temperatures during the winter months were provided by the Spanish National Institute of Meteorology (INM).

Water potential

Stem water potential (Ψ_S) was determined at predawn (0330–0530 h; Ψ_{Spd}) and midday (1200–1400 h; Ψ_{Smd}) with a pressure chamber (Skye Instruments) as described by Scholander et al. (1965). Briefly, two twigs of three plants per species were enclosed in a plastic bag and covered with aluminum foil for 2 h before measurements (Begg and Turner 1970, Garnier and Berger 1985, McCutchan and Shackel 1992, Martínez-Ferri et al. 2000).

Gas exchange measurements

In February 1998, photosynthetic light response curves were determined on two fully developed leaves (or twigs of *J. phoenicea*) per species under saturating natural light by decreasing PPF with combinations of neutral density filters. Measurements were made with an open-flow infrared gas analysis system (LCA 4, ADC Bioscientific, Herts, U.K.). The system was connected to a cylindrical cuvette (PLC 4C, ADC Bioscientific) equipped with microquantum sensors and thermocouples that monitored incident PPF and cuvette and leaf temperatures during gas exchange measurements. Temperature inside the cuvette never exceeded the external air temperature by more than 2 °C during measurements. Carbon assimilation (A) was calculated according to the equations of von Caemmerer and Farquhar (1981) and expressed on a projected leaf area basis, which was measured with an automatic image analyzer (SigmaScan 2.0, Jandel Scientific, San Rafael, Canada). Projected leaf areas of twigs of *J. phoenicea* and needles of *P. halepensis* were corrected by multiplying by $\pi/2$ as recommended by Cregg (1992).

Data were fitted to the rectangular hyperbolic model proposed by Chartier and Prioul (1976) with the program Photosyn Assistant Version 1.1.1 (Dundee Scientific, Dundee, U.K.). In this model, the initial slope of the curve is the apparent photosynthetic quantum yield (ϕ_e) and the upper asymptote is the light-saturated photosynthetic rate (A_{sat}).

Chlorophyll a fluorescence

In vivo chlorophyll a fluorescence signals of five attached current-year leaves (or twigs of *J. phoenicea*) of three plants per species were monitored simultaneously from predawn to dusk with two cross-calibrated portable fluorometers PAM-2000 (Heinz Walz, Effeltrich, Germany) equipped with leaf-clip holders to monitor incident PPF (PPF_{leaf}) and leaf temperature (T_{leaf}). Leaves were labeled for repeated measurements throughout the winter. Fluorescence parameters were determined by the saturation pulse method (Schreiber et al. 1994). Briefly, leaf samples were exposed to a weak modulated measuring beam to assess the initial minimal fluorescence following dark adaptation (F_o) or steady-state fluorescence in the light (F_t). Then, a flash (0.8 s) of saturating light (12,000 μmol m^{-2} s^{-1}) was given to assess maximal fluorescence, either in the dark, when PSII centers are closed (F_m), or in the light (F_m'). For quenching analysis, minimal fluorescence yield of a

pre-illuminated sample (F_o') was assessed in leaves darkened immediately after every saturation pulse and subsequently exposed to far-red light for 5.5 s. Measurements of F_m and F_o were taken at predawn to calculate maximal photochemical efficiency of PSII ($F_v/F_m = (F_m - F_o)/F_m$). Diurnal non-photochemical quenching of fluorescence ($qN = (F_m - F_m')/(F_m - F_o')$ was calculated from F_m (Buschmann 1995). Daily variation in the relative quantum yield of PSII photochemistry ($\Phi_{PSII} = (F_m' - F_t)/F_m'$), photochemical quenching ($qP = (F_m' - F_t)/(F_m' - F_o')$) and photochemical efficiency of the open reaction centers of PSII ($F_v'/F_m' = (F_m' - F_o')/F_m'$) were calculated according to Genty et al. (1989).

Pigment analyses

Three pooled leaf samples from three trees per species were taken before dawn and at noon and immediately stored in liquid nitrogen until pigments were extracted (Martínez-Ferri et al. 2000). Leaf samples (100 mg) were extracted in 5 ml of cool acetone in the presence of sodium ascorbate. After filtering through a 0.2-µm syringe nylon filter, 30 µl of the extract was injected into a Spherisorb ODS2 (Waters, Milford, CT) reverse-phase steel column (25 cm, 5 µm particle diameter) and chlorophylls and carotenoids separated by high performance liquid chromatography (HPLC; Waters). The HPLC was equipped with a Waters 996 photodiode array detector as described in Val et al. (1994). Solvents for HPLC (LabScan, Dublin, Ireland) were degassed before use by bubbling with helium. For peak identification and quantification, pure commercial standards (VKI, Hørsholm, Denmark) were used. The de-epoxidation state of the xanthophyll cycle pigments (DPS) was calculated as the ratio of antheraxanthin and zeaxanthin to the total xanthophyll cycle pool as described by Adams et al. (1995).

Statistical analyses

Data were analyzed with the software program STATISTICA 4.5 (Statsoft, Tulsa, OK). Species effects on chlorophyll fluorescence-based variables, PPF_{leaf} and T_{leaf}, were assessed by a multifactorial repeated measures analysis of variance (ANOVA). In this model, species was the between-groups factor and plant was nested under species. Within subjects, sampling date was considered a repeated measures factor and nested under month when differences between sampling months at a given point during the diurnal course were tested. Time of day was included as a third repeated measures factor when differences between predawn and midday were tested. Statistical analyses of chlorophyll fluorescence-based variables also incorporated a linear combination of climatic variables as a changing covariate to account for the effects of climatic variation across leaves, time of day and field campaigns. To calculate this covariate, climatic variables were subjected to a principal component analysis (PCA). Because a different batch of leaves was selected every year, the corresponding data sets were analyzed separately. Differences in the diurnal course of pigment composition among species were evaluated by ANOVA, with species as a between-groups factor and time of the day, sampling date and month as within-subjects factors. Differences in stem water potential among species during the day were tested by a two-way ANOVA. Differences were considered significant at $P < 0.05$, and all pairwise comparisons were completed with a least significance difference test (LSD). Assumptions of normality and homoscedasticity were determined with the Kolmogorov-Smirnov test and Cochran's C test, respectively. When necessary, data were transformed to attain a normal distribution.

Results

Weather conditions

Winter 1997–1998 was warmer than winter 1998–1999. Mean daily minimum temperatures from January to February ranged from –0.18 ± 0.29 to 1.23 ± 0.51 °C in winter 1997–1998, and from –2.36 ± 0.53 to –0.29 ± 0.64 °C in winter 1998–1999. Similarly, mean daily maximum temperatures ranged from 13.29 ± 0.63 to 18.75 ± 0.37 °C in winter 1997–1998, and from 10.39 ± 0.67 to 13.84 ± 0.61 °C in winter 1998–1999. Daily minimum temperatures were mostly below 2 °C from December to February. In both years, the lowest minimum temperatures were recorded in February when most of the days did not reach 0 °C.

Stem water potential

Stem water potential did not change significantly between sampling months of each winter in any of the species. None of the species suffered severe water stress during either winter, as indicated by the relatively high Ψ_{Spd} values, which were even higher than those observed in summer (Martínez-Ferri et al. 2000). Nevertheless a significant decline (~ 35%) in Ψ_S was observed between predawn and midday in all species except for *P. halepensis* during winter 1997–1998 (Figure 1). Both Ψ_{Spd} and Ψ_{Smd} were significantly higher in *P. halepensis* than in the other species (Figure 1).

Light response curves

Photosynthetic light responses measured in February 1999 differed among species. *Quercus ilex* ssp. *ballota* and *P. halepensis* had higher A_{sat} and ϕ_e than *Q. coccifera* and *J. phoenicea* (Figure 2).

Chlorophyll fluorescence parameters

Predawn F_v/F_m values were well below the optimum values in all species in both years (Figure 3A). A decrease in maximal photochemical efficiency (F_v/F_m) from December to February was observed in the *Quercus* species in both years (Figure 3A), whereas in *P. halepensis* and *J. phoenicea* the decrease in F_v/F_m was significant only during the coldest winter (1998–1999). Values of F_v/F_m were always significantly higher in *P. halepensis* than in the other species (Figure 3A). In winter 1998–1999, F_v/F_m of *Q. coccifera* decreased dramatically to < 0.4, which was significantly lower than F_v/F_m values in the other species.

All study species showed a progressive and significant decrease in Φ_{PSII} from predawn maximum values (F_v/F_m) to minima at midday in both winters (data not shown). There were no

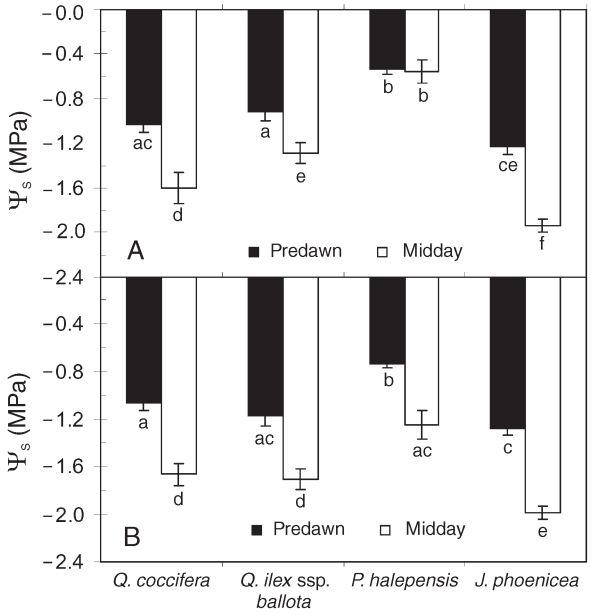

Figure 1. Predawn and midday stem water potential (Ψ_S) in twigs of *Q. coccifera*, *Q. ilex* ssp. *ballota*, *P. halepensis* and *J. phoenicea* measured in: (A) winter 1997–1998; and (B) winter 1998–1999. Error bars represent ± SE (n = 18–24). Statistical differences ($P < 0.05$) between species and time of day are indicated by different letters (two-way ANOVA, followed by LSD).

significant differences in any of the fluorescence parameters at midday across months during winter 1997–1998 (Figure 4). However, in winter 1998–1999, midday values of Φ_{PSII}, qP and F_v'/F_m' were significantly lower in February than in December (Figure 4). This decrease was accompanied by a significant increase in qN values (Figure 4C).

Among species, *P. halepensis* always exhibited significantly higher midday values of Φ_{PSII} than *J. phoenicea* and *Q. coccifera*, similar to those of *Q. ilex* ssp. *ballota* (Figure 4). Differences among species in the relative quantum yield of PSII photochemistry (Φ_{PSII}) were not matched by differences in leaf temperature or by differential light interception at midday (data not shown). The differences in Φ_{PSII} resulted from the combined variation in qP and F_v'/F_m' (Figure 4). At midday during both winters, Φ_{PSII} was positively correlated with the number of open PSII reaction centers (qP; $r^2 = 0.53$ and $r^2 = 0.82$ in winter 1997–1998 and winter 1998–1999, respectively), and with their efficiency (F_v'/F_m'; $r^2 = 0.78$ and $r^2 = 0.94$ in winter 1997–1998 and winter 1998–1999, respectively). *Pinus halepensis* displayed significantly higher midday values of photochemical efficiency (F_v'/F_m') than the other study species, and *Q. coccifera* reached the lowest F_v'/F_m' values observed in this study in winter 1998–1999. Differences in photochemical efficiency were not associated with similar differences in midday qN values (Figure 4C) and no significant correlation was found between qN and Φ_{PSII} or F_v'/F_m'.

Pigment concentrations and diurnal variation

In both years, pigment concentration per unit leaf area differed among the species. *Quercus ilex* ssp. *ballota* always had higher pigment concentrations than the other species followed closely by *Q. coccifera* during winter 1998–1999 (Table 1). During this period, *P. halepensis* displayed the lowest concentrations of carotenoids and chlorophylls, which were also low in *J. phoenicea*. The Chl a/b ratio was higher in *P. halepensis* than in the other species.

Although leaf-area-based pigment concentration varied from December to February, the variation was not in the same direction in the two years of study. During winter 1997–1998, species showed an increase in almost all pigments analyzed in February, whereas in winter 1998–1999 the reverse was true. Despite these differences between years, changes in xanthophyll cycle pigments and lutein concentration per chlorophyll unit (VAZ/Chl a+b and Lut/Chl a+b, respectively) were consistent in both years. In each sampling month, the VAZ/Chl a+b ratio was similar in all species except *P. halepensis*, in which the ratio was significantly lower (Figure 5B). In both years, the *Quercus* species showed an increase in VAZ/Chl

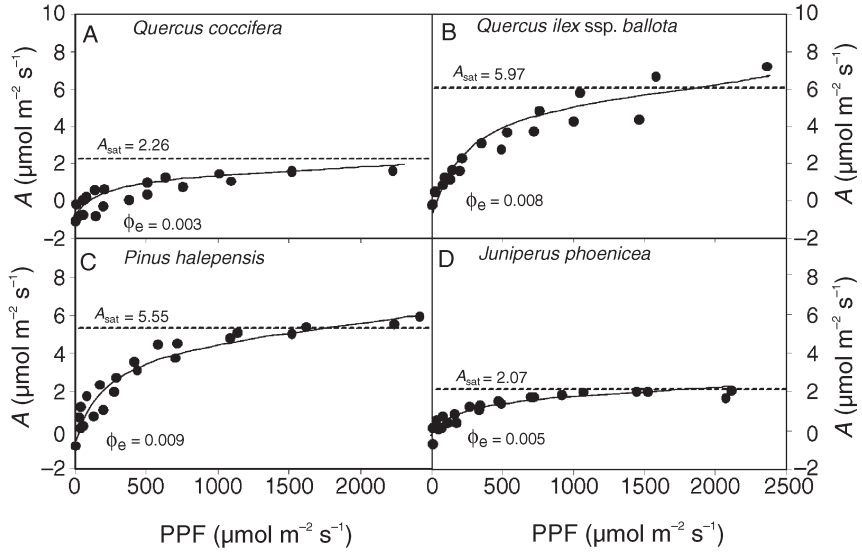

Figure 2. Photosynthetic light response curves fitted to data from two current-year leaves of (A) *Q. coccifera*, (B) *Q. ilex* ssp. *ballota*, (C) *P. halepensis* and (D) *J. phoenicea*, measured in February 1999. The dotted line represents the light-saturated photosynthetic rate (A_{sat}) and ϕ_e represents the apparent photosynthetic quantum yield. Abbreviation: PPF = photosynthetic photon flux.

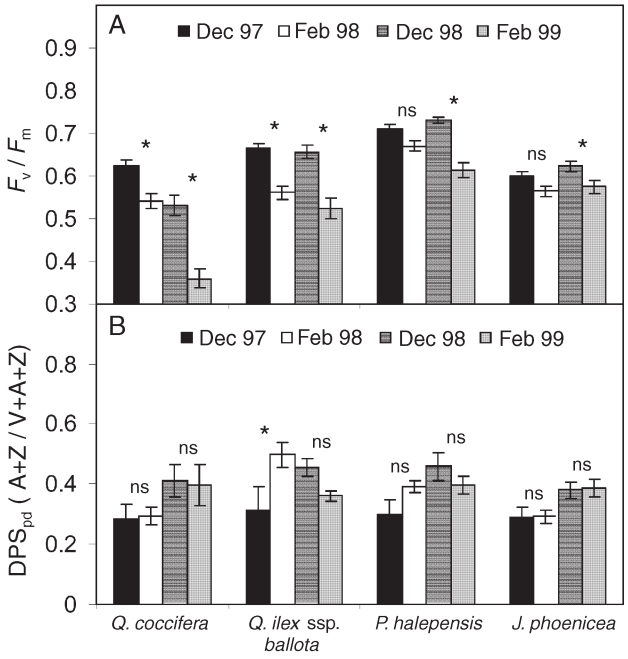

Figure 3. Variation during winter months (December 1997, 1998, February 1998, 1999) of predawn values of (A) maximal photochemical efficiency of Photosystem II (F_v/F_m; $n = 30–45$) and (B) de-epoxidation index (DPS_{pd}; $n = 6–9$) in current-year leaves of *Q. coccifera*, *Q. ilex* ssp. *ballota*, *P. halepensis* and *J. phoenicea*. Error bars represent ± SE. Symbols above December and February indicate: (*) a significant difference ($P < 0.05$) within each winter; and (ns) no significant difference. Abbreviations: V = violaxanthin; A = antheraxanthin; and Z = zeaxanthin.

a+b ratio from December to February (Figure 5B) that was paralleled by a significant increase in the Lut/Chl a+b ratio, which reached significantly higher values in the *Quercus* species than in *P. halepensis* and *J. phoenicea* in February (Figure 5C).

No significant variation in either VAZ/Chl a+b or Lut/Chl a+b was found from predawn to midday during either winter. Values of the de-epoxidation index (DPS) did not vary significantly from December to February in any species except in *Q. ilex* ssp. *ballota*, which showed an increase in DPS_{pd} from December 1997 to February 1998 (Figure 3B). There were no significant differences in DPS_{pd} among species in either winter (Figure 3B). All species experienced a marked and highly significant ($P < 0.001$) increase in DPS_{md} (Figure 5A), but there were no significant differences among species in the degree of photoconversion of the epoxidized forms at midday.

Discussion

Photoinhibition during the Mediterranean winter

Chronic photoinhibition occurred in our Mediterranean evergreen species under winter field conditions as made manifest by low predawn photochemical efficiency of PSII (F_v/F_m ranged from 0.4–0.7) in all species, suggesting a cumulative effect of low temperatures and high irradiance as the winter progressed. There were no symptoms of water stress, because predawn water potentials were moderately high (-1.5 MPa $< \Psi_{Spd} < -0.5$ MPa). In Mediterranean evergreen species (García-Plazaola et al. 1999*a*, 1999*b*, 2003) and in other types of ecosystems (Baker 1995, Verhoeven et al. 1996), low predawn F_v/F_m values during winter have been associated with the overnight retention of high amounts of zeaxanthin and antheraxanthin, attributed to the inhibitory effect of chilling temperatures on the enzymatic conversion of zeaxanthin and antheraxanthin to violaxanthin (Adams and Demmig-Adams 1994 and 1995, Adams et al. 1995). Our results with *J. phoenicea*, *P. halepensis*, *Q. coccifera* and *Q. ilex* are partly consistent with those findings. When minimum daily temperatures were below 2 °C throughout the sampling months, all species had significant amounts of zeaxanthin and antheraxanthin at predawn, resulting in high DPS_{pd} values (from 0.28–0.49). However, DPS_{pd} values did not vary significantly between sampling months in either winter, despite concomitant decreases in F_v/F_m and minimum temperatures throughout the winter. The high winter DPS_{pd} values and VAZ size do

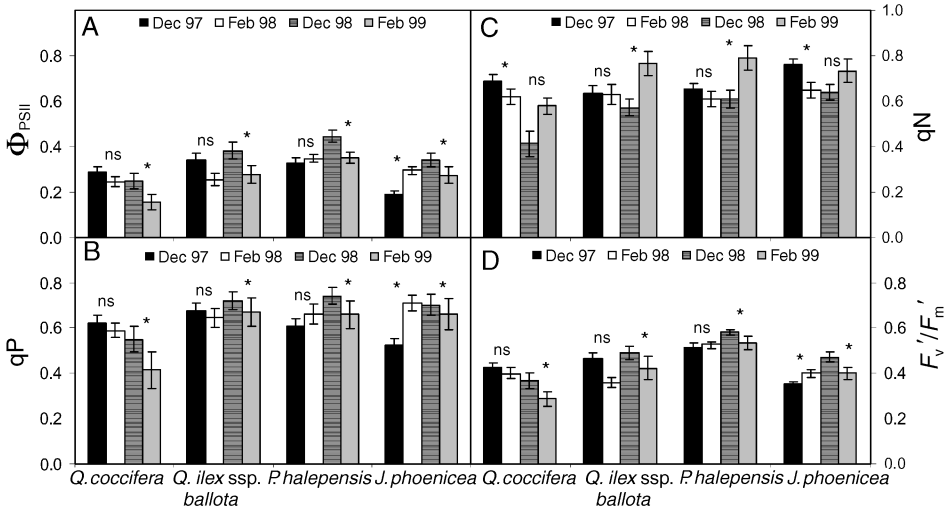

Figure 4. Variation during winter months of predawn values of (A) quantum yield of non-cyclic electron transport (Φ_{PSII}), (B) photochemical quenching (qP), (C) photochemical efficiency of the open reaction centres of PSII (F_v'/F_m') and (D) non-photochemical quenching of fluorescence (qN) in current-year leaves of *Q. coccifera*, *Q. ilex* ssp. *ballota*, *P. halepensis* and *J. phoenicea*. Error bars represent ± SE ($n = 30–45$). Symbols above December and February indicate: (*) a significant difference ($P < 0.05$) within each winter; and (ns) no significant difference.

Table 1. Pigment composition (μmol m^{-2} leaf area) in current-year leaves of *Quercus coccifera*, *Q. ilex* ssp. *ballota*, *Pinus halepensis* and *Juniperus phoenicea* during December and February of winter 1997–1998 and winter 1998–1999. Each value represents the mean (n = 12–18). Statistical differences between species (SP) and month each winter are indicated by different letters (2-way ANOVA followed by LSD). Significant interaction between SP and Month are denoted by an asterisk; ns indicates no significant differences.

Pigment	Month	Winter 1997–1998						Winter 1998–1999					
		Q. coccifera	*Q. ilex* ssp. *ballota*	*P. halepensis*	*J. phoenicea*	SP	Month	*Q. coccifera*	*Q. ilex* ssp. *ballota*	*P. halepensis*	*J. phoenicea*	SP	Month
Neoxanthin	Dec	25.508 a	35.063 b	17.366 c	19.194 c	$P < 0.01$	$P < 0.01$	34.602 a	45.162 b	12.746 c	19.442 d	$P < 0.01$	$P < 0.01$
	Feb	33.330 b	38.944 b	24.600 a	24.268 a			25.117 de	28.751 ae	6.178 c	8.350 c		
Violaxanthin (V)	Dec	34.906 ac	44.211 ab	33.402 a	36.248 a	ns	$P < 0.01$	66.413 a	81.684 a	25.558 b	39.256 bc	$P < 0.01$	ns
	Feb	51.152 b	48.308 bc	41.176 ab	52.398 b			62.621 ac	64.766 ac	17.382 b	26.339 b		
Antheraxanthin (A)	Dec	13.640 a	20.740 b	15.138 a	17.533 ab	$P < 0.01$	$P < 0.01$	31.688 a	46.114 b	17.472 c	25.669 a	$P < 0.01$	$P < 0.01$
	Feb	21.584 bd	35.859 c	20.864 b	25.205 d		*	27.391 a	27.766 a	7.659 d	13.429 bd		
Zeaxanthin (Z)	Dec	18.345 ace	27.393 b	11.463 ae	19.153 ace	$P < 0.05$	$P < 0.01$	36.644 a	55.666 b	12.219 c	25.096 a	$P < 0.01$	$P < 0.01$
	Feb	22.202 cb	39.742 d	11.623 e	16.852 ac		*	25.702 a	26.842 a	4.076 c	8.098 c		
VAZ	Dec	66.891 ad	92.344 b	60.003 ad	72.934 ad	$P < 0.01$	$P < 0.01$	134.745 a	183.464 b	55.249 c	90.020 d	$P < 0.01$	$P < 0.01$
	Feb	94.938 b	123.909 c	73.663 d	94.455 b			115.713 a	119.374 a	29.117 c	47.866 c		
Lutein	Dec	112.808 ae	145.235 b	86.685 c	85.876 c	$P < 0.01$	$P < 0.01$	173.831 a	207.885 b	72.417 cd	90.028 c	$P < 0.01$	$P < 0.01$
	Feb	163.369 d	179.027 d	118.650 a	95.926 ce		*	147.813 a	147.678 a	40.857 de	39.297 e		
β-Carotene	Dec	105.444 a	106.824 a	73.558 b	47.446 c	$P < 0.01$	$P < 0.01$	160.349 a	154.065 a	62.359 b	42.383 bd	$P < 0.01$	$P < 0.01$
	Feb	127.360 d	112.533 a	100.947 a	42.602 c		*	126.640 c	108.858 c	44.958 bd	26.615 d		
Chl a+b	Dec	372.462 ad	498.354 b	320.778 ac	296.267 c	$P < 0.01$	$P < 0.01$	444.409 a	561.500 b	217.470 c	271.187 cd	$P < 0.01$	$P < 0.01$
	Feb	428.848 d	500.643 b	427.832 d	352.228 ac			315.589 d	353.964 d	112.063 e	111.724 e		
Chl a/Chl b	Dec	2.392 a	2.255 b	2.617 c	2.197 b	$P < 0.01$	$P < 0.01$	2.603 a	2.553 a	2.990b	2.618 a	$P < 0.01$	$P < 0.01$
	Feb	2.600 c	2.582 c	2.941 d	2.394 a			3.130 b	2.933 b	3.714 c	3.159 b		

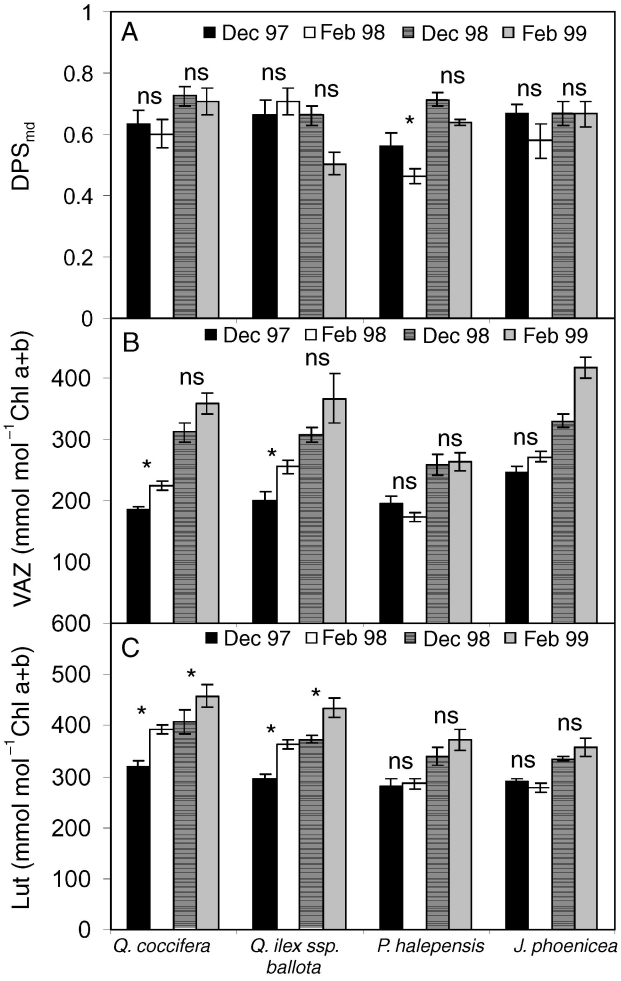

Figure 5. Variation during winter months of (A) midday de-epoxidation index (DPS_{md}), (B) pool xanthophyll cycle pigments on a total chlorophyll basis (VAZ) and (C) lutein concentration on a total chlorophyll basis (Lut) in current-year leaves of *Q. coccifera*, *Q. ilex* ssp. *ballota*, *P. halepensis* and *J. phoenicea*. Error bars represent ± SE (n = 12–18). An asterisk indicates significant differences between December and February ($P < 0.05$) within each winter; NS indicates no significant differences.

not explain the observed changes in maximal PSII photochemical efficiency (F_v/F_m) during the winter or the differences among species in degree of chronic photoinhibition.

During the warmer winter (1997–1998), the observed variation in F_v/F_m was probably related to changes in DPS (e.g., *Q. ilex*; Figure 3) or VAZ size or both (Figure 5). However, in the colder winter, the marked decrease in F_v/F_m observed in all study species from December 1998 to February 1999 was not associated with changes in DPS or VAZ size. This suggests that, when winter minimal temperatures are mostly below 0 °C, chronic photoinhibition in these species is not only attributable to the persistence of slowly reversible photoprotective mechanisms but may also involve some degree of cumulative damage (e.g., D1 protein degradation; Ottander et al. 1995) toward the end of the winter.

The study species behaved differently under winter conditions. *Quercus coccifera* showed a more marked decrease in F_v/F_m (~32 %) than *Q. ilex* ssp. *ballota* (~20 %), *P. halepensis* (~16 %) and *J. phoenicea* (~8 %) from December 1998 to February 1999, indicating differences in sensitivity to winter conditions or the operation of different photoprotective mechanisms in the study species. Recent studies have implicated the lutein epoxy cycle in photoprotection of Mediterranean evergreen species belonging to the genus *Quercus* (García-Plazaola et al. 2003) as a mechanism to maintain sustained energy dissipation. We found that the *Quercus* species had higher lutein concentrations than *P. halepensis* and *J. phoenicea* that can account for the more marked decrease in F_v/F_m observed in the *Quercus* species during winter. However, lutein concentration did not parallel the observed species differences in degree of photoinhibition: *P. halepensis* showed the highest values of photochemical efficiency of PSII, both at predawn (F_v/F_m) and at midday (Φ_{PSII}), followed closely by *Q. ilex* ssp. *ballota*, *J. phoenicea* and *Q. coccifera*. The higher PSII photochemical efficiency observed in *P. halepensis* and *Q. ilex* ssp. *ballota* is consistent with the higher photosynthetic capacity of *Q. ilex* ssp. *ballota* and *P. halepensis* (see light response curves) during the most stressful month (February 1999).

The higher values of photochemical efficiency observed in *P. halepensis* than in *Q. ilex* ssp. *ballota* were not associated with differentially lower values of qN, indicating that photoprotection in *P. halepensis* may be associated with a higher contribution of alternative non-photochemical mechanisms. Ottander et al. (1995) suggested that some photosystem I polypeptides in *P. sylvestris* are associated with photo-dissipating chlorophyll–xanthophyll aggregates that could facilitate energy transference between PSII and PSI. In this context, the higher Chl a/b ratios observed in *P. halepensis* could indicate the presence of more xanthophyll binding sites. This mechanism for avoiding photoinhibitory damage in winter may be more important in *P. halepensis* than in the other species. The photoprotective response of *Q. ilex* ssp. *ballota* to winter conditions seems to be associated with a high pigment concentration, particularly pigments with a β-cyclic de-epoxidized end-group structure (antheraxanthin, zeaxanthin and lutein) that may cause a conformational change in chlorophyll binding proteins leading to the development of qN (Gilmore 1997).

In *Q. coccifera* and *J. phoenicea*, the marked chronic photoinhibition (F_v/F_m) together with the high degree of PSII photoinactivation (F_v'/F_m') are in accordance with the low photosynthetic capacity observed in these species. However, the greater photoinhibition in *Q. coccifera* during winter 1998–1999 than during winter 1997–1998 suggests that this species is probably more sensitive to winter stress than *J. phoenicea*, which is consistent with its absence in the eastern Mediterranean basin and its infrequent occurrence or absence in continental areas experiencing cold winters (Rambal 1984, Tenhunen et al. 1985, Terradas 1999).

Species differences in photoinhibition are also likely to be the combined result of differences in leaf physiology and foliage morphology and architecture (Valladares 1999). The particular leaf morphology and crown architecture of pines leads to a relatively low exposure of foliar surface area to direct sun-

light because of the combination of curved and cylindrical leaves, steep leaf angles and significant mutual shading between adjacent leaves within clusters. In *Quercus* species, small broad leaves placed at regular internodes (Valladares et al. 2002) with high spatial variability in leaf angle, allow irradiance to be distributed to a large foliage area, thereby preventing most individual leaves from intercepting too much light (Werner et al. 1999). This leaf arrangement can be viewed as an effective compromise between efficient light harvesting and avoidance of excessive radiation in evergreen plants and may optimize annual primary production (Werner et al. 2001). Nevertheless, architectural photoprotection in *Quercus* species may be less effective than in *P. halepensis* because modulation of leaf angles is not rapid enough to avoid photochemical damage in the outer parts of the crown. The crown architecture of *J. phoenicea* precludes the possibility of architectural photoprotection in this species.

Winter photoprotection versus summer photoprotection

Our results support a previous classification of these species into photoinhibition-avoiding (*P. halepensis*) and photoinhibition-tolerant (*Q. coccifera*, *Q. ilex* ssp. *ballota* and *J. phoenicea*) species on the basis of their susceptibility to dynamic photoinhibition (Martínez-Ferri et al. 2000). Photoinhibition-avoiding species are able to maintain a sustained PSII photochemical efficiency, whereas photoinhibition-tolerant species tolerate a more pronounced dynamic photoinhibition.

Dynamic photoinhibition has been associated with fast reversible mechanisms (Demmig-Adams and Adams 1992, Long et al. 1994), mostly those associated with the formation of non-epoxy xanthophylls, leading to the development of non-photochemical quenching. However, the comparison between the results for summer (Martínez-Ferri et al. 2000) and winter (present study) has identified several different aspects of the photoprotective role of the xanthophyll cycle. We found lower qN values in winter than in summer, despite the similar degree of photoinactivation of PSII (F_v'/F_m') and the higher values of VAZ/Chl a+b and DPS_{md} in winter than in summer.

These results contradict previous reports of non-photochemical energy dissipation at low temperatures. It has been shown that, at low temperatures, the transthylakoid pH gradient (ΔpH) increases as a result of a low rate of proton consumption by ATPase (Gilmore 1997) and thus, the energy-dependent non-photochemical quenching related with the photoconversion of xanthophyll cycle pigments (Gilmore and Björkman 1995). Moreover, Brüggeman and Koroleva (1995) reported that, in response to chilling temperatures, increases in energy-dependent non-photochemical quenching are proportional to the product of ΔpH and zeaxanthin + antheraxanthin concentration. Consequently, at low temperatures, low pigment concentrations would imply high qN values.

Most studies on the photoprotective role of the xanthophyll cycle have relied on correlative evidence, although there are studies reporting a nonlinear and even non-uniform relationship between zeaxanthin concentration and non-photochemical quenching, which raises the question of whether enhanced VAZ in high-light-acclimated leaves is associated with non-photochemical quenching alone (e.g., Brugnoli et al. 1998, Förster et al. 2001). In this context, Hurry et al. (1997) suggested that only a few of the zeaxanthin molecules present in the light-harvesting antenna of PSII are involved in the development of non-photochemical quenching and that an increase in conversion of violaxanthin to zeaxanthin will not necessarily enhance photoprotective energy dissipation. This suggestion could explain why the higher DPS_{md} values observed during winter were not associated with higher qN values at midday.

There is evidence that a certain fraction of zeaxanthin molecules are associated with pigment-binding complexes that are solubilized in thylakoid and chloroplast envelope membranes, providing membrane protection against photo-oxidative damage (Havaux and Niyogi 1999). The larger quantity of VAZ observed in winter than in summer could confer greater stability on the membranes, protecting them from the deleterious effects of low temperatures (Powles 1984) through a structural photoprotective role (Gruszecki 1995). Therefore, winter photoprotection in the four study species seems to be associated with photophysical and structural aspects.

The photoinhibition-avoiding and photoinhibition-tolerant categories defined above can be extended to chronic photoinhibition observed in winter. The dynamic photoinhibition in winter did not differ from that observed in summer despite lower temperatures and increased chronic photoinhibition, suggesting that dynamic photoinhibition is a phenomenon independent from chronic photoinhibition.

In conclusion, Mediterranean-type ecosystems are exposed to drought stress during the late-summer drought and low temperature stress in winter (Mitrakos 1982). The degree and extent of both stresses vary widely among years and regions, but both induce over-excitation of the photosynthetic apparatus and the development of energy dissipating mechanisms. Therefore, the photoprotective ability of Mediterranean evergreen species may determine their capacity to withstand stresses. Although summer stress has traditionally been considered to be the main factor limiting plant survival of Mediterranean evergreen species (Damesin and Rambal 1995, Faria et al. 1998), winter stress is crucial when interpreting species distribution in northern and montane districts of the Mediterranean region (Treitach et al. 1997). Our results provide new information on the significance of winter stress on the performance of Mediterranean evergreen species. Photoprotective mechanisms were insufficient to protect the trees entirely from a chronic reduction in maximal photochemical efficiency of PSII during winter. This may have implications for the long-term carbon budget of Mediterranean evergreen species.

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