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Physiological response of eight Mediterranean maquis species to low air temperatures during winter

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Abstract

We analyzed the physiological response of the Mediterranean evergreen species (*Arbutus unedo* L., *Cistus incanus* L., *Erica arborea* L., *Erica multiflora* L., *Phillyrea latifolia* L., *Pistacia lentiscus* L., *Quercus ilex* L., and *Rosmarinus officinalis* L.) to winter low air temperatures. In occasion of two cold events, in February 2005 (T_{min} = 1.8 °C), and January 2006 (T_{min} = 3.1 °C and minimum T_{air} = –0.40 °C during the nights preceding the measurements), *R. officinalis*, *C. incanus*, and *E. multiflora* had the highest net photosynthetic rate (P_{N}) decrease (73 %, mean value) with respect to the winter P_{N} maximum, followed by *A. unedo* (62 %), *P. latifolia* and *P. lentiscus* (54 %, mean value), *E. arborea* (49 %), and *Q. ilex* (44 %). Among the considered species, *Q. ilex* was able to maintain P_{N} near the maximum for 150 min during the day, *A. unedo*, *P. lentiscus*, *E. arborea, P. latifolia*, *E. multiflora*, and *R. officinalis* for 60 min, and *C. incanus* for 30 min. The calculated mean winter daily P_{N} ranged from 7.9±0.6 (*Q. ilex*) to 2.8±0.5 (*R. officinalis*) μmol(CO$_2$) m^{-2} s^{-1}. During the study period, chlorophyll (Chl) content decreased by 36 % on an average in the two cold events, and the carotenoid (Car) to Chl ratio increased by 133 % in *Q. ilex*, having the highest value in January 2006. Principal component analysis underlined the highest cold resistance of *Q. ilex* by high P_{N} and high Car/Chl ratio. On the contrary, *R. officinalis* and *C. incanus* had the lowest cold resistance by the highest P_{N} decrease and the lowest Car/Chl (*C. incanus*). Thus, winter stress could be an additional limitation to Mediterranean evergreen species production, and the capacity of the species to maintain P_{N} near 90–100 % during winter is determinant for biomass accumulation.

Additional key words: carotenoids; chlorophyll; cold events; evergreen species; photosynthesis; stomatal conductance.

Introduction

Drought stress is one of the most important limiting factors of the Mediterranean evergreen species carbon gain, particularly when water deficit is associated with high air temperature and irradiance (Gratani and Bombelli 2000, Gratani and Varone 2004). Nevertheless, winter stress may represent an additional limitation to Mediterranean plant production (Larcher 2000). Although the mildness of the Mediterranean climate may allow a relatively high photosynthetic activity of evergreen species in winter (Larcher 2000, Oliveira and Peñuelas 2000), very cold winter and frost can be expected every decade in the Mediterranean Basin (Terradas and Savé 1992, Larcher 2000). The combination of high irradiance and sub-optimal growth air temperatures during winter causes a depression of the photosynthetic activity (García-Plazaola *et al*. 1999, Larcher 2000, Oliveira and Peñuelas 2002). Moreover, the short winter photoperiod negatively influences plant metabolism of Mediterranean species (Tretiach *et al*. 1997, Karavatas and Manetas 1999). Consequently, the photosynthetic activity of evergreen species reaches 1/3 to 2/3 of the yearly maximum rates in winter (Larcher 2000).

Any factor which reduces photosynthetic carbon fixation also reduces the species' productivity, and therefore the ability to compete in its habitat (Groom *et al*. 1991). Thus, photosynthesis may provide an indicator for functional limitations imposed by environmental stress (Larcher 1994, 2000). The maintenance of sufficiently high photosynthetic rates in winter may play a major role in explaining the fitness and distribution of the Mediterranean species (Antolin *et al*. 2005).

The main objective of this research was to analyze the physiological response of the Mediterranean evergreen species (*Arbutus unedo* L., *Cistus incanus* L., *Erica*

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Abbreviations: Car – carotenoid; Chl – chlorophyll; g_{s} – stomatal leaf conductance; g_{smax} – daily maximum stomatal leaf conductance; P_{N} – net photosynthetic rate; P_{Nmax} – daily maximum photosynthetic rate; PAR – photosynthetically active radiation; T_{air} – air temperature; T_{min} – monthly mean minimum air temperature.

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arborea L., *Erica multiflora* L., *Phillyrea latifolia* L., *Pistacia lentiscus* L., *Quercus ilex* L., and *Rosmarinus officinalis* L.) to winter low air temperatures. The following questions were addressed: (*1*) Can photosynthesis be a potential indicator of cold tolerance? (*2*) What is the magnitude of the photosynthetic response to low air temperatures? (*3*) Are the most drought tolerant species also the most cold-tolerant ones?

Materials and methods

Study area: The study was conducted in the Mediterranean maquis developing within the Castelporziano Estate (41°45'N, 12°26'E, Rome) (Gratani and Crescente 2000). The area climate is of Mediterranean type. Total annual rainfall was 721 mm mostly distributed in autumn-winter, and dry period was from May to August. The mean maximum air temperature of the hottest month (August) was 30.7±1.4 °C and the mean minimum air temperature of the coldest months (January and February) was 3.9±1.8 °C (data by the Castelporziano Meteorological Station for the period 1985–2005). The annual amount of global solar radiation can be estimated on an average at 4 250 MJ m^{-2} (Gratani *et al.* 2000) and most of the winter days were characterised by cloud-free sky.

Field measurements were carried out through winter months (January, February, and December) from 2001 to 2006. The winters 2005 and 2006 were characterised by low air temperatures and nights of minimum air temperatures below 0° C.

Leaf gas exchange was measured during the day (25 January, 7 February, and 11 December, 2001; 2 February, 2002; 3 December, 2003; 12 January, 2004) from 07:30 to 16:30 to determine daily maximum photosynthetic rate, P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}] and daily maximum stomatal leaf conductance, g_{smax} [mmol(CO_2) m^{-2} s^{-1}], according to Gratani *et al.* (2006). Mean daily photosynthetic rate per species was then calculated. On nine occasions in each of the considered months, gas exchange was measured.

Photosynthetically active radiation, PAR [μmol(photon) m^{-2} s^{-1}], net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], and stomatal conductance, g_s [mmol(H_2O) m^{-2} s^{-1}] were monitored with an infrared gas analyser, *Ciras-1* open system (*PP Systems*, UK). The analyser was equipped with a leaf chamber *PLC* narrow cuvette for measurements of *E. arborea*, *E. multiflora*, and *R. officinalis* and with a 2.5 cm^2 leaf area chamber (*Ciras-1 Parkinson* leaf cuvetes) for the other considered species. All measurements were carried out under natural conditions, on cloud-free days (Reich *et al.* 1995). During gas exchange measurements, air temperature, T_{air} [°C] was measured with a thermo-hygrometer (*HD8901*, *Delta Ohm,* Italy).

Field measurements were carried out *in situ* on the external exposed apical shoots (Munné-Bosch *et al.* 1999) of shrubs of *E. arborea*, *E. multiflora*, and *R. officinalis* (three shoots per shrub) and on "sun" leaves of shrubs of *Q. ilex*, *P. latifolia*, *P. lentiscus,* and *C. incanus* (three leaves per shrub).

Chlorophyll (Chl) and carotenoid (Car) contents were determined in leaf samples (five per species) occasionally during the study period. Immediately after collection, fully expanded leaves from the selected shrubs were kept cool in the dark for 3 h. Leaves were ground in acetone. The homogenates were centrifuged in a *4237R* refrigerated centrifuge (*A.L.C.*, Italy). Absorbance of the supernatants was measured with a *Jasco* model *7800 LCD* (Japan) spectrophotometer at the wavelengths of 645, 663, and 440 nm for Chl *a*, Chl *b*, and Car, respectively. Chl and Car amounts per fresh mass were calculated according to MacLachlan and Zalik (1963) and Holm (1954), respectively.

Statistics: Statistical differences in leaf traits were determined by analysis of variance (ANOVA) and Tukey test for multiple comparisons. Simple regression analysis was conducted to analyze correlation between P_N and g_s. Moreover, a multiple regression analysis was carried out, using P_N as dependent variable and Chl, Car, and Car/Chl as independent variables. The considered physiological leaf traits (P_N, g_s, Car/Chl, Chl, and Car) were analysed by Principal Component Analysis (PCA) on the basis of a matrix of the normalised data. The matrix was subjected to a rotated principal component analysis to summarise the main factors determining the variations of the analysed traits in the considered species (García-Plazaola *et al.* 2000). All statistical tests were performed using a statistical software package (*Statistica*, *Statsoft*, USA).

Results

Daily winter gas exchange trend: Diurnal trends of PAR and T_{air} were characterized by low values at 08:00 [752±92 μmol(photon) m^{-2} s^{-1} and 8.5±0.5 °C, respectively], a maximum at 12:00 [1 458±61 μmol(photon) m^{-2} s^{-1} and 17.4±0.6 °C], a significant ($p<0.05$) decrease at 15:00 [1 017±94 μmol(photon) m^{-2} s^{-1} and 13.2± 1.1 °C], and the lowest values at 16:30 [500±53 μmol(photon) m^{-2} s^{-1} and 8.1±0.4 °C] (Fig. 1).

The considered species had a typical one-peak time course of P_N during the day. P_{Nmax} was monitored late in the morning (between 11.00 and 12:30) at T_{air} of 15.9± 0.9 °C for *Q. ilex*, *P. latifolia*, and *A. unedo* [10±0.3 μmol(CO_2) m^{-2} s^{-1}, mean value], *P. lentiscus* and *E. arborea* [6.9±0.1 μmol(CO_2) m^{-2} s^{-1}, mean value] and at 17.8±0.6 °C for *C. incanus* [12.6±0.6 μmol(CO_2) m^{-2} s^{-1}], *R. officinalis* and *E. multiflora* [6.0±0.3 μmol(CO_2) m^{-2} s^{-1}, mean value] (Fig. 2*A*).

The capacity to maintain P_N near the maximum during the day varied among the considered species. *Q. ilex* maintained P_N between 90 and 100 % of the maximum rate for 150 min during the day, *A. unedo*, *P. lentiscus*, *E. arborea*, *P. latifolia*, *E. multiflora,* and *R. officinalis* for 60 min, and *C. incanus* for 30 min. The calculated mean daily P_N were: 7.9±0.6, 7.4±1.1, 6.9±0.5, 5.7±0.8, 5.0±0.4, 4.1±0.6, 3.4±0.5, and 2.8±0.5 μmol(CO_2) m^{-2} s^{-1} for *Q. ilex*, *C. incanus, A. unedo, P. latifolia, P. lentiscus, E. arborea, E. multiflora,* and *R. officinalis*, respectively.

g_s had daily trend similar to that of P_N: all the considered species reached g_{smax} in correspondence with P_{Nmax}. Among the considered species, *C. incanus*, *A. unedo*, and *P. latifolia* had the highest g_{smax} [208±20, 133±15, and 127±10 mmol(H_2O) m^{-2} s^{-1}, respectively], followed by *P. lentiscus* and *E. arborea* [123±2 mmol(H_2O) m^{-2} s^{-1}, mean value], *Q. ilex* [111±21 mmol(H_2O) m^{-2} s^{-1}], *E. multiflora* [66±3 mmol(H_2O) m^{-2} s^{-1}], and *R. officinalis* [49±5 mmol(H_2O) m^{-2} s^{-1}] (Fig. 2*B*).

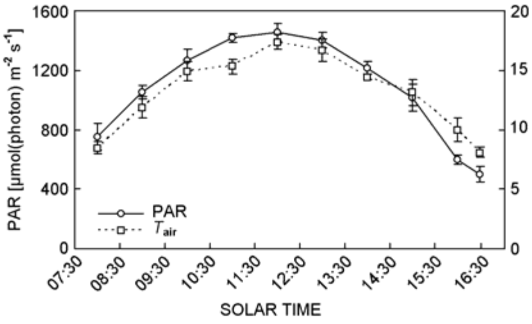

Fig. 1. Daily trend of photosynthetically active radiation (PAR) and air temperature (T_{air}) in winter (25 January, 7 February, 11 December, 2001; 2 February, 2002; 3 December, 2003; 12 January, 2004). *Standard errors* are shown.

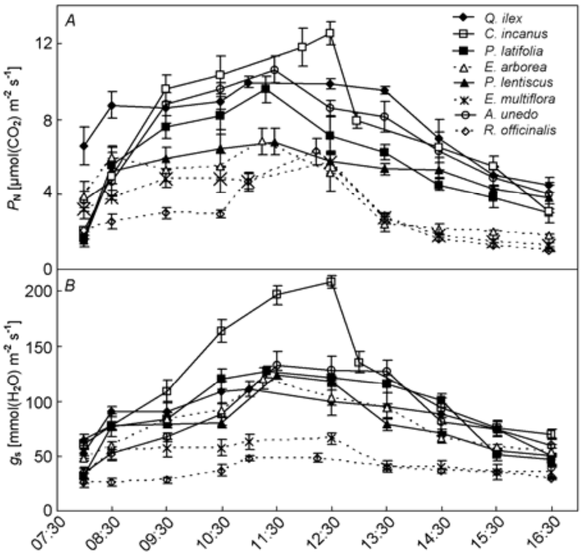

Fig. 2. Daily courses of (*A*) net photosynthetic rate, P_N and (*B*) stomatal conductance, g_s of the considered species for the study period (25 January, 7 February, and 11 December, 2001; 2 February, 2002; 3 December, 2003; 12 January, 2004). *Each bar* is the mean of nine measures during the day. *Standard errors* are shown.

Monthly winter gas exchange trend: During the study period the highest P_N for the considered species were found in December 2003 (T_{min} = 5.6 °C and T_{air} = 16.8±1.2 °C). *C. incanus* had the highest P_N [18.5±1.0 μmol(CO_2) m^{-2} s^{-1}], followed by *A. unedo* (14.6±1.0), *Q. ilex* and *P. latifolia* (12.3±0.3, mean value), *E. arborea* (8.4±1.0), and *P. lentiscus*, *R. officinalis,* and *E. multiflora* (8.2±0.2, mean value) [μmol(CO_2) m^{-2} s^{-1}] (Fig. 3*A*).

The lowest P_N values of the considered species were found in February 2005 (T_{min} = 1.8 °C) and January 2006 (T_{min} = 3.1 °C). In February 2005, P_N decreased with respect to the winter P_N maximum (December 2003) by 65 % in *C. incanus*, *R. officinalis*, and *E. multiflora* (mean value), 55 % in *A. unedo* and *P. latifolia* (mean value), 45 % in *P. lentiscus* and *E. arborea* (mean value), and 41 % in *Q. ilex*. Nevertheless, the absolute minimum P_N was found in January 2006 due to the lowest air temperatures during the nights preceding the gas exchange measurements (–0.4±0.1 °C): P_N decreased by 83 % in *R. officinalis*, 78 % in *E. multiflora* and *C. incanus* (mean value), 66 % in *A. unedo* and *P. lentiscus* (mean value), 54 % in *P. latifolia* and *E. arborea* (mean value), and 48 % in *Q. ilex*.

g_s had a similar monthly P_N trend (Fig. 3*B*) confirmed by the significant ($p<0.001$) correlation (r = 0.86) between P_N and g_s (Fig. 4). The highest g_s values were monitored in December 2003 ranging from 299±2 mmol(H_2O) m^{-2} s^{-1} (*C. incanus*) to 76±1 mmol(H_2O) m^{-2} s^{-1} (*R. officinalis*).

The absolutely lowest g_s values were found in January 2006; in particular, *P. lentiscus*, *A. unedo*, and *P. latifolia* had the largest g_s decrease (82 %, mean value), followed by *Q. ilex*, *C. incanus*, and *E. multiflora*, (79 % mean value), *R. officinalis* (54 %), and *E. arborea* (41 %).

Chl and Car contents: In correspondence with the maximum P_N and g_s (December 2003) the highest Chl values were found [g kg^{-1}]: 0.959±0.046 (*P. lentiscus*), 0.789±0.005 (*P. latifolia*), 0.770±0.007 (*Q. ilex*, *A. unedo*, and *C. incanus*, mean value), 0.738±0.004 (*E. arborea*), and 0.553±0.015 (*E. multiflora* and *R. officinalis*, mean value) (Fig. 5*A*).

The lowest Chl and the highest Car contents were found in January 2006 for all the considered species: *Q. ilex* had the highest Car/Chl (0.503±0.003), followed by *R. officinalis* (0.486±0.001), *P. latifolia* (0.484±0.002), *E. arborea* and *E. multiflora* (0.420±0.007, mean value),

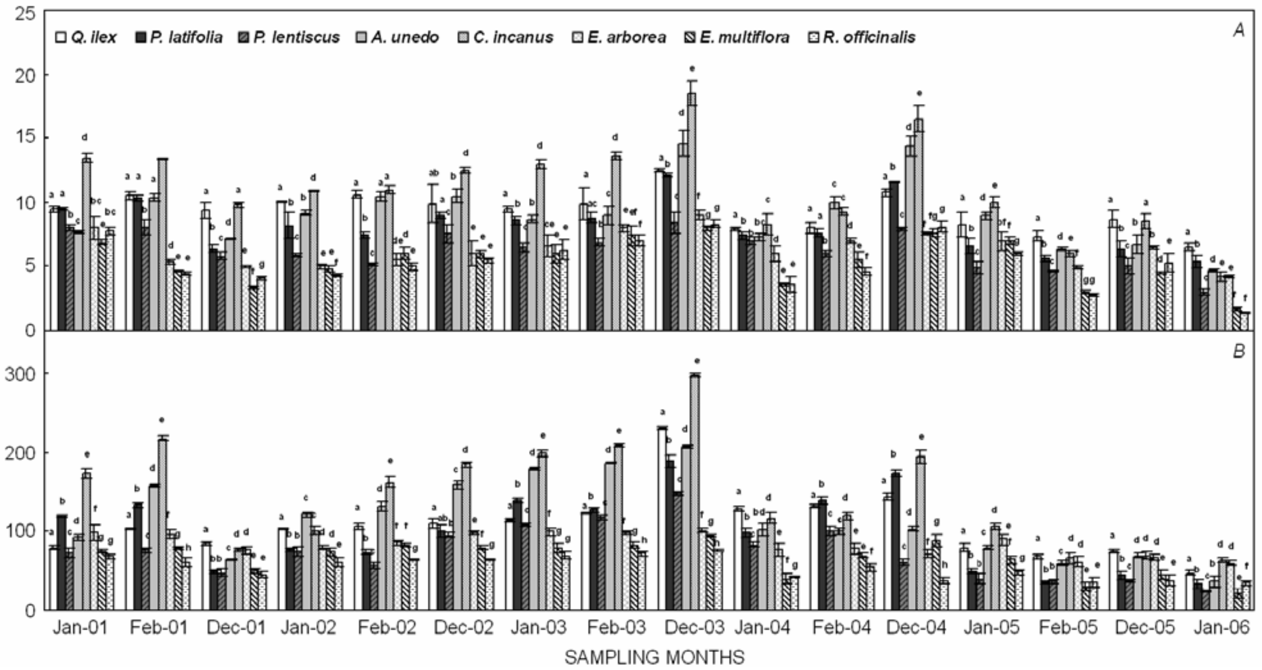

Fig. 3. Monthly courses of (*A*) net photosynthetic rate, P_N and (*B*) stomatal conductance, g_s of the considered species for the study period. *Each bar* is the mean of nine measurement days in each of the considered months. *Standard error*s are shown. Within the same month, inter-specific differences with the same letters are not significant (ANOVA, $p>0.05$).

Table 1. Results of multiple regression analysis using the net photosynthetic rate (P_N) as dependent variable and chlorophyll content (Chl), carotenoid content (Car), and carotenoid/chlorophyll ratio (Car/Chl) as independent variables. Chl and Car/Chl were the only variable extract by the analysis. Multiple R value, intercept value, un-standardized (Beta coefficient), standardized (B coefficient) regression coefficients, and significance levels of those coefficients (*p*) are shown.

Independent variable		Chl	Car/Chl
Multiple R value	0.650		
Intercept	6.296		
Beta regression coefficient		0.35	–0.35
B regression coefficient		8.2	13.6
p		0.001	0.001

A. unedo (0.393±0.003), and *P. lentiscus* (0.369±0.011). *C. incanus* had the lowest Car/Chl (0.336±0.002) (Fig. 5*B*). Multiple regression analysis between P_N (dependent variable) and Chl, Car, and Car/Chl (independent variables) showed that Chl and Car/Chl significantly ($p \leq 0.01$) explained 65 % of P_N variation (Table 1).

PCA using P_N, g_s, Car/Chl, Chl, and Car contents showed two principal axes explaining 86 % of the total variance. In particular, the first axis accounted for 60 % of the total variance and it was positively related to P_N, g_s, and Chl, and negatively to Car/Chl. The second axis accounted for 26 % and it was related negatively to Car content. The analysis showed a separation among the considered species along these two axes (Fig. 6): the narrow-leaf species were characterized by the lowest gas exchange rates and Chl content but a higher Car/Chl; nevertheless, among them, *E. arborea* had the highest P_N, g_s, and Chl content and the lowest Car/Chl. Among the broad-leaf species, *C. incanus* had the highest P_N, g_s, and Chl content but by the lowest Car/Chl, *Q. ilex* a high Car/Chl, and *P. lentiscus*, *P. latifolia*, and *A. unedo* a low P_N, g_s, and Chl content and a high Car/Chl.

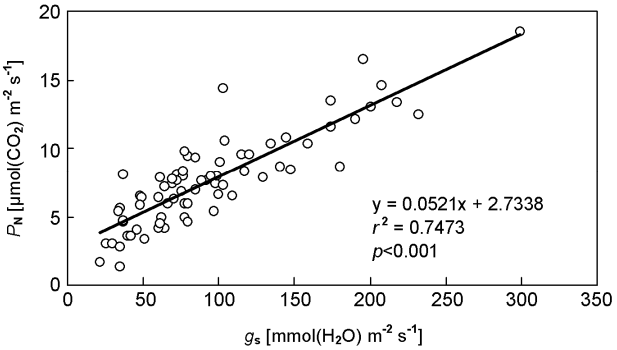

Fig. 4. Simple regression analysis of net photosynthetic rate (P_N) on stomatal conductance (g_s) of the considered species.

Discussion

The results on the whole underline the sensibility of evergreen species to low winter air temperatures. Considering the occurrence of two cold events (in February 2005 and in January 2006), *R. officinalis*, *C. incanus*, and *E. multiflora* had the highest P_N decrease (73 %, mean value) with respect to the winter P_N maximum (December 2003), followed by *A. unedo* (62 %), *P. latifolia* and *P. lentiscus* (54 %, mean value), *E. arborea* (49 %), and *Q. ilex* (44 %).

The daily winter P_N trend showed an increase in the first hours of morning up to 11:00–12:30 for all the considered species, when PAR was ≥1 416 μmol m^{-2} s^{-1}. *Q. ilex*, *E. arborea*, *P. latifolia, A. unedo,* and *P. lentiscus* reached P_{Nmax} at 15.9±0.9 °C, *C. incanus*, *R. officinalis*, and *E. multiflora* at 17.8±0.6 °C. Since the shorter winter photoperiod contributes to carbon fixation limitation (Nunes *et al.* 1992), the capacity of the species to maintain P_N near 90–100 % is determinant for biomass accumulation (Gratani and Varone 2004). Among the considered species, *Q. ilex* was able to maintain P_N near the maximum for 150 min during the day; moreover, in the first hours of the afternoon (PAR >1 000 μmol m^{-2} s^{-1}) it photosynthesized at 70 % of the maximum rate. *A. unedo*, *P. lentiscus*, *E. arborea*, *P. latifolia*, *E. multiflora*, and *R. officinalis* maintained P_{Nmax} for 60 min, and *C. incanus* for 30 min during the day. Thus, considering the mean daily P_N, the tested species had a mean daily P_N ranging from 7.9±0.6 μmol(CO_2) m^{-2} s^{-1} (*Q. ilex*) to 2.8±0.5 μmol(CO_2) m^{-2} s^{-1} (*R. officinalis*), which plays a major role in explaining the fitness and distribution of the Mediterranean species, according to Antolín *et al.* (2005).

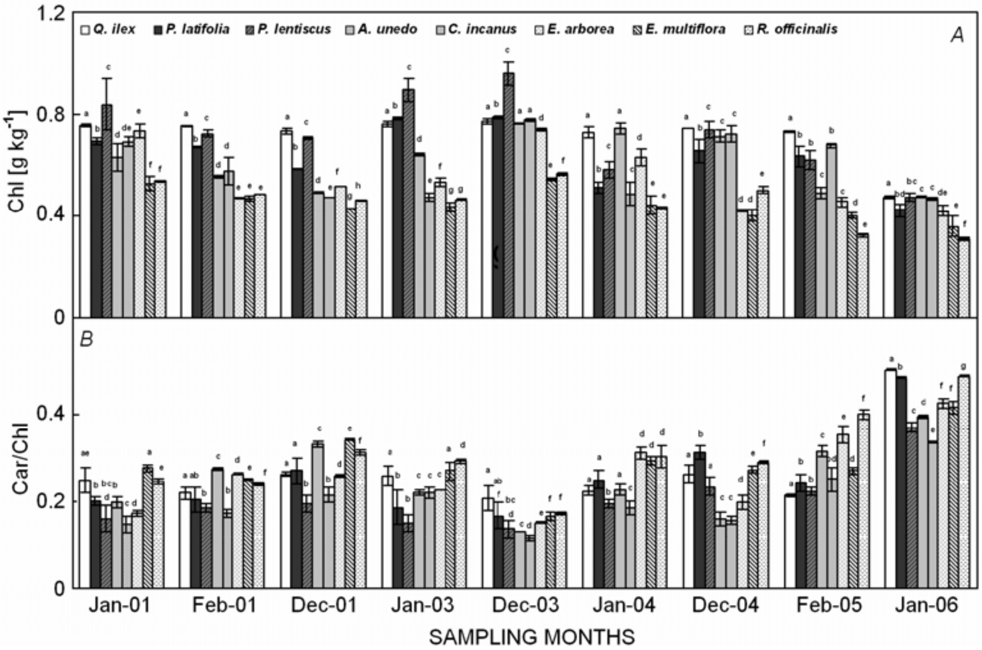

Fig. 5. Total chlorophyll (Chl) content (*A*) and ratio of carotenoids (Car) to Chl (*B*) for the study period. *Each bar* is the mean of five measurement days in each of the considered months. *Standard error* is shown. Within the same month, inter-specific differences with the same letters are not significant (ANOVA, $p>0.05$).

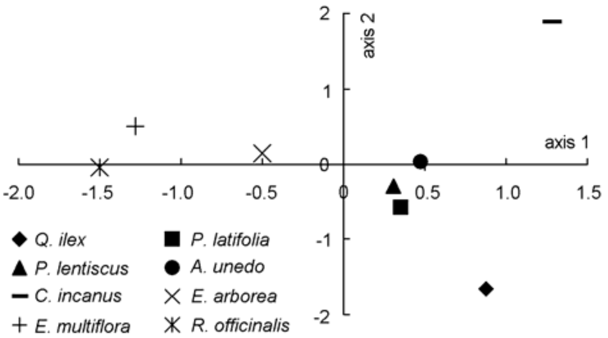

Fig. 6. Principal component analysis (PCA) of leaf functional traits (P_N, g_s, Chl, Car, and Car/Chl) measured during the study period.

During the study period, g_s showed a similar trend as P_N. On the occasion of two cold events (in February 2005 and in January 2006), the narrow-leaf species had a lower g_s decrease (56 % on an average) than the broad-leaf ones (78 % on average), and among them *Q. ilex* had the lowest g_s decrease (75 %).

The exposure to a combination of high irradiance and suboptimal growth temperatures during winter could determine photo-oxidative damage to the photosynthetic apparatus. Mediterranean evergreen species defend themselves against this risk by increasing antioxidant (ascorbate, glutathione, and α-tocopherol) and Car contents and/or decreasing Chl content (Gratani and Fiorentino 1988, Hansen *et al.* 1996, Oliveira and Peñuelas 2004). Chl content loss acts as a supplementary defence reducing photon absorbance (Kyparissis *et al.*

2000). During the study period the highest Chl decrease of the considered species was monitored in January 2006; moreover, the Car/Chl increased in all the considered species, and *Q. ilex* had the highest ratio (0.503±0.030).

PCA confirmed these results underlining the highest cold resistance of *Q. ilex* by the highest Car/Chl that contributes to a reduction of the photosynthetic machinery damage and reflects a lower reduction of photosynthesis due to photoinhibition (Ögren and Sjöström 1990, Oliveira and Peñuelas 2004). Photoinhibition may underline a mechanism of protection (Adams and Demmig-Adams 1995, Ball *et al.* 1995, Tretiach *et al.* 1997). Our study underlines the high adaptability of *Q. ilex* to climatic constraints: in fact, this is the most cold-tolerant species but also a drought-tolerant one (Gratani and Bombelli 2001, Gratani and Varone 2004, 2006).

On the contrary, *R. officinalis* and *C. incanus* had the lowest cold resistance by the highest P_N decrease; nevertheless, this decrease was associated with a high Car/Chl in *R. officinalis* and a low Car/Chl in *C. incanus*. Thus, winter stress might represent an additional limitation to Mediterranean evergreen species production. The intrinsic link between photosynthesis and biomass production suggests that photosynthesis and its response to heat and cold periods might determine the survival of Mediterranean species under new climatic constraint.

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