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Gender, mediterranean drought, and seasonality: photosystem II photochemistry in *Pistacia lentiscus* L.

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Abstract

In this work, photosystem II (PSII) photochemistry, leaf water potential, and pigment contents of male and female *Pistacia lentiscus* L. were investigated during a seasonal cycle at three different, arid locations: superior semiarid, inferior semiarid, and arid. The results showed that the gender, season, and the site conditions interacted to influence the quantum yield and pigment contents in *P. lentiscus*. Predawn leaf water status was determined only by the site and season. The annual patterns of PSII maximum quantum efficiency (F_v/F_m) were characterized by a suboptimal activity during the winter, especially, populations with the more negative water potential exhibited a lower chlorophyll (Chl) *a* content and chronic photoinhibition irrespective of a gender. We also demonstrated that both photochemical or nonphotochemical mechanisms were involved to avoid the photoinhibition and both of them depended on the season. This plasticity of photosynthetic machinery was accompanied by changes in carotenoids and Chl balance. In the spring, the female F_v/F_m ratio was significantly higher than in male individuals, when the sexual dimorphism occurred during the fruiting stage, regardless of site conditions. *P. lentiscus* sex-ratio in Mediterranean areas, where precipitations exceeded 500 mm, was potentially female-biased. Among the fluorescence parameters investigated, nonphotochemical quenching coefficient appeared as the most useful one and a correlation was found between Chl *a* content and F_v/F_m. These results suggest that functional ecology studies would be possible on a large scale through light reflectance analysis.

Additional key words: aridity; carotenoids; chlorophyll; fluorescence; photoinhibition; *Pistacia lentiscus* L.; seasonality; sex.

Introduction

The Mediterranean climate induces drought stress in plants, especially during the summer and the winter, which inhibits many physiological and biochemical processes (Zhang *et al.* 2005). The autumn allows assessing plant resilience depending on the intensity of summer drought. Drought affects either directly or indirectly photosynthetic apparatus functioning and it often modifies optical and fluorescence leaf properties (Lichtenthaler *et al.* 2000). Under such conditions, the mechanisms for a disposal of the energy excess do not work efficiently, which leads to changes in competing reactions among photochemistry, heat, and fluorescence

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Abbreviations: A – arid; Car – carotenoids; Chl *a*(*b*) – chlorophyll *a*(*b*); DM – dry mass; DOX – deoxyxylulose-5-phosphate pathway; DOXP – 1-deoxy-D-xylulose-phosphate; F_0 – minimal fluorescence yield in the dark-adapted state; F_m – maximal fluorescence yield in the dark-adapted state; F_m' – maximal fluorescence yield after light induction; F_v/F_m – maximum quantum efficiency of PSII reaction centers; ISA – inferior semiarid; MEP – methylerythritol 4-phosphate pathway; NPQ – nonphotochemical quenching of Chl fluorescence; PAR – photosynthetically active radiation; PSII – photosystem II; q_P – photochemical quenching; SE – standard error; SSA – superior semiarid; $Y_{1/2}$ – light radiation, which induced half inhibition of PSII maximal efficiency in dark acclimated leaf; Φ_{PSII} – actual quantum yield of PSII photochemistry; Ψ_w – predawn leaf water potential.
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(Naumann *et al.* 2007, Longenberger *et al.* 2009). As a result, two types of photoinhibition may occur: (*1*) temporary or dynamic, which is attained, when physiological processes are quickly reversible (Long *et al.* 1994); and (*2*) sustained or chronic photoinhibition is reached, when the photochemical machinery is either slowly reversible or permanently dysfunctional (Osmond 1994).

Fluorescence analysis has become a powerful and widely used technique among plant physiologists and ecophysiologists in order to provide information about the overall photosynthetic potential of the plant (Maxwell and Johnson 2000, Rose and Haase 2002, Einhorn *et al.* 2004) and an early stress detection (Lichtenthaler *et al.* 2005). In addition to instantaneous fluorescence parameter measurements, it is often desirable to evaluate plant photosynthetic machinery functioning through light-response curve measurements (Rascher *et al.* 2000).

In southern France, *P. lentiscus* L. is an interesting model species for evaluation of drought effects. A sclerophyllous, dioecious shrub grows at low altitude up to 2 m; it may attain a tree form in more humid and protected sites (Munne-Bosch and Peñuelas 2003). Male and female plants naturally co-occur in sites of contrasting aridities, such as superior semiarid (SSA), inferior semiarid (ISA), and arid (A), with a precipitations of 500–600, 400–500, and 300–400 mm, respectively.

Previous ecophysiological studies on *P. lentiscus* male and female plants showed that females strongly reduce the number of newly formed leaves at the fruiting stage. The subsequent, reduced photosynthetic capacity at the individual level was compensated by delaying abscission of old leaves contrary to male plants (Jonasson *et al.* 1997). Correia and Diaz Barradas (2000) concluded that female shrubs have lower CO_2 assimilation rates and stomatal conductances than those of male plants under natural stress conditions during Mediterranean summer months. *In situ* measurements of gas exchanges have also been carried out in *P. lentiscus* throughout a year by Flexas *et al.* (2001), but solely on female plants growing at two sites of contrasting temperature and precipitation.

We compared the PSII photochemistry and pigment contents of male and female plants of *P. lentiscus* over the entire seasonal cycle in SSA, ISA, and A sites. Our specific goals were: (*1*) to predict future plant community sex-ratio patterns; (*2*) to test the hypothesis that the compensatory effect of the photosynthetic capacity developed by female plants during fruit development is accompanied by physiological changes in the photosynthetic apparatus; (*3*) to find possible links between leaf fluorescence parameters and other plant functional traits in a view of perspective for large-scale ecosystem studies by remote-sensing approaches.

Materials and methods

Plants and growth conditions: This study was conducted in 3 localities in the vicinity of Marseille (France) located at different climatic sites (Fig. 1). Montespin (43°19'–5°30'E; 221 m a.s.l.) was at SSA, Montredon (43°13'N–5°20'E; 24 m a.s.l.) was at ISA, and Callelongue (43°12'N–5°21'E; 34 m a.s.l.) was at A site. The plant communities of the studied sites were growing on calcareous rocky surfaces. They were dominated by *P. lentiscus* and other typical evergreen, sclerophyllous shrubs, such as *Phillyrea angustifolia, Quercus coccifera*, and *Rhamnus alaternus*.

Thirty six individuals of *P. lentiscus* (18 males and 18 females) were chosen at each site. To avoid the age effect, individuals with similar diameters were selected.

Fluorescence measurements, leaf water potential (Ψ_w), and pigment analysis were done on fully expanded leaves of *P. lentiscus*. The measurements were conducted at predawn (*i.e.* before sunrise) during 2009: the winter (February 10–23), the spring (April 18–22), the summer (June 21–July 1), and the autumn (November 3–December 9). One measurement was done per the shrub and the season.

Leaf water potential (Ψ_w): One measurement of Ψ_w per tree was done at predawn in a field using a *Scholander pressure chamber* (*PMS Instrument Co.*, Corvallis, Oregon, USA). This was also indicator of soil Ψ_w due to absence of transpiration in darkness before sunrise.

Leaf pigments: Leaves were collected at predawn, then wrapped in aluminum foil to avoid degradation of pigments by light, and finally stored at –20°C until analysis in the laboratory. Chl and total carotenoids (Car) were extracted with acetone (80%) and filtered through Millipore membrane (0.45 µm pore size). Quantification was performed spectrophotometrically (*Thermo Electron Corporation BioMate 3S, Thermo Fisher Scientific*, France) by measuring their absorbance at 663, 646, and 470 nm according to Wellburn (1994).

Chl *a* fluorescence and light-response kinetics: Chl *a* fluorescence measurements (36 measurements per site and season) were performed with a portable pulse-amplitude modulated *Fluorometer (FMS2)* (*Hansatech King's Lynn,* Norfolk, UK) equipped with a leaf clip holder. Five incident photosynthetically active irradiance (PAR) intensities were used in order to simulate daily and seasonal environmental conditions: 360, 540, 900, 1,260; and 1,620 µmol(photon) m^{-2} s^{-1} were selected for the winter measurements, while 360, 540, 900, 1,440; and 2,160 µmol(photon) m^{-2} s^{-1} were the PAR levels used in the spring, summer, and autumn. A light-saturating pulse [8,000 µmol(photon) m^{-2} s^{-1}] was applied for each actinic light only when the steady-state fluorescence intensity was reached. This was checked during preliminary studies prior to the design of a specific, light kinetic program. Fluorescence measurements were conducted in

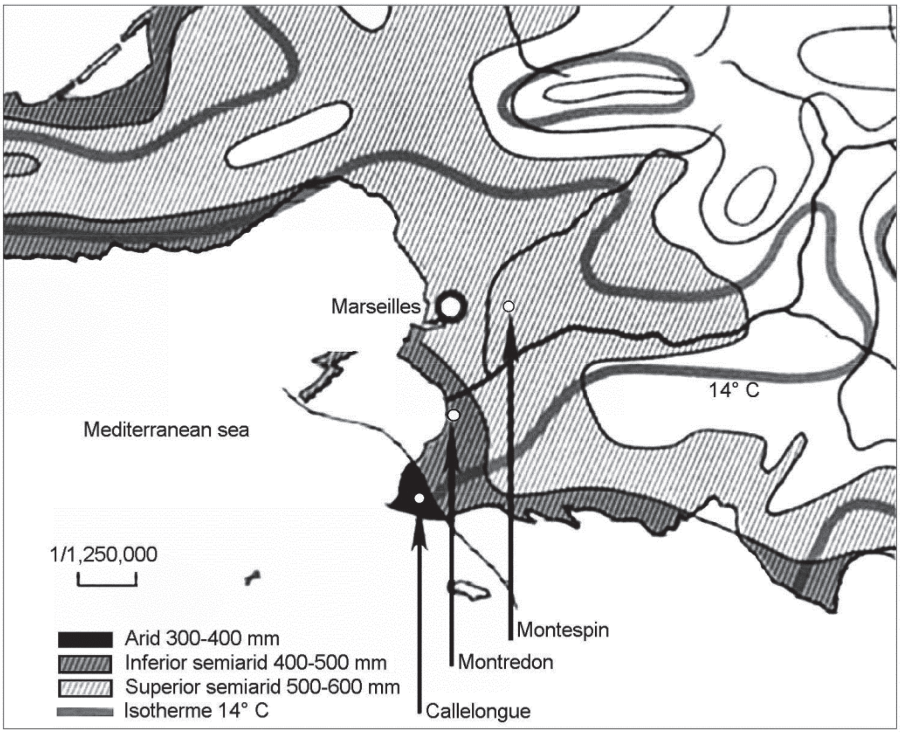

Fig. 1. Precipitation and thermal map of southeastern France with the location of the sites investigated: Montespin, Callelongue, and Montredon (Source: Carte de vegetation de Marseille: R. Molinier and A. Lavagne, publication du CNRS).

the central part of sun-exposed leaf lamina using dark-adaptation leaf clips. Maximum quantum efficiency of PSII reaction centers was determined from the ratio of variable to maximal fluorescence, *i.e.* $F_v/F_m = (F_m - F_0)/F_m$ (Kitajima and Butler 1975). F_0 is the minimum fluorescence level of a dark-adapted plant with the PSII primary acceptor 'open' (Q_A fully oxidized), which is obtained by the application of a low-intensity amber (594 nm) modulating beam. F_m is the maximal fluorescence level achieved upon the application of a saturating flash of light [8,000 µmol(photon) m^{-2} s^{-1}], such that all primary quinone electron acceptors in PSII are closed (Q_A fully reduced).

The photochemical efficiency in the light adapted state of PSII (Φ_{PSII}) was calculated from $(F_m' - F_s)/F_m'$ according to Genty *et al.* (1989), where F_s is the steady-state fluorescence, and F_m' is the maximal fluorescence during a saturating light flash.

Two quenching coefficients were assessed: the nonphotochemical quenching (NPQ), calculated as $(F_m - F_m')/F_m'$ (Bilger and Björkman 1990), which expresses the dissipation of excess energy as heat; and the photochemical quenching (q_P), which was calculated as $(F_m' - F_s)/(F_m' - F_0)$ (Bilger and Schreiber 1986) and gives an indication of the proportion of PSII reaction centers that are open (Maxwell and Johnson 2000).

Statistical analyses: In order to assess the variability of the predawn, physiological indicators (F_v/F_m, pigment content, and Ψ_w) according to sex, season, and site, as well as any interaction occurring among these factors, a three-way *ANOVA* was carried out. Differences between sites and seasons were tested through post-hoc tests (*Tukey*'s HSD), whereas differences between male and female plants were tested through a *Student*'s test. Additionally, the correlations between the different physiological variables were also performed.

Light responses were fitted to exponential (for Φ_{PSII} and q_P) and square root models (for NPQ). For each season, covariance analyses (*ANCOVA*), with light intensities as covariate, were performed to examine the individual effects of sex and site, sex *vs.* site interactions as well as the global effect of sex and site in the absence of interaction among these two factors. In order to achieve such comparison, these models were previously fitted to linear regression using log transformed data (for the exponential models), or square transformed data (for the square root models). Curve fitting and statistical analyses were performed using *Statgraphics Centurion XV* software.

Results

Ψ_w: The results showed a significant interaction between season and site (Fig. 2). Ψ_w was 1.4–1.7-fold lower at A site compared with SSA site during the winter, spring, and summer. In the autumn, the difference was only 1.2-fold lower. The *Student*'s test did not show any significant difference among both sexes.

F_v/F_m and leaf pigment content: Three-way *ANOVA* tests (Table 1) with F_v/F_m as dependent variable showed a significant effect of sex, season, and site. However, significant two-way interactions on F_v/F_m were observed between the sex, season, and site, but no three-way interaction was shown among these factors (Table 1). Hence, on one hand, the site effect on F_v/F_m was tested season-by-season and sex-by-sex as shown in Fig. 3. ISA site exhibited F_v/F_m of the dark-adapted leaves that was significantly below that of SSA and A sites in the winter and autumn for both male and female plants. The ratio of F_v/F_m of males from ISA stand was 0.767, while that from SSA and A were 0.799 and 0.793, respectively. On the other hand, the sex effect on F_v/F_m was likewise examined site-by-site and season-by-season also through one-way *ANOVA* and *Tukey*'s test. As shown in Fig. 4, the differences of F_v/F_m were mainly highlighted in the spring with female individuals exhibiting a higher ratio compared with males whatever the sites investigated. It should be noted that this female dominance persisted during the summer and the autumn among individuals of SSA site.

In view of the leaf pigment concentrations, there were significant two-way interactions among the sex, season, and site (Table 1). No three-way interactions were noted among these factors except for Chl *a*/*b* ratio. The test of the site effect (tested sex-by-sex and season-by-season) on Chl *a* and *b* contents revealed that these pigments from ISA site remained below those of A and SSA sites regardless the season and plant sex (Fig. 5*A*,*B*). This observation was confirmed for Car contents (Fig. 5*C*). However, the highest Car/total Chl ratio was found in ISA site all over the seasons and for both male and female individuals (Fig. 5*D*). For instance, the maximum of this ratio was recorded in female individuals during the summer: 0.37. Regarding the sex effect on pigment contents, site-by-site and season-by-season, one-way *ANOVA* showed that sex changes occured only in the winter. In particular, Chl *a* and *b* contents in male individuals were higher than those of females in A (Fig. 6*A*)

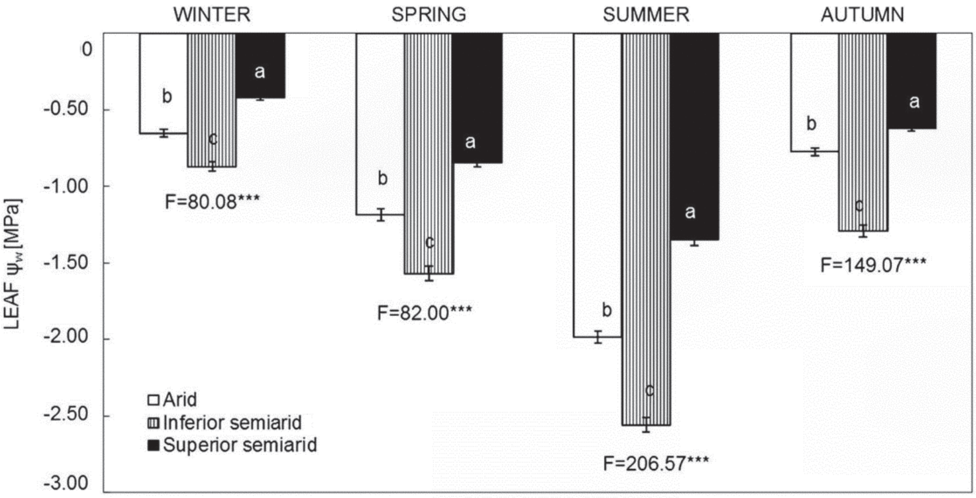

Fig. 2. *P. lentiscus* predawn leaf water potential (Ψ_w) over the seasonal cycle in arid, inferior semiarid, and superior semiarid sites. Means of n = 36 ± SE. *** – $P<0.001$ (one-way *ANOVA*).

Table 1. *ANOVA* analysis of maximum quantum efficiency of PSII (F_v/F_m), leaf water potential (Ψ_w), pigment contents and ratio measured at predawn from leaves of *P. lentiscus* (n = 36 per site). *** – $P<0.001$; ** – $P<0.01$; * – $P<0.05$, and ns – not significant.

Response variables		F_v/F_m	Chl *a* [mg g^{-1}(DM)]	Chl *b* [mg g^{-1}(DM)]	Total Chl	Chl *a*/*b*	Car	Chl/Car	Ψ_w [MPa]
Source	*Df*	*F*	*F*	*F*	*F*	*F*	*F*	*F*	*F*
Main effects									
Sex	1	11.90^{***}	6.83^{ns}	6.77^{**}	7.52^{**}	1.03^{ns}	1.81^{ns}	6.46^{*}	7.18^{**}
Season	3	110.65^{***}	77.59^{ns}	59.54^{***}	75.51^{***}	57.57^{***}	17.95^{***}	54.04^{***}	819.85^{***}
Site	2	19.46^{***}	176.62^{ns}	98.80^{***}	164.02^{***}	13.37^{***}	18.73^{***}	33.87^{***}	488.70^{***}
Interactions									
Sex *vs*. season	3	5.21^{**}	6.32^{***}	2.84^{*}	5.65^{***}	0.56^{ns}	1.00^{ns}	10.35^{***}	0.98^{ns}
Sex *vs*. site	2	0.3^{ns}	0.57^{ns}	0.51^{ns}	0.60^{ns}	2.16^{ns}	0.78^{ns}	0.70^{ns}	0.52^{ns}
Season *vs*. site	6	13.57^{***}	63.25^{***}	41.46^{***}	57.86^{***}	37.8^{***}	6.54^{***}	16.35^{***}	24.25^{***}
Sex *vs*. season *vs*. site	6	0.85^{ns}	1.43^{ns}	1.72^{ns}	1.43^{ns}	3.99^{***}	0.77^{ns}	1.74^{ns}	0.81^{ns}
Residuals	408								

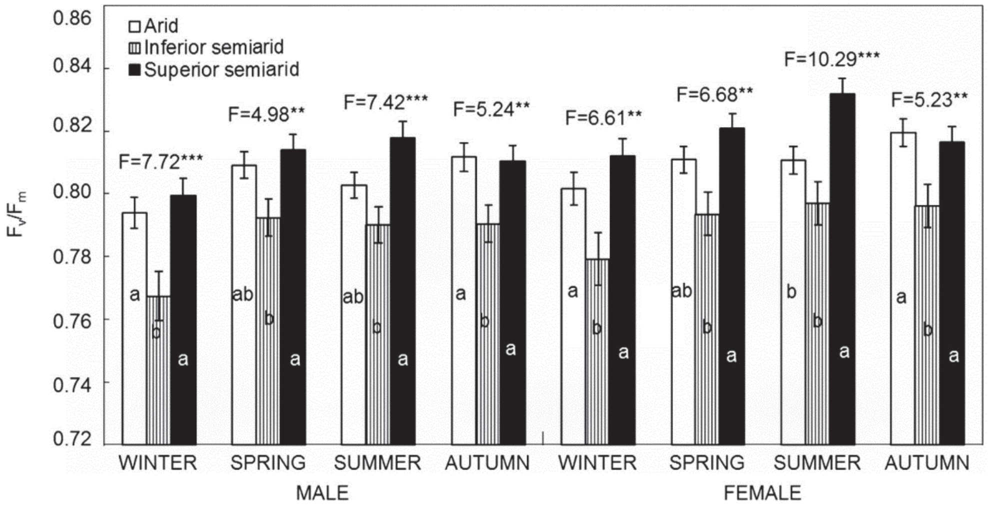

Fig. 3. Effect of arid, inferior semiarid, and superior semiarid sites on *P. lentiscus* male and female predawn leaf maximum quantum efficiency of PSII (F_v/F_m) over the seasonal cycle. Means of $n = 18 \pm$ SE. ** – $P<0.01$, *** – $P<0.001$ (one-way *ANOVA*).

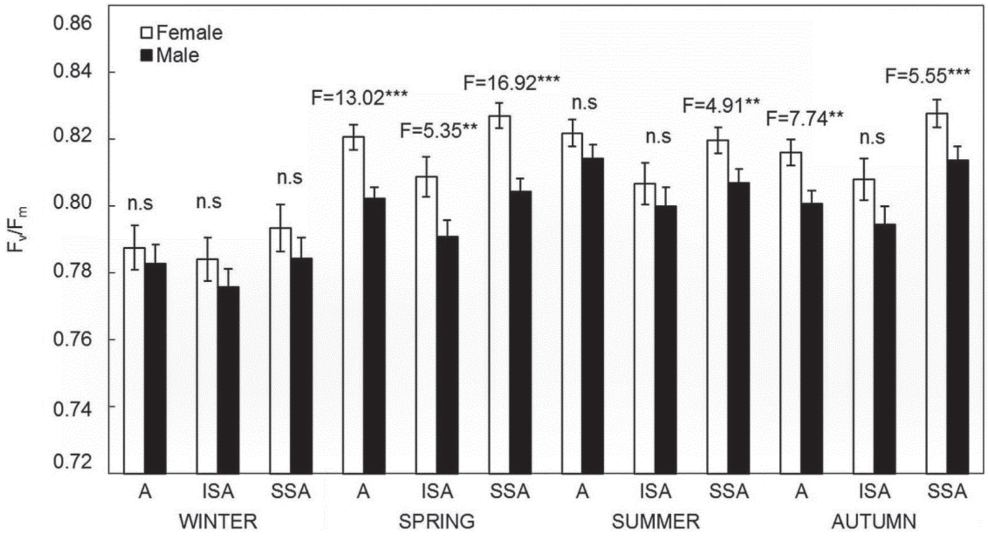

Fig. 4. Sex effect of *P. lentiscus* on predawn leaf maximum quantum efficiency of PSII (F_v/F_m) from arid, inferior semiarid, and superior semiarid sites over the seasonal cycle. Means of $n = 18 \pm$ SE. ** – $P<0.01$, *** – $P<0.001$ (one-way *ANOVA*). n.s – not significant.

and ISA (Fig. 6*B*) sites, respectively. On the contrary, the ratio Car/total Chl was higher in female individuals at all three sites during the winter (Fig. 6*C*).

Correlations between physiological parameters: A significant correlation was established between Ψ_w and the ratio Car/total Chl (Fig. 7*A*). This ratio decreased exponentially with the absolute value of Ψ_w. Car and Chl *a* were also correlated (Fig. 7*B*) as well as Chl *a vs*. F_v/F_m (Fig. 7*C*). No correlation was found between the ratio of Chl *a*/*b* and F_v/F_m.

Seasonal light-response models of the contrasting sites:

Spring models: Fig. 8*A* shows PSII actual quantum yield (Φ_{PSII}) as a function of actinic light intensities recorded *in situ*. Regression curves depending on the site and plant sex were adjusted to decreasing exponential functions with significant correlations. In order to compare the slopes of the different patterns, data were linearized prior to *ANCOVA* analysis that showed significant differences for the factors site and sex, but the interaction site *vs*. sex was not significant (Table 2). Thus, the global effect of both the sex and the site was tested through *ANCOVA* followed by *Tukey*'s test for examining the differences among sex and site patterns (Fig. 8*A*). The light radiations, which induced half inhibition of PSII maximal efficiency in the dark-acclimated leaves ($Y_{1/2}$) for male individuals were 2,567; 2,888; and 3,150 µmol(photon) m^{-2} s^{-1} for ISA, SSA, and A stands, respectively. For females, these light radiations were 3,150; 3,850; and 4,077 µmol(photon) m^{-2} s^{-1} in ISA, SSA, and A sites, respectively. Hence, individuals from ISA were more sensitive to photoinhibition than those of two other sites.
Correlation between q_P and light intensity was significant through an exponential model (Fig. 8*B*). The values exhibited some differences only between the sites as shown in Table 2. The decay constants of q_P data from ISA and SSA male individuals were similar but significantly higher than those from A stands. Especially,

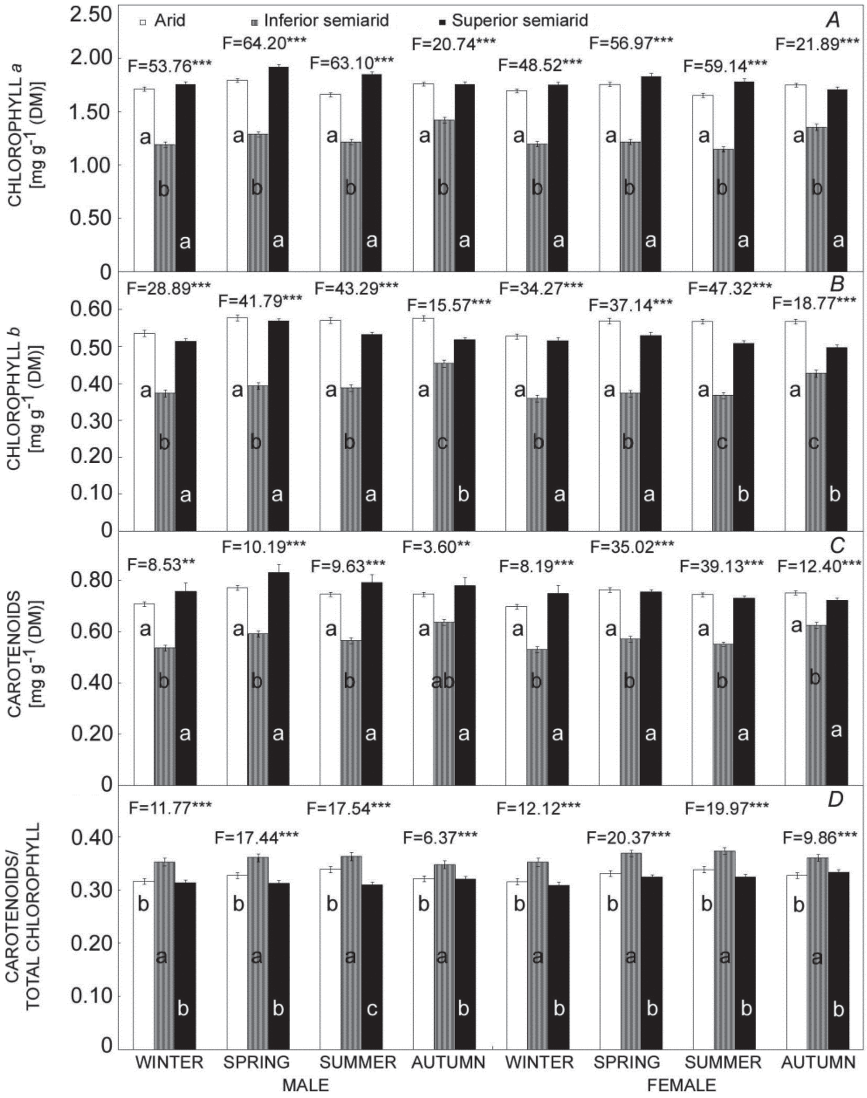

Fig. 5. Effect of arid, inferior semiarid, and superior semiarid sites on *P. lentiscus* male and female predawn leaves pigment contents: chlorophyll *a* (*A*), chlorophyll *b* (*B*), carotenoids (*C*), and carotenoids/chlorophyll ratio (*D*). The test was performed season-by-season and sex-by-sex (one-way *ANOVA*). ** – $P<0.01$ and *** – $P<0.001$.

the light intensity, which caused the closure of half maximal openness of PSII reaction centers in the dark-adapted state ($q_{P1/2}$), were 4,951 and 9,902 µmol(photon) m^{-2} s^{-1} for male populations of SSA and A sites, respectively.

NPQ patterns exhibited only a slight significant difference between the sites. For instance, under saturating light conditions [2,400 µmol(photon) m^{-2} s^{-1}], male NPQ values were 1.24 and 1.08 in ISA and SSA stands, respectively (Table 2, Fig. 8*C*).

Summer models: Some differences regarding the actual quantum yield were observed only among sites, there was no interaction of sex *vs*. site (Table 2). The decay constant (λ) of this yield for samples of SSA site was about one third that of the other sites (Table 3). Consequently, $Y_{1/2}$ of SSA male individuals was 5,335 µmol(photon) m^{-2} s^{-1}, whereas that of A and ISA stands was 2,235 µmol(photon) m^{-2} s^{-1}.

The values of q_P were similar for both male and female plants regardless the sites investigated (Tables 2, 3). Only the site effect was noticed, individuals of SSA exhibited the lowest q_P constant decays. NPQ values showed significant differences for the factors of the sex, the site, and the interaction site *vs* sex. Hence, the site effect on NPQ values was tested sex-by-sex through one-way *ANCOVA* (Fig. 1S, *see* supplementary material online). For both male and female individuals, the slopes of NPQ curves were similar for A and ISA stands, but higher than that of SSA individuals (Table 3).

Autumn models: PSII actual efficiency kinetics during autumn exhibited significant differences among the sexes, the sites, and the interaction site *vs*. sex was not significant. Similar patterns were obtained for both sexes except for samples of A stands (Tables 2, 3). In particular, $Y_{1/2}$ of male individuals was 4,077 µmol(photon) m^{-2} s^{-1}, while that of female plants was about 5,332 µmol(photon) m^{-2} s^{-1}. No sex effect was shown with q_P values (Tables 2, 3), but this was significant for the factor

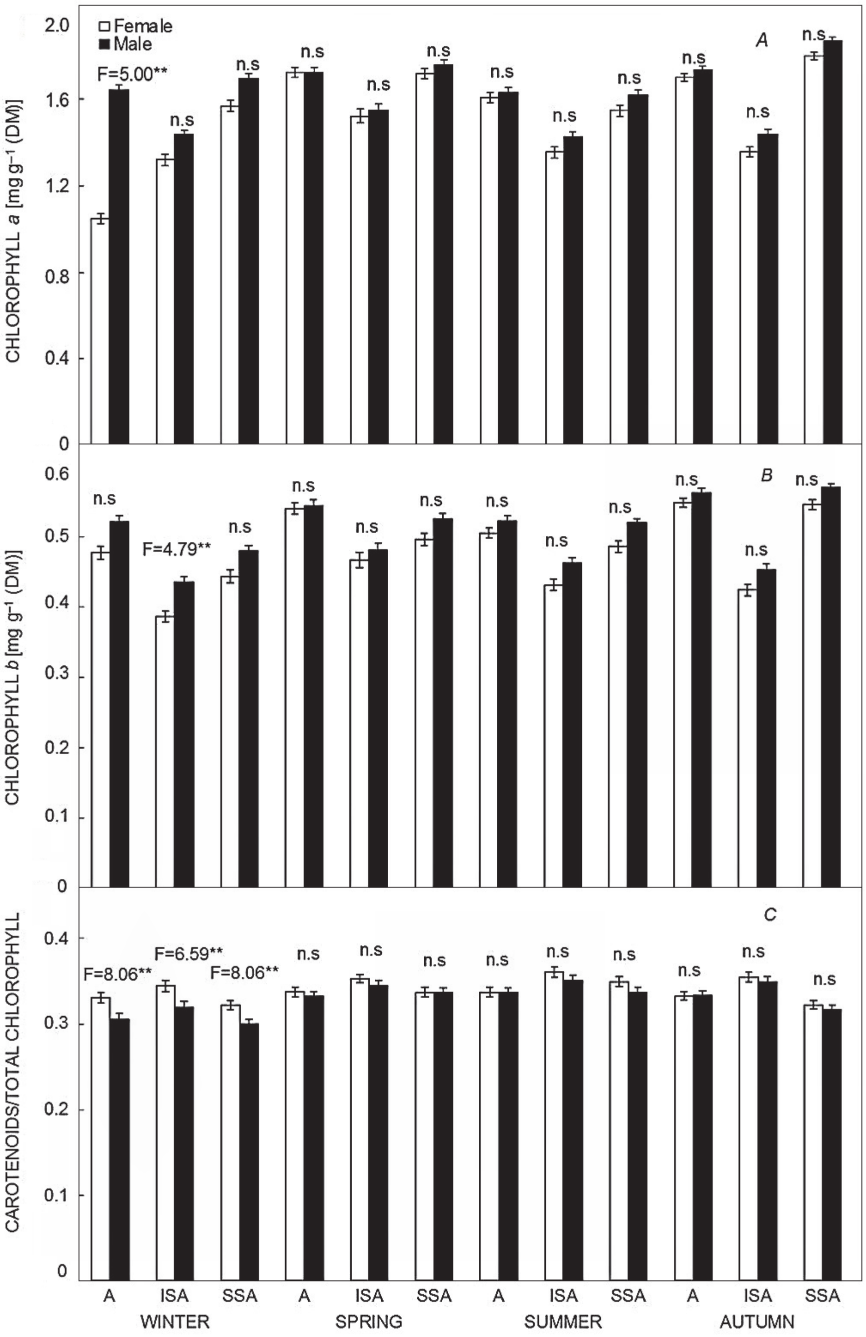

Fig. 6. Sex effect of *P. lentiscus* on predawn leaves pigment contents: chlorophyll *a* (*A*), chlorophyll *b* (*B*), and carotenoids/chlorophyll ratio (*C*). The test was performed site-by-site and season-by-season (one-way *ANOVA*). ** – $P<0.01$ and *** – $P<0.001$. n.s – not significant.

site and the interaction sex *vs.* site. When examining site effect on q_P sex-by-sex, significant differences were demonstrated (Fig. 2S, *see* supplementary material online). Regardless the sex, the proportion of the photon energy captured by open PSII reaction centers sharply decreased with increasing light intensity in SSA stand. Consequently, $q_{P1/2}$ from A and ISA were about 2–3 fold that of SSA individuals (Table 3). For the values of NPQ, the site and sex *vs.* site interaction effects were shown, but there was no sex effect (Tables 2, 3). Site effect on NPQ was also treated sex-by-sex from one-way *ANCOVA* (Fig. 3S, *see* supplementary material online), which concluded to a lesser extend of the increase of NPQ values from SSA individuals compared with those of the other sites (Table 3).

Winter models: Φ_{PSII} kinetics exhibited significant site and the sex effects as shown in Table 2. The interaction between the site and sex was also significant (Tables 2, 3). Hence, the site effect was performed sex-by-sex from one-way *ANCOVA* followed by *Tukey*'s test (Fig. 4S, *see* supplementary material online). This revealed that Φ_{PSII} slightly decreased with light intensity from SSA individuals compared with those of A and ISA sites. For

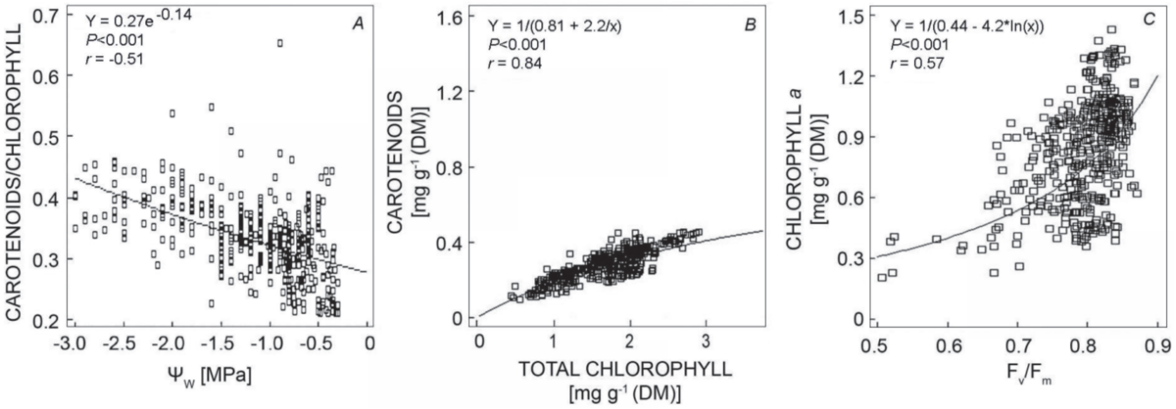

Fig. 7. Correlation curves between *P. lentiscus* predawn leaves physiological parameters: carotenoids/chlorophyll and leaf water potential (Ψ_w) (*A*), carotenoids and total chlorophyll (*B*), and chlorophyll *a* and maximum quantum efficiency (F_v/F_m) (*C*). *n* = 432.

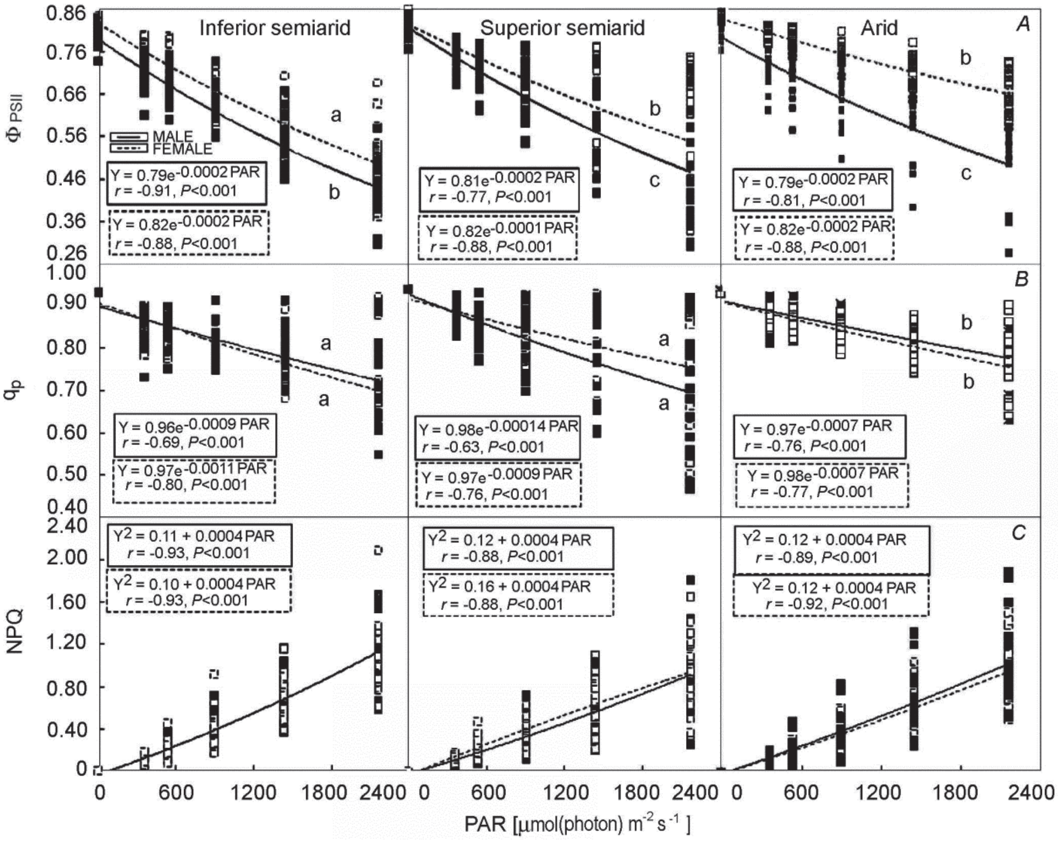

Fig. 8. Typical leaf response curves of actual PSII quantum yield (Φ_{PSII}) (*A*), photochemical quenching coefficient (q_P) (*B*), and nonphotochemical quenching coefficient (NPQ) (*C*) to light intensity under predawn conditions for male and female individuals of *P. lentiscus* from inferior semiarid (ISA), superior semiarid (SSA), and arid (A) sites during the spring. The global effect of site and sex was tested through *ANCOVA* ($P<0.05$) followed by *Tukey*'s post-hoc test. Significant differences are shown in letters: c < b < a. *n* = 18 for each light intensity.

instance, $Y_{1/2}$ for SSA female individuals was 2,888 μmol(photon) m^{-2} s^{-1}, while that of A and ISA were about 2,100 μmol(photon) m^{-2} s^{-1} (Table 3). The q_P values exhibited some differences between sites, but not among sexes and the site *vs*. sex interaction (Table 2). $q_{P1/2}$ were 5,776; 4,332; and 3,850 μmol(photon) m^{-2} s^{-1} for ISA, SSA, and A stands, respectively. Regarding the NPQ patterns, the differences were significant only between the sites (Table 2). For instance, at irradiation of 1,800 μmol(photon) m^{-2} s^{-1}, the lowest NPQ value was recorded in A stands (male NPQ = 0.74) compare with the other sites; *i.e.* 0.93 and 0.95 for male individuals from SSA and ISA sites, respectively.

Table 2. *ANCOVA* analysis ($P<0.05$) of the response curves of actual PSII quantum yield (Φ_{PSII}), photochemical quenching coefficient (q_P), nonphotochemical quenching coefficient (NPQ) to light intensity in predawn leaves of *P. lentiscus* from arid, inferior semiarid, and superior semiarid sites during the spring, the summer, the autumn, and the winter. *** – $P<0.001$; ** – $P<0.01$; * – $P<0.05$, and ns – not significant.

Season	Parameter	Site *F*	Sex *F*	Site *vs*. sex *F*	Site + sex *F*
Spring	Φ_{PSII}	66.8^{***}	13.25^{***}	0.27^{ns}	18.77^{***}
	q_P	7.77^{***}	2.28^{ns}	2.25^{ns}	
	NPQ	3.06^{*}	0.18^{ns}	2.62^{ns}	
Summer	Φ_{PSII}	152.20^{***}	3.49^{ns}	1.47^{ns}	
	q_P	16.50^{***}	0.96^{ns}	0.05^{ns}	
	NPQ	42.96^{***}	5.69^{**}	3.56^{*}	
Autumn	Φ_{PSII}	143.82^{***}	14.72^{***}	2.07^{ns}	61.30^{***}
	q_P	159.70^{***}	0.26^{ns}	6.24^{**}	
	NPQ	52.5^{***}	2.86^{ns}	6.67^{**}	
Winter	Φ_{PSII}	133.60^{***}	5.02^{*}	5.85^{**}	
	q_P	25.50^{***}	0.47^{ns}	0.69^{ns}	
	NPQ	6.17^{**}	0.08^{ns}	1.27^{ns}	

Table 3. Regression equations ($P<0.001$) of the response curves of actual PSII quantum yield (Φ_{PSII}), photochemical quenching coefficient (q_P), nonphotochemical quenching coefficient (NPQ) to light intensity in predawn leaf of *P. lentiscus* from arid, inferior semiarid, and superior semiarid sites during the summer, the autumn, and the winter. Φ_{PSII} and q_P data fitted against exponential decay function ($y = y_0 \exp - \lambda x$) and NPQ data against square root function ($y^2 = C + D x$). Site and sex global effect or site effect were tested through *ANCOVA*, $P<0.05$. * Interaction cases, site effect on the variables were tested sex-by-sex through one-way *ANCOVA*. Significant differences are shown in letters: c < b < a. A – arid; ISA – inferior semiarid; SSA – superior semiarid.

Season	Parameter	Male			Female		
		A	ISA	SSA	A	ISA	SSA
Summer	Φ_{PSII}	$y_0 = 0.77$ $\lambda = 3.10^{-4\,\mathrm{a}}$ $r = -0.83$	$y_0 = 0.77$ $\lambda = 3.10^{-4\,\mathrm{a}}$ $r = -0.80$	$y_0 = 0.83$ $\lambda = 1.10^{-4\,\mathrm{b}}$ $r = -0.76$	$y_0 = 0.76$ $\lambda = 3.10^{-4\,\mathrm{a}}$ $r = -0.78$	$y_0 = 0.77$ $\lambda = 4.10^{-4\,\mathrm{a}}$ $r = -0.78$	$y_0 = 0.82$ $\lambda = 1.10^{-4\,\mathrm{b}}$ $r = -0.76$
	q_P	$y_0 = 0.95$ $\lambda = 7.10^{-5\,\mathrm{a}}$ $r = -0.64$	$y_0 = 0.95$ $\lambda = 7.8.10^{-5\,\mathrm{a}}$ $r = -0.68$	$y_0 = 0.97$ $\lambda = 6.4.10^{-5\,\mathrm{b}}$ $r = -0.58$	$y_0 = 0.94$ $\lambda = 6.2.10^{-5\,\mathrm{a}}$ $r = -0.58$	$y_0 = 0.94$ $\lambda = 6.7.10^{-5\,\mathrm{a}}$ $r = -0.72$	$y_0 = 0.96$ $\lambda = 5.7.10^{-5\,\mathrm{b}}$ $r = -0.57$
	NPQ*	C = 0.21 $D = 5.10^{-4\,\mathrm{a}}$ $r = 0.88$	C = 0.22 $D = 5.10^{-4\,\mathrm{a}}$ $r = 0.87$	C = 0.22 $D = 4.10^{-4\,\mathrm{b}}$ $r = 0.82$	C = 0.25 $D = 5.10^{-4\,\mathrm{a}}$ $r = 0.85$	C = 0.27 $D = 6.10^{-4\,\mathrm{a}}$ $r = 0.86$	C = 0.25 $D = 3.10^{-4\,\mathrm{b}}$ $r = 0.76$
Autumn	Φ_{PSII}	$y_0 = 0.79$ $\lambda = 1.7.10^{-4\,\mathrm{b}}$ $r = -0.77$	$y_0 = 0.76$ $\lambda = 1.5.10^{-4\,\mathrm{b}}$ $r = -0.77$	$y_0 = 0.78$ $\lambda = 3.3.10^{-4\,\mathrm{a}}$ $r = -0.94$	$y_0 = 0.75$ $\lambda = 1.3.10^{-4\,\mathrm{c}}$ $r = -0.79$	$y_0 = 0.78$ $\lambda = 1.5.10^{-4\,\mathrm{b}}$ $r = -0.81$	$y_0 = 0.82$ $\lambda = 3.6.10^{-4\,\mathrm{a}}$ $r = -0.94$
	q_P^{*}	$y_0 = 0.99$ $\lambda = 1.0.10^{-4\,\mathrm{b}}$ $r = -0.77$	$y_0 = 0.99$ $\lambda = 7.6.10^{-5\,\mathrm{b}}$ $r = -0.83$	$y_0 = 0.97$ $\lambda = 1.9.10^{-4\,\mathrm{a}}$ $r = -0.77$	$y_0 = 1.08$ $\lambda = 8.1.10^{-5\,\mathrm{b}}$ $r = -0.79$	$y_0 = 0.99$ $\lambda = 7.5.10^{-5\,\mathrm{b}}$ $r = -0.83$	$y_0 = 0.98$ $\lambda = 2.5.10^{-4\,\mathrm{a}}$ $r = -0.77$
	NPQ*	C = 0.22 $D = 4.7.10^{-4\,\mathrm{a}}$ $r = 0.83$	C = 0.19 $D = 4.6.10^{-4\,\mathrm{a}}$ $r = 0.85$	C = 0.04 $D = 4.5.10^{-4\,\mathrm{b}}$ $r = 0.94$	C = 0.17 $D = 4.1.10^{-4\,\mathrm{ab}}$ $r = 0.87$	C = 0.20 $D = 4.7.10^{-4\,\mathrm{a}}$ $r = 0.85$	C = 0.06 $D = 4.4.10^{-4\,\mathrm{b}}$ $r = 0.92$
Winter	Φ_{PSII}^{*}	$y_0 = 0.72$ $\lambda = 3.3.10^{-4\,\mathrm{a}}$ $r = -0.90$	$y_0 = 0.72$ $\lambda = 3.2.\ 10^{-4\,\mathrm{a}}$ $r = -0.85$	$y_0 = 0.83$ $\lambda = 3.2.10^{-4\,\mathrm{b}}$ $r = -0.75$	$y_0 = 0.76$ $\lambda = 3.3.10^{-4\,\mathrm{a}}$ $r = -0.91$	$y_0 = 0.70$ $\lambda = 3.2.10^{-4\,\mathrm{b}}$ $r = -0.73$	$y_0 = 0.84$ $\lambda = 2.4.10^{-4\,\mathrm{c}}$ $r = -0.69$
	q_P	$y_0 = 0.96$ $\lambda = 1.8.10^{-4\,\mathrm{a}}$ $r = -0.79$	$y_0 = 0.97$ $\lambda = 1.2.10^{-4\,\mathrm{b}}$ $r = -0.75$	$y_0 = 0.93$ $\lambda = 1.6.10^{-4\,\mathrm{a}}$ $r = -0.55$	$y_0 = 0.97$ $\lambda = 1.8.10^{-4\,\mathrm{a}}$ $r = -0.79$	$y_0 = 0.97$ $\lambda = 1.3.10^{-4\,\mathrm{b}}$ $r = -0.65$	$y_0 = 0.94$ $\lambda = 1.6.10^{-4\,\mathrm{a}}$ $r = -0.55$
	NPQ	C = 0.02 $D = 4.10^{-4\,\mathrm{b}}$ $r = -0.90$	C = 0.05 $D = 5.10^{-4\,\mathrm{a}}$ $r = -0.78$	C = 0.03 $D = 5.10^{-4\,\mathrm{a}}$ $r = -0.92$	C = 0.01 $D = 5.10^{-4\,\mathrm{b}}$ $r = -0.90$	C = 0.04 $D = 5.10^{-4\,\mathrm{a}}$ $r = -0.81$	C = 0.03 $D = 5.10^{-4\,\mathrm{a}}$ $r = -0.90$

Discussion

The different locations investigated were highly contrasting in the recorded annual precipitations. Predawn Ψ_w, which also determined soil water availability for plants, confirmed the heterogeneity of all 3 sites (Fig. 2). In A and SSA, Ψ_w patterns were in accordance with sites of annual rainfalls, suggesting similar soil physico-chemical properties in terms of water retention. However, it should be noted that the more negative Ψ_w was recorded in ISA stands, although this site exhibited annual rainfalls, which were higher than that of A location (Fig. 1). This inconsistency might be explained by the influence of dew, which was an important factor in this site. Another observation was the absence of the sex effect and this might be interpreted as both male and female plants of *P. lentiscus* exhibited similar root architecture.

The ratio of F_v/F_m is widely used to detect changes in the photosynthetic apparatus as a result of stress (Kitajima and Butler 1975, Baker and Rosenqvist 2004). Björkman and Demmig (1987) considered that healthy, dark-adapted, C_3 species have the F_v/F_m ratio ranging from 0.80–0.86. The ratio measured in male individuals at predawn in the winter was: 0.79, 0.76, and 0.79 in A, ISA, and SSA sites, respectively (Fig. 3). We could conclude that *P. lentiscus* underwent a chronic photoinhibition during the winter, especially, populations in ISA environments. The winter chronic photoinhibition has been also shown for female plants of *P. lentiscus* (Flexas *et al.* 2001) as well as for several Mediterranean plants (Oliveira and Peñuelas 2000, Martinez-Ferri *et al.* 2004). The sustained downregulation of PSII efficiency may be a result of several factors, such as low temperature reduced enzyme activity (Martinez-Ferri *et al.* 2004), a substantial decrease of PSII components (Sarvikas *et al.* 2010), and water availability (Souza *et al.* 2004). Gas-exchange analysis showed that the decrease of *P. lentiscus* net photosynthesis was 54% during the winter (Varone and Gratani 2007). Among PSII constituents, photosynthetic pigments were analysed and this led to the conclusion that leaves of *P. lentiscus* from ISA stands exhibited the lowest content of Chl *a* and Chl *b* (Fig. 5*A,B*). The low content of Chl *a* can also be regarded as a protective adaptive mechanism in stressed plants (Kyparissis *et al.* 2000, Balaguer *et al.* 2002). Indeed, Chl loss under excessive sunlight reduces leaf photon absorption capacity, thus preventing over-excitation of photosystems (Anderson *et al.* 1992). However, adaptation to the winter chronic photoinhibition of *Cistus albidus* and *Quercus ilex* was not affected by Chl contents (Oliveira and Peñuelas 2000), which suggests that a decrease of Chl *a* content as the stress indicator depends on a species.

In the spring, *P. lentiscus* plants were not submitted to a drastic decrease of PSII photosynthetic activity, but a sexual dimorphism regarding PSII photochemistry was shown; the female F_v/F_m ratio was higher than that of males in all the sites investigated (Fig. 4). This was confirmed by the actual quantum yield of PSII (Fig. 8) indicating that females might assimilate more CO_2, although other processes, such as nitrate reduction and Mehler reaction, are also metabolic sinks of photochemical products. Indeed, the net photosynthesis of female plants of *P. lentiscus* growing under moderately high temperature conditions exhibited a maximum in the spring as previously described by Flexas *et al.* (2001). On one hand, *P. lentiscus* sets fruits in April, but their development and maturation continue until the autumn (Jordano 1988, Montserrat-Martí and Pérez-Rontomé 2002). On the other hand, fructification coincides with physiological, gender differences and thus, sex-related photosynthetic responses are not excluded as reported earlier for other species (Laporte and Delph 1996, Letts *et al.* 2008, Xu *et al.* 2008). In our study, the sexual differences occurred mainly during the favorable period of growth and this could mean that fruiting carbon demand of *P. lentiscus* rather involves source-regulated mechanisms. If so, it was tempting to identify leaf components that would be related to carbon demand regarding to plant sex. By examining photosynthetic pigment traits, no difference was found between both sexes in the spring for all the sites (Fig. 6).

Surprisingly, the measurements carried out in the summer did not show any chronic photoinhibition as the recorded F_v/F_m values were typical as those of nonstressed plants except for individuals of ISA site. This photosynthetic response was associated with a significant difference of the ratio Car/total Chl among all three sites investigated (Fig. 5*D*) indicating the plasticity of *P. lentiscus* photosynthetic machinery in contrasting environments. The highest values of the ratio Car/total Chl was recorded in ISA site as well as the more negative leaf Ψ_w (–2.5 MPa) during the summer. The increase of Car contents might be interpreted as prevention of oxidative damage during drought stress. Car in thylakoid membranes act as a protective system, since they abduct or prevent the formation of radicals or other reactive molecular species, especially those derived from oxygen (Krause 1988, Demmig-Adams *et al.* 1989). Moreover, these pigments quench the Chl triplets and stimulate the transition of oxygen singlets into triplets (Long *et al.* 1994). This mechanism of photosynthetic apparatus protection from oxidative damage is a common pathway for several species to cope with excessive light energy (Choudhury and Behera 2001). As well as in the summer, *P. lentiscus* plants were not stressed during the autumn at A and SSA stands.

Integrating the different physiological parameters all over 4 seasons led to establishment of several significant correlations (Fig. 7). Among these, Car were highly correlated with total Chl contents. This result is in accordance with the involvement of DOXP/MEP pathway in the coordinated biosynthesis of Car and the long alcoholic side-chain of Chl (Lichtenthaler 2007). Hence, geranyl diphosphate synthase (*GPPS1*) gene is presumably a key regulatory node that determines Car/total Chl balance as demonstrated from transcriptomic analysis of *Arabidopsis thaliana* (Meier *et al.* 2011). We found a strong correlation between Chl *a* content and the F_v/F_m ratio. Similar results have been obtained in other studies (Zarco-Tejada *et al.* 2000). This could mean that utilization of remote-sensing measurements of *P. lentiscus* leaves, which are based on Chl *a* reflectance, could evaluate the photoinhibition in large scale population.

Our fluorescence data showed high seasonal variation, which was also reported by Ain-Lhout *et al.* (2004) in many Mediterranean scrub species including *P. lentiscus*. During the winter, as above-mentioned by predawn measurements, *P. lentiscus* showed the chronic photoinhibition principally at ISA stands. Photochemical reactions within PSII result in the conversion of light into redox potential energy and this conversion is q_P. A decrease in q_P is attributed to an increase of the reduction state of the Q_A pool, which means that high q_P values in plants contribute to photoinhibition avoidance (Lu and Lu 2004). There is another decay mechanism for de-excitation of Chl *a* in competition with photochemistry. It is NPQ, which is often associated with processes such as the transthylakoid pH gradient (ΔpH), xanthophyll cycle, and the aggregation of PSII light-harvesting complex (Ivanov *et al.* 2008). As concerns the winter data, both male and female from ISA site exhibited the highest values of q_P (Table 3). Because Φ_{PSII} is the product of q_P and the antenna conversion efficiency, the chronic photoinhibition observed during the winter in ISA stands rather involves antenna conversion mechanisms of extra light absorbed. These facts seemed to corroborate the decrease of total Chl contents shown in leaves harvested at predawn, thus providing new insight in PSII functioning. Indeed, a decrease of Chl *a* content would reduce the proportion of PSII since the reaction centers are composed by a dimer of this molecule. In the case of *P. lentiscus*, the lost of Chl *a* rather involves antenna pigment content.

In the spring, PSII actual quantum yield changed in dependence on the sex and the site conditions. The quantum yield of male individuals decreased faster with increasing irradiation than that of female plants in all the investigated sites (Table 3). A significant sex effect was clearly shown, but when taking into account the values of q_P and NPQ, only a site effect was obtained mainly for q_P data. Hence, the lower quantum yield exhibited by individuals of ISA stand compared with those of the other sites was rather the result of photochemical quenching processes. The other highlight of these results is that neither q_P nor NPQ could explain the difference in PSII quantum yield between sexes in the spring.

During the summer, there was no sex effect, when considering the pattern of Φ_{PSII}. Samples of SSA individuals showed the lowest slope regarding Φ_{PSII} patterns as well as for q_P and NPQ patterns (Table 3). This suggested that the chronic photoinhibition recorded in ISA site involved both photochemical and nonphotochemical mechanisms mechanisms as the result of drought severity.

In the autumn, SSA stands showed the highest constant decay (λ) of actual Φ_{PSII} curves compared with those of the other sites (Table 3). As a consequence, no chronic photoinhibition was pointed out (Fig. 3) and the question raised was thus to understand the photoprotective mechanisms involved. Taking into account SSA quenching coefficient patterns, q_P showed the highest constant decays, while NPQ exhibited the lowest slopes.

In conclusion, the winter was the most critical season and the deleterious effect on *P. lentiscus* Φ_{PSII} increased with drought severity; this could mean a possible regression of populations of A zones with the predicted decrease of precipitation in Mediterranean region (Penuelas *et al.* 2005). *P. lentiscus* leaf protection against chronic photoinhibition was explained either by photochemical or nonphotochemical mechanisms and it was accompanied by changes in Car *vs.* Chl balance, which might constitute a central regulatory node in plant breeding programmes. A sexual dimorphism was also clearly shown in the spring in all sites. Hence, in addition to the ability of female plants to delay older leaves senescence, changes in PSII photochemistry were also involved. NPQ was shown as the most plastic of the fluorescence parameters investigated under drought condition. A correlation between total Chl *a* contents and the ratio of F_v/F_m was also found indicating a possible link between leaf level fluorescence measurements and canopy reflectance properties. However, further research should take into account such physiological traits as water-use efficiency and the quantum yield of CO_2 assimilation.

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