Acta Oecologica **21** (2) (2000) 97−107 / © 2000 Éditions scientifiques et médicales Elsevier SAS. All rights reserved
S1146609X00001211/FLA

Comparative photochemical and phenomorphological responses to winter stress of an evergreen (*Quercus ilex* L.) and a semi-deciduous (*Cistus albidus* L.) Mediterranean woody species

Graça Oliveira*, Josep Peñuelas

CREAF (Centre de Recerca Ecològica i Aplicacions Forestals), Facultat de Ciències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
** Corresponding author (fax: +34 93 5811312; e-mail: g.oliveira@creaf.uab.es)*

Received 8 June 1999; revised 25 January 2000; accepted 25 February 2000

Abstract — The impact of winter stress on plants from a Mediterranean area was evaluated through comparison of photosystem II (PS II) efficiencies and phenomorphological characteristics of two Mediterranean woody species – *Quercus ilex* (evergreen) and *Cistus albidus* (semi-deciduous). The studies were carried out in NE Spain, at two sites with different mean winter temperatures. The results showed that reductions of the efficiency of PS II may occur in Mediterranean plant communities during winter, and are especially remarkable on colder days. The extent and duration of the decrease in PS II efficiency (photoinhibition) depended not only on the climatic conditions, but also on the site, on the species considered and on the position of leaves in the plant canopy. Increased photoinhibition at the colder site was only clear for *C. albidus*. Nevertheless, the efficiencies of PS II were always higher in *C. albidus* than in *Q. ilex*. Phenological patterns, morphology and leaf inclination may protect *C. albidus* leaves from potentially photoinhibitory conditions in winter. Morphological and structural photoprotection is apparently not so well developed in *Q. ilex*, which probably relies more on increased protection at the photochemical level of its long-lived leaves. As has been reported in relation to summer-drought stress, *Q. ilex* has possibly developed a strategy of tolerance to photoinhibition, whereas *C. albidus* relies preferentially on avoidance features. © 2000 Éditions scientifiques et médicales Elsevier SAS

Chlorophyll fluorescence / evergreens / leaf characteristics / Mediterranean winter / phenology / photoinhibition / semi-deciduous

1. INTRODUCTION

Dry summers and cool winters are usually given as the main characteristics of the Mediterranean climate [31]. Summer drought, entailing high radiation intensities and air temperatures as well as water shortage, is generally considered the primary constraint to the productivity and dynamics of the vegetation in Mediterranean-climate regions [31, 44, 49]. It is recognised that Mediterranean woody species have developed various functional and structural characteristics that allow them to survive the summer stress period and to grow and reproduce during the favourable times of the year (e.g. [4, 12, 21, 40]).

However, a few authors suggest that winter cold stress plays a similarly important role in the development and distribution of Mediterranean evergreen species [29, 30]. Although many reports are available on the effects of natural winter conditions on plant growth and function (e.g. [2, 20, 38]), few concern Mediterranean species [19, 45, 46]. The mildness of the Mediterranean winter may allow a relatively high photosynthetic activity in evergreen plants [4, 42, 45]. However, there is evidence that winter conditions such as lower temperatures and shorter photoperiods condition plant metabolism and growth in Mediterranean ecosystems [4, 21, 24, 46]. Winter may thus represent an additional and important limitation to the annual productivity of these species, especially if we consider that this period normally precedes one of intense growth and reproduction effort, in spring, which probably relies on currently acquired and previously stored energy [31].

Mediterranean winters may be relatively cold, and often associated with cloudless skies. These conditions make photoinhibitory effects possible [1, 2, 3, 20], because photosynthesis may be impaired by low temperatures, and thus high light levels cannot be utilised. Photoinhibition is associated with various processes of dissipation of excitation energy and serves primarily as a strategy to allow the survival of the photosynthetic structures [1, 38]. It thus represents a mechanism of protection rather than a damage [1, 2, 36]. However, if photoinhibition causes a significant limitation to net carbon assimilation [34], then it may affect productivity and growth, and therefore species distribution [20, 46].

The aim of this work was to test the following hypotheses: i) Mediterranean plants are susceptible to photoinhibition under unfavourable conditions prevailing in winter – e.g. relatively low temperatures and high light intensities; ii) that susceptibility varies with the species considered, and with the position of leaves in the canopy; and iii) the same species displays different responses in sites with different climates.

We compared the physiological and phenological behaviour of two woody species – *Quercus ilex*, an evergreen sclerophyll, and *Cistus albidus*, a semi-deciduous species – during winter in Catalonia (NE Spain). The two functional types represented by these species rely on distinct phenological, structural and/or physiological features to overcome summer drought stress (*table I*), but the question arises as to whether those and/or other characteristics are effective against potential winter stress. Chlorophyll fluorescence parameters were used to detect any possible effects of daily and seasonal variation in air temperature and light intensity levels on the maximal and actual efficiencies of PS II in these species. To evaluate the effect of different leaf positions within the canopy, we studied leaves at different azimuths in each plant. Moreover, to investigate the influence of cold over the long-term, we chose two sites with different winter temperatures where both species occur. Chlorophyll fluorescence measurements were accompanied by morphological and phenological monitoring, for subsequent consideration of the structural and functional features of these species.

Table I. Structural, phenological and physiological characteristics of *Cistus* spp. and evergreen *Quercus* spp. usually considered as adaptations to overcome the Mediterranean summer drought stress (derived from the references within brackets).

Characteristics	*Cistus* spp.	*Quercus* spp.
Plant form	shrubs	shrubs and trees
Specific leaf mass	low [12]	high [8, 35]
Leaves	relatively thin [14]	relatively thick
Trichome density	high (both surfaces) [12]	abaxial surface only [43]
Root depth	shallow [13, 27]	deep [27]
Growth period	long [14]	short [8, 35]
Canopy reduction (summer)	considerable [14, 21, 49]	slight [35, 49]
Leaf longevity	< 1 year [14, 40]	> 1 year [35]
Leaf water potential	very low [12 , 27, 49]	relatively high [8, 27, 41, 49]
Stomatal control	moderate-high [12, 21]	high [8, 44]
Net photosynthesis rate	> in spring	> in spring and autumn
	< in summer [12, 21]	< in summer [8, 41, 45]

2. MATERIALS AND METHODS

2.1. Site description

Two sites were selected, one at the Park of Collserola (41º26’ N, 2º08’ E), the other at the Park of Sant Llorenç del Munt i l’Obac (41º38’ N, 2º01’ E), in Catalonia (NE Spain). The first site – ‘Collserola’ – closer to the coast, is on a south-facing slope with schistic soils (alt. 350 m); the plant community is a typical maquis, with woody species such as *Quercus ilex*, *Q. coccifera*, *Arbutus unedo*, *Erica arborea* and *Cistus* spp. Annual precipitation averages 700 mm and annual temperature 14 °C; mean winter and summer temperatures range from 8 to 11 °C and from 21 to 24 °C, respectively [22]. The second site – ‘Sant Llorenç’ – is at an altitude of 900 m, with weakly aggregated soils of calcareous conglomerates. Here, the plant community is dominated by *Q. ilex* and *Phillyrea latifolia*, accompanied by species such as *Cistus albidus*, *Buxus sempervirens*, *Thymus vulgaris* and *Rosmarinus officinalis*. Annual precipitation averages 700 mm and annual temperature 12 °C; mean winter and summer temperatures range from 5 to 8 °C and from 18 to 22 °C, respectively [22].

2.2. Environmental monitoring

Air temperature and precipitation data concerning the sampling period were obtained from the Collserola weather station (Servei Meteorològic de Catalunya) and from the local weather station at the Park of Sant Llorenç del Munt i l’Obac.

2.3. Plant material

In NE Spain, *Quercus ilex* grows on coastal or subcoastal areas of temperate Mediterranean climate, on all kinds of soil substrates [9]. It may form relatively dense woodlands or be an important component of shrubland communities (maquis). *Cistus* *albidus* is a pioneer shrub with densely haired leaves, frequently found in more xeric and disturbed sites. It grows on dry soils near the coast, where winters are mild and summers are rather hot [9].

At each study site, shrubs of each species (*Q. ilex* and *C. albidus*) were selected, all sufficiently close to each other to optimise the sampling procedures. At Collserola, *C. albidus* shrubs were always smaller (< 1.5 m high) than *Q. ilex* (< 2.5 m high). In each of three shrubs per species, twelve branches – six facing south and six facing north-west – were tagged for subsequent chlorophyll fluorescence and phenomorphological studies. At Sant Llorenç, the selected shrubs of *Q. ilex* and *C. albidus* were very close to each other, forming a large canopy not higher than 1.7 m. Five shrubs were selected – three on the south and two on the north sides of the canopy – and six branches were tagged on each of them. None of the selected branches was shaded by neighbouring plants at any time of the day, but north branches seldom received direct sunlight during the study period (winter).

2.4. Chlorophyll fluorescence measurements

Chlorophyll fluorescence parameters were measured in situ with a PAM-2000 fluorometer (Walz, Effeltrich, Germany), after Bilger et al. [5].

The same healthy leaves per shrub and azimuth were used throughout the daily sampling programmes at the two sites. Additionally, at Sant Llorenç clearly steep leaves (insertion angle > 60°) were measured from south branches of *C. albidus*. This type of leaves was assessed because the phenological data indicated that they represented a considerable proportion of the *C. albidus* canopies at that site (see Results). Thus, three groups of leaves were studied – horizontal leaves from north/north-west branches, and horizontal and steep leaves from south branches (hereafter referred to as north, south, and steep leaves, respectively). Within each group, the selected leaves were those receiving maximum solar radiation throughout the day. In the case of *Q. ilex*, the same leaves were often used on several sampling dates. This was not so for *C. albidus* due to greater fragility of its leaves. All measurements were made on the adaxial surface of current-year leaves. *Q. ilex* and *C. albidus* shrubs were measured alternately to avoid any influence on the comparison caused by diurnal changes in fluorescence yield.

The actual and the maximal efficiencies of photosystem II (PSII) (sensu [15]) were determined as ΔF/F'm (in light-exposed leaves) and Fv/Fm (in dark-adapted leaves), respectively. Determinations of ΔF/F'm and Fv/Fm were made on different days.

Five light-adapted leaves per branch azimuth (north/north-west and south) were measured in each species under natural conditions [15] during the central hours of the day. Simultaneously, photon flux density incident on the leaf (PFD) was measured by a sensor of the leaf clip holder (model 2030-B, Walz, Effeltrich, Germany).

Maximal efficiency of PS II, Fv/Fm, was used to quantify photoinhibition [36, 37] and measured after 20-min dark adaptation of the leaf using light-weight clamp cuvettes. These measurements were done at predawn and at various times during the day, in six leaves per leaf group of all the selected plants. On the Fv/Fm sampling dates, the diurnal courses of the photon flux density incident upon each of the studied leaves (PFD) was determined with the Micro Quantum/Temperature Sensor 2060-M (Walz, Effeltrich, Germany).

2.5. Phenomorphological measurements

For each selected shrub and azimuth (north/north-west and south branches), the same six to eight branches were monitored for phenological changes on two occasions: mid-February and mid-March (1998). Only current-year shoots were studied, i.e. those holding leaves emerged after the previous summer (*C. albidus*) or in the previous spring (*Q. ilex*). The development of new shoots and buds, the number of expanded leaves, and the occurrence of foliage damage (e.g. yellowing and desiccation in *C. albidus*, and desiccated or bleached spots in *Q. ilex*) were recorded. In *C. albidus*, leaf inclination was also taken into account, as it changes from vertical (ca. 80°) at the apex to horizontal (ca. 0°) at the base of the stem. Leaves were thus classified as 'steep' (> 50°) and 'horizontal' (< 50°).

In mid-March, leaf samples of both sites were collected from each shrub and azimuth for laboratory determinations. Total chlorophyll content was measured with a Chlorophyll Meter (SPAD-502, Minolta, Japan), and the values corrected for each species according to previous calibrations against standard analytical methods [32, 48]. These samples were also used for determination of specific leaf weight, calculated from leaf disks (64 mm^2) dried in an oven at 70 °C to constant weight.

2.6. Statistical analyses

For each species, phenomorphological data were compared between sites and leaf groups using two-way analyses of variance with repeated measurements; differences between means were tested through Tukey tests. Regression analysis was used to compare the relationships between ΔF/F'm and PFD; differences between the two species were tested with analyses of covariance of ΔF/F'm with PFD. For each site and species, the diurnal patterns of Fv/Fm were compared between leaf groups and between dates with one-way ANOVAs with repeated measurements. For each species, the relationship between Fv/Fm values and the corresponding air temperature and PFD values were analysed through correlation tests. All the statistical analyses were performed with the Statistica software package (StatSoft, Inc., 1995, Tulsa, USA).

3. RESULTS

3.1. Climatic conditions

During the winter study period (February-March 1998) minimum daytime air temperatures ranged from 4.2 to 14.3 °C at Collserola, and from –2.2 to 10.7 °C at Sant Llorenç (*figure 1*). Maximum air temperatures reached 23.4 and 22.3 °C at Collserola and Sant Llorenç, respectively. Minimum diurnal air temperatures were frequently below 5 °C at Sant Llorenç, while at Collserola they were generally above that value. During this period, cumulative precipitation was about 11 mm at Collserola and 15 mm at Sant Llorenç (*figure 1*) – and clear sunny days were very frequent. Although considerably dry for the season, this period was preceded by relatively high precipitation in late January (*figure 1*). Moreover, predawn shoot water potentials of the studied plants, measured with a Scholander-type pressure chamber in mid-March (ca. –0.6 MPa, both species) were very similar, or even higher than those reported for *Q. ilex* and *Cistus* spp. in winter [8, 13, 21]. These facts indicate that the plants were not experiencing water stress during the study period.

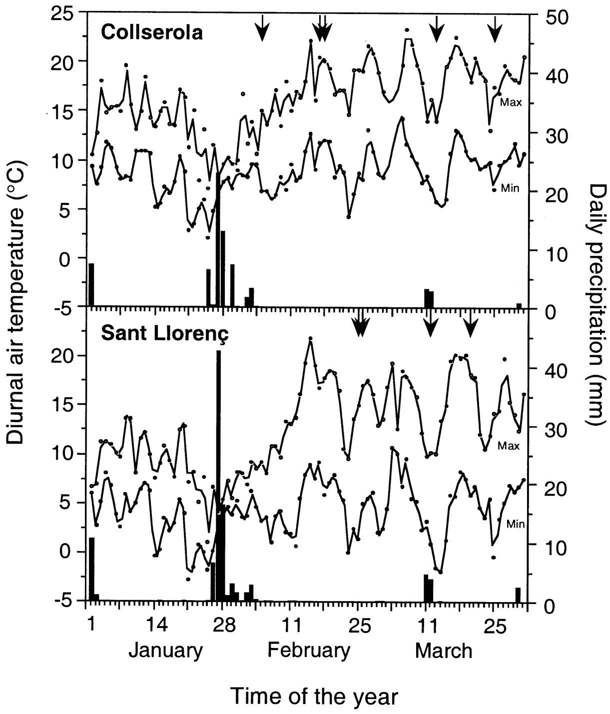


Figure 1. Diurnal maximum and minimum air temperatures (lines) and daily precipitation (bars) during the study period at Collserola and Sant Llorenç. Arrows indicate the dates of the chlorophyll fluorescence sampling presented in the Results.

Photon flux densities measured on various occasions at both sites (data not shown, but see *figure 3*) indicate consistently higher values at Sant Llorenç, this being possibly related to a frequent haze at the more coastal site, Collserola.

3.2. Chlorophyll fluorescence measurements

The relationship between the actual efficiency of PSII (ΔF/F'm) and PFD is depicted in *figure 2* for 3 d differing in diurnal air temperatures. As expected, ΔF/F'm decreased by late morning in south leaves, and in the afternoon in north-west leaves, remaining high and stable throughout the day in north leaves, which did not receive direct sunlight at any time of the day (results not shown). In *Q. ilex*, the ΔF/F'm responses to PFD were similar on these 3 d and lower than in *C. albidus* (*figure 2*). The differences in actual efficiency between the two species were significant on the warmer days ($P < 0.01$, ANCOVA), but tended to decrease with decreasing air temperatures, and disappeared on the coldest day, at Sant Llorenç (colder site).

Diurnal courses of the maximal efficiency of PSII (Fv/Fm), are displayed in *figure 3*. In this 3-d sequence, the second day was the coldest, so that the responses of plants prior to, during, and after the 'extreme-cold' event may be evaluated.

South leaves presented the greatest differences between days and species, in terms of Fv/Fm. Except for *C. albidus* at Collserola (17 February and 25 March, *figure 3A*), they always displayed the lowest values ($P < 0.05$), as compared to north/north-west, or steep leaves (*figure 3*). The highest recorded values were 0.83 for *C. albidus* (17 February, *figure 3A*) and 0.72 for *Q. ilex* (17 February, *figure 3A*). Minimum values (ca. 0.67 in *C. albidus* at Sant Llorenç, and 0.55 in *Q. ilex* at Collserola) were measured on the coldest days (*figure 3*). After the general Fv/Fm reduction on these days, recovery to values similar to, or even higher than on the previous sampling (first day) was particularly evident for *C. albidus* (*figure 3*).

Steep leaves of *C. albidus* (Sant Llorenç) behaved very similarly to north leaves of the same plants (*figure 3B*), but tended to have even higher predawn values. North leaves generally presented stable Fv/Fm

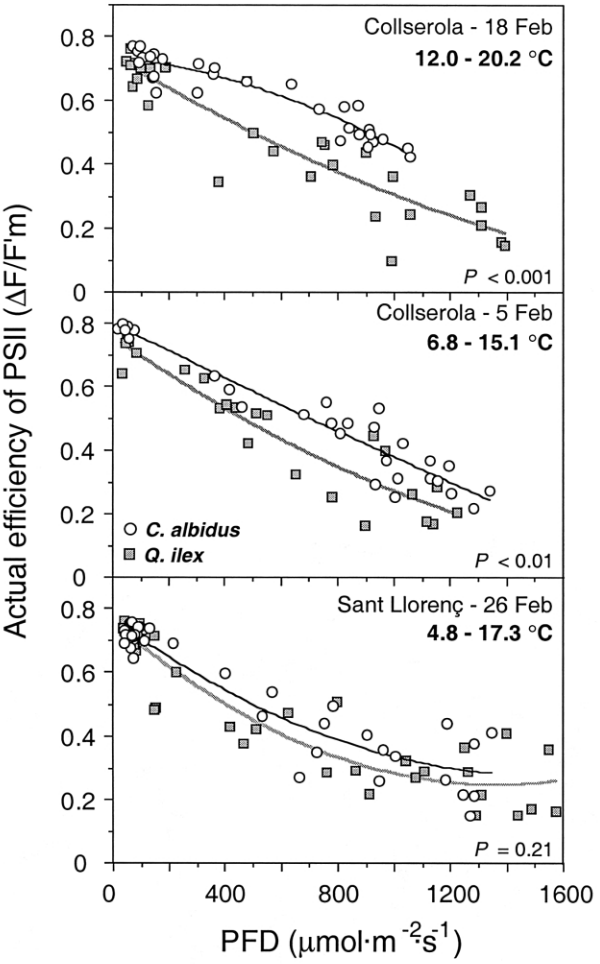


Figure 2. Dependence of the actual efficiency of PSII (ΔF/F'm) on incident photon flux density (PFD). Data from diurnal courses recorded on the 5 and 18 February (Collserola) and 26 February (Sant Llorenç). The range of diurnal air temperatures is displayed. Measurements were made on north (Sant Llorenç), north-west (Collserola) and south leaves (both sites), and each point represents the value of a single leaf. The significance level of the difference between the two species is shown for each day.

values throughout the day, but lower on the coldest day (*figure 3B*). The highest values were recorded on the third day – *C. albidus*, 0.81; *Q. ilex*, 0.80 (*figure 3B*) – and the lowest on the predawn of the coldest day, for *Q. ilex* (0.71, *figure 3B*).

The daily evolution of Fv/Fm in north-west leaves (Collserola) mirrored the variation in PFD, i.e. it remained high during the morning and declined in the afternoon, with increasing light intensities (*figure 3A*). This decline was more pronounced on the coldest day. Fv/Fm values in these leaves did not fully recover in the late afternoon of any of the sampled days.

Photoinhibition, as indicated by the level and duration of low Fv/Fm values in all the leaf groups, was more pronounced at Sant Llorenç than at Collserola for *C. albidus*, but the opposite trend was observed for *Q. ilex*. Nevertheless, Fv/Fm was always higher in *C. albidus* than in *Q. ilex* leaves (*figure 3*).

The relationship between the minimum daily Fv/Fm and the corresponding value of air temperature and PFD (either actual or cumulative) was investigated through simple and multiple regressions, for each species. Minimum Fv/Fm correlated significantly with air temperature ($P < 0.05$), but not with the concurrent PFD values ($P > 0.05$). However, significant negative correlations were found ($P < 0.001$) when the cumulative daily PFD was considered. The multiple regressions between minimum daily Fv/Fm and the corresponding air temperature and cumulative PFD were significant for both species ($r = 0.53$, for *Q. ilex*, $r = 0.55$ for *C. albidus*; $P < 0.001$).

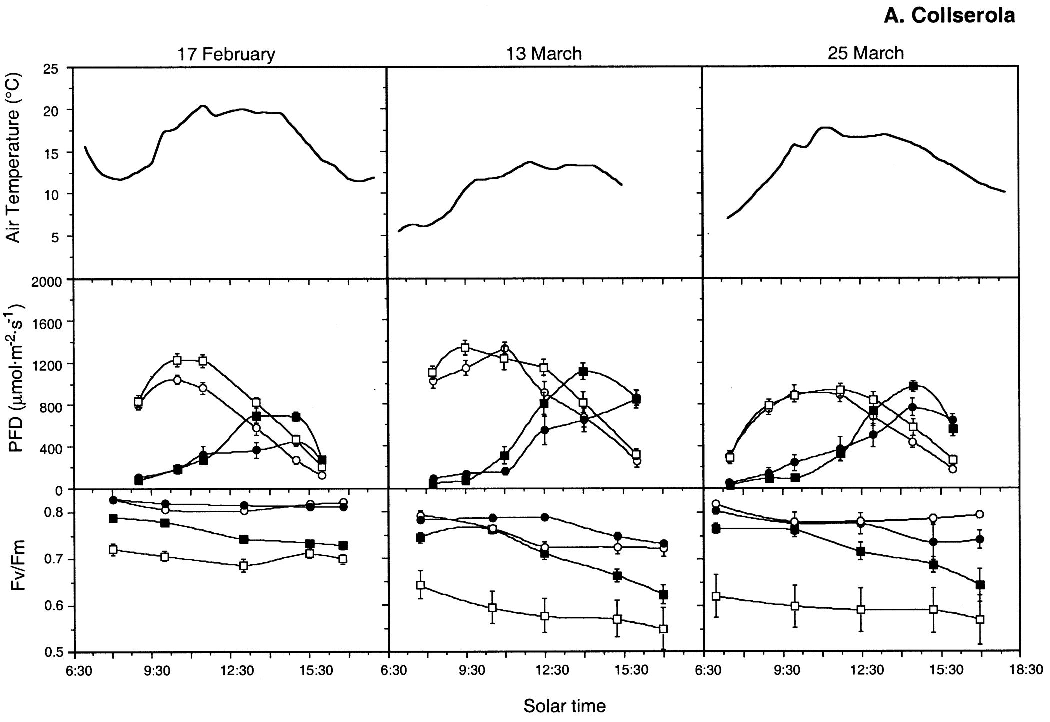
3.3. Phenomorphological measurements

Leaf demography of *C. albidus* changed during late winter at both sites (*figure 4*), with old leaf yellowing and shedding from the base of the shoot, and expansion of new, apical leaves. By the first sampling date (mid-February), apical leaves had recently emerged, indicating that growth was already occurring earlier in the season. In turn, *Q. ilex* shoots were phenologically dormant, only a few leaves being shed.

Differences in leaf number between azimuths were significant ($P < 0.05$) only at Collserola, in March (*C. albidus*), south shoots presenting more leaves than north-west ones (*figure 4*). The number of leaves per shoot was significantly lower ($P < 0.05$) at Sant Llorenç than at Collserola (*C. albidus*, *figure 4*).

In *C. albidus*, the proportion of steep leaves was significantly higher ($P < 0.001$) on south than on north/north-west shoots (*figure 5*). Although not significantly, it was consistently higher at Sant Llorenç than at Collserola. Except for the February sampling at Collserola, steep leaves in south shoots always outnumbered the horizontal ones (*figure 5*).

Differences in chlorophyll content between azimuths, although generally significant, were not consistent (*table II*). In *C. albidus*, the values were higher in south than in north-west leaves at Collserola, but the opposite trend was observed at Sant Llorenç. South and north-west *Quercus ilex* leaves had similar chlorophyll contents at Collserola, but not at Sant Llorenç. Compared to south leaves, north/north-west ones had lower specific leaf weight (SLW) in both species (*table II*). At Collserola, *C. albidus* shrubs had less sclerophyllous leaves than at Sant Llorenç, regardless of azimuth, but *Q. ilex* only showed this trend in north leaves.



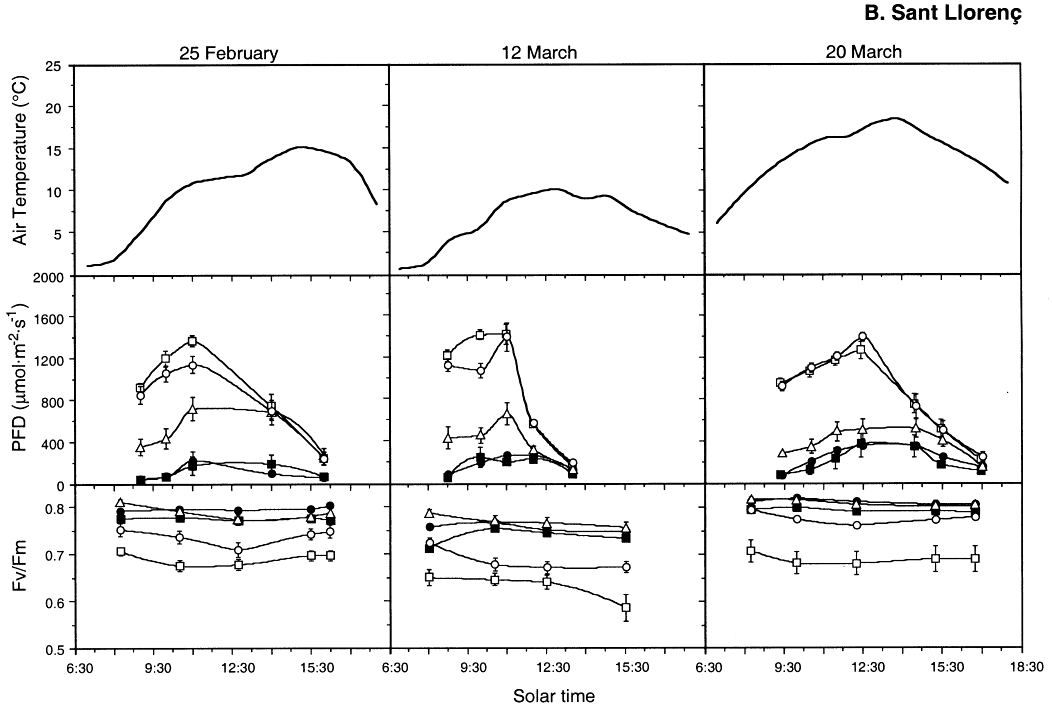


Figure 3. Daily courses of air temperature, PFD incident on the leaves and maximal PS II efficiency, Fv/Fm, of *C. albidus* (circles and triangles) and *Q. ilex* (squares) on three winter days at Collserola (A) and Sant Llorenç (B). Responses from different leaf groups are shown: south (open symbols), steep (triangles), north (B) and north-west (A) (closed symbols). Each symbol represents the average of the measurements on 12–18 leaves ± SE.

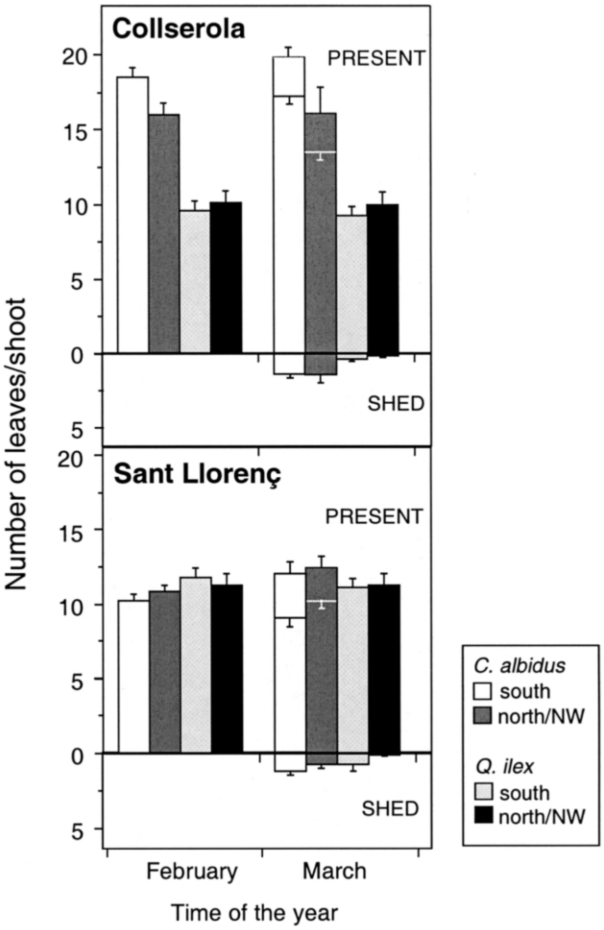


Figure 4. Leaf phenological events in *C. albidus* and *Q. ilex* at Collserola and Sant Llorenç (1998). Number of expanded leaves: present (total, up-directed bars), new (top section of up-directed bars) and shed (down-directed bars). Each bar and bar section represents the mean of 12–24 shoots on a current-year shoot basis. Vertical lines represent standard errors of the means.

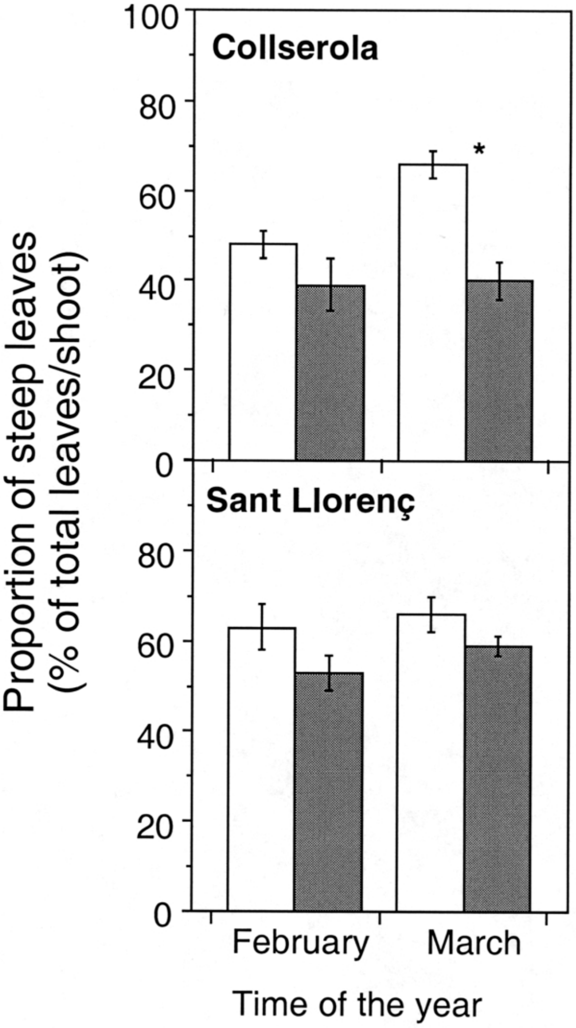


Figure 5. Proportion of steep leaves relative to the total number of leaves in *C. albidus* shoots, at Collserola and Sant Llorenç. Each bar represents the mean of 12–24 shoots, on a current-year shoot basis. Vertical lines represent standard errors of the means. The asterisk indicates a significant difference ($P < 0.001$) between branch azimuths: south (white bars) and north/north-west (shaded bars).

4. DISCUSSION

4.1. General photoinhibitory responses

These results confirm our initial hypothesis that photoinhibition may occur in Mediterranean plant communities during winter. In other words, Mediterranean winter temperatures are low enough to trigger photoinhibitory responses under high light conditions, similarly to what is frequently reported for evergreens in temperate and boreal regions (e.g. [2, 20, 38]). Increasing diurnal light intensities led to decreased efficiencies of PS II (ΔF/F'm and Fv/Fm), with further declines on colder days (*figures 2, 3*). As has also been found by other authors [2, 20], the Fv/Fm decline was better correlated with the cumulated dose of light energy reaching the leaves than the actual incident PFD at the time of the fluorescence measurement. However, no strong diurnal or seasonal increase in minimal fluorescence, Fo, parallel to Fv/Fm decline was recorded (data not shown), indicating that there was no chronic PSII damage [37, 38].

The extent and duration of photoinhibition depended not only on the climatic conditions, but also on the species considered and on the position of leaves in the plant canopy. Diurnal Fv/Fm decline was

Table II. Leaf chlorophyll content and specific leaf weight (SLW) of the studied plants in mid-March (means and standard errors, $n = 12–40$ leaves). Branch azimuth is indicated. For each species, site and parameter, means with the same superscript are not significantly different ($P > 0.05$) between orientations. * indicates significantly different ($P < 0.05$) means between sites, for each species and azimuth.

Parameter	*C. albidus*		*Q. ilex*	
	Collserola	Sant Llorenç	Collserola	Sant Llorenç
Total chlorophyll	51.7 ± 1.3 S^{a*}	33.1 ± 2.3 S^{b}	62.8 ± 2.1 S^{a*}	42.8 ± 3.2 S^{b}
(µg·cm^{-2})	44.3 ± 2.8 NW^{b}	46.0 ± 1.4 N^{a}	62.0 ± 1.9 NW^{a}	59.9 ± 1.7 N^{a}
SLW (mg dw·cm^{-2})	13.3 ± 0.3 S^{a*}	17.4 ± 0.6 S^{a}	21.5 ± 0.5 S^{a}	21.9 ± 0.3 S^{a}
	12.2 ± 0.3 NW^{b*}	13.8 ± 0.4 N^{b}	18.4 ± 0.4 NW^{b*}	20.9 ± 0.4 N^{a}

generally reversible (*figure 3*), the exceptions being presented by north-west leaves (*figure 3A*), and by all leaf groups on the coldest days.

Fv/Fm and ΔF/F'm values of *Q. ilex* (*figures 2, 3*) were similar to, or lower than those measured under summer conditions [17, 39, 42]. This may indicate some degree of sustained photoinhibition during winter in *Q. ilex* at our studied sites. In *C. albidus* however, Fv/Fm was considerably higher (*figure 3*) than was found during summer by other authors [49]. This suggests that summer leaves of semi-deciduous species are more susceptible to photoinhibition than winter leaves, possibly because other stress factors (e.g. drought, heat) concur with high light intensities during summer [24]. However, the minimum Fv/Fm values found in *C. albidus* (*figure 3*) were somewhat lower than those recorded by Karavatas and Manetas [24] in other *Cistus* species during winter.

4.2. Growth and structural features

The phenomorphological patterns of the studied plants (*figure 4*; *table II*) agreed with those previously described for Mediterranean evergreen and semi-deciduous species ([14, 19, 21, 35], see also *table I*). While *Q. ilex* remained phenologically dormant, *C. albidus* presented continuous growth during the studied winter period (*figure 4*).

Specific leaf weight (SLW) values of *Q. ilex* were similar to those described for mature individuals of this species in relatively open stands [8, 27]. In turn, *C. albidus* presented relatively higher SLW at both sites (*table II*) than in SW Portugal [14].

4.3. Species comparison

Generally, the semi-deciduous *C. albidus* was less prone to photoinhibition than *Q. ilex*, as shown by its consistently higher ΔF/F'm (*figure 2*) and Fv/Fm values (*figure 3*). Phenology and structure may provide important stress-avoiding characteristics which seem to be effective throughout the relatively short leaf lifespan of *C. albidus*. In *Cistus* spp., winter leaves – leaves emerging during autumn and winter – attain a mean age of 6 months [14]. Similarly to summer leaves [49], they are likely to avoid cold-induced photoinhibition by keeping a steep position (*figure 3B*). As the leaf grows older, it becomes less steep and progressively more affected by higher light intensities and hence photoinhibition. The protection mechanisms at the structural level are then no longer effective, either because of the age-dependent reduction of trichome density and radiation absorbing compounds at the leaf surface [23], or simply because excessive radiation loads cannot be effectively dissipated through the available processes at the physiological level. Indeed, horizontal leaves were always more photoinhibited than steep ones (*figure 3B*), though still with relatively high Fv/Fm ratios. This can be explained by the fact that the same leaves were not measured throughout the study in *C. albidus* (see Materials and Methods), i.e. measurements on the same horizontal leaves throughout the season would have presumably shown further photoinhibition.

Contrary to some other Mediterranean evergreen species [11], *Q. ilex* does not considerably change leaf inclination, although some proportion of the canopy may present more vertical leaf insertions [7]. Thus, any degree of structural protection from excessive incident light (at the leaf level) depends primarily on tissue organisation and composition, e.g. thick cuticles, high wax contents, etc. [10, 16]. In *Q. ilex*, the same leaves are submitted to both annual stresses (first summer drought, and then winter cold), and seem to rely more on a higher efficiency of protective processes at the photochemical level [1, 15, 17, 39]. Some leaf damage was observed after winter (data not shown), but leaves kept most of their green area and were not shed (*figure 4*). This suggests that photosynthesis of *Q. ilex* was not significantly impaired during the cold periods, as shown by other studies [28].

In relation to summer-drought stress, evergreen sclerophylls and semi-deciduous species are generally described as tolerators and avoiders, respectively [13, 19, 21, 49]. It seems that the same classification can be applied when winter-cold stress is considered, at least for the two species studied here. Moreover, the leaf characteristics usually described as adaptations towards summer stress are apparently efficient towards winter stress as well (*tables I, II*; *figure 5*).

4.4. Within-plant variations

The protective role attributed to increased leaf angles under summer-stress conditions [16, 47], was also found during the winter period: steep leaves of *C. albidus* presented reduced photoinhibition, if at all (*figure 3B*). This becomes even more important if we consider that steep leaves in this species are the youngest leaves, and that young leaves may have a reduced photodissipative capacity [6]. An additional benefit from steep angles may be a slight temperature increase during cold nights, as compared to horizontally-oriented leaves [25].

The differences in SLW and chlorophyll contents between south and north/north-west leaves were the ones generally expected, respectively from ‘sun’ and ‘shade’ leaves [41]. In evergreen shrubs, water relationships differ only slightly between sun and shade leaves [41], suggesting that they developed different mechanisms to withstand microclimatic conditions (avoidance and tolerance mechanisms, respectively in sun and in shade leaves) [41]. In our study, leaves not receiving full sunlight (i.e. north, north-west or steep leaves) generally showed higher maximal and actual efficiencies of PS II than sun-exposed (south) ones (*figures 2*, *3*), as found by other authors [3, 18, 20]. Nevertheless, on colder days, there was some degree of photoinhibition in other than south leaves (e.g. north and north-west, *figure 3*). Moreover, in *Q. ilex*, some predawn Fv/Fm values of north leaves were lower than mid-morning values (*figure 3B*), indicating incomplete recovery from the previous day’s photoinhibition. Groom et al. [20] report slower recovery after severe photoinhibition or in the dark at low temperatures, which could explain the results we obtained in north leaves (*figure 3B*).

4.5. Site comparison

As also found in a comparable study by Tretiach et al. [46], somewhat different ecological conditions brought about important differences in plant performance between the two studied sites, which were particularly evident during periods of more intense cold stress. From the climatic point of view, the two sites in our study differ in mean winter air temperatures (see Site description) and this difference was particularly evident during the study period (*figures 1*, *3*). Therefore, winter stress can be considered an important environmental constraint when they are compared. Such a comparison may serve as an approach to the long-term effects of such stress on species behaviour.

Both species decreased leaf chlorophyll contents by 32–36 % at Sant Llorenç (*table II*), possibly a strategy to reduce excessive light capture at this colder and more luminous site. It was found that leaves of *Cistus ladanifer* also present lower chlorophyll contents at colder sites [33]. Decreased chlorophyll contents may be an adaptive response against photoinhibition as they reduce the light harvesting capacity of the leaf [1, 26]. This would at least partially explain the differences found for this parameter between sites and azimuths (*table II*).

Moreover, *C. albidus* presented less, steeper and more sclerophyllous leaves at the colder site (*table II*). This appears as further evidence to consider *C. albidus* more susceptible to increasingly stressful winter conditions than *Q. ilex*, as shown by predawn Fv/Fm values at the two sites (*figure 3*), and that it has to reinforce its structurally photoprotective characteristics and semi-deciduous habit to overcome that constraint. In turn, and despite being more photoinhibited than *C. albidus*, *Q. ilex* presents more similar predawn Fv/Fm values (maximal PS II efficiency) under different site climates (*figure 3*), suggesting some degree of adaptation to Mediterranean cold conditions, as shown for *Quercus coccifera* by Mitrakos [30].

5. CONCLUSIONS AND FINAL REMARKS

The results confirmed our initial hypotheses: i) the studied Mediterranean plants were photoinhibited under winter conditions; ii) the extent of that photoinhibition was enhanced on colder days, and more intense in the evergreen species (*Q. ilex*) than in the semi-deciduous one (*C. albidus*). Moreover, leaf position in the canopy played a decisive role, south, well-exposed leaves being the most photoinhibited; iii) when comparing two sites differing in winter temperatures, *C. albidus* tended to present increased photoinhibition at the colder site, whereas *Q. ilex* showed rather similar photoinhibition levels. *C. albidus* apparently had to ‘change’ more than *Q. ilex* when facing a colder environment.

Although only for a relatively short life period, *C. albidus* leaves are able to maintain relatively high PS II efficiencies, presumably because their structural (e.g. leaf inclination) and morphological characteristics (e.g. high trichome density, high leaf reflectance) prevent excessive light energy from reaching (and damaging) the photosynthetic apparatus. It is conceivable that these mechanisms are no longer sufficiently effective when the leaves senesce. Continuous leaf renewal during winter possibly enables the shrub to avoid permanent photoinhibition. In turn, *Q. ilex* produces long-lived leaves which may be structurally more resistant to cold injury but are apparently less well protected against excessive light intensity loads. Therefore, their PS II efficiency is lower, but stable over large ranges of stress (summer drought and winter cold), probably at the expense of a strong investment in photodissipative processes, e.g. via the xanthophyll cycle [1, 17, 18]. *Q. ilex* seems to have developed a strategy of tolerance to photoinhibition, whereas *C. albidus* apparently avoids it.

Acknowledgments

To the administration of the 'Parc Natural de Sant Llorenç del Munt i l'Obac' for permitting free access to the study site, and to C. Castell for his collaboration and for providing the meteorological data of Sant Llorenç. To C. Werner for support and helpful comments on an earlier version of this manuscript. This research was supported by a grant from PRAXIS XXI (BPD/11810/97) to G.O. and by grants CICYT - CLI 97 - 0344 and AMB 97-0334 to J.P.

REFERENCES

[1] Adams III W.W., Demmig-Adams B., The xanthophyll cycle and sustained thermal energy dissipation activity in *Vinca minor* and *Euonymus kiautschovicus* in winter, Plant Cell Environ. 18 (1995) 117–127.

[2] Ball M.C., Hodges V.S., Laughlin G.P., Cold-induced photoinhibition limits regeneration of snow gum at tree-line, Funct. Ecol. 5 (1991) 663–668.

[3] Barker D.H., Logan B.A., Adams III W.W., Demmig-Adams B., Photochemistry and xanthophyll cycle-dependent energy dissipation in differently oriented cladodes of *Opuntia stricta* during the winter, Aust. J. Plant Physiol. 25 (1998) 95–104.

[4] Beyschlag W., Lange O.L., Tenhunen J.D., Photosynthese und Wasserhaushalt der immergrünen mediterranen Hartlaubpflanze *Arbutus unedo* L. im Jahreslauf am Freilandsort in Portugal. I. Tagesläufe von CO_2-Gaswechsel und Transpiration unter natürlichen Bedingungen, Flora 178 (1986) 409–444.

[5] Bilger W., Schreiber U., Bock M., Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field, Oecologia 102 (1995) 425–432.

[6] Bisba A., Petropoulou Y., Manetas Y., The transiently pubescent young leaves of plane (*Platanus orientalis*) are deficient in photodissipative capacity, Physiol. Plant. 101 (1997) 373–378.

[7] Burriel J.A., Calvet S., Sala A., Gracia C., Ángulo foliar en *Quercus ilex*: modulación por el ambiente, y contribución a la economia hídrica de la planta, Proceedings Congreso Forestal Español, Lourizán, (Pontevedra), 1993, pp. 225–232.

[8] Castell C., Terradas J., Tenhunen J.D., Water relations, gas exchange, and growth of resprouts and mature plant shoots of *Arbutus unedo* L. and *Quercus ilex* L., Oecologia 98 (1994) 201–211.

[9] Castroviejo S. Aedo C. Cirujano S. Laínz M. Montserrat P. Morales R. Muñoz Garmandia F. Navarro C. Paiva J. Soriano C. (Eds.), Flora Iberica – plantas vasculares de la Península Ibérica e Islas Baleares, Real Jardín Botánico, C.S.I.C., 1993.

[10] Catarino F.M., Correia O.A., Webb E., David M., Morphological and physiological responses of the Mediterranean evergreen sclerophyll, *Ceratonia siliqua*, to different light intensities, in: Margaris N.S., Mooney H.A.(Eds.), Components of Productivity of Mediterranean-Climate Regions - Basic and Applied Aspects, Dr W. Junk Publishers, The Hague, 1993, pp. 5–15.

[11] Comstock J.P., Mahall B.E., Drought and changes in leaf orientation for two California chaparral shrubs: *Ceanothus megacarpus* and *Ceanothus crassifolius*, Oecologia 65 (1985) 531–535.

[12] Correia O.A., Contribuição da fenologia e ecofisiologia em estudos da sucessão e dinâmica da vegetação mediterrânica, Ph.D. thesis, University of Lisbon, Lisbon, 1988, 196 p.

[13] Correia O.A., Catarino F.M., Seasonal changes in soil-to-leaf resistance in *Cistus* sp. and *Pistacia lentiscus*, Acta Oecol. 15 (1994) 289–300.

[14] Correia O.A., Martins A.C., Catarino F.M., Comparative phenology and seasonal foliar nitrogen variation in Mediterranean species of Portugal, Ecol. Mediterr. 18 (1992) 7–18.

[15] Demmig-Adams B., Adams III W.W., Barker D.H., Logan B.A., Bowling D.R., Verhoeven A.S., Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation, Physiol. Plant. 98 (1996) 253–264.

[16] Ehleringer J.R., Comstock J., Leaf absorptance and leaf angle: mechanisms for stress avoidance, in: Tenhunen J.D., Catarino F.M., Lange O.L., Oechel W.C.(Eds.), Plant Response to Stress. Functional Analysis in Mediterranean Ecosystems, NATO ASI Series, vol. G15, Springer, Berlin, 1987, pp. 55–76.

[17] Fleck I., Hogan K.P., Llorens L., Abadía A., Aranda X., Photosynthesis and photoprotection in *Quercus ilex* resprouts after fire, Tree Physiol. 18 (1998) 607–614.

[18] García-Plazaola J.I., Faria T., Abadía J., Abadía A., Chaves M.M., Pereira J.S., Seasonal changes in xanthophyll composition and photosynthesis of cork oak (*Quercus suber* L.) leaves under Mediterranean climate, J. Exp. Bot. 48 (1997) 1667–1674.

[19] Grammatikopoulos G., Kyparissis A., Manetas Y., Seasonal and diurnal gas exchange characteristics and water relations of the drought semi-deciduous shrub *Phlomis fruticosa* L. under Mediterranean field conditions, Flora 190 (1995) 71–78.

[20] Groom Q.J., Baker N.R., Long S.P., Photoinhibition of holly (*Ilex aquifolium*) in the field during the winter, Physiol. Plant. 83 (1991) 585–590.

[21] Harley P.C., Tenhunen J.D., Beyschlag W., Lange O.L., Seasonal changes in net photosynthesis rates and photosynthetic capacity in leaves of *Cistus salvifolius*, a European Mediterranean semi-deciduous shrub, Oecologia 74 (1987) 380–388.

[22] Institut Cartogràfic de Catalunya, Atles climàtic de Catalunya. Termopluviometria, Institut Cartogràfic de Catalunya, Departament de Medi Ambient, Barcelona, 1996.

[23] Karabourniotis G., Kotsabassidis D., Manetas Y., Trichome

density and its protective potential against ultraviolet-B radiation damage during leaf development, Can. J. Bot. 73 (1995) 376–383.

[24] Karavatas S., Manetas Y., Seasonal patterns of photosystem 2 photochemical efficiency in evergreen sclerophylls and drought semi-deciduous shrubs under Mediterranean field conditions, Photosynthetica 3 (1999) 41–49.

[25] King D.A., The functional significance of leaf angle in *Eucalyptus*, Aust. J. Bot. 45 (1997) 619–639.

[26] Kyparissis Y., Petropoulou Y., Manetas Y., Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents, J. Exp. Bot. 46 (1995) 1825–1831.

[27] Lansac A.R., Zaballos J.P., Martin A., Seasonal water potential changes and proline accumulation in Mediterranean shrubland species, Vegetatio 113 (1994) 141–154.

[28] Llusià J., Peñuelas J., Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions, Am. J. Bot. 87 (2000) 133–140.

[29] Miller P.C., Environmental constraints to vegetation form in Mediterranean type ecosystems, Ecol. Mediterr. 8 (1982) 411–416.

[30] Mitrakos K., Plant life under Mediterranean climatic conditions, Port. Acta Biol. Ser. A 16 (1980) 33–44.

[31] Mooney H.A., Parsons D.J., Kummerow J., Plant development in Mediterranean climates, in: Lieth H.(Ed.), Phenology and Seasonality Modeling, Ecological Studies 8, Chapman and Hall, London, 1974, pp. 255–268.

[32] Moran R., Poreth D., Chlorophyll determination in intact tissues using N,N-dimethyl-formamide, Plant Physiol. 65 (1982) 478–479.

[33] Núñez-Olivera E., Martínez-Abaigar J., Escudero J.C., Chlorophyll content of a Mediterranean shrub (*Cistus ladanifer* L.) over a latitude and altitude gradient in the Iberian Peninsula, Photosynthetica 30 (1994) 133–142.

[34] Ögren E., Sjöström M., Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy, Planta 181 (1990) 560–567.

[35] Oliveira G., Correia O., Martins-Loução M.A., Catarino F.M., Phenological and growth patterns of the Mediterranean oak *Quercus suber* L., Trees 9 (1994) 41–46.

[36] Öquist G., Anderson J.M., McCaffery S., Chow W.S., Mechanistic differences in photoinhibition of sun and shade plants, Planta 188 (1992) 422–431.

[37] Osmond C.B., What is photoinhibition? Some insights from comparisons of shade and sun plants, in: Baker N.R., Bower J.R. (Eds.), Photoinhibition of Photosynthesis: from Molecular Mechanisms to the Field, Bios Scientific Publishers, Oxford, 1994, pp. 1–24.

[38] Ottander C., Campbell D., Öquist G., Seasonal changes in photosystem II organization and pigment composition in *Pinus sylvestris*, Planta 197 (1995) 176–183.

[39] Peñuelas J., Filella I., Llusià J., Siscart D., Piñol J., Comparative field study of spring and summer leaf gas exchange and photobiology of the Mediterranean trees *Quercus ilex* and *Phillyrea latifolia*, J. Exp. Bot. 49 (1998) 229–238.

[40] Pereira J.S., Beyschlag G., Lange O.L., Beyschlag W., Tenhunen J.D., Comparative phenology of four Mediterranean shrub species growing in Portugal, in: Tenhunen J.D., Catarino F.M., Lange O.L., Oechel W.C.(Eds.), Plant Response to Stress. Functional Analysis in Mediterranean Ecosystems, NATO ASI Series, vol. G15, Springer, Berlin, 1987, pp. 503–513.

[41] Rhizopoulou S., Meletiou-Christou M.S., Diamantoglou S., Water relations for sun and shade leaves of four Mediterranean evergreen sclerophylls, J. Exp. Bot. 42 (1991) 627–635.

[42] Scarascia-Mugnozza G., de Angelis P., Matteucci G., Valentini R., Long-term exposure to elevated [CO_2] in a natural *Quercus ilex* L. community: net photosynthesis and photochemical efficiency of PSII at different levels of water stress, Plant Cell Environ. 19 (1996) 643–654.

[43] Skaltsa H., Verkokidou E., Harvala C., Karabourniotis G., Manetas Y., UV-B protective potential and flavonoid content of leaf hairs of *Quercus ilex*, Phytochemistry 37 (1994) 987–990.

[44] Tenhunen J.D., Lange O.L., Harley P.C., Beyschlag W., Meyer A., Limitations due to water stress on leaf net photosynthesis of *Quercus coccifera* in the Portuguese evergreen scrub, Oecologia 67 (1985) 23–30.

[45] Tretiach M., Photosynthesis and transpiration of evergreen Mediterranean and deciduous trees in an ecotone during a growing season, Acta Oecol. 14 (1993) 341–360.

[46] Tretiach M., Bolognini G., Rondi A., Photosynthetic activity of *Quercus ilex* at the extremes of a transect between Mediterranean and submediterranean vegetation (Trieste-NE Italy), Flora 192 (1997) 369–378.

[47] Valladares F., Pearcy R.W., Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*, Plant Cell Environ. 20 (1997) 25–36.

[48] Wellburn A.R., The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution, Plant Physiol. 144 (1994) 307–313.

[49] Werner C., Correia O., Beyschlag W., Two different strategies of Mediterranean macchia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought, Acta Oecol. 20 (1999) 15–23.