Original article

Self-shading in cork oak seedlings: Functional implications in heterogeneous light environments

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ABSTRACT

The high self-shading found in *Quercus suber* seedlings has been interpreted as a feature common for plants growing in high light environments. But many studies reveal that *Q. suber* has high survival rates under low-light conditions, so a high degree of self-shading could be the consequence of a foliage composed of many small leaves, with no drawbacks for coping with low light. A characterization of the light environment in a *Q. suber* stand together with a study of photosynthetic parameters of full sunlight-exposed (FSLE) and self-shaded (SS) leaves were carried out to tackle this apparent contradiction. Although the number of sunflecks longer than 120 min during the 3 months of measurements was low, the occurrence of at least one sunfleck longer than 120 min per day in the understory of the forest studied was very common. Sunflecks shorter than 30 min promoted an increase in net photosynthesis (A) in FSLE leaves, but not in SS leaves. However, sunflecks longer than 60 min led to a very strong decrease in A and in actual photosystem II efficiency (Φ_{PSII}) in FSLE leaves, when compared to sunflecks shorter than 30 min. In SS leaves, changes were, again, negligible. The multi-layered foliage of *Q. suber* seedlings allowed i) FSLE leaves to obtain the maximum photosynthetic yield for short sunflecks, and ii) SS leaves to increase their contribution to the photosynthesis of the whole plant for long sunflecks, thus, optimizing the use of light by FSLE and SS leaves during short and long sunflecks respectively. Therefore, shoot architecture of *Q. suber* seedlings involving high levels of self-shading allows to adequately cope with the low but highly heterogeneous light conditions of the understory, particularly when sunflecks of contrasting durations take place as it is frequently the case for evergreen Mediterranean forests.

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1. Introduction

Plant architectural traits are influenced by biotic and abiotic conditions, and thus to be strongly adapted to their habitat (Valladares and Pearcy, 1998). Specifically, leaves, as the primary photosynthetic organs, are found to be optimally adapted to the particular environmental conditions of each habitat regarding both their morphology (Traiser et al., 2004) and their arrangement within the shoot (Esteso-Martínez et al., 2006a). Several authors have explored the relationships between environment, leaf morphology and shoot architecture (Kikuzawa et al., 1996; Sisó et al., 2001; Valladares and Pearcy, 1998). However, the functional implications and the trade-offs involved in the different shoot architectures remain to be fully understood (Falster and Westoby, 2003).

Mutual shading among foliage elements is unavoidable, but plants differ in their levels of self-shading. Self-shading has been found to be a frequent feature of plants growing under dry and high irradiance conditions (Valladares and Pugnaire, 1999), such as those occurring over the Mediterranean summer (Corcuera et al., 2005). The structural avoidance of light capture (Morales et al., 2002; Valladares and Pearcy, 1998) is considered to be potentially as advantageous as the physiological mechanisms involved in energy dissipation (García-Plazaola et al., 2004; Morales et al., 2002). But self-shading can be detrimental for carbon gain under low-light conditions since it reduces light harvesting per unit of leaf surface area (Kikuzawa, 1995; Valladares and Brites, 2004; Yamada et al., 2000).

In a comparison of the architecture and leaf capture efficiency of eight *Quercus* species at the seedling stage, Esteso-Martínez et al. (2006a) found that species from temperate areas showed crown architectures different from those of genuinely Mediterranean oaks. Mesic species had a lower number of rather large leaves, which were

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arranged in a nearly mono-layered pattern. By contrast, the ever-green Mediterranean oaks had a higher number of small leaves, which were arranged all along the shoot in a multi-layered pattern that involved high levels of self-shading. Due to this architecture, light-harvesting efficiency in mesic species was significantly higher than in Mediterranean species. Mesic species, by displaying their foliage simultaneously as pseudo-whorls located at the end of long internodes, minimized self-shading, which in turn allowed for a near-optimal photosynthetic performance of most of the leaves of the crown under low-irradiance conditions as shown and discussed in plants from different environments (e.g. Yamada et al., 2000). By contrast, high self-shading has been considered as a typical feature of early successional and shade intolerant tree species (Kikuzawa et al., 1996).

Could the high level of self-shading in Mediterranean evergreen oaks be interpreted as a sign of dependence on regeneration in open habitats? It should be noted that Mediterranean oaks seedlings showed high survival under the canopy of mature forest trees and shrubs (Esteso-Martínez et al., 2006b). In such a situation, Gómez-Aparicio et al. (2008) showed that the protection from abiotic stress was even more critical than protection from herbivores.

Thus, an apparent contradiction emerges because Mediterranean oak seedlings have an architecture that is appropriate for high light conditions but they are reported to survive and perform relatively well under the low-light environment caused by nurse plants. This can be explained in two ways. First, by the occurrence of full sunlight events in the understory (i.e., sunflecks), which generates a hetero-geneous light environment (Valladares and Guzmán, 2006). There-fore, seedlings would experience dynamic and highly unpredictable light conditions throughout the seasons, which involve a number of different response mechanisms (Bazzaz and Wayne, 1994). Second, by considering aspects not directly related to the light environment but to other co-occurring environmental factors. Interestingly, most of the studies supporting a correspondence between these two main growth models (mono- and multi-layer) and the light environment have been performed in habitats where water deficit was not limiting for plant growth and survival (e.g. Miyazawa and Kikuzawa, 2005; Seiwa et al., 2006). However, Mediterranean climates impose the additional stress of water deficit to understory plants, which acting in combination with shade can render complex and counter intuitive situations in Mediterranean understories (Valladares and Pearcy, 2002; Valladares et al., 2008). Water saving, as a way of avoiding the risk of an excessive water deficit in plant tissues, has been related to the production of small leaves (Traiser et al., 2004) leading to a trade-off between water economy through leaf area reduction and light-harvesting efficiency through the increase of leaf number (Esteso-Martínez et al., 2006a). The arrangement of many small leaves with a very short petiole renders the most effective solution in terms of light capture, in a multi-layered foliage, with leaves homo-geneously distributed along the shoot (Horn, 1971). Thus, influence of this crown architecture on light harvesting can be interpreted as an epi-phenomenom induced by drought more than as an adaptation to the excess of light in open Mediterranean habitats.

In order to assess this apparent contradiction, two different studies were made: i) the characterization of the light environment in a *Quercus suber* stand and ii) a complete physiological study of photosyn-thetic-related parameters of the leaves within the crown and its relation to the physiological performance of the seedling were made.

2. Materials and methods

2.1. Canopy analysis

An analysis of the light environment in the understory of a natural forest of *Q. suber* was made prior to the design of the physiological experiments. The forest chosen occupies an area of 320 ha and is constituted by a mixed stand of *Q. suber* and *Quercus ilex* subsp. *ballota* in the Sistema Ibérico range (41° 35′ N, 1° 40′ W, 800–1100 m a.s.l.). This forest has not been affected by human activities (e.g. coppicing) at least for the last decades (Martínez-Cabeza, 1993).

The study of the structure of the canopy and light transmitted through canopy gaps was performed using hemispherical photo-graphs (Valladares and Guzmán, 2006), obtained with a digital camera (Nikon Coolpix 950) with a lens adapter providing a focal length equivalent to 8 mm in a 35 mm format. The camera was attached to a self-levelling device, which ensured a perfect hori-zontality of the optical plane. This device included a visual refer-ence, consisting in a red tag, to indicate the geographical north. The self-levelling device had three legs, 15 cm in length, which defined the height above ground. This height was approximately the size of a young seedling of *Q. suber*. A total amount of 23 photographs were obtained, being the location of the points decided by the generation of random pairs of azimuth and distance values, according to the method proposed by Skalski (1987).

The software Gap Light Analyzer (GLA V2.0, Frazer et al., 1999) was used in the interpretation of images. Site configuration setting was 41° 48′ N, 1° 59′ W, 572 m a.s.l., with 0 slope. This software allows obtaining two parameters related to the canopy structure and dosage of incoming light at the point where the photograph was taken, Sunfleck Distribution (SD) and Canopy Openness (CO).

SD was calculated for summer. This parameter was used to calculate the frequency of sunflecks (Frazer et al., 1999) that could influence seedling physiology during this period. Moreover, the number of sunflecks per day, the accumulated duration, and the relative contribution to summer direct light income for each sunfleck duration during summer (90 days) were calculated. Finally, CO was calculated in order to compare this canopy with other oak forests reported in the literature.

2.2. Plant material and experimental conditions

Seedlings were obtained from certified acorns of *Q. suber* from Spanish provenances. Pre-treatment of acorns before sowing was as described in Esteso-Martínez et al. (2006a). Sowing was performed during the early spring in 1.2 L containers, filled with a mixture of Humin substrat N3 (Neuhaus, Germany) and quartz sand. Nutrients were supplied by using a slow-liberation fertilizer (Osmocote Plus, Sierra Chemical, Milpitas, USA). Plants were kept in a shade tunnel (ca. 10% of full sunlight, mainly diffuse light) in order to adjust the light conditions to those experienced by the seedlings in the field during the growing period. Seedlings were well watered throughout the experiment. More than ninety individuals were randomly selected and tagged fixing the geographical orientation (N–S) of each plant with colour marks on the container.

2.3. Leaf physiological measurements

The physiological measurements were carried out around midday (12 h, solar time) during the late summer, along periods with a high meteorological constancy in terms of radiation and temperature. The situation emulated by the different experiments resembled natural light conditions and light changes affecting seedling physi-ology under field conditions. Specifically, photosynthetic photon flux density (PPFD) during the physiological measurements was 1870 ± 90 $\mu mol\ m^{-2}\ s^{-1}$ and 150 ± 2 $\mu mol\ m^{-2}\ s^{-1}$ (ELE International, Bedfordshire, UK) outside and inside the shade tunnel respectively. The PPFD value in the shade tunnel was similar to the average value measured for this parameter at midday in the understory of the studied forest (150 ± 12 $\mu mol\ m^{-2}\ s^{-1}$, $n = 23$). Prior to the measurements, a full sunlight-exposed (FSLE) and a self-shaded (SS)

leaf per seedling were chosen by taking a picture of the plant from an angle and azimuth equivalent to the sun ones (in the date and hour of the experiment). Fig. 1 shows a picture of one of the *Q. suber* seedlings used in this study. Both kinds of leaves received the same PPFD in the shade tunnel. In that way, it could be estimated and detected the most exposed and the most shaded leaves in a larger plant number than using Yplant calculations (Pearcy and Yang, 1996). The accuracy of this method was checked against the results given by Yplant in a set of 5 seedlings, following the methodology described in Esteso-Martínez et al. (2006a). Yplant yields an estimation of the light-harvesting efficiency for a three-dimensional model of plants by the simulation of the sun path during a given day. At each node within the crown, the internode and petiole angles and azimuths, the angle and azimuth of the leaf surface normal, and the azimuth of the midrib were recorded with a compass protractor. Leaf, petiole, and internode lengths were measured with a ruler and petiole and internode diameters were measured with digital callipers. A node or internode in Yplant is not identical to an actual node or internode since true nodes were skipped if the leaves had been shed and if no branching occurred at them. An internode may therefore contain one or more actual true nodes. The nodes were numbered proceeding from the base to the top of the plant and along each branch. By recording the mother node (the node from which a subsequent node arises) for each node, the proper topology of the shoot could be reconstructed by Yplant using the geometrical information. Leaf shape was established from x–y coordinates of the leaf margins. Leaf size was then scaled in the crown reconstruction from the measured leaf length. Total leaf area and total displayed area were also calculated with Yplant.

With the aim of emulating the effect of sunfleck duration on leaf seedling physiology, plants were placed outside the shade tunnel (sunfleck start) during eight exposition times, namely 0 (within the

Fig. 1. *Quercus suber* seedling showing a self-shading pattern, which generates FSLE and SS leaves.

shade tunnel), 5, 10, 15, 30, 45, 60 and 120 min, and then placed again in the shade tunnel (sunfleck end). Once outside the shade tunnel, PPFD received by FSLE leaves averaged 1871 ± 90 µmol m^{-2} s^{-1} while SS leaves received an average of 260 ± 80 µmol m^{-2} s^{-1}. Before the sunfleck start, net CO_2 assimilation (A), stomatal conductance (g_S), the dark-adapted maximum potential PSII efficiency (F_V/F_M) and the actual PSII efficiency (Φ_{PSII}) were measured in five FSLE and SS leaves respectively. These values were considered as a reference for each leaf illumination condition. Once simulated sunfleck periods reached their maximum duration, measurements were repeated. These values were considered as representative of the maximum effect of the sunfleck on leaf photosynthetic performance. Finally, once the sunfleck ended, measurements were done again after 1 h under shade conditions, as a way of studying the extent of recovery from sunfleck effects.

Gas exchange measurements were performed with a portable gas exchange system (CIRAS-1, PP Systems, UK) on attached leaves. Measurements were performed at controlled CO_2 external concentration ($C_a = 350$ ppm) and ambient relative humidity. Net CO_2 uptake (A) and stomatal conductance (g_S) were registered, and intrinsic water-use efficiency (WUE $= A/g_S$) was calculated. Whole plant net photosynthesis (A_{tot}) was estimated using gas exchange data and the total leaf area for FSLE and SS leaves obtained from the output of the Yplant model for 5 seedlings (see above). A theoretical situation, where all the leaf area would be as exposed as FSLE leaves, was also considered. Chl fluorescence parameters were measured on attached leaves with a PAM 2000 portable pulse amplitude modulation fluorometer (Heinz Walz, Effeltrich, Germany). Plants were covered with a black bag and kept in darkness for 30 min to estimate the minimum (F_0) and maximum (F_M) Chl fluorescence. F_0 was measured by switching on the modulated light at 0.6 kHz; PPFD was below 0.1 µmol m^{-2} s^{-1} at the leaf surface. F_M was measured at 20 kHz with a 1 s pulse of 6000 µmol m^{-2} s^{-1} of white light. The variable fluorescence in the dark (F_V) is defined as $F_M - F_0$. The Chl fluorescence at steady-state photosynthesis (F_S) was measured with the leaves in their original orientation and lighting, and a second pulse of high-intensity white light was used to determine the maximum Chl fluorescence in the light-adapted state (F_M). The experimental protocol for the analysis of the Chl fluorescence quenching was essentially as described by Genty et al. (1989) with some modifications (Morales et al., 2000). These involved the measurements of F_0, which were measured in presence of far-red light (7 µmol m^{-2} s^{-1}) in order to fully oxidise the PSII acceptor side (Belkhodja et al., 1998; Morales et al., 1998). The dark-adapted, maximum potential PSII efficiency was calculated as F_V/F_M (Abadía et al., 1999; Morales et al., 1991). The actual PSII efficiency (Φ_{PSII}) was calculated as $(F_M - F_S)/F_M$ (Genty et al., 1989).

The study of light-mediated changes of photosynthetic pigments was made in leaf disks, which were cut with a calibrated cork borer from the same leaves in which Chl fluorescence had been measured. The disks were wrapped in aluminium foil, frozen in liquid-N_2, and stored (still wrapped in foil) at -20 °C. Leaf pigments were later extracted with acetone in the presence of Na–ascorbate and stored as described previously (Abadía and Abadía, 1993). Pigments extracts were thawed on ice, filtered through a 0.45 µm filter and analyzed by an isocratic HPLC method (Larbi et al., 2004). All chemicals used were HPLC quality. This method is able to quantify a specific group of xanthophylls (oxygen-containing carotenoids), involved in the so-called xanthophylls cycle. Zeaxanthin (Z, no epoxides) is formed by de-epoxidation of violaxanthin (V, two epoxides) via antheraxanthin (A, one epoxide). The de-epoxidation state was defined as $(A + Z)/(V + A + Z)$. This index is an estimation of the actual number of molecules within the xanthophylls cycle partially or totally de-epoxidized over the maximum possible. A low $(A + Z)/(V + A + Z)$ ratio would indicate a displacement toward V, whereas a large one would indicate that Z and/or A account for
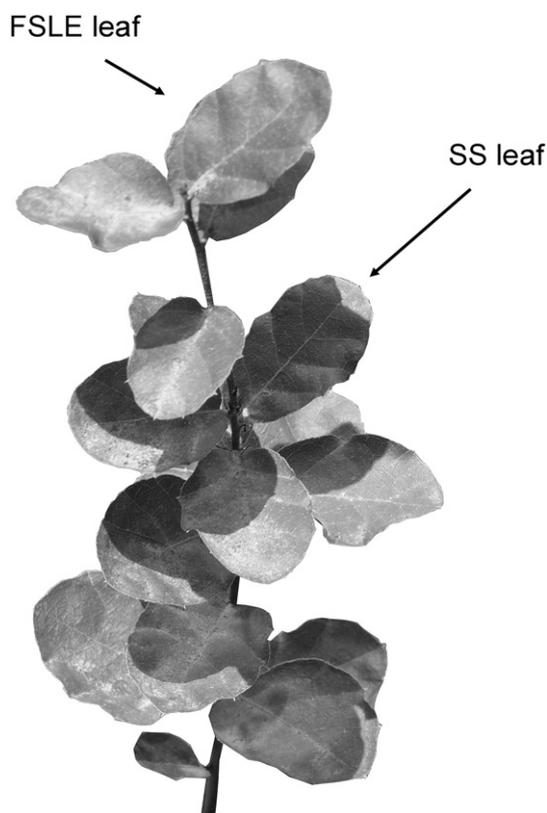
a large part of the pool of the xanthophylls cycle pigments. An important mechanism to avoid the deleterious effects of light excess is thermal dissipation within the PSII antenna (Abadía et al., 1999). This dissipation process involves the de-epoxidized xanthophylls Z and A (Gilmore and Yamamoto, 1993).

2.4. Statistical analysis

Data are expressed as mean values of five measurements ± standard error. Student's *t*-tests were used to compare the values obtained from physiological measurements for FSLE and SS leaves for each sunfleck duration. On the other hand, one-way ANOVAs were performed to compare leaf physiological parameters among exposition times for each leaf type. Multiple comparisons were carried out among exposition times for the physiological variables using the post hoc Tukey's Honestly Significant Difference test. All statistical analyses were carried out using SAS version 8.0 (SAS, Cary, NC, USA).

3. Results

3.1. Canopy analysis

Canopy openness (CO) in the studied forest was 22.4 ± 1.6%, with a maximum and minimum, respectively, of 38.0% and 11.3% for the 23 points under study. The calculated number of sunflecks, as well as their duration and contribution to direct incoming sunlight in the understory, are shown in Table 1. It is observed that the most frequent light comes as short duration sunflecks. However, and in spite of their low frequency (on average one per day), the main source of direct incoming sunlight during summer is attributable to sunflecks longer than 120 min.

3.2. Leaf physiological measurements

A, g_S and WUE for FSLE and SS leaves of seedlings of *Q. suber* before the exposition to full sunlight and after the exposition to high PPFD conditions of different duration are shown in Fig. 2. No significant differences ($P \leq 0.05$) were observed between FSLE and SS leaves before the sunfleck started. At the end of exposure to high light, A values were always higher in FSLE leaves, the differences being the higher the shorter the sunfleck duration. Stomatal conductance (g_S) was similar for FSLE and SS leaves at any given sunfleck duration, although some slight but significant differences ($P \leq 0.05$) were found at 15 and 30 min. Therefore, WUE was always higher ($P \leq 0.05$) in FSLE leaves for any sunfleck duration. The recovery after 60 min in the shade tunnel of A, g_S and WUE for FSLE and SS leaves subjected to sunflecks of 120 min is shown in Table 2. Data indicate that the recovery was not complete, in relation to the reference values. It should be noted that, with regard to A values, SS leaves only showed a 25% decrease relative to their reference values, whereas FSLE leaves showed a 79% decrease relative to their reference values after 120 min of sunfleck and 60 min in the shade tunnel (Table 2, $P \leq 0.05$).
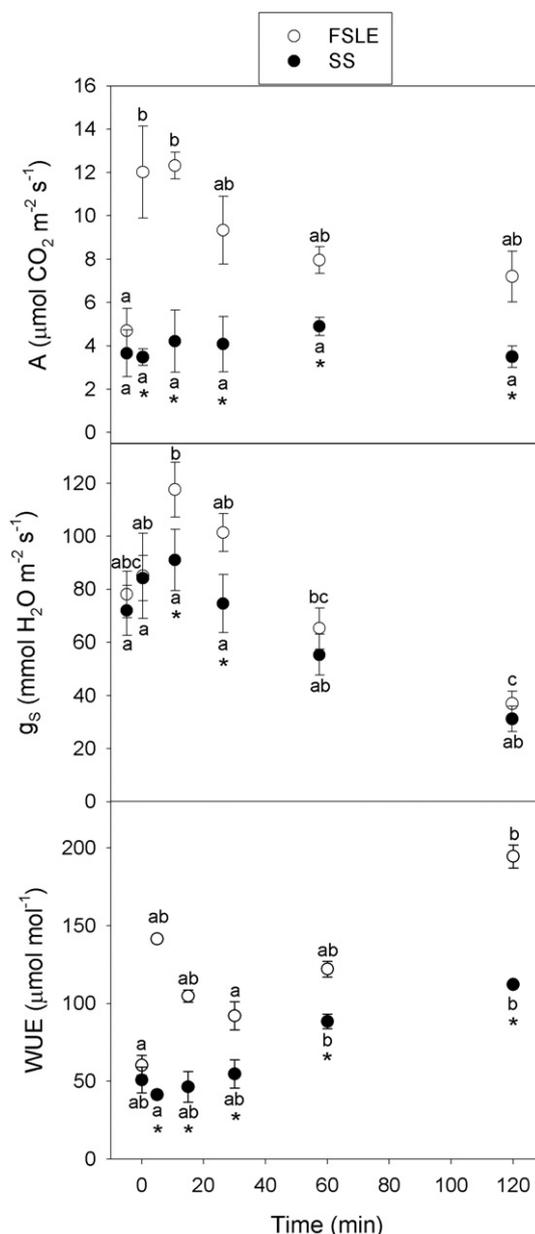

Fig. 2. Relationship between the time of exposure to full sunlight and net photosynthesis (A), stomatal conductance (g_S) and intrinsic water-use efficiency (WUE), in full sunlight-exposed (FSLE) and self-shaded (SS) leaves of seedlings of *Q. suber* exposed to increasing periods of full sunlight. Data are mean ± s.e. Asterisks indicate statistically significant differences between FSLE and SS leaves (Student's *t*-test, $P \leq 0.05$). Different letters indicate significant differences among exposition times for each leaf type (Tukey test, $P \leq 0.05$).

Table 1
Absolute number of calculated sunflecks during the 3 months of measurements, number of calculated sunflecks per day, accumulated duration and relative contribution to summer direct light income (%) for each duration of sunfleck studied during summer. Number of calculated sunflecks is mean ± s.e.

Duration of sunfleck	<5 min	5 min	10 min	15 min	30 min	45 min	60 min	>120 min
Number of sunflecks	3116 ± 223	138 ± 9	31 ± 3	10 ± 0.2	4 ± 0.1	1 ± 0.0	3 ± 0.1	95 ± 18
Number of sunflecks day^{-1}	33.87	1.50	0.34	0.11	0.04	0.01	0.03	1.03
Accumulative duration of sunflecks (min)	5393	690	310	150	120	45	180	22 709
Relative contribution to summer direct light income (%)	6.87	0.88	0.40	0.19	0.15	0.06	0.23	28.95

Table 2

Net photosynthesis (A), the percentage of decrease in net photosynthesis in relation to the values before the exposition to high light conditions (*Decrease in A*), the total contribution to net photosynthesis of the whole plant (A_{tot}), stomatal conductance (g_S), intrinsic water-use efficiency (WUE), the maximum potential PSII efficiency (F_V/F_M) and the actual PSII efficiency (Φ_{PSII}) for full sunlight-exposed (FSLE) and self-shaded (SS) leaves of *Q. suber* exposed to 120 min of full sunlight and 60 min of recovery in the shade tunnel under low-light conditions. Asterisks indicate statistically significant differences between FSLE and SS leaves (Student's *t*-test, $P \leq 0.05$).

	FSLE	SS
A (μmol CO_2 m^{-2} s^{-1})	0.98 ± 0.09	2.74 ± 0.70*
Decrease in A (%)	79 ± 8	25 ± 6*
A_{tot} (μmol CO_2 s^{-1})	0.0054 ± 0.0004	0.0227 ± 0.0058*
g_S (mmol H_2O m^{-2} s^{-1})	46.6 ± 3.0	58.4 ± 8.0*
WUE (μmol CO_2 mol^{-1} H_2O)	21.0 ± 0.5	46.9 ± 5.6*
F_V/F_M	0.58 ± 0.01	0.70 ± 0.01*
Φ_{PSII}	0.50 ± 0.01	0.61 ± 0.01*

The total contribution to net photosynthesis of the whole plant (A_{tot}) in FSLE and SS leaves, according to the total leaf area for FSLE and SS leaves obtained from the output of the Yplant model for 5 seedlings, is shown in Fig. 3 (uppermost panel). The contribution of FSLE leaves to whole plant net photosynthesis was higher than that of SS leaves when sunfleck duration was below 30 min. The differences were not statistically significant beyond 30 min of sunfleck
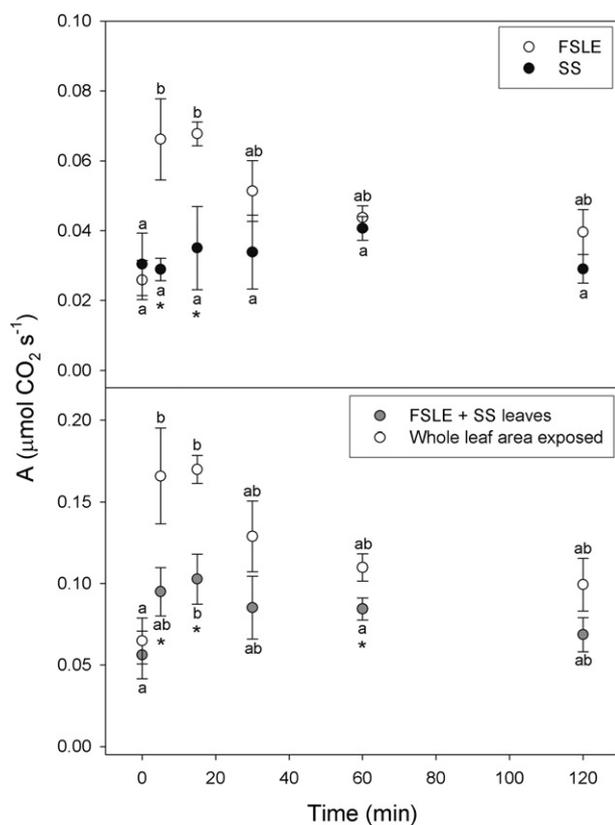

Fig. 3. Relationship between the time of exposure to full sunlight and the total contribution to net photosynthesis of the whole plant (A_{tot}) in full sunlight-exposed (FSLE) and self-shaded (SS) leaves of seedlings of *Q. suber* exposed to increasing periods of full sunlight (uppermost panel). The lower panel shows the sum of FSLE and SS leaves contributions (white symbols) compared with a theoretical situation where all the leaf area were as exposed as FSLE leaves (grey symbols). Data are mean ± s.e. Asterisks indicate statistically significant differences between FSLE and SS leaves (Student's *t*-test, $P \leq 0.05$). Different letters indicate significant differences among exposition times for each leaf type in the uppermost panel and for each one of the both situations in the lower panel (Tukey test, $P \leq 0.05$).

duration. The sum of both kinds of leaves contributions compared with a theoretical situation where all leaves were as exposed as FSLE leaves is shown in Fig. 3 (lower panel). The exposition of all leaves to full sunlight would yield much higher rates of net assimilation of CO_2 than the actual situation for sunflecks of short duration. In fact, the increase in net assimilation of CO_2 at the whole plant level, in relation to the values measured just before the exposition to high light conditions, was ca. 61% for the theoretical situation where all leaves were exposed as FSLE leaves, whereas it was only ca. 40% for the actual situation (Fig. 3, lower panel). However, for sunflecks above 60 min, the differences between the net CO_2 assimilation of a theoretical situation where all leaves were as exposed as FSLE leaves and the actual situation were lower than for sunflecks of short duration (ca. 40% and 33%, respectively, in relation to the values before the sunfleck). The recovery after 60 min in the shade tunnel of the sum of the contributions to photosynthesis (A_{tot}) of both kinds of leaves subjected to sunflecks of 120 min is shown in Table 2. Data indicate that the recovery of A_{tot} was ca. 75% for SS leaves, but only ca. 21% for FSLE leaves, in relation to the reference values measured before the sunfleck started (0,0258 ± 0,0056 and 0,0303 ± 0,0089 μmol CO_2 s^{-1} for FSLE and SS leaves, respectively). As a consequence, the net CO_2 assimilation calculated for the whole plant was reduced ca. 79% in the theoretical situation and ca. 50% in the actual situation.

The changes of Φ_{PSII} associated with increases in the duration of the sunfleck in SS and FSLE leaves of *Q. suber* are shown in Fig. 4. In SS leaves, it can be observed a slight although statistically significant decrease in Φ_{PSII} from 0 to 5 min, remaining fairly constant beyond 5 min. On the other hand, there was an important and sustained decrease in Φ_{PSII} in FSLE leaves. The F_V/F_M values for both kinds of leaves measured half an hour after the end of high light exposure are shown in Fig. 5. FSLE and SS leaves registered values not statistically different ($P \leq 0.05$) for sunfleck durations shorter than 60 min. However, a significant difference ($P \leq 0.05$) was found in F_V/F_M values for 60 min and 120 min sunflecks between both kinds of leaves. The decrease in F_V/F_M values showed by FSLE leaves after a 120 min sunfleck was ca. 24% when compared to the reference value, whereas it was ca. 11% in the SS leaves. The recovery of F_V/F_M and Φ_{PSII} values after 60 min in the shade tunnel for FSLE and SS leaves subjected to sunflecks of 120 min is shown in Table 2. Data indicate that the
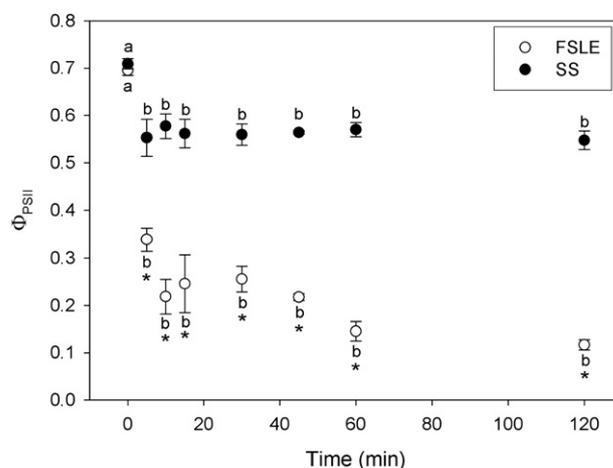

Fig. 4. Relationship between the time of exposure to full sunlight and the actual PSII efficiency (Φ_{PSII}) in full sunlight-exposed (FSLE) and self-shaded (SS) leaves of seedlings of *Q. suber* exposed to increasing periods of full sunlight. Data are mean ± s.e. Asterisks indicate statistically significant differences between FSLE and SS leaves (Student's *t*-test, $P \leq 0.05$). Different letters indicate significant differences among exposition times for each leaf type (Tukey test, $P \leq 0.05$).

Fig. 5. Relationship between the time of exposure to full sunlight and the maximum potential PSII efficiency (F_V/F_M) in full sunlight-exposed (FSLE) and self-shaded (SS) leaves of seedlings of *Q. suber* after exposure to increasing periods of full sunlight. Data are mean ± s.e. Asterisks indicate statistically significant differences between FSLE and SS leaves (Student's *t*-test, $P \leq 0.05$). Different letters indicate significant differences among exposition times for each leaf type (Tukey test, $P \leq 0.05$).
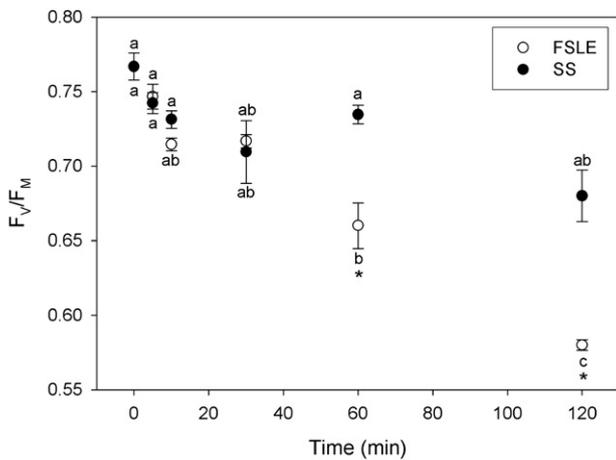
recovery for F_V/F_M values was lower (ca. 0.5% and 24% for FSLE and SS leaves, respectively) than those found for Φ_{PSII} values (ca. 66% and 38% for FSLE and SS leaves, respectively), in relation to the reference values measured before the sunfleck started.

The increases in the de-epoxidation state of xanthophyll cycle pigments, $(A + Z)/(V + A + Z)$, associated with increases in sunfleck duration in FSLE and SS leaves of *Q. suber*, are shown in Fig. 6. Although both FSLE and SS leaves showed an increase in $(A + Z)/(V + A + Z)$, the changes found in FSLE leaves were more pronounced than those found in SS leaves. The rest of photosynthetic pigments (chlorophylls and carotenoids other than $V + A + Z$) did not show significant differences in their composition between FSLE and SS leaves (Table 3). The linear correlation ($R^2 = 0.74$, correlation coefficient $= -0.856$, $P \leq 0.05$) between Φ_{PSII} and $(A + Z)/(V + A + Z)$, associated with increases in sunfleck duration in FSLE and SS leaves of *Q. suber*, is shown in Fig. 7. There was an inverse relationship
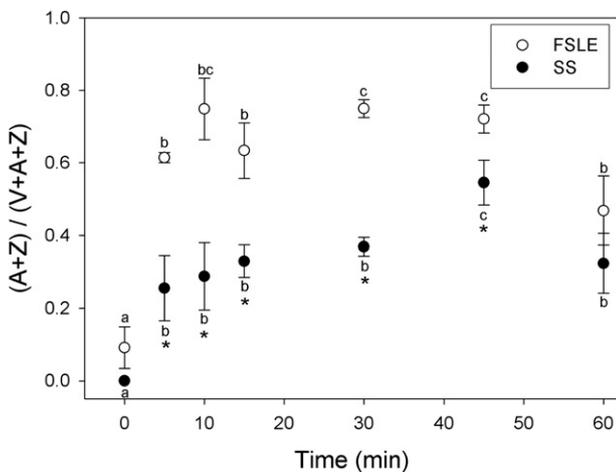

Fig. 6. Relationship between time of exposure to full sunlight and the de-epoxidation state of the xanthophyll cycle pigments, $(A + Z)/(V + A + Z)$, in full sunlight-exposed (FSLE) and self-shaded (SS) leaves of seedlings of *Q. suber* exposed to increasing periods of full sunlight. Data are mean ± s.e. Asterisks indicate statistically significant differences between FSLE and SS leaves (Student's *t*-test, $P \leq 0.05$). Different letters indicate significant differences among exposition times for each leaf type (Tukey test, $P \leq 0.05$).

between the thermal dissipation of the excess of energy (related to $(A + Z)/(V + A + Z)$ increases) and the efficiency with which electrons are being used in photochemistry (Φ_{PSII}), showing a direct competition between these processes.

4. Discussion

The studied forest structure allowed for high levels of PPFD in the understory during summer. Canopy openness values in this forest were within the range found for other oak forests, including subtropical mountain (Olvera-Vargas and Figueroa-Rangel, 1998) and humid temperate forests (Collins and Battaglia, 2002). Understory light features of the study forest regarding the number and duration of sunflecks were very heterogeneous (Table 1). The inverse relationship between the duration and the number of sunflecks involved, as a consequence, the occurrence of a very high number of very short sunflecks (≤ 5 min) and a low number of sunflecks of very long duration (≥ 120 min). However, although the number of very long sunflecks was low, at least one long duration sunfleck per day was recorded on average in the understory of the studied forest of *Q. suber*. Thus, most *Q. suber* seedlings can be expected to experience not only low light but also a few but intense and long sunflecks everyday in their natural environments.

The central question of this study was whether the high self-shading among neighbour leaves, well documented for seedlings of *Q. suber* (Esteso-Martínez et al., 2006a), involves a poor performance in low light, as it has been suggested for other species (Kikuzawa et al., 1996). In this sense, the arrangement of the leaves in a single layer, minimizing self-shading, could imply a better net CO_2 assimilation under low-light conditions (Yamada et al., 2000) or under a light environment with many short sunflecks. Our results do not confirm this argument. Actual and theoretical situation at which the whole leaf area was full exposed to the diffuse understory light (points at time 0 in Fig. 3) rendered no significant differences. It has been repeatedly shown that acclimation to the different light environments within a canopy induces strong functional differentiation among the leaves of a given plant (Abadía et al., 1996; García-Plazaola et al., 2004). However, the leaves of *Q. suber* exposed to high light conditions and those that remained shaded under a sunfleck did not show differences neither in terms of photosynthetic pigment composition (Table 3) nor in their photosynthetic response to light (data not shown). But this view is challenged when the heterogeneity in light environment is considered.

The sunflecks of short duration (≤ 30 min) led to an increase in net photosynthesis in FSLE leaves (Fig. 2), due to the very high increase in PPFD and the subsequent relatively low decrease in the electron transport rate in relation to the reference values (data not shown). This fact had, as a consequence, a beneficial effect for the contribution of FSLE leaves to whole plant CO_2 net assimilation (Fig. 3). On the contrary, SS leaves showed no significant changes in net photosynthesis during these short sunflecks (Fig. 2), having a negligible effect on the photosynthesis of the whole plant (Fig. 3). Moreover, FSLE leaves showed a strong increase in $(A + Z)/(V + A + Z)$ (up to ca. 0.7), whereas this increase was lower for SS (up to ca. 0.35), which suggests a high level of thermal dissipation of the excess of energy in FSLE leaves that can be related to the high increase in PPFD reaching these leaves during sunflecks. Therefore, the exposition of seedlings of *Q. suber* to sunflecks shorter than 30 min promoted a differential response between FSLE and SS leaves.

Sunflecks longer than 60 min led to a very strong decrease in Φ_{PSII} in FSLE leaves of seedlings of *Q. suber* (down to 0.11 in sunflecks longer than 120 min), whereas this decrease was very slight in SS leaves (Fig. 4). Decreases in Φ_{PSII} in FSLE with respect to SS leaves during long sunflecks (Fig. 4) are probably a function of $Z + A$-mediated engagement of energy dissipation (Fig. 6). This would lead

Table 3
Evolution of the concentration of photosynthetic pigments for full sunlight-exposed (FSLE) and self-shaded (SS) leaves of seedlings of *Q. suber* exposed to increasing periods of full sunlight. Chl *a* and *b* are given in μmol m^{-2}, and neoxanthin (Nx), lutein (Lx), β-carotene (β-car) and V + A + Z in mmol pigment mol^{-1} Chl. Data are mean $\pm$ s.e. Differences on concentration of photosynthetic pigments between FSLE and SS leaves were not significant (Student's *t*-test, $P \leq 0.05$).

Leaf type	Time (min)	Chl *a*	Chl *b*	Chl *a*/Chl *b*	Nx	Lx	β-car	V + A + Z
FSLE	0	376.6 $\pm$ 22.4	105.4 $\pm$ 6.5	3.6 $\pm$ 0.1	28 $\pm$ 3	130 $\pm$ 8	120 $\pm$ 6	38 $\pm$ 2
	5	332.7 $\pm$ 36.4	96.1 $\pm$ 8.9	3.5 $\pm$ 0.2	29 $\pm$ 2	135 $\pm$ 9	107 $\pm$ 10	43 $\pm$ 3
	10	342.3 $\pm$ 69.9	97.1 $\pm$ 2.0	3.5 $\pm$ 0.1	28 $\pm$ 6	136 $\pm$ 24	109 $\pm$ 25	48 $\pm$ 8
	15	412.1 $\pm$ 19.6	103.0 $\pm$ 0.4	3.8 $\pm$ 0.2	25 $\pm$ 4	122 $\pm$ 4	111 $\pm$ 2	42 $\pm$ 1
	30	286.9 $\pm$ 4.2	79.4 $\pm$ 3.1	3.6 $\pm$ 0.2	31 $\pm$ 2	136 $\pm$ 2	111 $\pm$ 4	48 $\pm$ 1
	45	314.6 $\pm$ 16.0	87.5 $\pm$ 3.2	3.6 $\pm$ 0.1	30 $\pm$ 4	143 $\pm$ 6	106 $\pm$ 2	51 $\pm$ 5
	60	392.6 $\pm$ 27.6	108.6 $\pm$ 7.3	3.6 $\pm$ 0.1	27 $\pm$ 3	129 $\pm$ 7	107 $\pm$ 7	43 $\pm$ 3
SS	0	335.7 $\pm$ 27.7	104.6 $\pm$ 7.2	3.2 $\pm$ 0.1	27 $\pm$ 4	123 $\pm$ 7	106 $\pm$ 6	34 $\pm$ 4
	5	351.7 $\pm$ 24.7	100.8 $\pm$ 6.7	3.5 $\pm$ 0.1	29 $\pm$ 3	129 $\pm$ 9	104 $\pm$ 9	39 $\pm$ 2
	10	340.0 $\pm$ 41.8	93.8 $\pm$ 5.9	3.6 $\pm$ 0.2	28 $\pm$ 1	124 $\pm$ 5	97 $\pm$ 7	36 $\pm$ 3
	15	336.8 $\pm$ 29.8	95.7 $\pm$ 8.3	3.5 $\pm$ 0.1	31 $\pm$ 5	126 $\pm$ 14	102 $\pm$ 10	41 $\pm$ 5
	30	284.5 $\pm$ 18.6	81.8 $\pm$ 4.1	3.5 $\pm$ 0.1	26 $\pm$ 4	133 $\pm$ 2	108 $\pm$ 8	41 $\pm$ 3
	45	321.7 $\pm$ 28.7	88.2 $\pm$ 6.1	3.6 $\pm$ 0.1	28 $\pm$ 3	136 $\pm$ 8	98 $\pm$ 7	39 $\pm$ 6
	60	351.0 $\pm$ 18.5	102.9 $\pm$ 6.7	3.4 $\pm$ 0.1	28 $\pm$ 2	131 $\pm$ 6	107 $\pm$ 5	36 $\pm$ 4

to a decrease in F_V'/F_M' and possibly decreases in qP (i.e., increases in the PSII reduction state). Only when sunflecks are longer than 60 min, a decreased F_V/F_M may also contribute to reduce Φ_{PSII} values in FSLE leaves (Fig. 5). Therefore, the decreases found in net CO_2 uptake in FSLE leaves (Fig. 2) when they were exposed to high light conditions for more than 60 min were possibly caused by Z + A-mediated engagement of energy dissipation (competing with PSII photochemistry) and only in part the result of a decreased F_V/F_M ratio, because FSLE leaves did not recover their reference net CO_2 uptake and F_V/F_M values even 60 min after their reintroduction in the shade tunnel (Table 2). The lack of recovery of FSLE leaves was also observed, in a higher or lower extent, in all the other parameters measured (Table 2), when compared to the reference values (Figs. 2, 3 and 4). Therefore, the exposition of seedlings of *Q. suber* to sunflecks longer than 60 min also promoted a differential response between FSLE and SS leaves. Specifically, FSLE leaves showed a decrease in the activity of their photosynthetic machinery under these environmental conditions, whereas SS leaves could maintain the photosynthetic activity at similar values than reference values. It should be noted that the in case of a second sunfleck of long duration, FSLE leaves will cope with it from an initially worse situation, in contrast to SS leaves, due to their lack of recovery long time after an intense and long sunfleck.
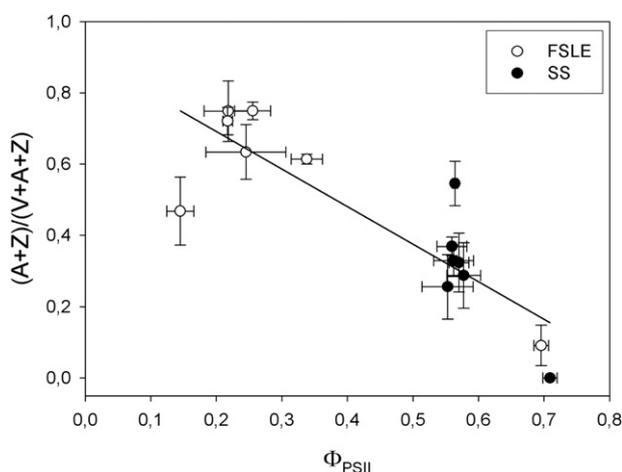

Fig. 7. Relationship between the actual PSII efficiency (Φ_{PSII}) and the de-epoxidation state of the xanthophyll cycle pigments, (A + Z)/(V + A + Z), in full sunlight-exposed (FSLE) and self-shaded (SS) leaves of seedlings of *Q. suber* exposed to increasing periods of full sunlight. Data are mean $\pm$ s.e.

The large PPFD increase during the sunflecks in FSLE leaves was associated to large increases in (A + Z)/(V + A + Z) and also to Φ_{PSII} decreases. Under the lower PPFD occurring in SS leaves during the sunflecks compared to FSLE leaves, the relationship between (A + Z)/(V + A + Z) and Φ_{PSII} was maintained (Fig. 7), suggesting that this mechanism of thermal dissipation competing with PSII photochemistry may be operating in a relative large PPFD-window during the sunflecks. Clearly, the extent of this mechanism was larger at high (FSLE leaves) than at low PPFD (SS leaves) (Fig. 7). Our data confirm previous findings showing that it is a combination of light intensity and zeaxanthin (Z) and antheraxanthin (A) that promotes thermal dissipation (Abadía et al., 1999; Morales et al., 1998, 2000), and not only the presence of Z + A in the PSII antenna (Morales et al., 1990, 1991), because this relationship disappeared when parameters of Chl fluorescence measured in darkness (i.e., dark-adapted F_V/F_M ratio) were plotted against (A + Z)/(V + A + Z) (see Figs. 5 and 6). These results support the contention that ΔpH and Z + A control together PSII antenna thermal energy dissipation. This seems to be the case in FSLE and SS leaves both during short and long sunflecks.

Thus, the potential benefit of self-shading in the physiological performance of the seedlings of *Q. suber* living in the understory of the natural forest studied must be carefully evaluated. Just after the change in the light regime, the theoretical situation where all leaves were as exposed as FSLE leaves would yield a higher net CO_2 assimilation at the whole plant level than the actual situation, although this advantage tended to be smaller after 60 min of exposition to full sunlight conditions (Fig. 3). Higher values of photosynthesis in FSLE leaves are the consequence of higher PPFD values, and the latter in the 60 and 120 min sunflecks had a negative influence on the leaf F_V/F_M values, because they showed a marked decrease that were not completely recover even 60 min after the end of the sunfleck. From our point of view, this would be the main advantage of the existence of two different light environments at leaf level. From a quantitative point of view, the decrease in A at the end of the sunfleck in the theoretical situation where the whole leaf area were full sun exposed was ca. 79%, in relation to the value before the sunfleck. However, this decrease was only ca. 50% in the actual situation. Thus, the multi-layered spiral foliage of *Q. suber* seedlings allows for the combination of two different strategies: i) to take advantage of the ability of FSLE leaves to achieve maximum photosynthetic yield when the sunfleck is short ($\leq$30 min) and ii) to increase the contribution of SS leaves to the photosynthesis of the whole plant when the sunfleck is long ($>$60 min, Fig. 3).

Interestingly, plants under natural conditions are exposed to the simultaneous effects of several potentially stressful environmental factors (Valladares and Pearcy, 2002). Valladares et al. (2008)

confirmed the co-occurrence of multiple factors affecting leaf performance of Q. ilex seedlings. When the impacts of shade, drought, and high light were considered, the beneficial effect of shade was eclipsed by reduced soil moisture during dry years. It should be noted that the conclusions derived from this investigation must be interpreted with caution since plants were kept under optimal irrigation conditions. Thereby, seedling performance under field conditions is subjected to multiple stress factors, which were out of scope of this study, but should be evaluated in further investigations. The importance of severe water constraints in Mediterranean areas must be taken into account in the interpretation of the functional implications of the shoot architecture of Q. suber seedlings. It remains to be explored whether crowns composed by many small leaves, a common feature for plants growing in these habitats (Traiser et al., 2004), tend to be associated with leaf arrangements such as those found in Q. suber seedlings as our study suggests.

In conclusion, shoot architecture of Q. suber seedlings involving high levels of self-shading allows to adequately cope with the low but highly heterogeneous light conditions of the understory, particularly when sunflecks of contrasting durations take place as it is frequently the case for evergreen Mediterranean forests.

Acknowledgments

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