The expression of light-related leaf functional traits depends on the location of individual leaves within the crown of isolated *Olea europaea* trees

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• **Background** The spatial arrangement and expression of foliar syndromes within tree crowns can reflect the coupling between crown form and function in a given environment. Isolated trees subjected to high irradiance and concomitant stress may adjust leaf phenotypes to cope with environmental gradients that are heterogeneous in space and time within the tree crown. The distinct expression of leaf phenotypes among crown positions could lead to complementary patterns in light interception at the crown scale.

• **Methods** We quantified eight light-related leaf traits across 12 crown positions of ten isolated *Olea europaea* trees in the field. Specifically, we investigated whether the phenotypic expression of foliar traits differed among crown sectors and layers and five periods of the day from sunrise to sunset. We investigated the consequences in terms of the exposed area of the leaves at the tree scale during a single day.

• **Key Results** All traits differed among crown positions except the length-to-width ratio of the leaves. We found a strong complementarity in the patterns of the potential exposed area of the leaves among day periods as a result of a non-random distribution of leaf angles across the crown. Leaf exposure at the outer layer was below 60 % of the displayed surface, reaching maximum interception during morning periods. Daily interception increased towards the inner layer, achieving consecutive maximization from east to west positions within the crown, matching the sun’s trajectory.

• **Conclusions** The expression of leaf traits within isolated trees of *O. europaea* varies continuously through the crown in a gradient of leaf morphotypes and leaf angles depending on the exposure and location of individual leaves. The distribution of light-related traits within the crown and the complementarity in the potential exposure patterns of the leaves during the day challenges the assumption of low trait variability within individuals.

**Key words**: Crown structure, leaf traits, leaf exposure, direct light, *Olea europaea*, isolated trees, high irradiance, leaf angles, daily pattern, variation within individuals.

INTRODUCTION

Trees are able to adjust foliar trait phenotype in response to the environment and within-crown gradients. The spatial heterogeneity across the tree crown could promote the expression of phenotypic differences in foliar traits and the prevalence of a set of coordinated traits (individual’s phenotypic syndrome). The phenotypic syndrome meets the limitations of the experienced environment that affect the fitness of the whole plant (Jurik et al., 1979; Howell et al., 2002; Falster and Westoby, 2003; Pearcy et al., 2005; Marks and Lechowicz, 2006; Valladares and Niinemets, 2008; Wilson and Nussey, 2010). The variability in leaf traits within the tree crown is functionally relevant. The plasticity in structural traits such as leaf angle and size can have profound consequences for the acquisition of the light resource (Howell et al., 2002; Falster and Westoby, 2003). Indeed, plasticity of leaf inclination angles alters light properties within the tree crown, enhancing whole-individual carbon gain (Uemura et al., 2006). Modifying light transmission and light properties can confer on trees advantages such as a higher degree of control of photosynthesis, transpiration and energy balance (Pearcy and Yang, 1996). Considering the modular nature of plants, the phenotypic response of the whole tree could be enhanced by the integrated expression of leaf phenotypes among functional units of the tree crown (de Kroon et al., 2005; Granado-Yela et al., 2011). The most and least exposed leaves within the tree crown (hereafter referred to as the outer and inner layer, respectively) constitute a classic example of functional subunits that are spatially segregated and specialized in the harvesting of a heterogeneous light resource (Sack et al., 2006). Nevertheless, the realized phenotypic diversity will necessarily be constrained by other abiotic and biotic factors. As a
consequence, the expression of leaf attributes related to light interception may be adjusted to the patterns of irradiance and concomitant stress of a local environment.

Intrinsically, tree growth generates local differences in the environment experienced by each portion of the crown. On isolated trees, the efficiency of direct light interception is strongly dependent on the environmental conditions and on leaf attributes, which in turn are affected by crown shape and structure (Ackerley and Bazzaz, 1995; Farnsworth and Niklas, 1995; Planche and Sinoquet, 1998; Farque et al., 2001; Falster and Westoby, 2003). Considering the direct component of the light in the sky, the potential light capture achieved by a flat leaf (or any flat surface with defined tilt and orientation angles) during a certain day and at any given latitude is determined by the angle of incidence of the sunbeams to the leaf blade. The mismatch between the sunbeam and the normal vector to the plane of the leaf imposes a reduction in the potential exposed surface due to diurnal changes in the azimuth and altitude of the sun (Granado-Yela et al., 2011). However, the estimation of the potential reduction in the exposed leaf surface is trivial once the spatial position of the leaf is defined for a given latitude and time. In high-irradiance environments with other sources of stress, such as high temperatures and low water availability, increases in factors that entail self-shading constitute crucial attributes of plant architecture (Howell et al., 2002; Peacey et al., 2005). In fact, minimizing the negative impact of high direct radiation on photosynthesis by means of steep leaf angles appears to compensate for potentially low daily carbon gains in high-irradiance environments (Falster and Westoby, 2003).

The leaf’s spatial position (inclination and orientation angles), in addition to the degree of aggregation of leaves, influences the potential of a single leaf to intercept direct sunlight and the penetration of light to lower layers of the crown (Falster and Westoby, 2003). Leaf shape also has an impact on the distribution of light within the crown. Elongated leaf shapes (high length-to-width ratio) can contribute to light-capture efficiency, decreasing self-shading among leaves and enhancing light penetration to deeper layers (Peacey et al., 2005; Sarlikioti et al., 2011). Adjustments of leaf traits related to the irradiance experienced by each portion of the crown can lead to spatial specialization and temporal complementarity of productive processes such as carbon fixation, accumulation and carbon export (Granado-Yela et al., 2011). Leaf attributes such as low specific leaf area, high leaf length-to-width ratio and high inclination angles at the more exposed layer of the crown can reduce heat loads while having a positive effect on carbon gain at the whole-individual level (Ehleringer and Werk, 1986; Valladares and Pugnaire, 1999; Werner et al., 2001, 2002).

Despite the expected within-crown heterogeneity, field designs under natural conditions aimed at characterizing leaf traits and daily patterns of leaf exposure to direct radiation are often restricted to saplings or to extreme positions of the light gradient within the crown (Valladares et al., 2005; Gratani et al., 2006; Sack et al., 2006; Rubio de Casas et al., 2007, 2011). However, a higher degree of phenotypic plasticity is expected to occur in reproductive adults under natural conditions across the whole crown (Cavender-Bares et al., 2004; Bouvet et al., 2005). Therefore, our main aim was to provide a detailed characterization of foliar trait variation across crown positions in order to identify the degree of leaf specialization in a spatiotemporal context and to assess the functional relationship among crown positions. Moreover, estimates of potential leaf exposure at the crown scale are required in individual-based models of vegetation dynamics (e.g. Moorcroft et al., 2001; Falster et al., 2011). We hypothesized that foliar differentiation would lead to divergent phenotypes and to distinct leaf exposure patterns across the tree crown. To test this hypothesis, we carried out systematic field measurements on a set of functional leaf traits that are related to light interception. Specifically, we characterized leaf angle, area, dry mass and shape (leaf length-to-width ratio) at different crown depths within the crown of ten Olea europaea trees growing in two populations with contrasting climates but similar radiation levels. In particular, we investigated whether functional leaf traits were complementary among crown positions. Indeed, variability in leaf angles among crown positions for a given location describe the geometrical relationship between leaf position and the angle of incidence of the incoming radiation, and thus sets the upper limit of potential exposure to direct radiation when self-shading is ignored. Moreover, combined with the investment in area per unit dry mass it can give insight to identify complementary strategies that supposedly optimize light capture from the leaf to the whole-crown level (Granado-Yela et al., 2011).

Materials and methods

Species and study site

We studied reproductive individuals of Olea europaea ssp. europaea (wild olive tree), an evergreen, sclerophyllous, long-lived tree distributed throughout the Mediterranean Basin. This species develops flat leaves that do not track sun movements and was found to have signs of foliar differentiation between the extremes of the light gradient within the tree crown (Granado-Yela et al., 2011; Rubio de Casas et al., 2011). The present study was conducted in two natural populations located on a Mediterranean island (San Luis [SL], Menorca, Spain) and in the central Iberian Peninsula (Aldea del Fresno [AF], Madrid, Spain). The two populations occurred at equivalent latitudes, thus experiencing similar daylength and sun elevation angles (Table 1). In each population, we selected five individuals that had previously borne fruits with the aim of sampling reproductive adults. Sampling was carried out on isolated trees during one midsummer day in each population (July to August, 2011) to ensure the complete development of leaves, as recommended for woody species under most of the Mediterranean climates in the northern hemisphere (Garnier et al., 2001). However, the AF population was located on sloping terrain (~30°, south aspect) and direct sun irradiance decreased on the surrounding terrain during the final hours of daylight. For each population, photosynthetically active radiation (PAR) was extrapolated from the web portal supported by the NASA LaRC POWER Project. Tree canopy structure was comparable across individuals and populations in spite of small differences in the light environment between populations (Table 1). Trees in the SL population were slightly taller than trees in the AF population (Table 1).
1400 (10 trees leaves were measured. The total number of sampled leaves was where leaves were present (Fig. 1). Sampling points for the S and W) and subdivided into three layers: the outermost layer hereafter) was labelled according to its cardinal direction (N, E, semi-ellipsoid was considered). Each sampling axis (‘sector’ length-to-width ratio). To account for allometric differences between individuals, plant size (biovolume) was estimated as:

\[
\text{biovolume} = \frac{2}{3}\pi(D/2)(d/2)h
\]

where \( D \) and \( d \) are the diameters used to define the canopy ellipsoid and \( h \) is plant height. We used biovolume as a size proxy because the total aerial size of wild olives can be approximated with a semi-ellipsoid (Rubio de Casas et al., 2011). Due to missing data, the lowest \( n \) considered was 1219 for \( L_{\text{width}} \), \( L_{\text{length}} \) and \( L_{\text{index}} \).

**Potential exposure of the surface of the leaves**

To examine the potential ability of each leaf to capture light, we calculated the silhouette area of the leaf blade (\( \text{SAL} = \text{projection of the leaf blade} \times \text{leaf area; cm}^2 \)) as a

### Table 1. Studied populations, radiation and tree dimensions per population (mean ± 1 s.e.)

<table>
<thead>
<tr>
<th>Location</th>
<th>Coordinates</th>
<th>Altitude (m a.s.l.)</th>
<th>Annual mean direct radiation (( \mu \text{mol m}^{-2} \text{s}^{-1} ))^a</th>
<th>Plant height^b (m)</th>
<th>Short diameter^b (m)</th>
<th>Long diameter^b (m)</th>
<th>Inner layer^b (m)</th>
<th>Middle layer^b (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldea del Fresno, Madrid, Spain</td>
<td>40°19′ N, 4°14′ W</td>
<td>690</td>
<td>923-57 ± 90-15</td>
<td>2-90 ± 0-13</td>
<td>3-31 ± 0-36</td>
<td>3-65 ± 0-49</td>
<td>1-36 ± 0-12</td>
<td>1-08 ± 0-09</td>
</tr>
<tr>
<td>San Luis, Menorca, Spain</td>
<td>39°49′ N, 4°16′ E</td>
<td>91</td>
<td>1038-49 ± 97-32</td>
<td>3-30 ± 0-18</td>
<td>4-23 ± 0-36</td>
<td>4-66 ± 0-25</td>
<td>1-30 ± 0-06</td>
<td>0-83 ± 0-05</td>
</tr>
</tbody>
</table>

\(^a\text{Monthly averaged midday insolation incident on a horizontal surface. These data were obtained from the NASA Langley Research Center Atmospheric Science Data Center surface meteorological and solar energy (SSE) web portal supported by the NASA LaRC POWER Project.}\n
\(^b\text{\( n = 5 \).}\)
measure of leaf area intercepting light, and the silhouette-to-leaf area ratio (STAR = projected area/leaf area), which relates the relative area of the leaf to the angle of incidence of the incoming radiation (Carter and Smith, 1985; Oker-Blom and Smolander, 1988; but see Granado-Yela et al., 2011). Both variables combine the trajectory of the sun and the spatial position of an isolated leaf without considering leaf overlapping. Nonetheless, they define the maximum potential area and proportion of the leaf displayed to capture light at a given time due to the angle of incidence of the incoming radiation to a normal vector of the leaf surface (STAR = 1, orthogonal incidence and full potential exposure). Comparisons between SAL and STAR values estimated over the period of 1 day further define when the potential maximum is achieved during the day. To account for light interception variance during the day period we calculated SAL and STAR among crown positions in five evenly distributed periods (SAL_t and STAR_t) and as the integral of each leaf over time (from sunrise to sunset) on the day of the measurements (SAL_d and STAR_d). To assess SAL_d and STAR_d, we calculated the integral of the leaf projection from sunrise to sunset for each leaf among crown positions. Calculations considered leaf angles (L_azi, corrected for magnetic declination at each population), location (geographic coordinates) and the sun’s position for a given time [eqn (3) in Escribano-Rocafort et al., 2014]. We integrated this equation over time for one of the measurement days (31 July), every 2 min. The equation used is sensitive enough to determine whether the exposure occurs in the adaxial or abaxial surface of the leaf (indicated by positive and negative signs, respectively). To assess the variation within the day period, SAL and STAR were calculated for five evenly distributed periods during the day (SAL_period, STAR_period; T1–T5, in which T3 equals solar noon) to account for representative sun elevation and azimuth angles in distinct day periods (Fig. 1).

Statistical analyses

We investigated differences in SAL_d, STAR_d, SAL_t, STAR_t and leaf traits among crown positions and day periods using a linear mixed effects model. All final models were fitted using restricted maximum likelihood using the nlme package in R software (R Development Core Team, 2008). We determined model selection based on Akaike’s information criterion (AIC), obtaining the most explanatory model, weighted by the number of estimated parameters, which includes all significant effects. We included in all models the variables biovolume and population as random intercept to account for differences in leaf exposure due to allometry and local conditions (Rubio de Casas et al., 2011). The most complex model included crown sector (with four levels: N, E, S and W), crown layer (three levels: outer, middle and inner) and day period as fixed factors. The most complex random term was plant biovolume nested within population. With the aim of assessing differences between and within day periods, we used linear mixed models for SAL_d and STAR_d, adding the fixed term day period (ordered factor with five levels: T1–T5). Multiple comparison tests were performed with the multcomp R package (Bretz et al., 2004). Leaf angles were transformed to radians and the remaining leaf traits were log-transformed to improve normality in the analyses. Normality and homoscedasticity assumptions were checked graphically in the final models. A detailed summary of all models and frequency histograms of leaf angles for each crown sector and layer is provided in the Supplementary Data Tables S1–S10 and Figure S1.

RESULTS

Crown structure and leaf traits

Leaf traits differed significantly among crown positions except for Lindex, SAL_d and STAR_d (Table 2). A detailed summary of all models is provided in the Supplementary Data Tables S1–S10. All crown sectors and layers had a mean L_azi oriented towards the south-east except for the middle and inner layer of the N and W sectors, which were facing north-east and south-west respectively (Table 3). Leaf tilt angles in the outer and middle layer in the S and W sectors were more vertical than the rest (L_tilt ~60°). The inner layer had the lowest L_tilt angles (~45°) except in the S sector, where L_tilt values were similar to those displayed in the middle layer (Table 3). Frequency histograms for leaf angles for each crown sector and layer are provided in Supplementary Data Figure S1. Despite an effect of the interaction between crown sector and crown layer, L_azi was similar among layers and sectors, with maximum differences within the W sector (2.59 ± 0.05 cm² in the outer layer compared with 2.92 ± 0.08 cm² in the middle layer) (Table 3). Specific leaf area revealed significant differences between layers (Table 3). The outer layer in the N, E and W sectors had the lowest SLA values (~47 cm² g⁻¹) followed by leaves in the outer layer in the S sector (52.08 ± 0.86 cm² g⁻¹). Leaves in the inner layer had the highest values of SLA. The Lindex value did not show an interaction between crown sector and layer (Table 2). Leaves in the E and S sectors were more elongated than those in the N sector. The values of Lindex were higher in the outer layer than in the middle and inner layers (Table 3).

### Table 2. Summary statistics for the best linear mixed model fitted to leaf traits and integrated SAL_d and STAR_d across the tree crown with the fixed effects of crown sector, crown layer and the interaction between them

<table>
<thead>
<tr>
<th>Sector</th>
<th>numDF</th>
<th>L_azi (°)</th>
<th>L_tilt (°)</th>
<th>L_area (cm²)</th>
<th>LDM (g)</th>
<th>L_width (cm)</th>
<th>L_length (cm)</th>
<th>SLA (cm² g⁻¹)</th>
<th>L_index</th>
<th>SAL_d (cm²)</th>
<th>STAR_d</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3</td>
<td>23.15***</td>
<td>2.49</td>
<td>0.89</td>
<td>3.40**</td>
<td>2.71*</td>
<td>2.05</td>
<td>13.51***</td>
<td>8.19***</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>6.49***</td>
<td>29.87***</td>
<td>1.16</td>
<td>38.14***</td>
<td>32.07***</td>
<td>15.80***</td>
<td>233.86***</td>
<td>80.04***</td>
<td>23.38***</td>
<td>26.17***</td>
</tr>
<tr>
<td>S</td>
<td>6</td>
<td>10.92***</td>
<td>4.46***</td>
<td>3.56**</td>
<td>2.97**</td>
<td>2.84***</td>
<td>2.98**</td>
<td>4.28***</td>
<td>1.44</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

numDF, numerator degrees of freedom.
Biovolume was included in all models as a random factor.
The standard deviation of the random components of each model and further details are provided in Supplementary Data Tables S1–S10.

*P < 0.05; **P < 0.01; ***P < 0.001; underscored values are marginally non-significant (0.05 < P < 0.1).
### Table 3

<table>
<thead>
<tr>
<th>Crown sector</th>
<th>Crown layer</th>
<th>L_{azi} (°)</th>
<th>L_{tilt} (°)</th>
<th>L_{area} (cm²)</th>
<th>SLA (cm²·g⁻¹)</th>
<th>L_{index} %</th>
<th>STAR_{d} (cm²)</th>
<th>STAR_{t} (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Outer</td>
<td>11.14 ± 2.8b</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
<td>76.3 ± 1.22c</td>
<td>76.3 ± 1.22c</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>21.29 ± 3.2ab</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
<td>76.3 ± 1.22c</td>
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<td>76.3 ± 1.22c</td>
<td>76.3 ± 1.22c</td>
</tr>
<tr>
<td>E</td>
<td>Outer</td>
<td>10.61 ± 2.1b</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
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<td>76.3 ± 1.22c</td>
</tr>
<tr>
<td>S</td>
<td>Outer</td>
<td>10.61 ± 2.1b</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
<td>76.3 ± 1.22c</td>
<td>76.3 ± 1.22c</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>21.29 ± 3.2ab</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
<td>76.3 ± 1.22c</td>
<td>76.3 ± 1.22c</td>
</tr>
<tr>
<td></td>
<td>Inner</td>
<td>10.16 ± 2.1b</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
<td>76.3 ± 1.22c</td>
<td>76.3 ± 1.22c</td>
</tr>
<tr>
<td>W</td>
<td>Outer</td>
<td>17.49 ± 2.8a</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
<td>76.3 ± 1.22c</td>
<td>76.3 ± 1.22c</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>27.18 ± 2.1abc</td>
<td>27.1 ± 0.06ab</td>
<td>72.8 ± 1.66a</td>
<td>76.3 ± 1.22c</td>
<td>56.5 ± 1.98d</td>
<td>76.3 ± 1.22c</td>
<td>76.3 ± 1.22c</td>
</tr>
</tbody>
</table>

Levels with different letters indicate significant differences (p < 0.05).

**Potential leaf exposure across the tree crown over time**

The integrated values of SAL_{d} and STAR_{d} (product of the interaction of leaf angles and the sun’s trajectory during the chosen day) were only significant among crown layers (Table 2). Leaves of the inner layer had greater SAL_{d} and STAR_{d} values than leaves in the middle and outer layers (Table 3), thus experiencing a smaller potential reduction in the intercepting surface due to the sun’s trajectory and leaf angles. On average, leaves in the inner layer exposed a surface area of 0.14 cm² and had 4% higher STAR_{d} than the outer layer during the chosen day. Values of SAL_{t} and STAR_{t} showed a significant effect of the triple interaction among crown sector, layer, and day period (Table 4). Leaves in each sector and layer were distinctly exposed to direct radiation during the selected periods. The outer layer of the crown had sustained adaxial exposure from T1 to T4. However, SAL_{d} values remained < 2 cm² in all crown sectors and reached their maximum between T2 and T3 (mid-morning and midday; Fig. 2A, dashed lines with squares). Therefore, leaves in the outer layer experienced a sustained reduction of the exposed surfaces during the day. On the contrary, middle and inner layers displayed distinct SAL_{d} patterns across crown sectors over the course of the day, achieving full interception and hence experiencing a lower reduction than the outer layer (Fig. 2A, dotted lines with triangles, and solid lines with circles). In the N sector, the inner and middle layers had similar daily SAL_{d} patterns to the outer layer. The remaining sectors had greater SAL_{d} values (> 2 cm²) and variance during the day. Leaves in the middle and inner layers in the E, S and W sectors reached maximum SAL_{d} in consecutive periods during the day from T2 to T4, enabling spatio-temporal complementarity (Fig. 2A, left to right). The variation in SAL_{d} pattern over the selected periods increased from the outer layer to the middle and inner layers of the crown across crown sectors, except for the N sector (Fig. 2A). Likewise, as leaf area did not differ among crown positions, the STAR_{d} patterns were similar to SAL_{d} (Fig. 2B). Maximum STAR_{d} values reached 0.6 in the outer layer, whereas middle and inner layers had a maximum STAR_{d} of 0.8 (except in the N sector; Fig. 2B).

Crown layers and sectors as a function of leaf angles (L_{azi} and L_{tilt}) had similar STAR_{d} values over time (integrated values from sunrise to sunset). Average leaf angles for each crown sector and layer for STAR_{d} differed by around 5–6% between the inner and outer layers during the day. However, the sun’s trajectory from east to west determines that all leaves oriented in these directions (L_{azi} ~ 90° or 270°) will experience the greatest variance in the exposed surface during the day—specifically, if they display high L_{tilt} angles, as shown by the outer layer (except the S sector), the inner and middle layers of the E sector (all with north-east to south-east orientations) and the inner and middle layers of the W sector (oriented to the west). These positions in the crown maximize potential light interception (the angle of incidence of the sunbeam is perpendicular to the plane of the leaf) either in the morning or the afternoon periods due to the displayed L_{azi}. In spite of a small variation in STAR_{d} values in the different crown sectors during the day, leaves in the middle and inner layers of the N sector and in all layers of the S sector experienced a greater reduction in the maximum surface to intercept light (STAR_{d} < 1) and less variability over time.
DISCUSSION

The results showed a high variance in leaf traits within the tree crown. The apparent non-random distribution of leaf angles across crown positions resulted in complementary patterns of potential leaf exposure to direct radiation during the day among crown sectors and layers. Although leaf angles differed among crown positions, the potential exposed leaf area was similar among sectors, with small differences between crown layers during the day. However, displayed leaf angles determined distinct temporal patterns of leaf exposure in contrasting day periods. Leaves in the outer layer had the greatest reduction in exposed surface compared with leaves in the inner and middle layers (specifically around midday). Leaf exposure was sustained during the day, with maxima skewed towards morning periods. On the contrary, leaves in the middle and inner layers achieved greater interception and high variance among day periods depending on the crown sector. These findings suggest that leaf phenotypes are adjusted in a particular manner across the spatial volume of the crown and that the leaf’s potential exposure depends on the location of the leaf within the tree crown. Moreover, differential leaf trait expression across the crown has shown a significant correlation with light interception patterns, carbon assimilation and export between contrasting light conditions within the crown (Granado-Yela et al., 2011).

To our knowledge, the present study is the first to report such non-random distribution of leaf orientation and inclination angles...
in trees. The observed angles are congruent with phenotypic integration of potential exposed surfaces across the canopy. The effects of non-random leaf orientation and inclination angles in high-irradiance environments have been shown to enhance water-use efficiency at high solar angles and to provide equivalent or higher carbon gain compared with other possible orientations (Jurik et al., 1990; James and Bell et al., 2000) and even linked to reproduction (Werk and Ehleringer, 1986). The environmental heterogeneity across the tree crown of isolated trees depends on exogenous conditions but also on modification of the within-canopy environment caused by the crown architecture. As a consequence, crown performance depends not only on the environment in which the tree grows but, to a large extent, on the modification of the environment caused by tree crown growth and its architecture (Rubio de Casas et al., 2007, 2011).

Differences in leaf inclination and orientation angles across tree crown positions can yield complex patterns of light interception and lead to a complementary strategy of leaf exposure to direct radiation during the day. Despite the fact that our measurements neither considered self-shading from adjacent layers and other phytocenomen nor the diffuse portion of the radiation, the inner and middle layer had near-optimal leaf inclination angles to maximize light interception in the studied location (L tilt ~40°; Mehleri et al., 2010). Moreover, L tilt varied, matching on average the crown sector in which the leaf was located. This configuration enabled a consecutive achievement of maximum interception during the day by each crown sector and a potentially constant assimilation even during high-irradiance periods, such as around midday. On the contrary, the outer layer of leaves experienced less variation during the day in the light interception patterns, due to high L tilt values and L mat with eastern and south-eastern orientations. Such relative spatial positions can enable sustained carbon assimilation throughout the day despite the diurnal variation in solar light intensity and angle of incidence (Granado-Yela et al., 2011). Our results showed that leaves in the outer layer potentially experience a decrease that is 5–6 % greater in the exposed leaf area than in the inner layer during the day, due to the cosine law, and that they do not achieve full exposure at any given time. Accordingly, the outer layer of leaves had lower SLA values than the inner and middle layers. Optimization theories suggest that the investment in SLA across crown positions should be inversely proportional to the interception light (Field, 1983; Farquhar, 1989). The observed configuration of the outer layer of the crown (more vertical leaves, low L tilt) may contribute to the avoidance of dynamic photoinhibition by ensuring that full leaf exposure is not reached at any given time. However, avoidance of direct sunlight by the outer leaves in the S sector during T3 would result in long-lasting, high-intensity irradiance pulses reaching the inner and middle layers. Such pulses may be at least as harmful for the middle and inner layers as they are for the outer layer. Consequently, inner and middle layers in the S sector had similar L tilt angles to the outer layer. This is congruent with previous findings of minimal photoinhibition during midday and subsequent recovery of assimilation in the afternoon (Granado-Yela et al., 2011). Factors that increase self-shading constitute crucial attributes of plant survival in high-irradiance environments despite their ability to adjust physiologically to stress (Valladares and Pearcy, 1997; Howell et al., 2002; Pearcy et al., 2005). If the outer layer allows a higher fraction of the radiation to penetrate into deeper layers, the use of light and whole-crown photosynthesis might be more efficient (Watson and Witts, 1959; Verhagen et al., 1963; Terashima and Hikosaka, 1995). Nevertheless, until self-shading and the whole portion of the radiation, i.e. direct and diffuse light, are taken into consideration, the optimization of the light interception strategy through the expression of multiple leaf syndromes at the crown scale will remain a working hypothesis. Even so, we believe that our work provides substantial evidence of a distinct expression of leaf phenotypes across the tree crown that encourages further research to assess the light environment within crowns and the implications in terms of light use efficiency at the individual scale.

The expression of leaf traits within isolated trees of O. europaea varies continuously through the crown in a gradient of leaf morphotypes and leaf angles depending on the exposure and location of individual leaves. The apparent non-random distribution of leaf angles across the tree crown yields complementary patterns of potential exposure of the leaves among crown positions through a single day. The distinct expression of leaf traits within trees suggests spatio-temporal integration of the leaves across the tree crown. Indeed, plants adapt morphologically and physiologically to subtle environmental differences at smaller scales than the individual level (de Kroon et al., 2005; Esteso-Martínez et al., 2006), which could result in interactions between functionally differentiated subunits and potentially in an increase in performance at the organism level (de Kroon et al., 2005; Granado-Yela et al., 2011). However, tree crowns are complex multifunctional structures that might be subjected to several pressures and constraints (Pearcy et al., 2005). A deeper understanding of the function of the tree crown requires additional research to characterize the local environment within the tree crown and to determine the evolutionary and ecological implications of the spatial variation of leaf traits within individuals.

**SUPPLEMENTARY DATA**

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Tables S1–S10: Details of linear mixed models of leaf traits. Summary statistics for the best linear mixed model fitted to leaf traits, the fixed effects of crown sector and crown layer, the interactions between them and biovolume as a random effect. Figure S1: Histograms
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LITERATURE CITED


wares/CRAN/src/contrib/Descriptions/multcomp.html (last accessed 8 February 2016).


APPENDIX

Abbreviations

L\textsubscript{area}, area of one side of leaf (cm\textsuperscript{2}).
L\textsubscript{width}, leaf lamina maximum width (cm).
L\textsubscript{length}, leaf maximum length (cm).
LDM, leaf dry mass (g).
SLA, specific leaf area (cm\textsuperscript{2} g\textsuperscript{-1}).
L\textsubscript{index}, leaf length-to-width ratio. Dimensionless; higher values indicate elongated leaves.
L\textsubscript{tilt}, leaf lamina inclination angle (\(^\circ\)). 90\(^\circ\), vertical inclination, 0\(^\circ\), leaves lie horizontal.
L\textsubscript{azi}, Leaf lamina azimuth angle (\(^\circ\)). Deviation angle from true north of the projection of a normal vector to the leaf’s lamina surface.
SAL, silhouette area of the leaf blade (cm\textsuperscript{2}). Negative values indicate underside exposure.

STAR, silhouette area of the leaf blade-to-area ratio. Dimensionless; negative values indicate underside exposure.
SAL\textsubscript{t}, silhouette area of the leaf blade during chosen periods (T1–T5, cm\textsuperscript{2}).
STAR\textsubscript{t}, silhouette area of the leaf blade-to-area ratio during chosen periods (T1–T5). Dimensionless, 0–1.
SAL\textsubscript{d}, silhouette area of the leaf blade integrated over a day period (cm\textsuperscript{2}).
STAR\textsubscript{d}, silhouette area of the leaf blade-to-area ratio integrated over a day period. Dimensionless.
T1 – T5, day period. Equivalent periods were chosen at each location from midday (T3, maximum sun’s elevation angle) to towards sunrise (T1) and sunset (T5).
AF, Aldea del Fresno population.
SL, San Luis population.