Correlation between leaf age and other leaf traits in three Mediterranean maquis shrub species: *Quercus ilex*, *Phillyrea latifolia* and *Cistus incanus*

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Abstract

The anatomical and morphological leaf traits as well as leaf inclination and orientation per different leaf age cohort of *Quercus ilex*, *Phillyrea latifolia* and *Cistus incanus* growing in the Mediterranean maquis along Rome’s coast-line (Italy) were investigated. Specific leaf weight (SLW), total leaf thickness (*L*), leaf density index and leaf inclination (*a*) changed according to leaf age. The maximum values were measured at full leaf expansion, underlining the strong influence of *a* on the reduction of solar radiation incident on leaf surface and the importance of the received solar radiation by leaf structure during leaf age. *C. incanus* summer leaves had the lowest surface area, the highest SLW (15 ± 2 mg cm\(^{-2}\)) and *L* (244 ± 15 µm) with respect to winter leaves, reducing the evaporative leaf surface during drought. Older leaves of 2–4 years *Q. ilex* and *P. latifolia*, shaded by new leaves had lower *a* than 1 year old leaves. *a* is a linear function of SLW. By the seasonal leaf dimorphism and the characteristic leaf folding the adjustment of leaf inclination angle from −37° in winter leaves to +44° in summer leaves increased reduction of incident solar radiation during drought. Leaf folding may be related to the less xeromorphic leaf structure of *C. incanus*. The index of xeromorphism, measured at full leaf expansion and resulting from the surface area of the polygon plotted joining the value of the seven considered xeromorphic leaf traits in the radar graph, is the highest in *P. latifolia* (0.88), and the lowest in *C. incanus* (0.44). © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Evergreen Mediterranean shrubs; Leaf age; Leaf inclination angle; Specific leaf weight; Leaf thickness; Leaf density; Xeromorphic index

1. Introduction

Evergreen sclerophylls and drought semi-deciduous species which prevail in regions with Mediterranean climate represent different adaptive strategies (Christodoulakis, 1989; Kutbay and Kilinci, 1994). Evergreen sclerophyllous species which have high construction cost of leaf protective structures (Chabot and Hicks, 1982; Williams et al., 1989) are long-lived (Miller and Stoner, 1979; Mooney, 1981), while semi-deciduous are short-lived and their adaptations include leaf anatomical differences between summer and win-
Seasonal and leaf age changes may be a source of variation in the relationship between the different leaf traits (Reich et al., 1991). Although there are numerous studies dealing with leaf morphological trends of Mediterranean evergreen species in response to environmental changes, their functional interpretation should be carefully considered (Reich, 1993; Smith et al., 1998).

Increasing drought stress may determine a shortening of leaf life-span (Field and Mooney, 1983; Jordan, 1983; Wolfe et al., 1988). We advanced the hypothesis that real evergreen sclerophyllous shrub species, by their long leaf life-span and the high degree of xeromorphism, will be at a competitive advantage in respect to semi-deciduous species, considering the forecasted increase of air temperatures and drought in the Mediterranean climate. The high degree of xeromorphism may permit evergreen sclerophyllous species to adapt to a changing environment maintaining their position in the community. The approach was to measure the relative importance of those leaf traits which are important life-history traits of plants with respect to their environment (leaf life-span, leaf inclination, leaf orientation) and those which reflect the species adaptation (specific leaf weight (SLW), total leaf thickness (L), leaf density, plasticity index) during leaf age. To obtain evidence supporting this hypothesis Quercus ilex, Phillyrea latifolia, typical evergreen sclerophyllous shrubs, and Cistus incanus, a drought-semi-deciduous shrub were compared; these are species largely represented in the Mediterranean maquis.

In a Mediterranean climate water stress during summer is often accompanied by high air temperatures, high irradiance and high leaf to water vapor deficit. It has been demonstrated that the combination of these factors favour leaf photoinhibition (Björkman and Powles, 1984; Valladares and Pearcy, 1997). Since leaves of most evergreen maquis species cannot effectively utilize the full irradiance, leaf inclination in all probability, provides the most powerful means of regulating light interception (De Lucia et al., 1991; Poulson and De Lucia, 1993; Ryel et al., 1993; Björkman and Demming-Adams, 1995; Myers et al., 1997; Kao and Tsai, 1998). Plants with steeper \( x \) may not utilise more efficiently the reduced incident flux, but also reduce the potential for photoinhibition (Powles and Björkman, 1981; Ludlow and Björkman, 1984; Werner et al., 1999). The evolution of leaf structure is assumed to have occurred in concert with the evolution of leaf orientation properties (Smith et al., 1998), therefore the authors would like to analyse how leaf inclination of the examined species is associated with leaf structure.

2. Materials and methods

2.1. Study area

The study was conducted in the Mediterranean maquis developing along the coast near Rome, in the Castelporziano Estate (41°45' N; 12°26' E). The area’s climate is of Mediterranean type and most of its annual rainfall is distributed in autumn–winter (Table 1). The average minimum air temperature of the coldest month (February) was 4.1°C and the average maximum air temperature of the hottest month (August) was 30.8°C (data by the Castelporziano Meteorological Station, 1987–1998, Table 1). Detailed descriptions of vegetation is available in Gratani and Marinucci (1985).

All field measurements were made on randomly chosen Q. ilex L., P. latifolia L. and C. incanus L. plants (three per species), representative of the population.

2.2. Leaf age

Leaf age was analysed in situ monitoring the number of nodes, according to Reich et al. (1992), since flushing patterns and C. incanus leaf dimorphism were known (Gratani and Crescente, 1997). In order to determine whether leaf traits differed in function of leaf age, different leaf age cohorts were collected in the field from February 1998 to May 1999. Particularly 2 and 4 month old leaves (summer leaves) and 3, 5 and 8 month old leaves
Table 1
Monthly average maximum air temperature ($T_{\text{max}}$), monthly average minimum air temperature ($T_{\text{min}}$), monthly average air temperature ($T_m$) and total monthly rainfall ($R$) for the period 1987–1998 and for the study period (from February 1998 to May 1999)*

<table>
<thead>
<tr>
<th></th>
<th>Average 1987–1998</th>
<th>Study period</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\text{max}}$ (°C)</td>
<td>$T_{\text{min}}$ (°C)</td>
<td>$T_m$ (°C)</td>
</tr>
<tr>
<td>Jan</td>
<td>13.6</td>
<td>4.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Feb</td>
<td>14.1</td>
<td>4.1</td>
<td>9.1</td>
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<tr>
<td>Mar</td>
<td>15.9</td>
<td>5.8</td>
<td>10.8</td>
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<tr>
<td>Apr</td>
<td>17.8</td>
<td>7.9</td>
<td>12.8</td>
</tr>
<tr>
<td>May</td>
<td>22.2</td>
<td>11.7</td>
<td>16.9</td>
</tr>
<tr>
<td>Jun</td>
<td>26.2</td>
<td>14.6</td>
<td>20.4</td>
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<td>Jul</td>
<td>30.0</td>
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<tr>
<td>Aug</td>
<td>30.8</td>
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<td>Sep</td>
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<tr>
<td>Dec</td>
<td>13.6</td>
<td>5.4</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td><strong>Total annual precipitation</strong></td>
<td><strong>727</strong></td>
<td></td>
</tr>
</tbody>
</table>

* Data of the Meteorological Station of Castelporziano, Rome.
(winter leaves) of *C. incanus*; 8 month, 1, 2 and 3 year old *Q. ilex* leaves; 8 month, 1, 2, 3 and 4 year old *P. latifolia* leaves were examined. In each sampling occasion forty leaves were collected per different leaf age cohort. These data were chosen to include both expanding and fully expanded leaves.

2.3. Photosynthetic active radiation (PAR)

PAR was measured on 02/02/98, 06/03/98, 06/07/98 and 03/08/98, using a Li-COR 190SB Quantum Sensor. The reduction of PAR incident on a sloping leaf surface (RI) was calculated, per species and per different leaf age cohort by: RI = 100 \( (1 - I_s/I_0) \), where \( I_0 \) was the fraction of PAR intercepted by a horizontal surface and \( I_s \) the fraction of PAR incident on a sloping leaf surface (Ehleringer, 1989).

2.4. Leaf orientation and leaf inclination

Leaf movement analysis was determined by diurnal measurements of leaf orientation (or azimuth, \( \eta \)) and leaf inclination (\( \alpha \)) (Nobel et al., 1993) and were conducted in the morning (09:30 h in winter, 07:30 h in summer), at midday (12:00 h in winter and in summer) and in the afternoon (14:30 h in winter, 16:30 h in summer) (Prichard and Forseth, 1988), during the coldest (02/02/98 and 06/03/98) and the hottest (06/07/98 and 03/08/98) months of the year, in order to observe plant behaviour in response to severe environmental conditions (low temperatures and PAR in winter; high temperatures, PAR and drought in summer). Forty randomly chosen sun leaves representative of the population of each species were labelled, with nylon tape at the base of the petioles, and monitored in each sampling occasion. \( \eta \) and \( \alpha \) for the different leaf age cohorts were recorded from February 1998 to May 1999.

Leaf inclination and leaf orientation were obtained by measuring the angle from the horizontal and azimuth (0° = N, 90° = E, 180° = S, 270° = W) of the adaxial leaf surface (Prichard and Forseth, 1988). Angle measurements were made by a hand-held clinometer (Suunto Co. Model PM-5/360PC); azimuth was measured by a compass (Suunto Co. Model KB-14/360R), according to Prichard and Forseth (1988). The repeatability of both measurements was ± 5°. Leaf orientation modified the angle of incidence (i) between the direct solar beam and the leaf lamina. The cosine of the angle of incidence was a measure of the fraction of the direct solar radiation beam that struck a planar surface.

*C. incanus* leaves were characteristically folded along the midrib. The degree of concavity was measured by a goniometer on prints of ink-dipped leaf cross sections (\( n = 120 \)) (Begg, 1980, modified).

2.5. Leaf morphology

Leaf samples (40 observations per species and per different leaf age cohort) were dried at 90°C for 3 days to constant weight. Leaf surface area (SA), excluding petiole, was measured using the Image Analysis System (Delta-T Devices, LTD). SLW was calculated as the ratio of leaf dry weight to unifacial leaf area (Reich et al., 1992). Leaf density index (LDI) was calculated by the ratio of leaf dry weight and leaf volume (g cm\(^{-3}\)), in order to express leaf compactness (Christodoulakis and Mitrakos, 1987).

2.6. Leaf anatomy

Leaf sections were hand-cut from fresh fully expanded leaves (4 month old summer leaves and 8 month old winter leaves of *C. incanus*; 1 year old leaves of *Q. ilex* and *P. latifolia*), dehydrated in 90% ethanol and analysed by light microscopy (Bolhär-Nordenkampf and Draxler, 1993). The following parameters were measured (40 observations per species): total leaf thickness (\( L \)), palisade and spongy layer thickness, thickness of the adaxial and abaxial epidermis, thickness of the adaxial and abaxial cuticle. Total leaf thickness was also measured per different leaf age cohort.

The ratio of palisade parenchyma thickness to the mesophyll thickness, that reveals xeromorphyc habitus (Xh), was calculated according to Pykkö (1966) and Christodoulakis and Mitrakos (1987).

Plasticity index (Pi) at full leaf expansion was calculated according to Carpenter and Smith
(1981) by (sun leaf thickness — shade leaf thickness)/sun leaf thickness.

2.7. Statistics

All statistical tests were performed using a statistical software package (Statistica, Statsoft USA). The distribution of $\eta_i$ and $x$ were compared to a uniform distribution by $\chi^2$ test. The differences of $\eta_i$ and $x$ during the day and between winter and summer were tested by one-way analysis of variance (ANOVA) and Tukey test for multiple comparison. Diurnal trend of $\cos(i)$ was compared to the $\cos(i)$ of a horizontal plane (depending only on solar movement) by $\chi^2$ test. Differences in mean morphological and anatomical leaf traits were determined by $t$-test, ANOVA and Tukey test for multiple comparison. Linear, quadratic and logarithmic regression relationships for all analysed leaf traits were tested.

3. Results

3.1. Leaf age

The typical evergreen sclerophyllous species, $Q. ilex$ and $P. latifolia$, had a leaf life-span (LLS) of 2–4 years: 84 and 16% leaves of $Q. ilex$ are shed respectively after 2 and 3 years; 41, 54 and 5% leaves of $P. latifolia$ are shed respectively after 2, 3 and 4 years. The seasonal leaf dimorphic shrub $C. incanus$ produced smaller leaves (summer leaves) at the beginning of May, persisting through summer and falling mostly in autumn (leaf life-span of 4 months); full leaf expansion was reached at 4 months. Larger leaves (winter leaves) were produced at the middle of September, persisting through winter and falling in spring (leaf life-span of 8 months). $P. latifolia$ and $Q. ilex$ full leaf expansion was reached at 1 year old leaves.

3.2. Leaf orientation, leaf inclination and incident $\text{PAR}$

$\eta_i$ depended strictly on phyllotaxy: $C. incanus$ and $P. latifolia$ had opposite pattern and $Q. ilex$ a whorled pattern of leaf attachment. Opposite leaves of $C. incanus$ and $P. latifolia$ were at 90°, from one to the other on the azimuthal projection, while $Q. ilex$ leaves were about 72°. To compare these patterns, leaves were monitored in all four cardinal directions. Azimuth was random with respect to sun and did not change daily, seasonally (Fig. 1) or per different leaf age cohort (the deviation from randomness and the differences of means were not significant).

$C. incanus$, $P. latifolia$ and $Q. ilex$ leaves showed a constant $x$ throughout the day (Fig. 2) (the differences of means were not significant).

$x$ changed per different leaf age cohort ($P < 0.01$) (Table 2). One year old leaves of $P. latifolia$ showed the steepest leaf inclination (59 ± 7°) in summer, decreasing with increasing leaf age ($P < 0.01$) and $Q. ilex$ showed the same trend. $C. incanus$ showed a different $x$ trend ($P < 0.01$) of winter and summer leaves: $x$ was negative (−37 ± 12°) in winter leaves while it was positive (44 ± 13°) in summer leaves. $x$ was significantly correlated with leaf age ($P < 0.01$) (Table 3): $x$ increased until full leaf expansion, then it decreased. RI showed the same trend, in particular $P. latifolia$ had the highest RI in summer (47%). $x$ was a linear function of SLW ($P < 0.05$). $C. incanus$ showed, moreover, a particular trend of leaf margin: winter leaves had a marginal leaf folding along the midrib axis (29 ± 9°) higher in summer leaves (76 ± 11°) ($P < 0.01$) (Fig. 3).

Diurnal trends of $\cos(i)$ was similar for the three species and per different leaf age cohort, depending solely on solar movement ($P < 0.01$), therefore no form of heliotropism was present.

3.3. Leaf morphology

The average leaf traits of the analysed species per different leaf age cohort are shown in Tables 2 and 4.

SLW varied significantly ($P < 0.01$) among the species and it increased until full leaf expansion was reached (maximum SA), decreasing with the increase of leaf age. Fully expanded summer leaves of $C. incanus$ were 20% smaller than winter leaves ($P < 0.05$). SLW at full leaf expansion was significantly higher ($P < 0.01$) in $Q. ilex$ and $P. latifolia$. The distribution of $\eta_i$ and $x$ were compared to a uniform distribution by $\chi^2$ test. The differences of $\eta_i$ and $x$ during the day and between winter and summer were tested by one-way analysis of variance (ANOVA) and Tukey test for multiple comparison. Diurnal trend of $\cos(i)$ was compared to the $\cos(i)$ of a horizontal plane (depending only on solar movement) by $\chi^2$ test. Differences in mean morphological and anatomical leaf traits were determined by $t$-test, ANOVA and Tukey test for multiple comparison. Linear, quadratic and logarithmic regression relationships for all analysed leaf traits were tested.

4. Discussion

The typical evergreen sclerophyllous species, $Q. ilex$ and $P. latifolia$, had a leaf life-span (LLS) of 2–4 years: 84 and 16% leaves of $Q. ilex$ are shed respectively after 2 and 3 years; 41, 54 and 5% leaves of $P. latifolia$ are shed respectively after 2, 3 and 4 years. The seasonal leaf dimorphic shrub $C. incanus$ produced smaller leaves (summer leaves) at the beginning of May, persisting through summer and falling mostly in autumn (leaf life-span of 4 months); full leaf expansion was reached at 4 months. Larger leaves (winter leaves) were produced at the middle of September, persisting through winter and falling in spring (leaf life-span of 8 months). $P. latifolia$ and $Q. ilex$ full leaf expansion was reached at 1 year old leaves.

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latifolia compared to that of *C. incanus*. Leaves of *P. latifolia* were characterised by the smallest SA, almost three times lower than *Q. ilex*. SLW significantly correlated with leaf age (*P* < 0.01), L (*P* < 0.01), LDI (*P* < 0.01) and *a* (*P* < 0.05), confirming the dependence among the leaf traits (Table

Fig. 1. Distribution of leaf orientation (η) frequency of *Cistus incanus*, *Phillyrea latifolia* and *Quercus ilex* sun leaves during the day in winter (02/02/98 and 06/03/98) and in summer (06/07/98 and 03/08/98). In winter: morning = 09:30 h (ηs = 135°), midday = 12:00 h (ηs = 174°), afternoon = 14:30 h (ηs = 212°). In summer: morning = 07:30 h (ηs = 85°), midday = 12:00 h (ηs = 177°), afternoon = 16:30 h (ηs = 269°). ηs = sun azimuth. The distribution of leaf orientation was random. Standard error is shown.
Fig. 2. Distribution of leaf inclination (α) frequency of Cistus incanus, Phillyrea latifolia and Quercus ilex sun leaves during the day in winter (02/02/98 and 06/03/98) and in summer (06/07/98 and 03/08/98). In winter: morning = 09:30 h (α = 26°), midday = 12:00 h (α = 37°), afternoon = 14:30 h (α = 28°). In summer: morning = 07:30 h (α = 30°), midday = 12:00 h (α = 72°), afternoon = 16:30 h (α = 35°). α = sun zenith. P. latifolia α was steeper in summer than in winter (P < 0.01). Standard error is shown.

3). LDI showed the same SLW trend increasing until full leaf expansion. In essence, longer leaf life-span combined with higher SLW, L, LDI and steeper α.

3.4. Leaf anatomy

The microscopical analysis of fully expanded leaves revealed significant variations among the
species (Table 5). *P. latifolia* showed a particularly thick adaxial cuticle, more than two time higer than *C. incanus*. *Q. ilex* leaves showed hairs on the abaxial surface while *C. incanus* on both the adaxial and the abaxial surfaces (data not shown). On the average *Q. ilex* and *P. latifolia* showed a densely packed mesophyll with few air spaces; on the contrary *C. incanus* showed well developed intercellular spaces (data not shown). *P. latifolia* had the highest total leaf thickness (Table 4). Xh of the three species was on the average 0.55 (Table 5). Total leaf thickness increased until full leaf expansion was reached, decreasing progressively with leaf age increase (*P* < 0.01). L significantly correlated with SLW (*P* < 0.01) (Table 3).

The plasticity index (Pi), calculated on sun and shade fully expanded leaves was the highest in *Q. ilex* (0.65) and the lowest in *C. incanus* (0.29).

### 4. Discussion

Evergreen sclerophyllous shrub species growing in the Mediterranean climate are basically xerophytes; their leaves are steeply inclined and possess similar basic anatomical organization (Walter and Kreeb, 1970; Kummerow, 1973; Catarino et al., 1981; Mitrakos and Christodoulakis, 1981; Turner, 1994; Karabourniotis, 1998). The results are in accordance with this general trend: a higher xeromorphic habitus, according to Pyykkö (1966) and Christodoulakis and Mitrakos (1987) steeper leaf inclination in summer in response to drought, according to Mooney (1982) and Comstock and Mahall (1985) thicker leaf cuticle according to Mitrakos and Christodoulakis (1981). Generally there is a great difference in sun and shade leaf

### Table 2

<table>
<thead>
<tr>
<th>LA</th>
<th>x</th>
<th>RI</th>
<th>SA</th>
<th>DW</th>
<th>SLW</th>
<th>L</th>
<th>LDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2°</td>
<td>29 ± 8</td>
<td>13</td>
<td>2.9 ± 0.3</td>
<td>25 ± 5</td>
<td>8.5 ± 0.9</td>
<td>235 ± 16</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>4°</td>
<td>44 ± 13</td>
<td>32</td>
<td>4.0 ± 0.7</td>
<td>59 ± 11</td>
<td>14.7 ± 1.5</td>
<td>244 ± 15</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>3°</td>
<td>−34 ± 10</td>
<td>17</td>
<td>3.5 ± 1.0</td>
<td>41 ± 12</td>
<td>11.9 ± 1.7</td>
<td>201 ± 14</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>5°</td>
<td>−35 ± 10</td>
<td>18</td>
<td>4.7 ± 0.8</td>
<td>63 ± 13</td>
<td>13.3 ± 1.6</td>
<td>216 ± 13</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>8°</td>
<td>−37 ± 12</td>
<td>20</td>
<td>5.0 ± 0.5</td>
<td>70 ± 16</td>
<td>14.0 ± 1.3</td>
<td>220 ± 14</td>
<td>0.64 ± 0.03</td>
</tr>
</tbody>
</table>

* LA, leaf age; x, leaf inclination; RI, reduction of PAR (μmol m<sup>2</sup> s<sup>−1</sup>) incident on the sloping leaf surface; SA, surface area; DW, dry weight; SLW, specific leaf weight; LDI, leaf density index; L, total leaf thickness. Standard error is shown.

### Table 3

Summary of regression relationships between all the analysed leaf traits (*n* = 14)*

<table>
<thead>
<tr>
<th>x–y (leaf traits)</th>
<th>Relationship</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA–L**</td>
<td>y = −0.1348x² + 8.3684x + 213.55</td>
<td>0.78</td>
</tr>
<tr>
<td>LA–SA</td>
<td>y = 0.0213x + 5.1227</td>
<td>ns</td>
</tr>
<tr>
<td>LA–SLW**</td>
<td>y = 3.4096 Ln(x) + 9.6506</td>
<td>0.78</td>
</tr>
<tr>
<td>LA–LDI</td>
<td>y = 0.0014x + 0.5889</td>
<td>ns</td>
</tr>
<tr>
<td>LA–x**</td>
<td>y = −0.0298x² + 1.2224x + 34.996</td>
<td>0.62</td>
</tr>
<tr>
<td>L–SA</td>
<td>y = 0.0104x + 2.4849</td>
<td>ns</td>
</tr>
<tr>
<td>L–SLW**</td>
<td>y = 0.0623x</td>
<td>0.90</td>
</tr>
<tr>
<td>L–LDI</td>
<td>y = 0.0006x + 0.4449</td>
<td>ns</td>
</tr>
<tr>
<td>L–x</td>
<td>y = 0.0868x + 16.108</td>
<td>ns</td>
</tr>
<tr>
<td>SA–SLW</td>
<td>y = 0.5436x + 14.776</td>
<td>ns</td>
</tr>
<tr>
<td>SA–LDI*</td>
<td>y = 0.0905 Ln(x) + 0.4686</td>
<td>0.57</td>
</tr>
<tr>
<td>SA–x</td>
<td>y = 0.4935x + 38.321</td>
<td>ns</td>
</tr>
<tr>
<td>SLW–LDI**</td>
<td>y = 0.2115 Ln(x) + 0.0102</td>
<td>0.78</td>
</tr>
<tr>
<td>SLW–x*</td>
<td>y = 1.2507x + 18.821</td>
<td>0.60</td>
</tr>
<tr>
<td>LDI–x</td>
<td>y = 55.329x + 7.1915</td>
<td>ns</td>
</tr>
</tbody>
</table>

* LA, leaf age (months); L, total leaf thickness (μm); SLW, specific leaf weight (mg cm<sup>−2</sup>); SA, leaf surface area (cm<sup>2</sup>); LDI, leaf density index (g cm<sup>−2</sup>); x, leaf inclination (°).

** The relationships were significant at *P* < 0.01; and

* at *P* < 0.05, unless noted ns (not significant, *P* > 0.05).
Table 4 and Smith, 1981) and comparison to other woody species (Carpenter analysed species have a high plasticity index in fitness (Bradshaw, 1965; Osmond, 1987; Fitter plasticity increases the species fitness and adaptiveness (Chabot et al., 1979; Williams and Black, 1993). High phenotypic plasticity increases the species fitness and adaptiveness (Bradshaw, 1965; Osmond, 1987; Fitter and Hay, 1993; Murchie and Horton, 1997). The analysed species have a high plasticity index in comparison to other woody species (Carpenter and Smith, 1981) and Q. ilex has the absolute highest Pi.

Nevertheless the analysis of morphological and anatomical leaf traits reveal different adaptive strategies. Q. ilex and P. latifolia maintain their leaves until 3–4 years while C. incanus has a LLS of 4 (summer leaves) to 8 months (winter leaves). Therefore the evergreenness character of C. incanus is maintained by sequentially formed leaf age cohorts rather than by extended leaf longevity. Leaf life-span is an important life-history trait of plants with respect to their development and response to light, nutrient availability, drought and herbivory (Chapin, 1980; Gray and Schlesinger, 1983; Reich et al., 1987; Codey, 1988;

Table 5
Anatomical leaf traits at full leaf expansion of Cistus incanus, Phillyrea latifolia and Quercus ilex

<table>
<thead>
<tr>
<th></th>
<th>C. incanus</th>
<th>P. latifolia</th>
<th>Q. ilex</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_d (μm)</td>
<td>7.2 ± 0.7^a</td>
<td>16.8 ± 1.7^b</td>
<td>9.7 ± 1.8^c</td>
</tr>
<tr>
<td>E_d (μm)</td>
<td>22.3 ± 3.5^a</td>
<td>19.9 ± 3.1^a</td>
<td>14.1 ± 1.4^b</td>
</tr>
<tr>
<td>P (μm)</td>
<td>107.2 ± 9.1^a</td>
<td>141.2 ± 15.7^b</td>
<td>163.2 ± 16.0^c</td>
</tr>
<tr>
<td>S (μm)</td>
<td>93.1 ± 12.1^a</td>
<td>138.0 ± 16.6^b</td>
<td>106.4 ± 15.5^c</td>
</tr>
<tr>
<td>E_a (μm)</td>
<td>10.8 ± 1.0^a</td>
<td>15.8 ± 1.8^b</td>
<td>9.3 ± 0.8^c</td>
</tr>
<tr>
<td>C_a (μm)</td>
<td>3.3 ± 1^a</td>
<td>6.7 ± 0.7^b</td>
<td>7.2 ± 0.9^c</td>
</tr>
<tr>
<td>Xh</td>
<td>0.54 ± 0.04^a</td>
<td>0.51 ± 0.05^a</td>
<td>0.61 ± 0.06^b</td>
</tr>
<tr>
<td>Pi</td>
<td>0.65 ± 0.04^a</td>
<td>0.55 ± 0.04^b</td>
<td>0.29 ± 0.02^c</td>
</tr>
</tbody>
</table>

^a C_d, adaxial cuticle thickness; E_d, adaxial epidermis thickness; P, palisade parenchyma thickness; S, spongy parenchyma thickness; E_a, abaxial epidermis thickness; C_a, abaxial cuticle thickness; Xh, palisade parenchyma thickness/mesophyll thickness; Pi, plasticity index. Means with the same letter are not significantly different (P > 0.05). Standard error is shown.

Table 4

<table>
<thead>
<tr>
<th>LA</th>
<th>x (°)</th>
<th>RI (%)</th>
<th>SA (cm^2)</th>
<th>DW (mg)</th>
<th>SLW (mg cm^-2)</th>
<th>LDI (g cm^-2)</th>
<th>L (μm)</th>
<th>LDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. latifolia</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>8</td>
<td>45 ± 8</td>
<td>29</td>
<td>3.0 ± 0.6</td>
<td>61 ± 12</td>
<td>20.9 ± 2.5</td>
<td>330 ± 15</td>
<td>0.61 ± 0.02</td>
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</tr>
<tr>
<td>12</td>
<td>59 ± 7</td>
<td>47</td>
<td>3.6 ± 0.6</td>
<td>77 ± 14</td>
<td>21.3 ± 2.2</td>
<td>338 ± 18</td>
<td>0.63 ± 0.03</td>
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</tr>
<tr>
<td>24</td>
<td>48 ± 9</td>
<td>33</td>
<td>3.5 ± 0.7</td>
<td>74 ± 13</td>
<td>21.2 ± 2.0</td>
<td>338 ± 17</td>
<td>0.63 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>39 ± 5</td>
<td>22</td>
<td>3.5 ± 0.5</td>
<td>72 ± 13</td>
<td>20.6 ± 1.8</td>
<td>332 ± 15</td>
<td>0.62 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>31 ± 6</td>
<td>14</td>
<td>3.3 ± 0.5</td>
<td>66 ± 15</td>
<td>19.9 ± 2.0</td>
<td>328 ± 14</td>
<td>0.61 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Q. ilex</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>51 ± 11</td>
<td>35</td>
<td>9.9 ± 2.6</td>
<td>209 ± 66</td>
<td>20.9 ± 1.7</td>
<td>305 ± 18</td>
<td>0.68 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>52 ± 11</td>
<td>37</td>
<td>10.0 ± 2.8</td>
<td>213 ± 77</td>
<td>21.3 ± 1.9</td>
<td>310 ± 21</td>
<td>0.69 ± 0.05</td>
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</tr>
<tr>
<td>24</td>
<td>38 ± 10</td>
<td>21</td>
<td>9.9 ± 2.7</td>
<td>205 ± 69</td>
<td>20.7 ± 1.6</td>
<td>309 ± 19</td>
<td>0.67 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>33 ± 9</td>
<td>16</td>
<td>9.7 ± 2.5</td>
<td>193 ± 58</td>
<td>19.9 ± 1.6</td>
<td>308 ± 18</td>
<td>0.65 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

^a LA, leaf age; x, leaf inclination; RI, reduction of PAR (μmol m^-2 s^-1) incident on the sloping leaf surface; SA, surface area; DW, dry weight; SLW, specific leaf weight; LDI, leaf density index; L, total leaf thickness. Standard error is shown.
Reich et al., 1992). Even when growing in the same site, different plant species show adaptive differences in LLS and related plant traits (Reich et al., 1992; Gower et al., 1993). The highest LLS of the examined evergreen sclerophyllous species with respect to the semi-deciduous species may be necessary to cover the higher leaf production costs and achieve a net profit in carbon gain (Mooney, 1981). SLW is considered a useful index of xeromorphism (Witkowski and Lamont, 1991). The results show that SLW significantly correlates with total leaf thickness, particularly in palisade parenchyma thickness. Many authors have demonstrated that reflectance increases and transmittance decreases with an increase in leaf thickness (Gausman et al., 1973; Knapp and Carter, 1998). Palisade layers may act more as ‘light conducting pipes’ into the leaf, whereas spongy mesophyll layers may scatter light more efficiently (Vogelmann, 1993). The thickness of the cuticle often corresponds with the degree of leaf xeromorphism (Bolhär-Nordenkampf and Draxler 1993), and it is considered part of the leaf construction cost (Koike 1988). In the examined species SLW changes according to leaf age and the maximum SLW is reached at full leaf expansion, generally corresponding with the maximum photosynthetic rates (Gratani, unpublished; Woolhouse, 1967). SLW decreases during senescence. An adaptive feature of evergreen leaves may be that older leaves provide a sink for nutrient storage during nutrient uptake (Kutbay and Kilinc¸, 1994). C. incanus summer leaves have the lowest SA and the highest SLW with respect to winter leaves, reducing the evaporative leaf surface during summer. Moreover the maximum litter-fall in May–June, just before summer drought, reduces the system’s nutrient loss due to a lack of leaching by rainwater, according to Nuñez-Olivera et al. (1993).

Q. ilex and P. latifolia have steeper z than C. incanus. The higher z reduces the leaf amount of solar radiation (Ludlow and Björkman, 1984; Kao and Forseth, 1991, 1992) in the evergreen sclerophyllous species that maintain their leaves throughout the year. The higher z may be also a means of preventing photoinhibition of water-stressed leaves and it may provide a mechanism for reducing transpiration rates during drought by lowering leaf temperatures (Comstock and Mahall, 1985). Older leaves are shaded by new and consequently have a lower z.

C. incanus leaf folding may be related to the less xeromorphic structure of leaves. Water stress may induce leaf folding as a means of improving water use efficiency and avoiding photoinhibition (Chiarello et al., 1987; Werner et al., 1999). By the seasonal leaf dimorphism and leaf folding, the adjustment of leaf inclination angle from −37° in winter to +44° in summer leaves increases RI during drought.

The examined species at full leaf expansion were compared by analysing the degree of xeromorphism, expressed by calculating the surface area of the polygon plotted joining the value of the considered leaf xeromorphic traits (Fig. 4).
The area of the polygon in the radar graph integrates many xeromorphic leaf traits, resulting in an integrative index of xeromorphism. Considering all the analysed leaf traits (LLS, SLW, LDI, L, z, Xh, Pi) the two sclerophyllous shrub, which show the larger area (0.88 and 0.87, respectively, for P. latifolia and Q. ilex) are the more xeromorphic species and the semi-deciduous which has the lowest (0.44) is the less xeromorphic species.

The results on the whole enable one to underline the strong influence of leaf inclination on the received solar radiation and the influence of incident light level on leaf structure during leaf age. Moreover it is possible to define adaptive strategies of Q. ilex, P. latifolia and C. incanus: the typical evergreen sclerophyllous species show longer LLS, steeper z, and higher SLW, LDI, L, that is the opposite in the semi-deciduous species. As global change effects on Mediterranean climate are likely to provide an increase of air temperature and drought (Houghton et al., 1990; Peñuelas, 1996), it is important to understand the morphological and physiological responses of these species in order to predict possible changes in species dominance and in landscape structure (Filella et al., 1998). The results indicate that Q. ilex and P. latifolia will be at a competitive advantage on C. incanus, considering that drought stress increase may determine a shortening of leaf life-span and that C. incanus is the species with the lower LLS, the lower SLW and the lower Pi (lower adaptativeness). A high degree of xeromorphism may be more advantageous to increasing aridity in the regions with Mediterranean climate.

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