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Contrasting foliar responses to drought in *Quercus ilex* and *Phillyrea latifolia*

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Abstract

Leaf morphology, longevity, and demography were examined in *Quercus ilex* and *Phillyrea latifolia* growing in a holm oak forest in Prades mountains (northeast Spain). Four plots (10 × 15 m) of this forest were submitted to an experimental drought during three years (soil moisture was reduced about 15 %). Leaf area, thickness and leaf mass per area ratio (LMA) were measured in sun and shade leaves of both species. Leaf longevity, the mean number of current-year shoots produced per previous-year shoot (Sn/Sn-1), the mean number of current-year leaves per previous-year shoot (Ln/Sn-1), and the percentage of previous-year shoots that developed new ones were measured once a year, just after leaf flushing. LMA and leaf thickness increased since leaf unfolding except in summer periods, when stomatal closure imposed low photosynthetic rates and leaves consumed their reserves. LMA, leaf area, and leaf thickness were higher in *Q. ilex* than in *P. latifolia*, but leaf density was higher in the latter species. Drought reduced the leaf thickness and the LMA of both species *ca*. 2.5 %. Drought also increased leaf shedding up to *ca*. 20 % in *Phillyrea latifolia* and decreased it up to *ca*. 20 % in *Q. ilex.* In the later species, Sn/Sn-1 decreased by 32 %, Ln/Sn-1 by 41 %, percentage of shoots developed new ones by 26 %, and leaf area by 17 %. Thus the decrease of leaf number and area was stronger in the less drought-resistant *Q. ilex*, which, under increasingly drier conditions, might lose its current competitive advantage in these Mediterranean holm oak forests.

Additional key words: climate change, leaf longevity, leaf mass per area ratio, Mediterranean forest.

Introduction

Water availability in the Mediterranean regions is likely to be reduced in the near future by the predicted increases of temperatures, and the consequent increases of evapotranspiration rates, (Piñol *et al.* 1998, Peñuelas *et al.* 2002, 2005). Among the different species, different morphological and physiological responses are expected under the new climatic conditions.

It has often been observed that thickening and high concentration of mechanical cells and thicker cell walls give Mediterranean sclerophyllous leaves a high leaf mass per area ratio (LMA) (Cowling and Campbell 1983). Several studies have shown an increment of LMA under drought (Salleo and Lo Gullo 1990, Wookey *et al.* 1995, Carter *et al.* 1997, Yin 2002), and in Mediterranean species such as *Quercus ilex*, low LMA values during the wettest periods have often been observed (Gratani 1996, Harley *et al.* 1987, Kyparisis and Manetas 1993), and also an important leaf damage and fall of leaves is observed in *Q. ilex* under severe water stress (Pesoli *et al.* 2003). Higher LMA can be produced by increases in leaf density, in leaf thickness or both. High leaf thickness is often related to a great tolerance to drought (Chandra *et al.* 2004), but recent studies showed an important decrease in leaf thickness under low irradiance and an important increase in leaf density under low soil water availability (Witkowsky and Lamont 1991, Groom and

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Abbreviations: LMA - leaf mass per area ratio; Ln/Sn-1 - number of current-year leaves produced per previous-year shoot; Sn/Sn-1 - number of current-year shoots produced per previous-year shoot.
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Lamont 1997, Niinemets 2001). Therefore, it seems that sclerophyll leaf structure is not linked to any particular environmental stress and that can respond differently in sun than in the shade leaves.

Some Mediterranean plants have higher leaf longevity and higher LMA than plants from more mesic environments, but in sites with a long dry period, drought resistant semi deciduous plants with lower LMA values, shorter lifespan, and greater CO_2 uptake rates are more frequent (Harley *et al.* 1987, Kyparissis and Manetas 1993, Werner *et al.* 1999).

Under the expected drier conditions for the next decades, it is likely that more drought tolerant species will be favored in detriment of more mesic species. Holm oak (*Q. ilex* L.) is a drought-adapted tree widely distributed in the Mediterranean basin, *Phillyrea latifolia* L. is a tall shrub common in holm oak forests that is more drought tolerant than *Q. ilex* (Lloret and Siscart 1995, Peñuelas *et al.* 1998, 2000, Ogaya and Peñuelas 2003). In previous studies we found that an experimental field drought (15 % decreased soil water availability) produced only very slight decreases of instantaneous photosynthetic rates in these species (Ogaya and Peñuelas 2003) and instead strong decreases in biomass accumulation especially in *Q. ilex* (Ogaya *et al.* 2003). We wondered whether part of this apparent contradiction was related to leaf morphology and total leaf area. We studied the effects of an experimental drought on morphology, longevity and demography of sun and shade leaves in these two co-occurring Mediterranean woody species with contrasting performances under low water availabilities. We aimed *1*) to gain a better knowledge of the leaf morphology, longevity and demography of these Mediterranean species that could provide further understanding on the apparent contradiction between clear drought effects on their plant growth (Ogaya *et al.* 2003) and scarce drought effects on their foliar photosynthetic rates (Ogaya and Peñuelas 2003) and *2*) to conduct a better prediction of the performance of these co-dominant holm oak forest species in a near future climate scenario of drier conditions.

Materials and methods

Study site: The study was carried out in a holm oak forest growing at Prades Mountains in North-Eastern Iberian peninsula (41°13'N, 0°55'E), on a south-facing slope (25 % slope) at 930 m a.s.l. The soil is a stony xerochrept on a bedrock of metamorphic sandstone, and its depth ranges between 35 and 90 cm. Summer drought is pronounced and usually lasts for 3 months.

This holm oak forest is very dense (1.66 trees m^{-2}), trees are about 4 - 8 m high and has a mean stem diameter (measured at 50 cm height) of 5 cm. This forest is dominated by *Quercus ilex* L., *Phillyrea latifolia* L., and *Arbutus unedo* L. with abundant presence of other evergreen species well adapted to drought conditions (*Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L.), and occasional individuals of deciduous species such as *Sorbus torminalis* (L.) Crantz and *Acer monspessulanum* L.

Experimental design: Eight 15 × 10 m plots were delimited at the same altitude along the slope. Half the plots received the dry treatment and the other half were used as control plots. The dry treatment consisted of partial rain exclusion by suspending PVC strips and funnels at a height of 0.5 - 0.8 m above the soil, similarly to the experimental systems used by Borghetti *et al.* (1998) and Hanson *et al.* (2003). Strips and funnels covered approximately the 30 % of the total plot surface. Moreover, a 0.8 m deep ditch was excavated along the entire top edge of the upper part of the treatment plots to intercept runoff water supply. Dry treatment was started in March 1999.

Temperature and precipitation were monitored each half-hour by an automatic meteorological station installed between the plots in a forest gap. Soil moisture was measured every two weeks throughout the experiment by time domain reflectometry (*Tektronix 1502C*, Beaverton, USA) (Zegelin *et al.* 1989) due to three stainless steel cylindrical rods, 0.25 m long, permanently fully driven into the soil at four randomly selected places in each plot. We also determined the soil moisture at permanent wilting point measuring soil desorption curves with a *WP4 Dew Point Hygrometer* (*Decagon Devices, Inc.*, Pullman, Washington) (Perfect *et al.* 2004).

Measurements of leaf morphology, longevity, demography, and litterfall: Five *Quercus ilex* and five *Phillyrea latifolia* trees were randomly selected in each plot. In each selected tree, one current-year leaf and one leaf of the previous year were collected in the upper layer of the canopy (sun leaves) and in the lower layers of the canopy (shade leaves) every annual season from summer 1998 to winter 2001 (the midday photosynthetic photon flux density was on average 1406 ± 20 and 139 ± 4 $\mu mol\ m^{-2}\ s^{-1}$ for sun and shade leaves, respectively). Leaf area of collected leaves was measured in a *LI-COR 3100 Area Meter* (*Li-Cor Inc.*, Lincoln, USA), leaf thickness was measured using a Palmer micrometer, and leaf dry mass was measured by weighing the leaves after they had reached constant mass in an oven at 70 °C. Leaf mass per area ratio (LMA) was calculated by dividing leaf dry mass by leaf area, and leaf density was calculated by dividing leaf dry mass by leaf volume (leaf area × leaf thickness).

To follow leaf demography, five *Quercus ilex* and five *Phillyrea latifolia* trees were randomly selected in each plot in summer 1998. Two well-developed branches were marked with plastic tags in each tree, one branch in the upper layer of the canopy (sun leaves), and the other branch in the lower layer of the canopy (shade leaves). All the current-year shoots were measured and their leaves were counted as a cohort 1998, and also all the new shoots and new leaves growing over the cohort 1998 shoots were measured and counted in the two following years (1999 and 2000). In summer 1999 two other well-developed branches (one on the sun and one on the shade) were marked in the same selected trees, and shoots and leaves growing during year 2000 on these branches were measured and counted too. Leaf demography of all counted leaves was followed in each annual season (spring, summer, autumn, and winter) from summer 1998 to summer 2002.

Mean number of current-year shoots produced per previous-year shoot (Sn/Sn-1), mean number of current-year leaves per previous-year shoot (Ln/Sn-1), and the percentage of previous-year shoots that developed new shoots were analyzed each year to estimate the production of new leaves.

Litterfall was collected in 20 circular baskets (of 27 cm diameter with a 1.5 mm mesh) randomly distributed on the ground of each one of the eight plots. The fallen litter was collected every fifteen days from winter 1999 to winter 2001 and every two months from winter 2001 to summer 2002, oven-dried and weighed.

To establish the proportion of fallen leaves relative to total leaf biomass, the latter was estimated by allometric relationships between tree leaf biomass (LB) and stem diameter at 50 cm height (D50) measured in *Q. ilex* ($\ln \mathrm{LB} = 3.48 + 1.70 \times \ln \mathrm{D50}$; $r^2 = 0.91$; $n = 12$) and *P. latifolia* ($\ln \mathrm{LB} = 1.43 + 2.43 \times \ln \mathrm{D50}$; $r^2 = 0.81$; $n = 13$) growing in the area of study (outside the plots). The stem diameter was measured in all *Q. ilex* and *P. latifolia* trees growing in the plots.

Statistical analyses: Analysis of variance (*ANOVA*) was conducted with the mean of the LMA, leaf area, leaf thickness, and leaf density in each plot as dependent variables and with species, treatment application, season, and canopy position as independent factors. One way *ANOVA* was conducted by each cohort of leaves (1998, 1999, and 2000). Analyses of covariance (*ANCOVA*) were used to test the species and treatment differences in the relationships between LMA and leaf thickness, and between LMA and leaf density. *ANCOVA* was conducted with sun and shade leaves separately. Another *ANOVA* was conducted with the mean of the length of the shoots, mean number of current-year shoots produced per previous-year shoot, mean number of current-year leaves per previous-year shoot, and percentage of developed previous-year shoots in each plot as dependent variables and with species, treatment application, leaf cohort, and canopy position as independent factors. Data of percentage of developed previous-year shoot was transformed to arcsin $(p)^{1/2}$ to reach the normality assumptions of the *ANOVA*. We used Kaplan-Meyer non-parametric method for the computation of leaf survival curves for each leaf cohort. We thereafter used the Log-Rank test to assess treatment differences. Another *ANOVA* was conducted with the leaf fall expressed as percentage of the leaf biomass remaining in the canopy (calculated from allometric relationships) as a dependent variable and species, year, and treatment application as independent factors. Data of percentage of the leaf fall was transformed to arcsin $(p)^{1/2}$ to reach the normality assumptions of the *ANOVA*. All analyses were performed with the *Statview* software package (*Abacus Concepts* 1998) and the *Statistica* software package (*StatSoft* 2001).

Results

Meteorological data and soil water status: Climatic data during these two years of experimental set-up was typical from Mediterranean sites. Mean annual temperature was slightly higher in the year 2000 (12.4 °C) than in the year 1999 (12.2 °C). Rainfall was also higher in 2000 (727 mm) than in 1999 (610 mm). In 2000 rainfall was concentrated in late spring and late autumn whereas in 1999 it was concentrated in early spring and early autumn (Fig. 1).

Soil moisture showed great fluctuations during this period but was 3.3 % higher in 2000 than in 1999 (Fig. 1) in agreement with meteorological data. Minimum values were reached in summer, as a consequence of summer drought, and maximum values in spring and autumn, coinciding with heavy rainfall periods (Fig. 1). Differences in soil moisture between control and dry plots were significant on most of the samplings throughout the two years (Fig. 1). Control plots had on average 16 % higher soil moisture than dry plots in 2000 and 13 % in 1999. Permanent wilting point in the soil of studied plots is reached when water content is lower than 7.6 % (v/v), and soil moisture in the plots was always higher (Fig. 1).

Morphological measurements: LMA and leaf thickness continuously increased since leaf unfolding until the next spring (the maximum increase occurred the first spring while leaf was fully developing) and then slightly decreased the next summer. LMA, leaf area, and leaf thickness were higher in *Q. ilex* than in *P. latifolia* during

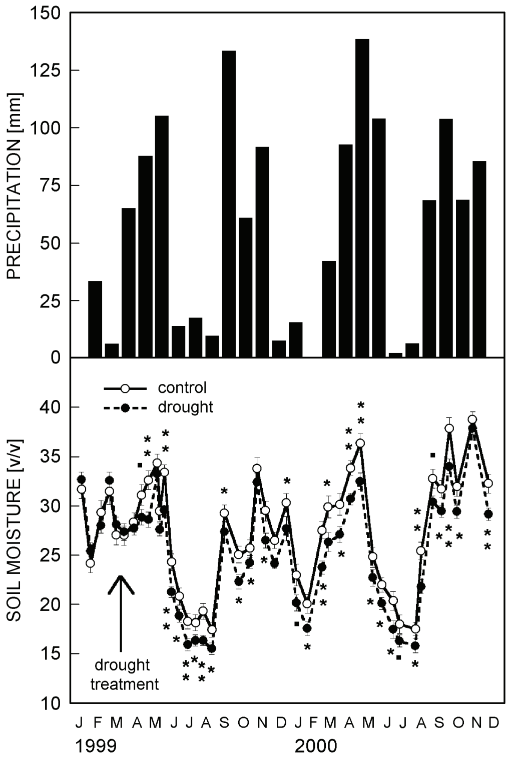

Fig. 1. Seasonal course of soil moisture (0.25 m depth) and precipitation at the Prades (NE Iberian peninsula) study site. *Vertical bars* indicate standard error of the mean (SE; $n = 4$ plot average of 4 TDR measurements per plot). *One square* indicates ($P < 0.1$) significant differences between the two treatments, *one asterisk* ($P < 0.05$), and *two asterisks* ($P < 0.01$).

all seasons ($P < 0.001$), but leaf density was higher in *P. latifolia* than in *Q. ilex* ($P < 0.001$). Sun leaves of both species showed higher LMA, thickness, and lower area than shade ones ($P < 0.001$) (Figs. 2, 3, and 4), but density values were similar in sun and shade canopy positions and in the two treatments (data not shown).

In 1998 and 1999 leaf cohorts, leaves from control plots showed *ca.* 3 % higher LMA ($P < 0.01$) and leaf thickness ($P < 0.05$) than leaves from dry plots in both species and in both years, and in 1999, higher leaf area only in shade leaves of *Q. ilex* (Figs. 2 - 4). In 2000 leaf cohort, leaf area of *Q. ilex* also tended to be higher in leaves from control plots than in leaves from dry ones (Fig. 4), and LMA and leaf thickness were slightly higher in sun leaves from control plots than in sun leaves from dry plots (3.6 %, $P = 0.068$, and 2.9 %, $P = 0.062$ for LMA and thickness, respectively) (Figs. 2 and 3).

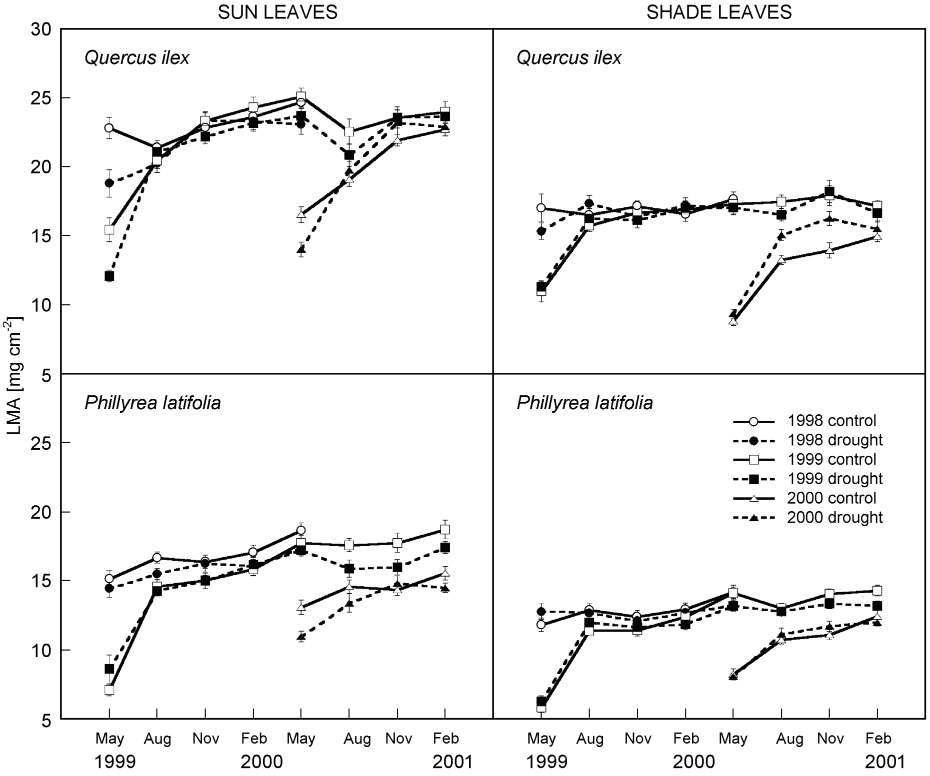

Fig. 2. Seasonal course of leaf mass per area ratio (LMA) in leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. *Error bars* indicate SE ($n = 4$ plot averages of 5 measurements per plot).

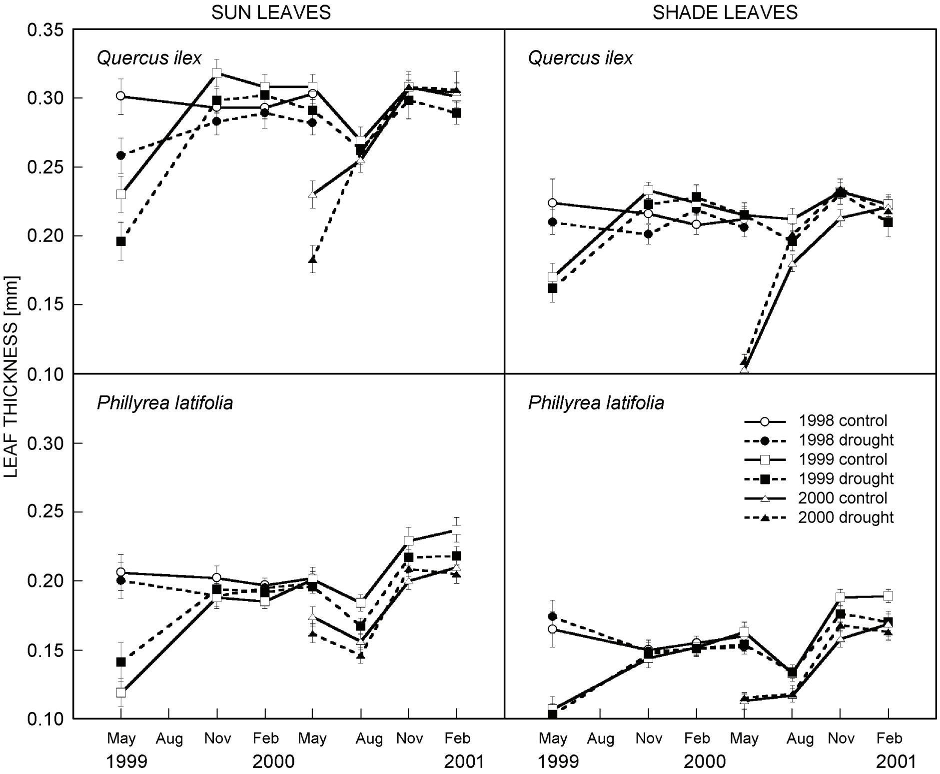

Fig. 3. Seasonal course of leaf thickness in leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. *Error bars* indicate SE ($n = 4$ plot averages of 5 measurements per plot).

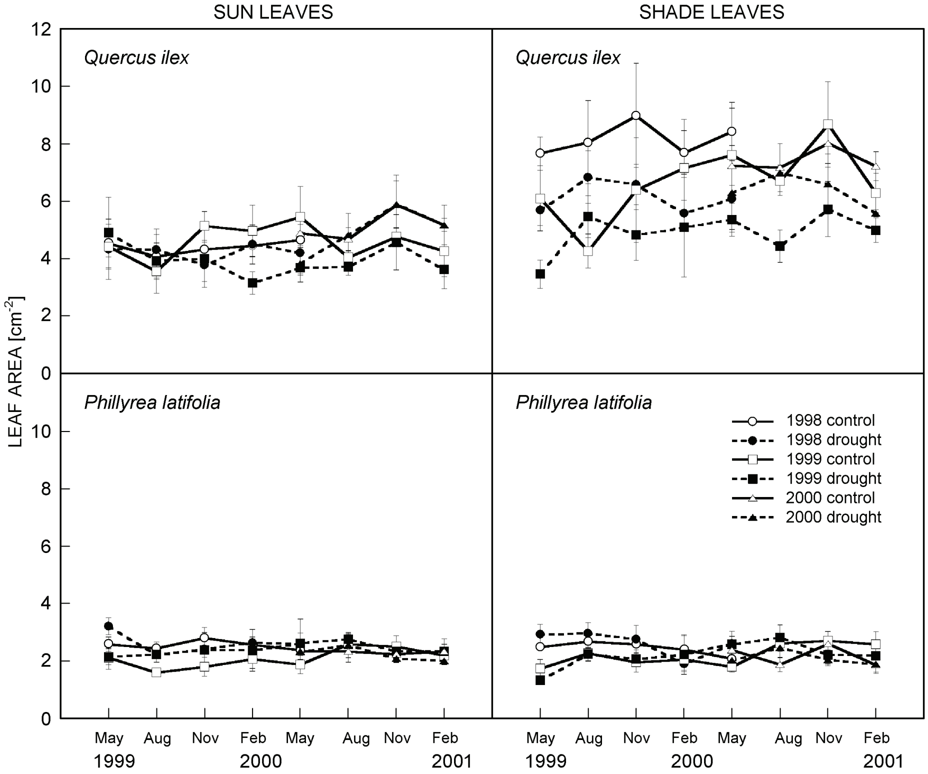

Fig. 4. Seasonal course of leaf area in leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. *Error bars* indicate SE ($n = 4$ plot averages of 5 measurements per plot).

However, shade *Q. ilex* leaves of 2000 cohort showed slightly higher LMA and leaf thickness in dry plots than in control ones.

LMA-leaf thickness relationships showed a good fit in all cases. Their slopes were very similar in both species and canopy position. LMA-leaf density relationships showed worse fits and lower slopes than LMA-leaf thickness relationships, especially for shade leaves of *Q. ilex* (Table 1). The slopes of LMA-leaf density relationships were higher in sun leaves than in shade ones ($P < 0.001$). No significant differences were found between control and dry plots for any of these two types of relationships.

Leaf longevity and demography: Shade leaves lasted longer than sun leaves ($P < 0.001$), and *Q. ilex*

Table 1. Relationships between leaf mass per area ratio and leaf thickness, and leaf density in sun and shade leaves of *Quercus ilex* and *Phillyrea latifolia*. All relationships are significant ($P < 0.01$), $n = 42$.

	Position	Species	Regression equations	r^2
LMA	sun	*Q. ilex*	y = -0.03 + 75.83x	0.68
[mg cm^{-2}]	sun	*P. latifolia*	y = -0.20 + 81.36x	0.60
	shade	*Q. ilex*	y = 2.92 + 61.79x	0.69
	shade	*P. latifolia*	y = 0.22 + 77.36x	0.66
Leaf	sun	*Q. ilex*	y = -2.39 + 31.60x	0.36
thickness	sun	*P. latifolia*	y = -0.83 + 19.67x	0.48
[mm]	shade	*Q. ilex*	y = 13.14 + 3.75x	0.01
	shade	*P. latifolia*	y = 1.01 + 13.64x	0.35

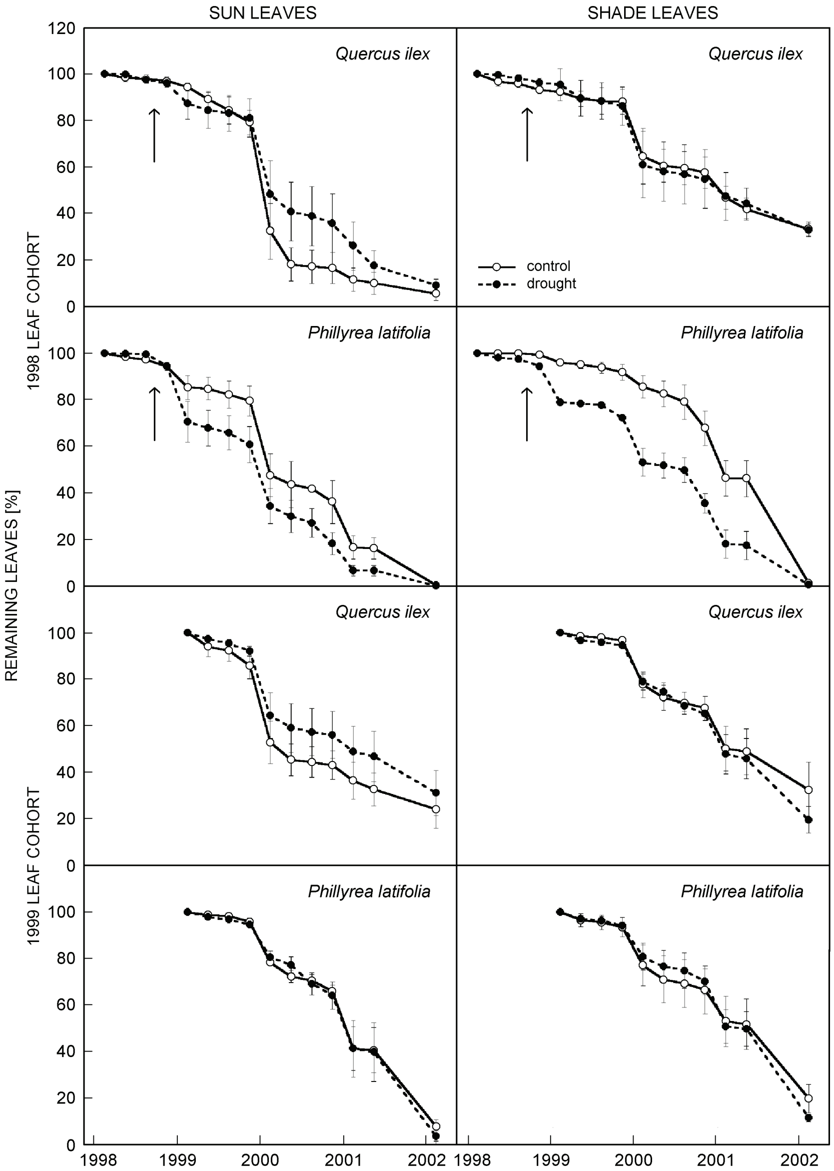

Fig. 5. Seasonal course of the percentage of remaining sun and shade leaves of *Quercus ilex* and *Phillyrea latifolia* flushed in 1998 and 1999. *Error bars* indicate SE ($n = 4$ plot averages of 10 - 31 shoots).

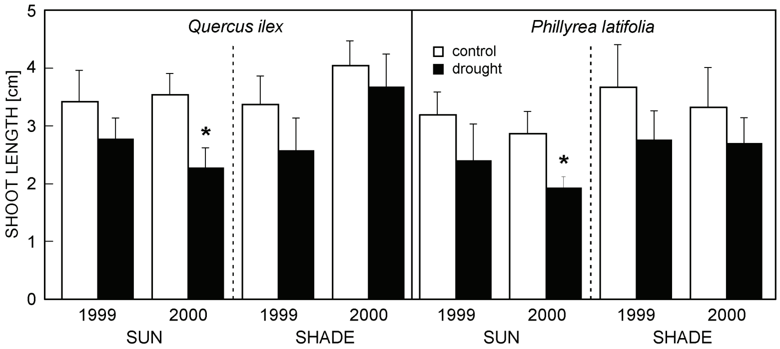

Fig. 6. Length of sun and shade *Quercus ilex* and *Phillyrea latifolia* shoots flushed in 1999 and 2000. *One asterisk* indicates ($P < 0.05$) significant differences between the two treatments. *Error bars* indicate SE (n = 4 plot averages of 10 - 31 shoots).

experimented on average, similar percentage of remaining leaves than *P. latifolia*. In the 1998 cohort, the percentage of remaining leaves differed in control and dry plots depending on the species. *P. latifolia* showed higher percentage of remaining leaves in control plots than in dry plots (*ca*. 20 %, $P < 0.001$), whereas *Q. ilex* showed slightly higher percentages in dry plots than in control plots (*ca*. 20 %), when only sun leaves were considered ($P = 0.037$) (Fig. 5). Leaves of 1999 cohort showed similar values in both species and in the two treatments (Fig. 5).

Current-year shade-shoots tended to be longer than current-year sun-shoots ($P = 0.064$), but no differences were established between the two species (Fig. 6). Shoot length was higher in control plots than in dry ones during the two years, 1999 and 2000 (21.4 and 25.2 % in *Q. ilex* and *P. latifolia*, respectively; $P = 0.002$).

Mean number of current-year shoots produced per previous-year shoot (Sn/Sn-1), and mean number of current-year leaves per previous-year shoot (Ln/Sn-1) were higher in the sun than in the shade during the overall experimental period ($P < 0.050$ in both variables for 1999 and 2000) (Fig. 7). Sn/Sn-1 and Ln/Sn-1 were higher in *P. latifolia* than in *Q. ilex* during 1999 ($P = 0.01$ for the two variables). On the contrary, Sn/Sn-1 and Ln/Sn-1 were higher in *Q. ilex* in the year 2000 ($P < 0.001$ for the two variables). In the year 2000 these variables were also higher in control plots than in dry ones ($P = 0.019$ and 0.005 for Sn/Sn-1 and Ln/Sn-1, respectively) (Fig. 7).

In 1999, the percentage of developed shoots was higher in the sun than in the shade ($P = 0.021$), and higher in *P. latifolia* than in *Q. ilex* ($P = 0.031$) (Fig. 7), but in the year 2000, the percentage of developed shoots on branches marked in 1999 was higher in *Q. ilex* than in *P. latifolia* ($P < 0.001$). Drought treatment had little influence on the percentage of developed shoots. Only in *Q. ilex* there was a trend towards a lower percentage of developed shoots in drought plots than in control ones ($P = 0.074$).

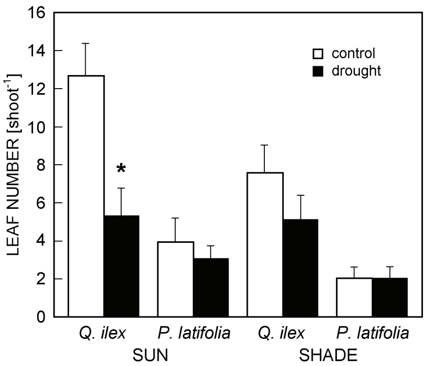

Fig. 7. Mean number of current-year leaves produced per previous-year shoot (Ln/Sn-1) in sun and shade *Quercus ilex* and *Phillyrea latifolia* shoots. The shoots were flushed in 1999 and were measured in 2000. *One asterisk* indicates ($P < 0.05$) significant differences between the two treatments. *Error bars* indicate SE (n = 4 plot averages of 10 - 31 shoots).

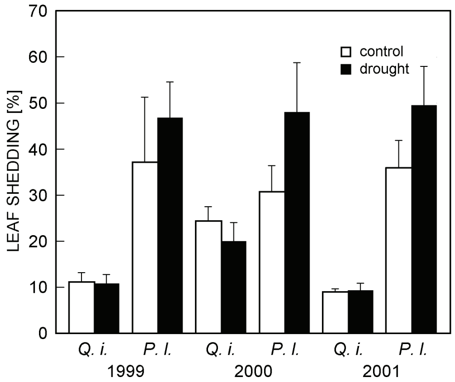

Fig. 8. Percentage of annual leaf shedding of *Quercus ilex* and *Phillyrea latifolia* referred to their total amount of leaves in each plot. *Error bars* indicate SE (n = 4 plots). Drought treatment had an overall significant effect on *P. latifolia* ($P = 0.05$).

Litterfall and overall plant leaf demography: Leaf shedding was greater in *P. latifolia* than in *Q. ilex* during the overall studied period ($P < 0.001$). *Q. ilex* experienced higher rates in leaf shedding in the year 2000 than in 1999 or 2001, but *P. latifolia* showed similar rates during the three years. The leaf shedding of *P. latifolia* was greater in drought plots than in control ones ($P = 0.05$) (Fig. 8). On the other hand, in 1999 the sum of leaves from 1998 and 1999 cohorts was higher in *P. latifolia* than in *Q. ilex* ($P = 0.063$) (Fig. 9). In shoots marked in 1999, the sum of leaves from 1999 and 2000 cohorts was higher in *Q. ilex* than in *P. latifolia* ($P < 0.001$), and higher in control plots than in drought ones when only *Q. ilex* was considered ($P = 0.002$) (Fig. 9). *Q. ilex* showed higher sum of leaves and proportion of young leaves in control plots than in drought ones both for sun and shade leaves in the 1999 shoots, *i.e.* those grown after the drought treatment started (February 1999), but *P. latifolia* only showed this trend in the upper canopy during 2000 year (Fig. 9).

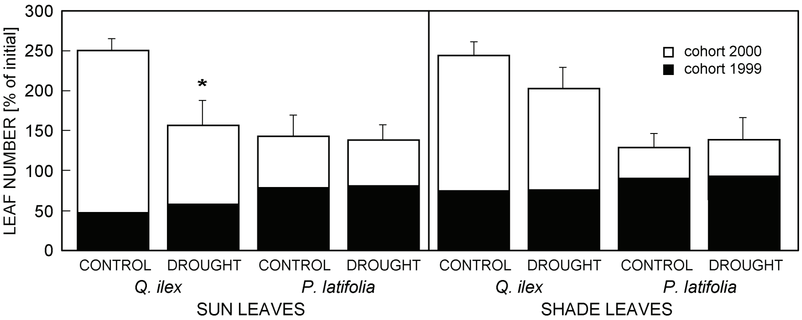

Fig. 9. Number of leaves relative to the initial number of leaves in shoots marked in 1999 and measured in year 2000. *One asterisk* indicates ($P < 0.05$) significant differences between the two treatments. *Error bars* indicate SE ($n = 4$ plot averages of 10 - 31 shoots).

Discussion

Lower LMA induced by drought: High LMA has often been related to a capacity to resist low water availabilities, but in this study we report lower LMA values in the more drought resistant species *P. latifolia* than in *Q. ilex*, and in dry plots than in control ones. Decrease in LMA can be produced by decrease in leaf thickness, leaf density, or both (Witkowsky and Lamont 1991). Leaf thickness was lower in *P. latifolia* than in *Q. ilex*, in shade leaves than in sun leaves, and in dry plots than in control ones, but leaf density was slightly higher in *P. latifolia*, and showed similar values in the two canopy positions, and treatment applications. There was a strong correlation between LMA and leaf thickness, such as it has been found in other studies (Wilson *et al.* 1999), with very similar slopes in the two species. The LMA-density relationships were weaker. The capacity to resist water stress of leaves with high LMA is usually attained, in a great part, due to a thick cuticle and high cell wall content (Witkowsky and Lamont 1991), which implies an increment in leaf density that was not found in this study. The lower LMA values in the dry plots are likely due to lower photosynthetic rates (Ogaya and Peñuelas 2003), and lower carbon allocation to leaves. These decreases in LMA values in the dry plots were produced by the decreases in leaf thickness. Lower leaf area and leaf thickness could be explained by the decline in cell expansion imposed by drought during leaf growth (Hsiao *et al.* 1985, Niinemets and Kull 1998), when the area of shoots and needles are most sensitive to resources availability (Pokorný *et al.* 2004).

Many temperate species experience an increase in LMA when water availability decreases (Salleo and Lo Gullo 1990, Wookey *et al.* 1995, Carter *et al.* 1997), and there is a gradient of increase in LMA from temperate forests to Mediterranean ones (Niinemets 2001). An increase of LMA for a given leaf lifespan when water availability decreases (Reich *et al.* 1999), with a consequent decrease in maximum photosynthetic rates (Reich *et al.* 1998) are common leaf traits observed in several biomes. However, there is instead a decrease in LMA values and lifespan in a gradient from Mediterranean forests to more xeric vegetation (Harley *et al.* 1987, Kyparissis and Manetas 1993, Werner *et al.* 1999). The drought-induced LMA decreases would indicate a similar trend towards more xeric traits than those currently found in Mediterranean holm oak forests. Water availability in the dry plots of the studied forest seemed also to be insufficient to generate higher LMA in the leaves of plants growing there. Some authors have described the advantages of sclerophylly under different climatic constraints in a wide range of biomes, but sclerophylly is not an adaptation specific to drought conditions (Salleo *et al.* 1997, Salleo and Nardini 2000), and shows important limitations under long and severe drought. In any case, the advantage of hard and coriaceous leaves under low water availability seems to be derived from a high density of these leaves, and changes in LMA derived from changes in leaf thickness may not cause important differences in resistance to drought.

Decreases in plant leaf area induced by drought: Contrasting performances between the two studied species were found in relation to the leaf number and area. As in many previous studies, a decrease in the overall plant leaf area was found in both Mediterranean species in response to the experimental drought (Poole and Miller 1981, Rambal and Leterme 1987). But while *P. latifolia* experienced a reduction in longevity of leaves from 1998 cohort, the less drought-resistant species *Q. ilex* experienced similar or even higher longevity in dry plots when only sun leaves were considered. However, in *Q. ilex* values of Sn/Sn-1, Ln/Sn-1 and percentage of previous shoots which developed new shoots were lower in dry plots than in control ones during both 1999 and 2000. Moreover, *Q. ilex* reached higher leaf flushing and litterfall rates in the wetter 2000 year, whereas *P. latifolia* experienced a more conservative behaviour, less dependent on water availability. In *Q. ilex*, the lower sum of leaves from 1999 and 2000 cohorts under drought indicated a smaller increase in the overall plant leaf area. Moreover, in *Q. ilex* the proportion of young leaves, with higher photosynthetic capacity, and with higher carbon sequestration rates (Niinemets and Lukjanova 2003), was lower in dry than in control plots.

Both species presented slightly shorter current-year shoots in dry plots, very likely as a consequence of the slightly lower photosynthetic rates measured in these plots (Ogaya and Peñuelas 2003) and the consequent lower carbon sequestration. The decrease in the total plant leaf area, stronger in *Q. ilex* than in *P. latifolia*, could imply an important decrease in height and radial growth (Krause and Raffa 1996), and may be also partly responsible for lower carbon sequestration under drought as shown by the lower biomass accumulation previously described in the study site (Ogaya *et al.* 2003).

Leaf demography, carbon sequestration and changes in species dominance: In the predicted climate change scenario (IPCC 2001), the lower water availability expected in Mediterranean forests could be accompanied by a decrease in the total amount of plant leaf area due to a decrease in the synthesis of new leaves. This response would be especially important in the more drought sensitive species such as *Q. ilex*. An increase of temperatures is also expected in Mediterranean areas for the next decades (IPCC 2001), and lower leaf longevity has been described in *Q. ilex* when temperature increases due to higher respiration rates to maintain leaves, that are not compensated by the expected increment of atmospheric CO_2 concentration (Gracia *et al.* 2001). Lower water availability and higher temperatures could thus have a synergic effect decreasing the number of leaves and the total tree leaf area.

Lower overall leaf area imply an important decrease in carbon sequestration and in biomass accumulation in Mediterranean ecosystems, but this decrease will be different in the different species, since it will depend on their sensitivity to drought. Lower biomass increment under drought have already been observed in this experiment, being the decrease, as expected from this greater decrease in total leaf number and area, larger in *Q. ilex* than in the more drought-tolerant *P. latifolia* (Ogaya *et al.* 2003). These results highlight the importance of leaf demography to understand changes in growth otherwise difficult to explain by the leaf gas exchange measurements conducted in these two species, which were not substantially different between treatments (Ogaya and Peñuelas 2003). All these changes may lead, at the long term, to a change in the dominance of these species, and even to substitutions, with the consequent changes in the structure and functioning of these holm oak Mediterranean forests.

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