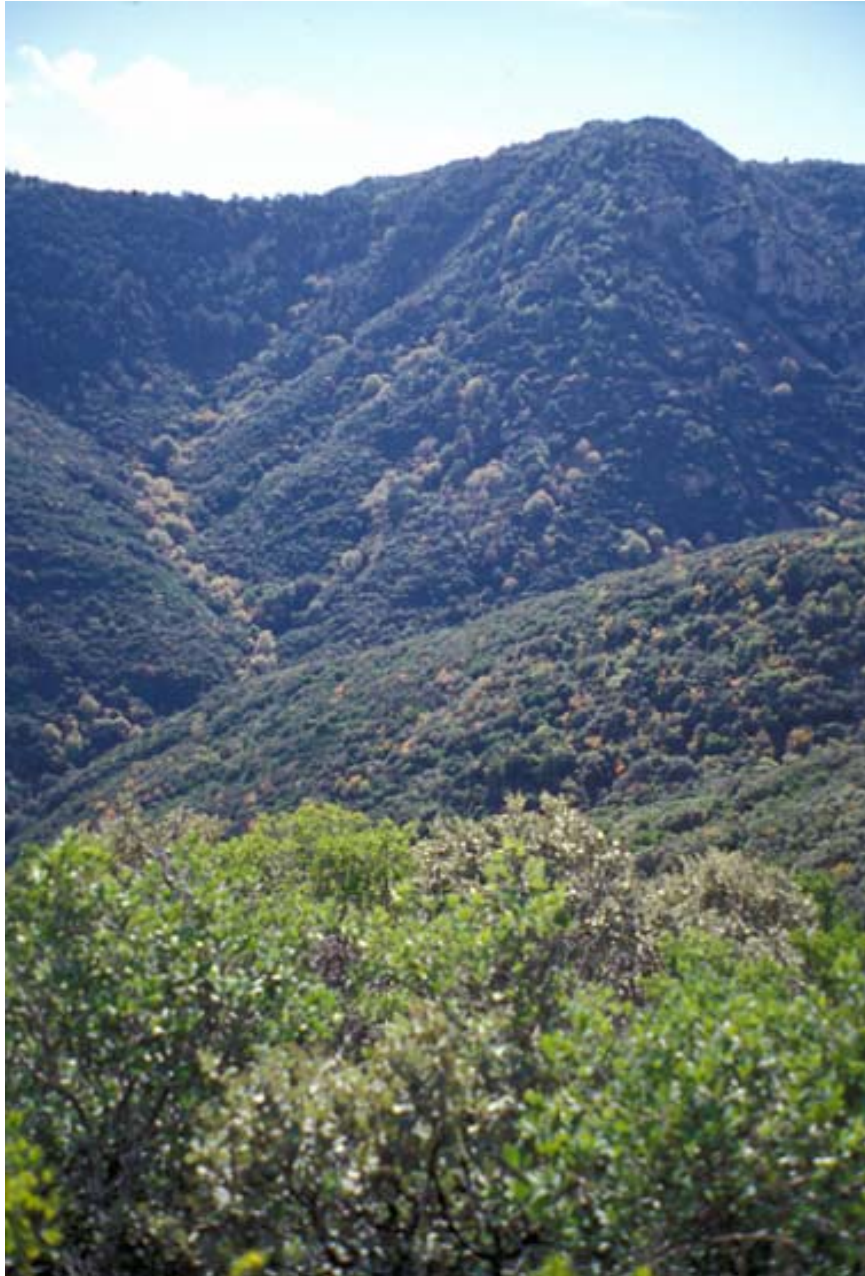


**Plant ecophysiological responses to a field experimental drought in the
Prades holm oak forest.**



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Bellaterra, Juliol de 2003



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Prades holm oak forest.**

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Introducció general.

L'augment del consum de recursos (principalment combustibles fòssils) per part de la població humana ha comportat un augment de la concentració atmosfèrica de gasos amb efecte hivernacle, la qual cosa ha comportat un augment de temperatura (Fig. 1). Per a les properes dècades es preveuen encara majors augments de la temperatura i variacions en el règim de precipitacions a escala planetària com a resultat del increment continuat d'aquests gasos hivernacle (IPCC 2001). Tot plegat es tradueix, i sembla que encara ho farà més intensament en les properes dècades, en importants efectes sobre els sers vius i els ecosistemes (per exemple Peñuelas & Filella 2001).

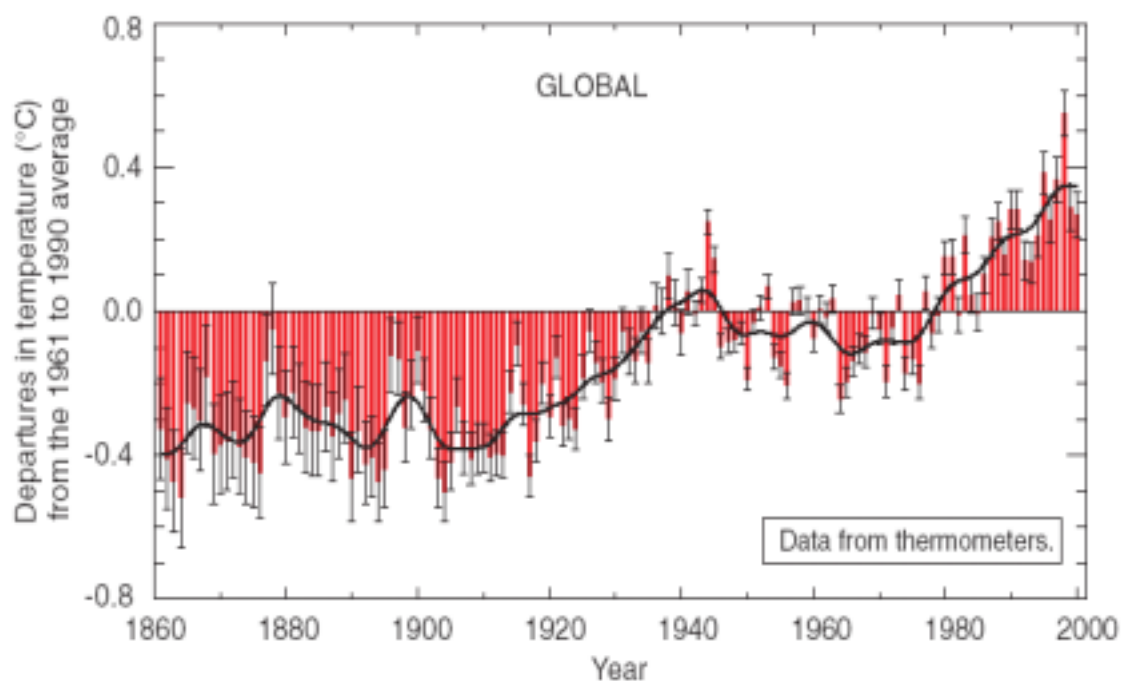


Fig. 1. Evolució de la temperatura mitjana anual durant l'últim segle. Font: IPCC 2001.

Per a la conca mediterrània concretament, es preveu un augment de temperatura i una disminució en la disponibilitat d'aigua produïda per una major evapotranspiració i per una disminució de les precipitacions (IPCC 2001) (Fig. 2), la qual cosa accentuarà els trets propis dels ecosistemes mediterranis. Els ambients mediterranis presenten sovint dos tipus d'estrès: unes condicions seques durant l'estiu i baixes temperatures a l'hivern (Mitrakos 1980). L'eixut estival caracteritzat per una baixa precipitació, temperatura i radiació solar elevades, i un alt dèficit de pressió de vapor d'aigua és freqüentment considerat com un

factor clau en ambients mediterranis (DiCastrì & Mooney 1973). La disponibilitat hídrica constitueix generalment un dels factors més determinants en el desenvolupament i distribució de les plantes (Kramer 1983, Woodward 1988), i a aquestes zones mediterrànies és d'esperar que de disminuir encara més l'aport hídric (Fig. 2.), la disponibilitat d'aigua sigui un factor cada cop més limitant.

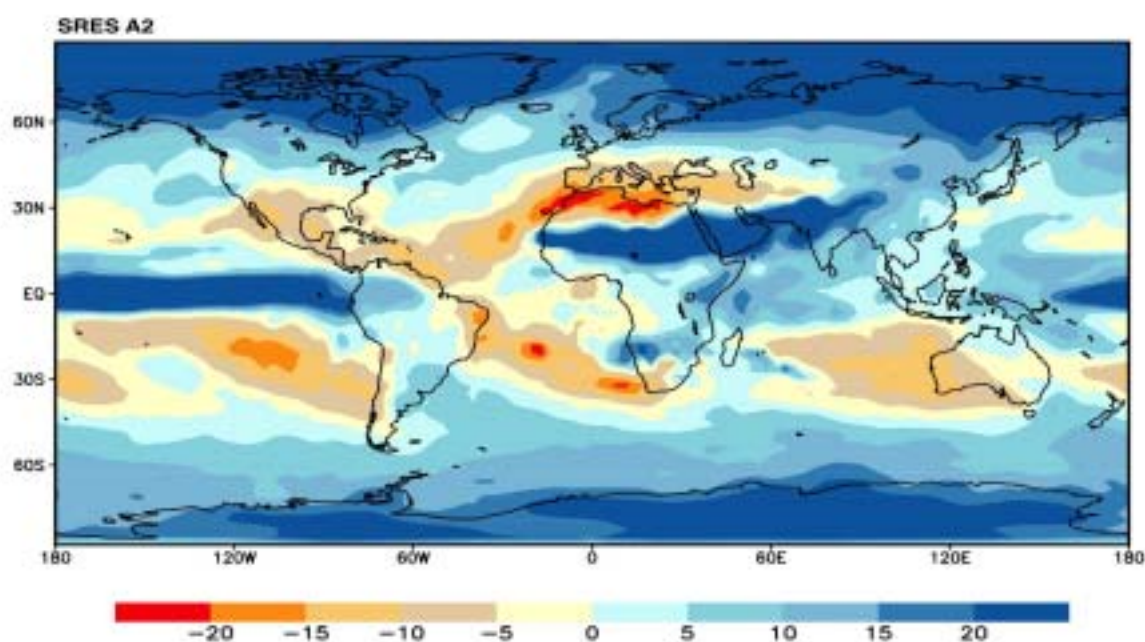


Fig. 2. Predicció del canvi de la precipitació mitjana anual de 2071 a 2100 relatiu a la de 1990. Font: IPCC 2001.

Les condicions climàtiques exerceixen una gran influència en la fisiologia de les plantes. En espècies perennifòlies de la conca mediterrània, varis treballs han mostrat una baixa taxa fotosintètica durant l'eixut de l'estiu degut a un tancament estomàtic per evitar excessives pèrdues d'aigua per transpiració (Harley et al. 1987, Tenhunen et al. 1990, Filella et al. 1998, Peñuelas et al. 1998). Tanmateix, una baixa taxa fotosintètica (Tretiach et al. 1997, Larcher 2000) associada a una fotoinhibició parcial del fotosistema II (Larcher 2000, Oliveira & Peñuelas 2000) ha estat descrita durant les baixes temperatures hivernals. El període d'aparició de determinats processos fenològics també depèn de les condicions climàtiques com per exemple la temperatura (Fitter et al. 1995, Peñuelas & Filella 2001). Una altra característica de la vegetació mediterrània és la presència de fulles amb una

llarga longevitat i una elevada relació massa/àrea que diversos autors l'han relacionada amb una adaptació a condicions àrides (Salleo & Lo Gullo 1990, Kyparisis & Manetas 1993, Wookey et al. 1995), a condicions de baixa disponibilitat de nutrients (Turner 1994) i a una elevada radiació solar (Yun & Taylor 1986). Totes aquestes adaptacions fisiològiques a les condicions climàtiques comporten variacions en el creixement dels vegetals. Diversos treballs han posat de manifest una forta relació entre el creixement diametral dels troncs dels arbres i diferents paràmetres climàtics com la disponibilitat d'aigua o la temperatura (Fritts 1976, Orwig & Abrams 1997). En boscos mediterranis s'ha constatat un menor creixement diametral quan l'aport hídric disminueix (Zhang & Romane 1991, Caritat et al. 1996, Borghetti et al. 1998, Costa et al. 2001). I apart d'aquests efectes sobre els individus, els canvis en les condicions climàtiques també tenen efectes sobre l'habilitat competitiva de les espècies, sobre l'estructura de les poblacions i sobre els processos ecosistèmics com ara la productivitat, la descomposició de la matèria orgànica o la respiració del sòl, entre molts altres (Hanson et al. 2003).

Les diferents espècies dominants dels boscos mediterranis no presenten la mateixa sensibilitat front a un determinat estrés ambiental. *Quercus ilex* és una espècie arbòria àmpliament distribuïda en els boscos mediterranis. *Phillyrea latifolia* i *Arbutus unedo* són espècies arbustives de port arbori associades a l'alzinar. *Phillyrea latifolia* ha mostrat en diverses ocasions una menor resistència a les baixes temperatures (Tretiach et al. 1997) i una major tolerància a la sequera que *Q. ilex* (Gratani 1993, Lloret & Siscart 1995, Peñuelas et al. 1998, 2000, 2001, Gratani & Bombelli 2000).

Davant la incertesa de la futura disponibilitat d'aigua i de les conseqüències que pot comportar per la vegetació mediterrània, els experiments de manipulació en sistemes naturals poden ser una eina molt útil per identificar les respostes graduals (així com els límits d'aquestes respostes) que poden desenvolupar les diferents espècies dels ecosistemes mediterranis front al dèficit hídric.

Totes aquestes consideracions, sumaryzades en una previsió de condicions més àrides per a les properes dècades i un desconeixement dels seus efectes biològics, en especial de les diferències en les respostes de les diverses espècies i de l'efecte sobre les poblacions i els ecosistemes mediterranis, han donat lloc a la realització d'un projecte de sequera experimental en un bosc mediterrani per a estudiar-ne els efectes ecofisiològics,

demogràfics i ecosistèmics (projecte CLIPRADES). El present treball, emmarcat dins l'esmentat projecte, pretén determinar les diferents respostes ecofisiològiques de les principals espècies dominants (*Quercus ilex*, *Phillyrea latifolia* i *Arbutus unedo*) a unes condicions més àrides que les actuals. Al capítol 1 es comparen les pautes fotosintètiques de *Quercus ilex* i *Phillyrea latifolia* al llarg de l'any, i al capítol 2 les diferents respostes fotosintètiques d'aquestes dues espècies a les condicions de sequera experimental. Al capítol 3, es tracten les respostes de *Quercus ilex*, *Phillyrea latifolia* i *Arbutus unedo* a la sequera pel que fa a la seva fenologia reproductiva, i al capítol 4 les respostes a la sequera de *Quercus ilex*, *Phillyrea latifolia* pel que fa a la morfologia i demografia foliar. Finalment, al capítol 5 es comparen els patrons de creixement diametral dels tronc de *Quercus ilex*, *Phillyrea latifolia* i *Arbutus unedo* i la seva resposta al tractament de sequera. És a dir, en aquesta memòria de tesi doctoral presento els treballs que he realitzat al llarg dels darrers quatre anys amb l'objectiu general de caracteritzar les respostes ecofisiològiques de les espècies dominants de l'alzinar de Prades a unes condicions de major aridesa com les previstes per a les properes dècades (IPCC 2001).

Altres treballs no presentats a la memòria.

Durant el període de realització d'aquest treballs, també he desenvolupat diverses tasques en altres treballs igualment relacionats amb l'ecofisiologia d'espècies mediterrànies i la seva relació amb la disponibilitat hídrica. Aquests treballs han quedat reflectits en diverses publicacions, de les quals aquí refereixo les recollides al "Science Citation Index":

- Peñuelas J, Piñol J, Ogaya R, Filella I. 1997. Estimation of plant water concentration by the reflectance Water Index WI (R900/970). *Int. J. Remote Sensing* 18: 2869-2875.
- Piñol J, Filella I, Ogaya R, Peñuelas J. 1998. Ground-Based spectroradiometric estimation of live fine fuel moisture of Mediterranean plants. *Agric. For. Meteorol.* 77: 207-224.

- Viegas DX, Piñol J, Viegas MT, Ogaya R. 2001. Estimating live fine fuels moisture content using meteorologically-based indices. *Int. J. Wildland Fire* 10: 223-240.
- Serrano L, Peñuelas J, Ogaya R, Savé R. Seasonal patterns of plant and tissue-water relations of two co-occurring evergreen Mediterranean species in response to summer drought. En preparació.
- Lloret F, Peñuelas J, Ogaya R. Seedling versus sprout recruitment of two Mediterranean trees (*Phillyrea latifolia* and *Quercus ilex*) under drought conditions. En preparació.

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Chapter 1.

Comparative seasonal gas exchange and chlorophyll fluorescence of two dominant woody species in a Holm Oak Forest.

Romà Ogaya & Josep Peñuelas

Abstract.

Diurnal courses of net CO₂ uptake rate, stomatal conductance (g_s), maximum photochemical efficiency of PSII (Fv/Fm) and apparent photosynthetic electron transport rate (ETR) were measured in *Quercus ilex* and *Phillyrea latifolia* in a holm oak forest throughout the seasons of the year. These measurements were complemented with response curves of photosynthetic rates to PPFD and CO₂ concentrations. *P. latifolia* was

better adapted to drought and warm conditions and showed higher net CO₂ uptake, g_s and Fv/Fm values than *Q. ilex* in summer, but in autumn and specially in winter *P. latifolia* was more sensitive to low temperatures and experimented lower net CO₂ uptake, g_s , ETR and Fv/Fm values than *Q. ilex*. The maximum net CO₂ uptake values for *P. latifolia* occurred under summer high temperatures whereas maximum net CO₂ uptake values for *Q. ilex* occurred under winter low temperatures. However, during summer midday both species presented null or slightly negative net CO₂ uptake rates. Since in the summer season both species experienced similar ETR values, the lower net CO₂ uptake values of *Q. ilex* suggest that *Q. ilex* presented greater photorespiration rates. During winter, very low Fv/Fm values were found especially for *P. latifolia*, indicating that maximal photochemical efficiency of PSII is very sensitive to low temperatures. However, they were not accompanied by low net CO₂ uptake rates showing that cold photoinhibition determined a potential but not an actual decrease in photosynthetic performance. Under well watered conditions and with high CO₂ concentration and saturated PPFD, *Q. ilex* was able to increase its photosynthetic rates whereas *P. latifolia* had lower plasticity to make a profit of optimal environmental conditions. These results show different strategies between these two dominant co-occurring species. They also indicate that the warmer and drier conditions expected for the Mediterranean region in the near decades as a result of the climate change will favour drought resistant species with lower photosynthetic rates such as *P. latifolia* in detriment of more mesic species such as *Q. ilex*.

Keywords: chlorophyll fluorescence, cold, drought, electron transport rate, Mediterranean trees, photochemical efficiency, photoinhibition, photosynthetic rates, *Phillyrea latifolia*, *Quercus ilex*, stomatal conductance.

Introduction.

Mediterranean environments are often characterized by a double stress: summer drought and winter cold (Mitrakos 1980). Summer drought, characterized by low precipitation and

high temperature, high irradiance and high water vapour pressure deficit (Terradas & Savé 1992, Di Castri 1973, Pereira & Chaves 1995), is frequently considered as a key factor in Mediterranean environments. Many studies have described low photosynthetic rates during summer drought due to a great stomatal control of transpiration water loss (Tenhunen et al. 1981, 1990, Lange et al. 1982, Lösch et al. 1982, Harley et al. 1987, Gratani 1993, 1995, Faria et al. 1998, Filella et al. 1998, Peñuelas et al. 1998, Gratani & Bombelli 1999, Karavatas & Manetas 1999, Llusià & Peñuelas 2000). On the other hand low photosynthetic rates during winter (García-Plazaola et al. 1997, Tretiach et al. 1997, Larcher 2000) and partial photoinhibition of PSII under low temperatures (Larcher 2000, Oliveira & Peñuelas 2000, 2001) have also been described.

Co-occurring Mediterranean woody species have often different climatic constraints to photosynthesis under summer drought (Castell et al. 1994, Peñuelas et al. 1998) and low temperatures in winter (Larcher 2000, Oliveira & Peñuelas 2000, 2001). Mediterranean climate is likely to become warmer and with drier summers (IPCC 2001), and the different Mediterranean species will not develop the same physiological responses to the environmental changes. Information of these physiological responses may allow to predict future changes in species development and distribution, community structure and ecosystem functioning.

Holm oak (*Quercus ilex* L.) is a tree species well adapted to drought and widely distributed in the Mediterranean basin. *Phillyrea latifolia* L. is a tall shrub species associated with the holm oak forest, that has been described as more drought resistant (Gratani 1993; Lloret & Siscart 1995, Peñuelas et al. 1998, 2000, 2001, Gratani & Bombelli 2000) and less cold tolerant (Tretiach 1993) than *Q. ilex*. The aim of this work was to characterize photosynthetic performance of *Q. ilex* and *P. latifolia* in the different annual seasons, including the dry summer and the relatively cold winter, in order to discuss the adaptative strategies of these two species to a changing climate and the possible consequences in their future distribution.

Material and Methods.

Study site.

The study was carried out in a natural holm oak forest growing at Prades Mountains in North-Eastern Spain (41° 13' N, 0° 55' E) at 950m above sea level, on a south-facing slope (25% slope). The soil is a stony xerochrept on a bedrock of metamorphic sandstone, and its depth ranges between 35 and 90 cm. The average annual temperature is 12°C and the annual rainfall 658 mm. Summer drought is pronounced and usually lasts for 3 months. Table 1 shows the average temperature, rainfall and radiation for each season during the period of study. The data was provided by an automatic meteorological station placed in the study site.

Table 1. Mean values of radiation, temperature, and cumulative precipitation during the four annual seasons in the study site.

	Spring	Summer	Autumn	Winter
Radiation (MJ m⁻² day⁻¹)	21.2	21.4	7.6	11.4
Temperature (°C)	14.4	19.5	9.1	6.6
Precipitation (mm)	335.4	76.6	257.8	57.4

The vegetation in the area studied is a typical machia dominated by tall shrubs and short trees about 3 or 4 m high with a canopy not completely closed. The most abundant species are *Quercus ilex* L. and *Phillyrea latifolia* L., but there is an important presence of other species well adapted to Mediterranean drought conditions (*Arbutus unedo* L., *Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L.).

Gas exchange and fluorescence measurements.

Gas exchange and chlorophyll fluorescence were measured during 2-6 consecutive days in the four annual seasons: spring summer. autumn, and winter in current-year leaves (fully developed in spring). Sun leaves (from the upper layer of the canopy, fully exposed to the sun, and southfacing orientation) and shade leaves (from the lower layers of the canopy) were measured under clear-sky conditions.

Net CO₂ uptake rate and stomatal conductance (g_s) were measured with a portable gas exchange system ADC4, with a PLC4B chamber (ADC Inc., Hoddesdon, Hertfordshire, UK). Four different plants per species were measured three times a day: morning, midday and afternoon. Current-year leaves were measured in each plant and

canopy position. Both net CO₂ uptake and g_s were expressed on a projected leaf area basis measured with a Li-Cor 3100 Area Meter (Li-Cor Inc., Nebraska, USA).

The maximum photochemical efficiency of PSII (F_v/F_m) and the apparent photosynthetic electron transport rate (ETR) were measured with a PAM-2000 fluorometer (Walz, Effeltrich, Germany). ETR was estimated as

$$\text{ETR} = F/F_m' \times \text{PPFD} \times 0.84 \times 0.5$$

where F/F_m' (actual photochemical efficiency of PSII) was calculated according to GENTY et al. (1989), 0.84 was the coefficient of absorption of the leaves, and 0.5 was the fraction of electron involved in the photoexcitation produced by one quantum, as two photosystems are involved. Chlorophyll fluorescence was measured on five current-year leaves of each one of four plants per species and canopy position three times a day: morning, midday and afternoon. In summer the measurements were made on five leaves of each one of three plants per species and canopy position five times a day: dawn, morning, midday, afternoon and evening. The maximum PSII photochemical efficiencies (F_v/F_m) were measured after keeping leaves in the dark for at least 20-25 min.

As complementary measurements, four net CO₂ uptake rate response curves to CO₂ for each one of the two species, and four A response curves to PPFD for each one of the two species and for each one of the two types of leaves (sunlit and shaded ones) were conducted with a portable gas exchange system CIRAS2 (PP Systems, Hitchin, Hertfordshire, UK) in autumn 2001.

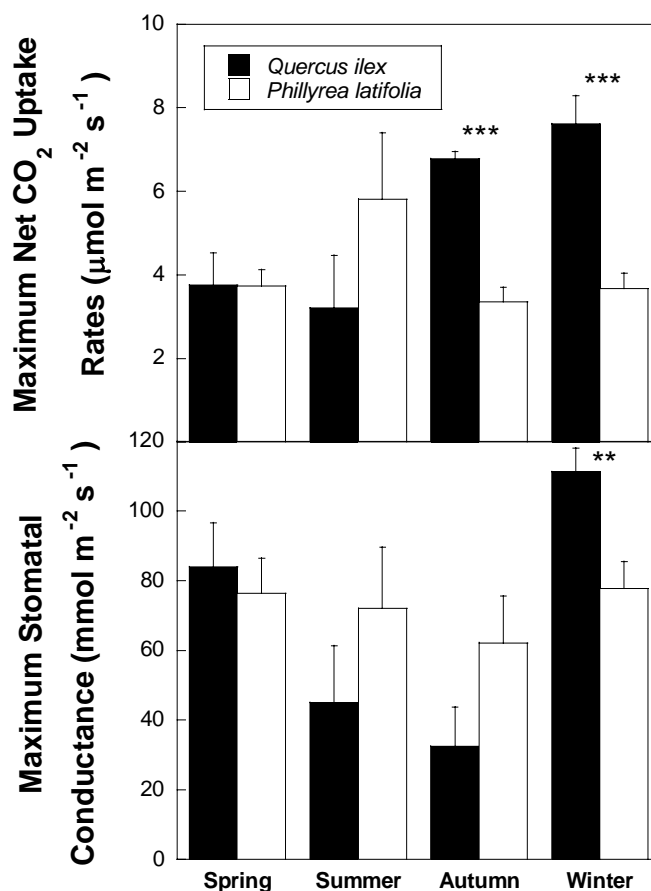


Figure 1. Maximum net photosynthetic rates and stomatal conductance in sun leaves of *Quercus ilex* and *Phillyrea latifolia* in the four annual seasons. Vertical bars indicate standard error of the mean (n=3-4). Two asterisks indicate $p < 0.01$, and three asterisks $p < 0.001$.

Statistical analyses.

In each season differences of net CO₂ uptake, g_s , Fv/Fm and ETR between *Quercus ilex* and *Phillyrea latifolia* were tested by analysis of variance (ANOVA) with species as independent factor. Regression analyses were conducted to examine the relationships between net CO₂ uptake and PPFD, g_s , ETR, and temperature. Analyses of covariance (ANCOVA) were used to test the differences of these relationships between *Q. ilex* and *P. latifolia* and among seasons by using net CO₂ uptake as a dependent factor and g_s and ETR as covariates. Non-linear regression analyses were also conducted to examine the CO₂ and PPFD response curves. Analyses of covariance (ANCOVA) were used to test the differences of these relationships between *Q. ilex* and *P. latifolia*, net CO₂ uptake was the

dependent variable and CO₂ the covariate. CO₂ and PPFD variables were log transformed to reach the normality assumptions of the ANCOVAs. For Michaelis-Menten type relationships, only the lineal unsaturated part of the curves was analyzed with ANCOVAs. The saturated values were analyzed with ANOVAs or t-tests. All analyses were performed with the Superanova software package (Abacus Concepts Inc., 1991) and the Statview software package (Abacus Concepts Inc., 1998).

Results.

While *P. latifolia* presented similar g_s values, and maximum net CO₂ uptake rates throughout all seasons (with slightly higher rates in summer), *Q. ilex* had higher maximum net CO₂ uptake rates in autumn and winter. On the other hand, both species presented highest g_s values in winter (Fig. 1). During spring both species showed similar maximum net CO₂ uptake and g_s values. But in autumn and winter *Q. ilex* experienced larger maximum net CO₂ uptake rates, and in winter larger g_s values than *P. latifolia* (Fig. 1). However *P. latifolia* usually showed higher maximum net CO₂ uptake and g_s values than *Q. ilex* in summer.

In the summer season, net CO₂ uptake declined during midday both in sun and shade leaves, even reaching negative values, and g_s declined only in sun *P. latifolia* leaves because shade *P. latifolia* and *Q. ilex* leaves experienced low values throughout the whole day (Fig. 2). Water use efficiency (WUE), calculated as net CO₂ uptake / transpirational rate declined at midday in both species but was overall higher in *P. latifolia* than in *Q. ilex* ($p=0.012$) (Fig. 2). Summer net CO₂ uptake rates were higher in sun leaves than in shade leaves in both species except at midday, when all sun and shade leaves showed very low net CO₂ uptake rates, often even negative. The g_s values were higher in sun leaves except at midday, and WUE showed similar values in the two types of leaves of the two species except in afternoon for *P. latifolia* which presented slightly higher WUE in shade than in sun leaves (Fig. 2).

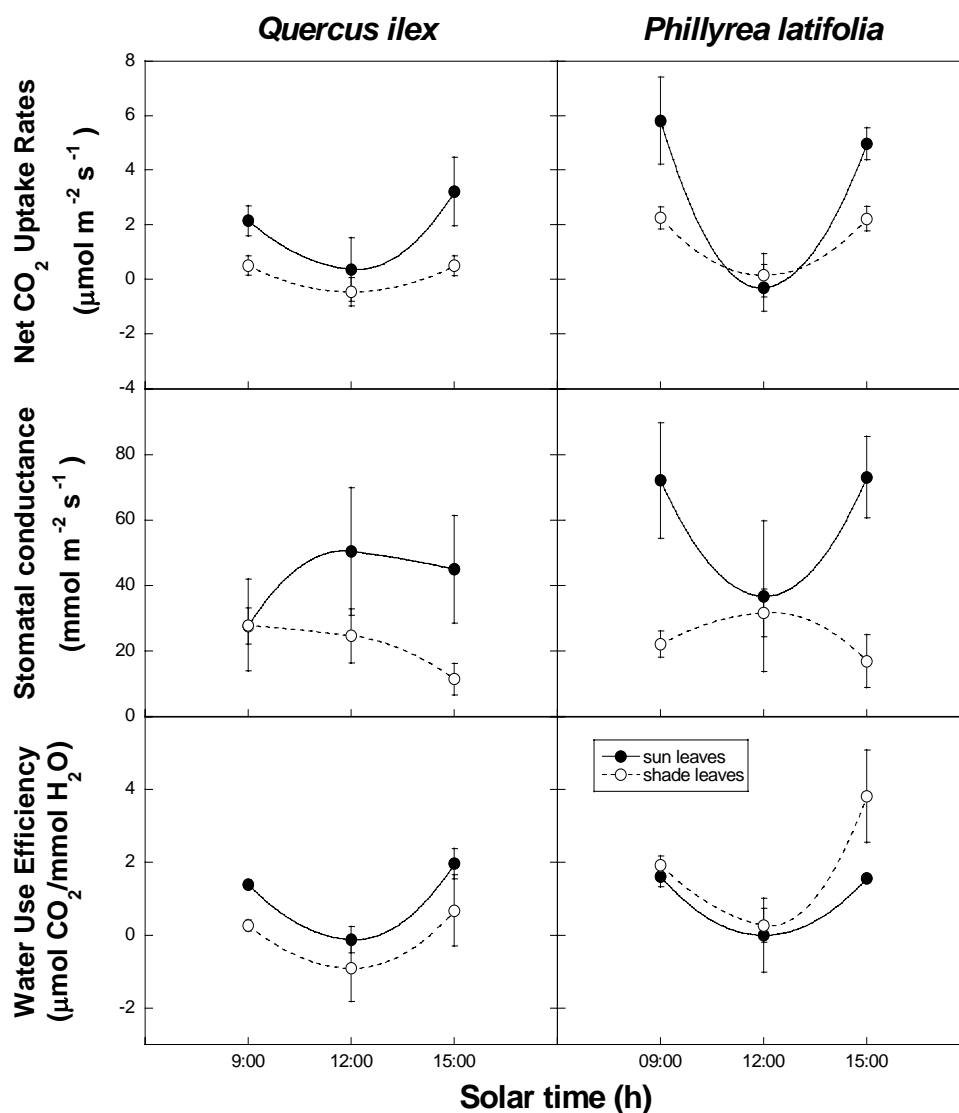


Figure 2. Daily course of net photosynthetic rates, stomatal conductance and water use efficiency (net photosynthetic rates / transpiration rates) in sun leaves of *Quercus ilex* and *Phillyrea latifolia* during summer. Vertical bars indicate standard error of the mean (n=3).

In congruence with the different photosynthetic performance of these two woody Mediterranean species, Fv/Fm presented higher values for *P. latifolia* in summer and for *Q. ilex* in winter. In spring Fv/Fm and ETR values were very high compared to the rest of the seasons and very similar in both species, whereas in summer, autumn and especially in winter sun leaves showed low Fv/Fm and ETR values, especially in *P. latifolia*, (Fig. 3). In winter even shade leaves showed lower Fv/Fm values than in the other seasons (Fig. 3). Fv/Fm and ETR values experimented a slight decrease around midday in sun leaves during

spring and autumn, but not during winter when they were already very low in the morning (Fig. 3). In summer ETR values were low (such as in autumn and winter) and similar in both species, but Fv/Fm of sun leaves decreased around midday and this decrease was higher in *Q. ilex* than in *P. latifolia* (Fig. 3). Net CO₂ uptake and ETR values were correlated in spring and autumn, but not in summer and winter. On the other hand, there were no significant differences in net CO₂ uptake-ETR relationships between the two species, but there was a trend towards higher slopes in *Q. ilex* during autumn and winter, and higher slopes in *P. latifolia* during summer (Fig. 4).

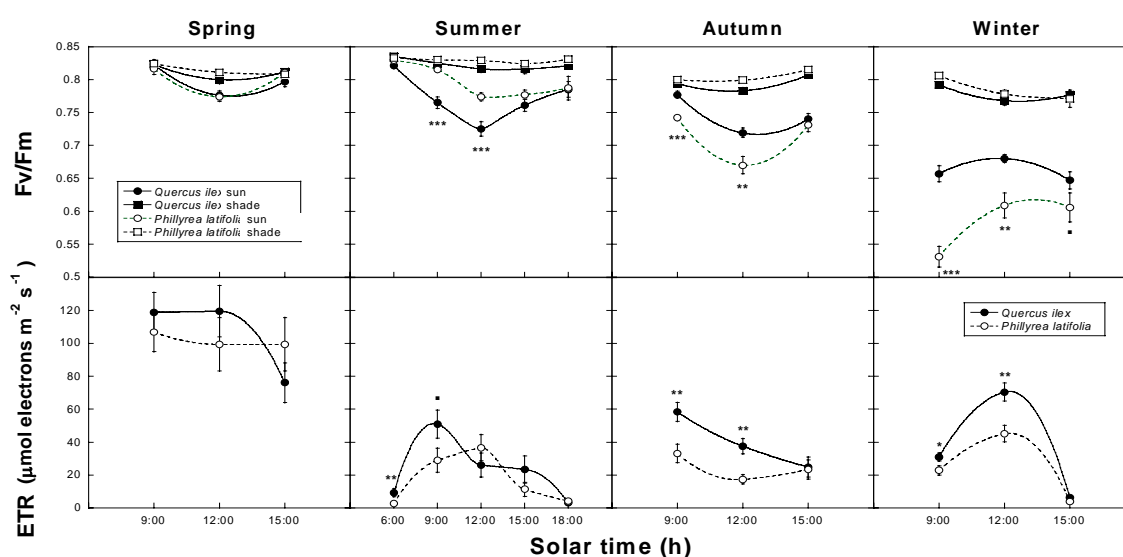


Figure 3. Daily course of maximum photochemical efficiency (Fv/Fm) and apparent photosynthetic electron transport rate (ETR) in sun leaves of *Quercus ilex* and *Phillyrea latifolia* during the four annual seasons. Vertical bars indicate standard error of the mean (n=15-20). One square indicate $p < 0.1$, one asterisk $p < 0.05$, two asterisks $p < 0.01$, and three asterisks $p < 0.001$.

The relationship between net CO₂ uptake and g_s showed a linear fit in all seasons (Fig. 5). In both species the relationships were very similar during spring and summer, but *Q. ilex* relationships experienced higher slopes than those of *P. latifolia* in autumn and winter ($p < 0.001$ and $p < 0.01$, respectively).

In sun leaves net CO₂ uptake, g_s and ETR relationships with leaf temperature differed depending on the different seasons ($p = 0.025$, $p = 0.079$ and $p < 0.0001$ for net CO₂ uptake, g_s and ETR, respectively). In both species leaf optimal temperature ranged from

low values (12°C for *Q. ilex* and 15°C for *P. latifolia*) in winter to high values (32°C for *Q. ilex* and 35°C for *P. latifolia*) in summer and were higher in *P. latifolia* than in *Q. ilex* (Fig. 6). This Fig. 6 clearly illustrates the contrary performance of these coexisting dominant species. The maximum net photosynthetic rates for *Q. ilex* occur under winter low temperatures whereas the maximum net photosynthetic rates for *P. latifolia* occur under summer high temperatures. This Fig. 6 also highlights that the maximum ETR and therefore the maximum capacities for photosynthetic performance of both species occur in spring linked to the highest irradiances and the optimal temperature and water availability conditions.

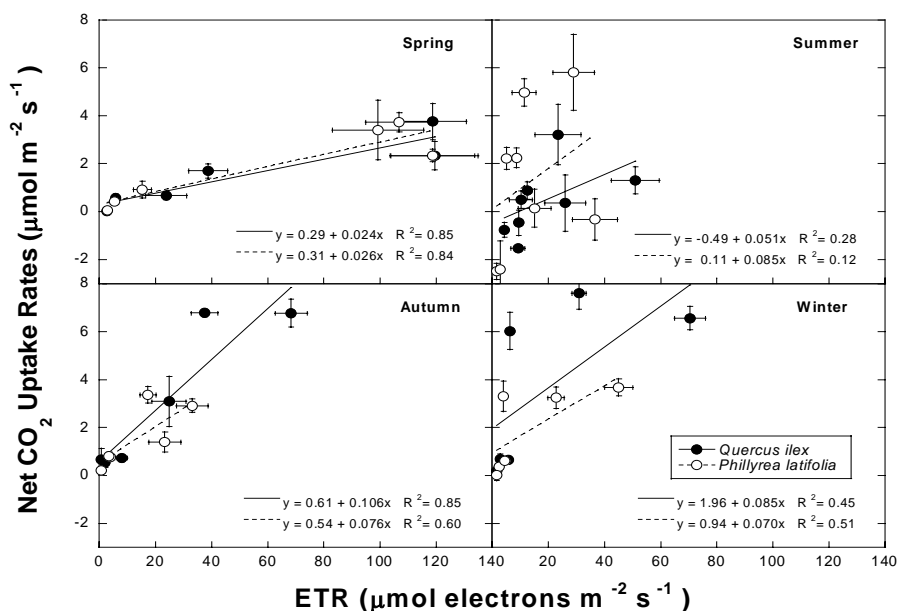


Figure 4. Relationship between net CO₂ uptake rates and apparent photosynthetic electron transport rate (ETR) in *Quercus ilex* and *Phillyrea latifolia* during the four annual seasons. Data include sun and shade leaves. Bars indicate standard error of the mean (n=3-4 for net photosynthetic rates and n=15-20 for ETR). Values correspond to measurements in morning, midday and afternoon for shade and sun leaves.

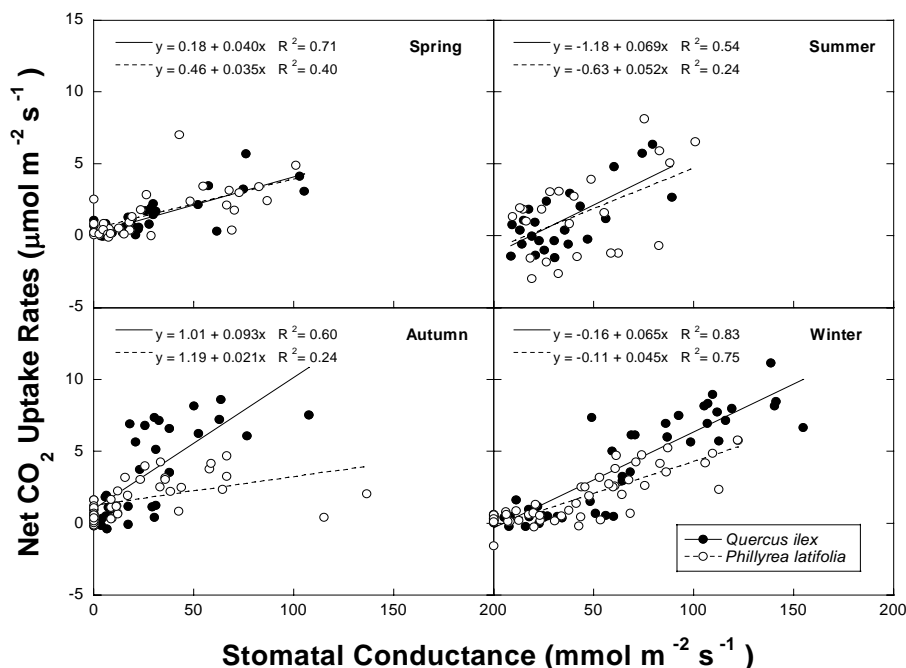


Figure 5. Relationship between net CO₂ uptake rates and stomatal conductance in *Quercus ilex* and *Phillyrea latifolia* during the four annual seasons. Data include sun and shade leaves. Each point is an individual measurement.

CO₂ and PPFD response curves, conducted in autumn where plants are under moderate temperatures and high water availability, showed good fits to logarithmic and Michaelis-Menten relationships, respectively. CO₂ and PPFD response curves showed earlier saturation and lower net CO₂ uptake values at saturating PPFD in *P. latifolia* than in *Q. ilex* ($p < 0.0001$) (Fig. 7, Fig. 8). For both species, there was a trend to earlier saturation of net photosynthetic rates at lower PPFD levels in shade than in sun leaves. However, sun and shade leaves of both species showed similar saturation net CO₂ uptake rates (Fig. 8).

Discussion.

Equinoctial periods, without drought and cold stresses, are widely described as the most favourable seasons to photosynthetic activity of Mediterranean vegetation (Savé et al. 1999). In this work, high net CO₂ uptake and gs values were also reached in winter in *Q.*

ilex despite the low temperatures. As this was not the case for *P. latifolia*, *Q. ilex* showed higher photosynthetic rates than *P. latifolia*, suggesting a higher tolerance of photosynthesis of *Q. ilex* to low temperatures in agreement with a previous report of Tretiach (1993). During hot and dry summer both species experienced low net CO₂ uptake rates during midday due to stomatal closure, avoiding excessive water loss during the central hours of the day (Tenhunen et al. 1981, 1990, Lange et al. 1982, Lösch et al. 1982, Harley et al. 1987, Gratani 1993, 1995, Faria et al. 1998, Karavatas & Manetas 1999, Llusà & Peñuelas 2000). However, *P. latifolia* reached higher net CO₂ uptake and WUE values than *Q. ilex* in the morning and in the afternoon showing a better adaptation to drought and warm conditions (Filella et al. 1998, Peñuelas et al. 1998, Gratani & Bombelli 2000).

Maximal photochemical efficiency of PSII was very sensitive to low temperatures (Larcher 2000, Oliveira & Peñuelas 2000, 2001), and in second term to summer low water availability. Cold stress reduced Fv/Fm in both sun and shade leaves, but Fv/Fm had a stronger decrease in sun leaves due to the combined cold and high irradiance stress, while low water availability also reduced Fv/Fm values only when combined with high irradiance. When no cold or drought stress occurred Fv/Fm values remained high all the day in sun and shade leaves.

Actual photochemical efficiency of PSII was high in spring and lower in the other periods, probably because spring was the only season where irradiance was high and no cold or drought stress occurred. In the coldest days *Q. ilex* had higher ETR and Fv/Fm values than *P. latifolia*, but in summer, despite of the great Fv/Fm in *P. latifolia*, both species showed similar ETR values or they were even higher in *Q. ilex*. Other reports have also shown higher net CO₂ uptake and *g_s* in *P. latifolia* than in *Q. ilex* during summer drought, and also similar low ETR values (Filella et al. 1998, Peñuelas et al. 1998). Those authors have hypothesized higher photorespiration rates in *Q. ilex* to explain this mismatch between net CO₂ uptake and ETR.

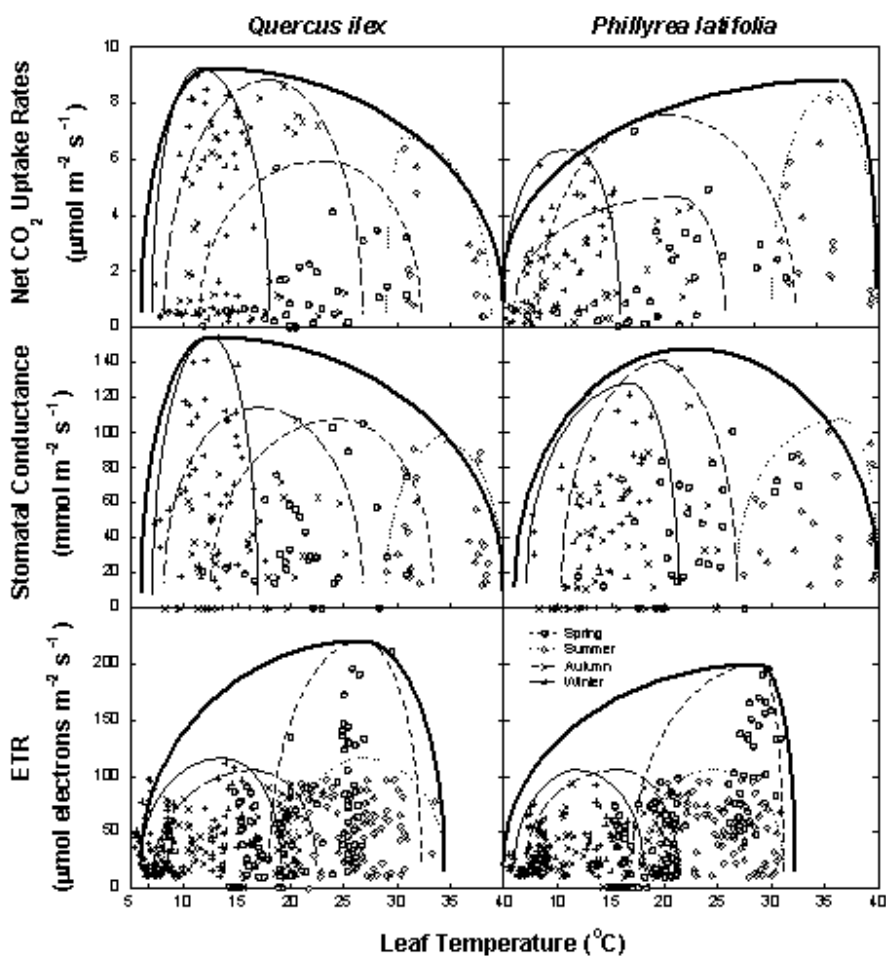


Figure 6. Relationship between net photosynthetic rates, stomatal conductance, apparent photosynthetic electron transport rate (ETR) and leaf temperature in sun and shade leaves of *Quercus ilex* and *Phillyrea latifolia* during the four annual seasons. Each point is an individual measurement.

Strong irradiation combined with cold or drought result in an activation of some photoprotective mechanisms such as the de-epoxidation of the xanthophyll cycle that lowers the photochemical efficiency of PSII (Demmig-Adams & Adams 1992, Long et al. 1994). During the coldest seasons Fv/Fm values were low and no recover took place during the night, probably because re-epoxidation did not occur during the night if there was frost (Adams & Demmig-Adams 1995). In summer a high difference between predawn and midday de-epoxidation values have been described (Martínez-Ferri et al. 2000), which is in agreement with the recovery of Fv/Fm values found here during the night. During the summer drought the low Fv/Fm and ETR values coincided with low net

CO₂ uptake rates, but in coldest season the low photochemical efficiencies did not imply low net CO₂ uptake rates. Thus winter photoinhibition only determined a potential decrease in net CO₂ uptake rates, but it was summer stomatal closure that produced a strong actual decrease on net assimilation rates.

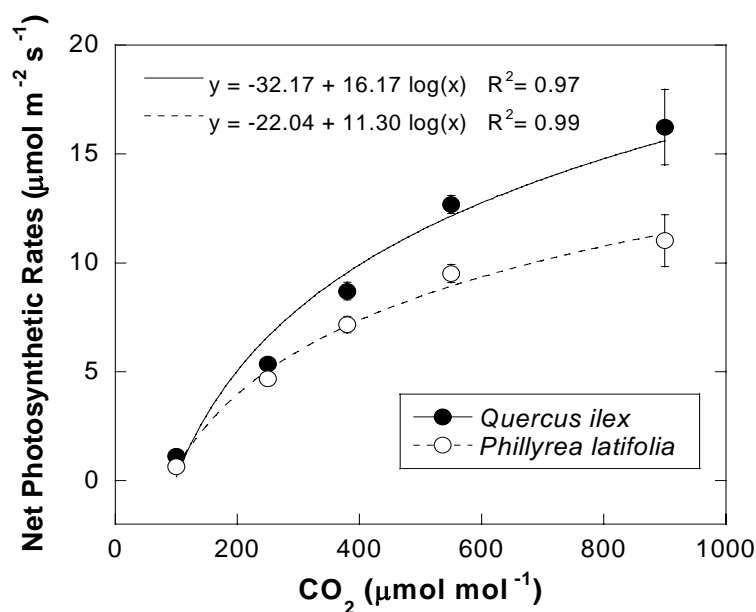


Figure 7. Responses of PPFD-saturated photosynthetic rates to different CO₂ concentrations in sun leaves of *Quercus ilex* and *Phillyrea latifolia*. Each point is an individual measurement. (These response curves were measured in autumn).

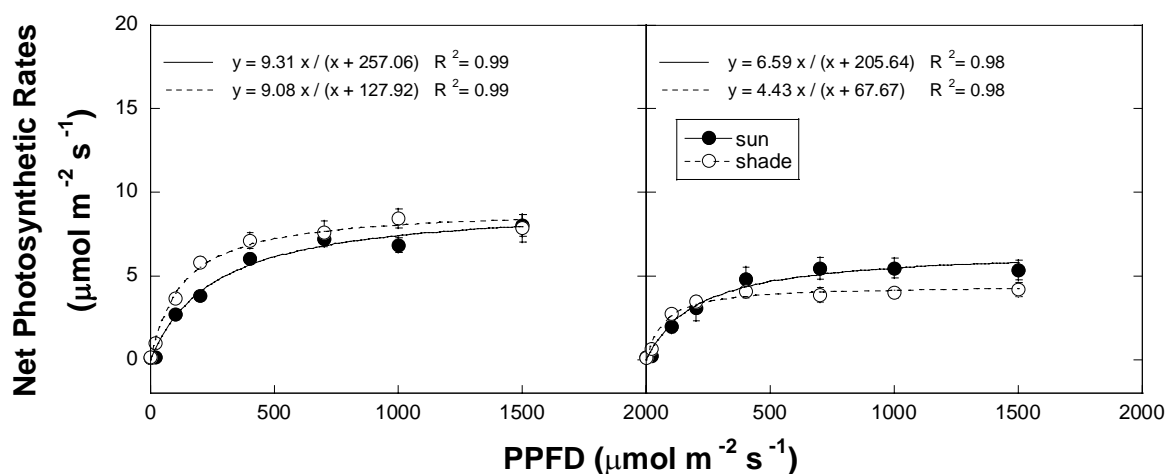


Figure 8. Responses of CO₂-saturated photosynthetic rates to different PPFD fluxes in sun and shade leaves of *Quercus ilex* and *Phillyrea latifolia*. Each point is an individual measurement. (These response curves were measured in autumn).

Likely higher photorespiration rates in *Q. ilex* (Heber et al. 1990, Scarascia-Mugnozza et al. 1996) may explain the trend towards a lower slope in the net CO₂ uptake-ETR relationship in *Q. ilex* than in *P. latifolia* observed during summer drought (Filella et al. 1998, Peñuelas et al. 1998). On the other hand, the trend to higher net CO₂ uptake-ETR relationship slope in *Q. ilex* than in *P. latifolia* during the colder seasons can also be explained by the lower photorespiration rates in *Q. ilex* in cold seasons than in summer. Photorespiration is suggested as a possible mechanism of dissipating excess photochemical energy in water-stressed and heat-stressed plants (Peñuelas & Llusià 2002) and it decreases exponentially when temperatures decrease (Cornic & Briantais 1991, Kozaki & Takeba 1996). In any case, in winter, *P. latifolia* experienced higher photoinhibition rates (lower ETR values) than *Q. ilex*. But the net CO₂ uptake-ETR relationship slope was still lower because of the stronger decrease in net CO₂ uptake rates.

Linear regressions between net CO₂ uptake and g_s measured throughout the whole day showed a good linear fit in all seasons, even though in other studies of *Quercus* species with data only from the morning, it has been observed a better fit with a rectangular hyperbola (Mediavilla et al. 2001). The proportionality between g_s and net CO₂ uptake indicates again that photosynthesis of the two species is strictly controlled by stomatal apertures. *P. latifolia* seemed to be able to cope better with summer heat and drought than *Q. ilex* because *P. latifolia* had higher net CO₂ uptake and also g_s values despite of similar net CO₂ uptake- g_s rates in both species. On the other hand, *P. latifolia* showed lower slopes in net CO₂ uptake- g_s relationships than *Q. ilex* during the colder seasons. The lower g_s values in *P. latifolia* than in *Q. ilex* implied lower net CO₂ uptake rates, but *P. latifolia* also presented a higher photoinhibition as another factor involved in its low net CO₂ uptake rates in colder seasons without a direct effect on g_s values. Thus, *Q. ilex* seemed to cope better with winter cold.

Temperatures were very different between seasons (Table 1), and photosynthetic parameters such as net CO₂ uptake, g_s and ETR were strongly influenced by these seasonal changes in temperature. In both species, it seems that optimum temperature for net CO₂ uptake and g_s is seasonally adjusted depending on the range of temperatures that occurred in each season. *P. latifolia* showed again higher tolerance to high temperatures and lower

tolerance to winter cold than *Q. ilex*, it had higher optimum temperature for net CO₂ uptake rates both in summer and winter. This higher tolerance is further indicated by the maximum response curves (Fig. 6) that show decreasing net CO₂ uptake values with high temperatures in *Q. ilex* and, on the contrary, increasing net CO₂ uptake values with high temperatures in *P. latifolia*.

CO₂ and PPFD response curves were measured in autumn, a season with well watered conditions and moderate temperatures. *Q. ilex* was able to reach higher net CO₂ uptake rates under PPFD and CO₂ saturating conditions. *P. latifolia*, which was more drought resistant than *Q. ilex*, presented, however, lower plasticity to obtain high photosynthetic rates under non stressed environmental conditions.

The two dominant species of this Mediterranean forest, *Q. ilex* and *P. latifolia* presented different photosynthetic performances in response to the variable temperature and water conditions throughout the year. The increase of temperatures and summer drought predicted by global circulation models (IPCC 2001) could favour more drought and heat resistant species such as *P. latifolia* compared to more mesic ones such as *Q. ilex*.

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Chapter 2.

Comparative field study of *Quercus ilex* and *Phillyrea latifolia*: photosynthetic response to experimental drought conditions.

Romà Ogaya & Josep Peñuelas

Abstract.

Quercus ilex and *Phillyrea latifolia* growing in a holm oak forest in Prades mountains (NE Spain) were subjected to experimental drought conditions. Soil water availability was reduced about 15% by plastic strips and funnels that partially excluded rain throughfall and by ditch exclusion of water runoff. Diurnal courses of maximum photochemical efficiency of PSII (Fv/Fm), apparent photosynthetic electron transport rate (ETR), net photosynthetic rate (A), transpiration rate (E) and water use efficiency (WUE) were measured in sunlit and shade leaves of both species during two years. Moreover, the responses of photosynthetic rates to PPFD and CO₂ concentrations were also measured. *Q. ilex* experienced lower E rates and higher A rates and WUE than *P. latifolia* throughout the experimental period, but during summer drought these differences disappeared. *Q. ilex* exhibited a less cold sensitive behavior whereas *P. latifolia* showed a more heat-drought resistant behavior. Under severe summer drought conditions none of the two species was able to reach a positive carbon gain. Drought treatment produced a slight decrease in Fv/Fm values of *Q. ilex* plants and a strong decrease in Fv/Fm values of *P. latifolia* only in winter 2000, when drought stress coincided with cold stress. Drought treatment produced also a slight decrease in ETR values of both species. During midday, A and E rates decreased in drought plots in both species associated to lower photochemical efficiencies. In those drought plots, only *P. latifolia* was able to increase WUE by reducing transpiration losses during midday. Both species tended to present higher A rates for a given soil humidity in drought than in control plots. However, whereas *Q. ilex* A rates increased with soil humidity, *P. latifolia* A rates did not increase above 17% soil humidity, showing no water availability response above such threshold. It is very likely that mesic species such as *Q. ilex* lose competitive advantage in the drier environment forecasted for next decades than the more xeric *P. latifolia*.

Keywords: carbon sequestration, climate change, chlorophyll fluorescence, electron transport rate, photochemical efficiency, photoinhibition, photosynthetic rates, stomatal conductance.

Introduction.

General circulation models predict drier conditions for the Mediterranean basin due to an increase of air temperatures and water deficit (Houghton et al., 2001). The increase in water deficit could induce ecophysiological changes in different species affecting their growth and survival (Tenhunen et al. 1987) and, in the long-term, their distribution and abundance (Mitrakos 1980, Gucci et al. 1999).

In the Mediterranean basin, many publications have described low photosynthetic rates of evergreen species during summer drought due to the stomatal control of water loss by transpiration (Harley et al. 1987, Tenhunen et al. 1990, Filella et al. 1998; Peñuelas et al., 1998; Llusà and Peñuelas, 2000), and low photosynthetic rates (Tretiach et al. 1997, Larcher 2000) associated to a partial photoinhibition of PSII (Larcher 2000, Oliveira & Peñuelas 2000, 2001) during winter cold. However, there are few studies of co-occurring Mediterranean woody species for photoinhibition and photosynthetic rate responses to experimental field drought conditions and for more than a single growing season.

Quercus ilex and *Phillyrea latifolia* are plant species frequently co-occurring in the Mediterranean maquis and in the *Quercus ilex* evergreen forests. In particular, *Q. ilex* is widely distributed in the sub-humid areas of the Mediterranean Basin whereas *P. latifolia* in warmer and drier Mediterranean areas (Tretiach 1993, Lloret & Siscart 1995, Peñuelas et al. 1998, 2000). We aimed to determine the photosynthetic response of *Q. ilex* and *P. latifolia* to experimental field drought conditions during two years in order to elucidate the adaptative strategies of these two species to a changing climate. We expected drought effects on the photosynthetic activity of both species throughout the different seasons, but we also expected a more favorable photosynthetic response of the more drought- and hot-resistant *P. latifolia* during summer drought and a more favorable response of the more mesic- and less cold-sensitive *Q. ilex* during winter.

Material and Methods.

Study site.

The study was carried out in a natural holm oak forest growing at Prades Mountains in North-Eastern Spain (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. The average annual temperature is 12°C and the annual rainfall 658 mm. Summer drought is pronounced approximately from mid-June to mid-September.

The vegetation of the studied area is a typical machia characterized by 3 or 4-m tall shrubs. This machia is constituted by *Quercus ilex* L., *Phillyrea latifolia* L., *Arbutus unedo* L., *Erica arborea* L., *Juniperus oxycedrus* L. and *Cistus albidus* L.

Experimental design.

Four (15 x 10 m) plots were randomly distributed in the studied area. Half the plots were subjected to a drought treatment and the other half were control plots. The drought treatment consisted of rain exclusion by suspending PVC strips and funnels at a height of 0.5-0.8 m above the soil. Strips and funnels covered approximately 30% of the total plot surface. Also a 1 m deep ditch was excavated along the entire top edge of the upper part of the treatment plots to intercept runoff water. Water intercepted by strips, funnels, and ditches was conducted outside the plots, below the bottom edge of the plots. Drought treatment was conducted from March 1999 to January 2001.

Temperature, photosynthetic active radiation, air humidity, and precipitation were monitored each half-hour by an automatic Meteorological station installed in a gap between the plots. Soil moisture was measured every two weeks throughout the experiment by time domain reflectometry (Tektronix 1502C, Beaverton, Oregon, USA) (Zegelin et al. 1989). Three stainless steel cylindrical rods, 25 cm long, were permanently fully driven into the soil at four randomly selected places in each plot. The time domain reflectometer was connected to the ends of the rods in each measurement.

Chlorophyll fluorescence and gas exchange measurements.

Chlorophyll fluorescence and gas exchange were measured during 2-6 consecutive days in the study period (one for each season of the year during two years). Sunlit leaves (from the

upper layer of the canopy) and shade leaves (from the lower layers of the canopy) were measured under clear-sky conditions.

The maximum photochemical efficiency of PSII (F_v/F_m) and the apparent photosynthetic electron transport rate (ETR) were measured with a PAM-2000 fluorometer (Walz, Effeltrich, Germany). ETR was estimated as

$$\text{ETR} = \Delta F/F_m' \times \text{PPFD} \times 0.84 \times 0.5$$

where $\Delta F/F_m'$ (actual photochemical efficiency of PSII) was calculated according to Genty et al. (1989), 0.84 is the coefficient of absorption of the leaves, and 0.5 is the fraction of electron involved in the photoexcitation produced by one quanta, since two photosystems are involved. Chlorophyll fluorescence was measured on five current-year leaves of each one of two plants per species and canopy position ($5 \times 2 \times 2 \times 2 = 40$ leaves) in each plot twice a day: morning (8.00-10.00 h, solar time) and midday (11.00-13.00 h, solar time). The maximum PSII photochemical efficiencies (F_v/F_m) were measured after keeping leaves in the dark for at least 25 min.

Net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) were measured with a portable gas exchange system ADC LCA4, with a PLC4B chamber (ADC Inc., Hoddesdon, Hertfordshire, UK). Water use efficiency (WUE) was calculated as A/E in μmol fixed CO_2 per mmol transpired H_2O , and Vapour Pressure Deficit (VPD) was calculated from air temperature and relative humidity. One current-year leaf of two different plants per species and canopy position ($1 \times 2 \times 2 \times 2 = 8$ leaves) in each plot were measured two times a day: morning (8.00-10.00 h, solar time) and midday (11.00-13.00 h, solar time). A, E, and g_s values were expressed on a projected leaf area basis measured with a Li-Cor 3100 Area Meter (Li-Cor Inc., Nebraska, USA).

As complementary measurements, net photosynthetic rate (A) response curves to PPFD and CO_2 were conducted with a portable gas exchange system CIRAS2 (PP Systems, Hitchin, Hertfordshire, UK) during four consecutive days in autumn 2001. Four *Q. ilex* and four *P. latifolia* individuals were selected. One sunlit leaf per plant was used for the CO_2 response curves, and one sunlit leaf and one shade leaf per plant were used for the PPFD response curves.

In one terminal twig of two different plants per species in each plot, leaf water potential before dawn was determined using a Scholander pressure chamber (PMS, Corvallis, Oregon, USA). Leaf water potential was measured on several dates (at least once every season) between February 1999 and November 2000.

Statistical analyses.

Repeated measurements ANOVA was conducted with soil moisture values in each plot as dependent variable and treatment as independent factor. Overall ANOVAs were conducted with the mean values of Fv/Fm, ETR, A, E and WUE in each plot as dependent variables and with species, treatment, season, time of the day and canopy position as independent factors. Moreover, in each season, canopy position, and time of the day, differences of the mean of the above-mentioned variables between control and drought plants were tested in each species by analysis of variance (ANOVA) with treatment as the independent factor.

Non-linear regression analyses were also conducted to examine the PPFD and CO₂ response curves. Analyses of covariance (ANCOVA) were used to test the differences of these relationships between control and drought plants in both species, and between sunlit and shade leaves. Other ANCOVAs were conducted to test the differences in the relationships A-soil moisture, leaf water potential, A-VPD, and A-g_s between control and drought plants in both species. When necessary (for CO₂ and soil moisture), variables were log transformed to reach the normality assumptions of the ANCOVAs. For Michaelis-Menten type relationships, only the linear unsaturated part of the curves was analysed with ANCOVAs. The saturated values were analyzed with ANOVAs or t-tests.

All analyses were performed with the Superanova software package (Abacus Concepts Inc., 1991) and the Statview software package (Abacus Concepts Inc., 1998).

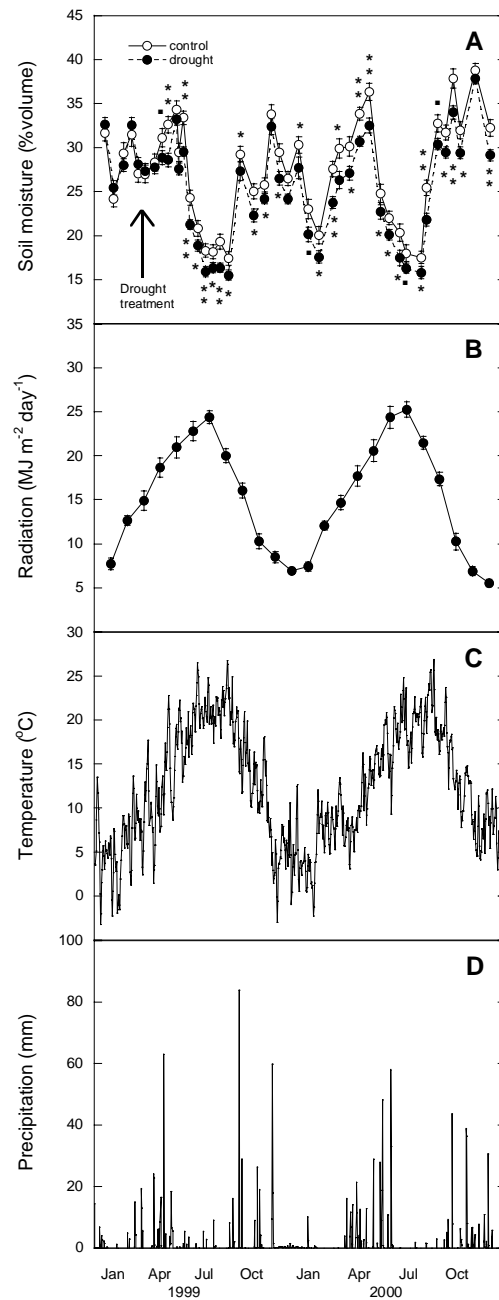


Figure 1. Seasonal course of soil moisture (0.25 m depth) (A), mean daily values of radiation (B), mean monthly temperature (C), and precipitation (D) at the study site. Vertical bars indicate standard errors of the mean (n=2 plot averages of 4-8 TDR measurements per plot in A and n=28-31 days in (B)). One square indicates (p<0.1) significant differences between the two treatments, one asterisk (p<0.05), and two asterisks (p<0.01).

Results.

The climate of the area studied is of Mediterranean type; the mean annual temperature during the study period was 12.3 °C, and mean total rainfall 668 mm (Fig. 1). Soil moisture showed variations during the study period (Fig. 1); the lowest values (about 15%) were reached in both summers and in early autumn 2000, the maximum values (about 35%) were present in spring and autumn-winter 2001, following the rainfall distribution. On average, control plots had 15% higher soil moisture than drought plots.

Both species exhibited lower Fv/Fm values in sunlit than in shade leaves, the differences being larger in winter (Fig. 2). On few occasions control plants had significantly higher Fv/Fm values than drought ones (Fig. 2), but only in winter 2000 there was an overall drought effect on sunlit leaves when both species and both times of the day were considered all together ($p < 0.01$), this effect being larger in *P. latifolia* (Fig. 2). Moreover, sunlit leaf Fv/Fm was higher in *Q. ilex* than in *P. latifolia*. ETR values were higher in spring periods than in the other periods, and in *Q. ilex* higher than in *P. latifolia* during all the experimental period (data not shown).

Both species showed negative A values in summer (August) 1999 (after a long drought period, Fig. 1), but not in summer 2000 (Fig. 3) surely because measurements were conducted earlier in summer 2000 (July) when water availability was not yet very low, especially in that year 2000 which had rains in late spring (Fig. 1). In October 2000 during midday, A values were higher in control plots than in drought ones in *Q. ilex* ($p < 0.05$), but not in *P. latifolia* (Fig. 3).

The relationships between A and CO₂, and A and PPFD showed higher A rates for *Q. ilex* both in sunlit and shade leaves than for *P. latifolia* (Figs. 4 and 5). A rates decreased due to the drought treatment in both A-CO₂ ($p = 0.036$) and A-PPFD relationships ($p = 0.004$ and $p < 0.0001$ in the unsaturated and saturated parts of the curves, respectively) only in sunlit leaves of *P. latifolia*. Shade leaves of both species showed lower A rates in control plants than in drought ones ($p = 0.021$ and $p = 0.006$ in the saturated parts of the curves in *Q. ilex* and *P. latifolia*, respectively) (Fig. 5).

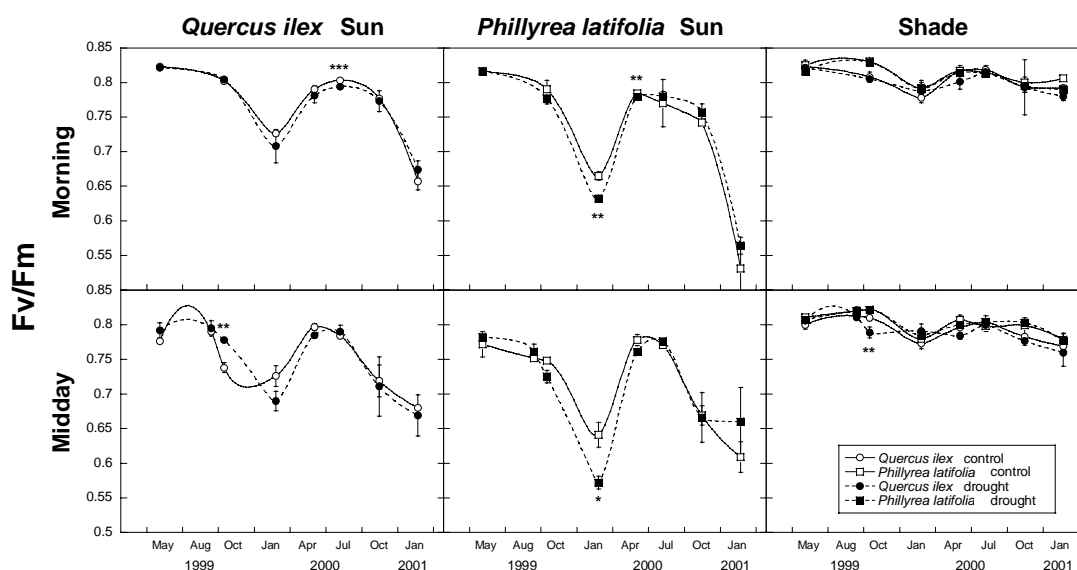


Figure 2. Seasonal course of maximum photochemical efficiency (Fv/Fm) in the morning and the midday in sunlit and shade leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. Error bars indicate standard error of the mean (n=2 plot averages of 10 measurements per plot). * p<0.1, ** p<0.05, *** p<0.01 statistical significance of the difference between drought and control treatment (ANOVA) for the signaled species.

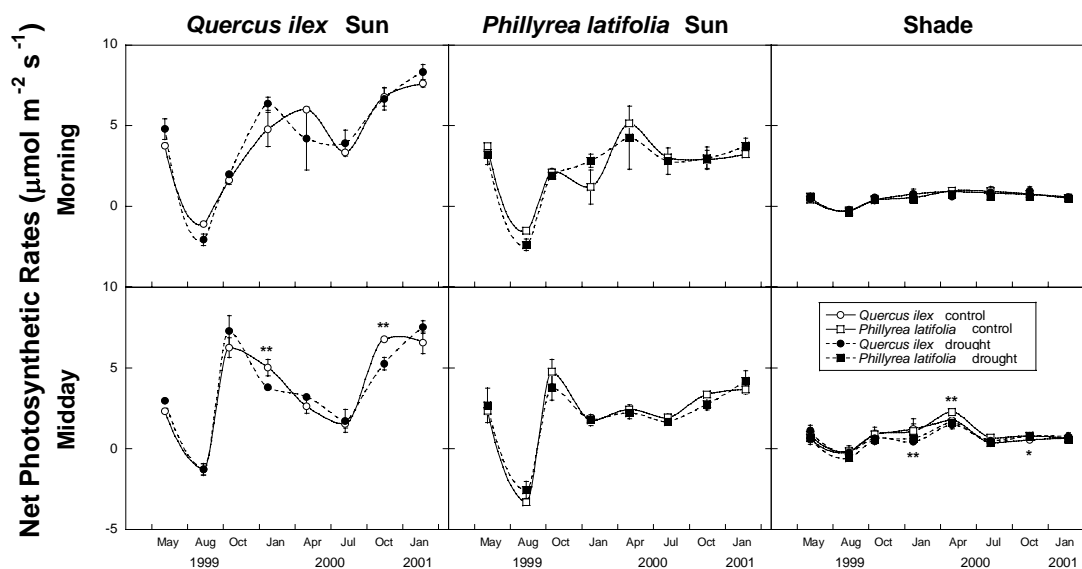


Figure 3. Seasonal course of net photosynthetic rates during the morning and midday in sunlit and shade leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. Error bars indicate standard error of the mean (n=2 plots and 2 measurement per plot). * p<0.1, ** p<0.05, *** p<0.01 statistical significance of the difference between drought and control treatment (ANOVA) for the signaled species.

Q. ilex reached maximum A rates at lower temperatures than *P. latifolia* (Fig. 6). There were no significant differences between control and drought plants, but in *Q. ilex*, the maximum A rates were reached at higher temperature under the drought treatment (12.5 °C and 16.5 °C in control and drought plots, respectively), whereas in *P. latifolia* maximum A rates were reached at similar temperatures in the two treatments (22.1 °C and 21.2 °C in control and drought plots, respectively). On the other hand, A rates reached values near 0 when T was between 34.8 °C and 36.5 °C, without significant differences between the two species and the two treatments (Fig. 6).

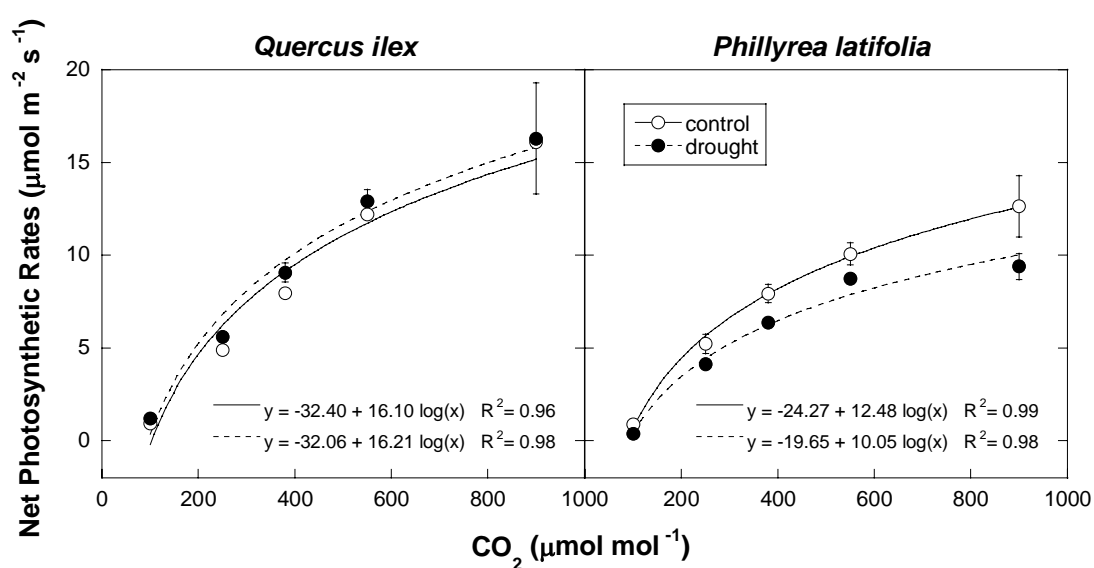


Figure 4. Responses of PPFD-saturated photosynthetic rates to different CO₂ concentrations in sunlit leaves of *Quercus ilex* and *Phillyrea latifolia* in control and drought plots. Each point is the mean of the measurements at the same CO₂ concentration in all response curves (n=4). (These response curves were measured in Autumn 2001).

There was an increase in net photosynthetic rates with increasing soil water availability in *Q. ilex* but not in *P. latifolia* (Fig. 7). In drought plots, both species had higher A values for a given soil moisture in the lower range of soil moistures but only *Q. ilex* kept this trait in the higher range of soil moisture (Fig. 7). A rates were very dependent on leaf water potential (which was strongly correlated with soil moisture), and very low values of both A and water potential were reached during summer 1999 (Fig. 7). A-VPD relationships showed higher slopes in *Q. ilex* than in *P. latifolia*, but no differences were found between control and drought plots (Fig. 8). A-g_s relationships had also higher slopes

in *Q. ilex* than in *P. latifolia*, and in *P. latifolia*, drought plants had higher slopes than control plants ($p=0.020$) (Fig. 8).

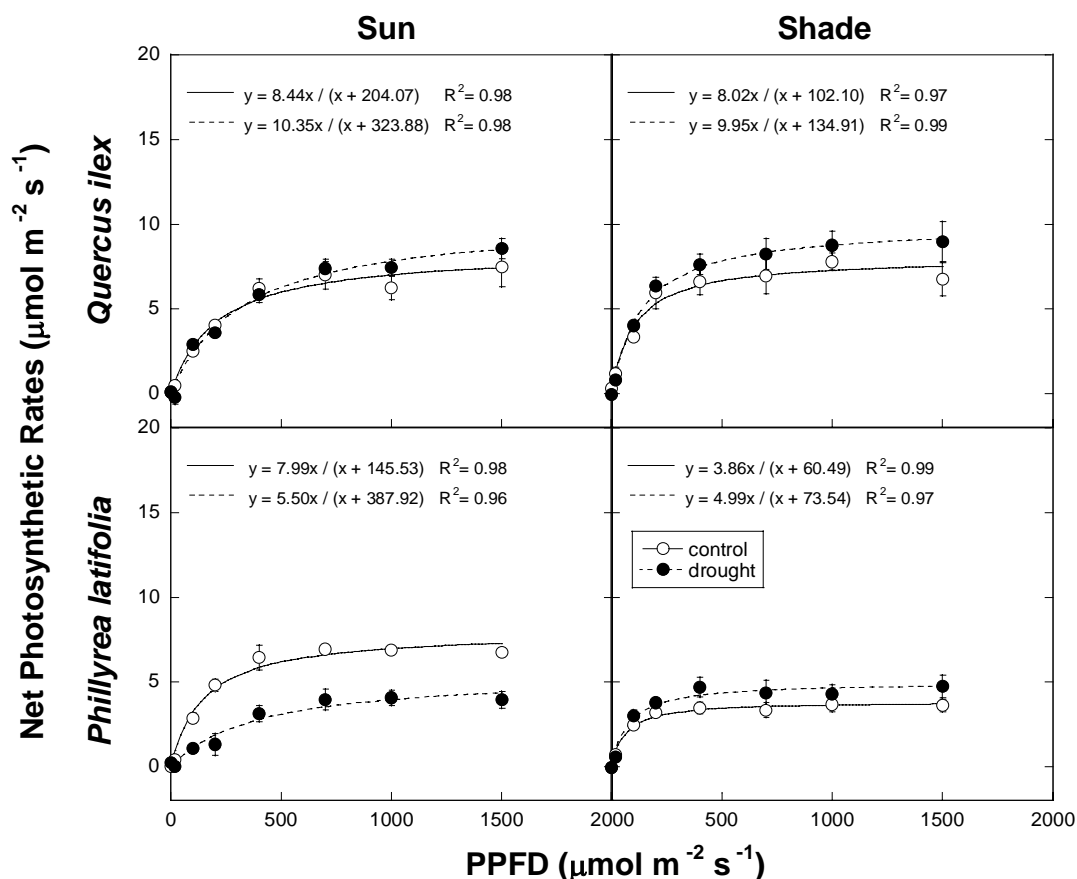


Figure 5. Responses of CO_2 -saturated photosynthetic rates to different PPFD fluxes in sunlit and shade leaves of *Quercus ilex* and *Phillyrea latifolia* in control and drought plots. Each point is the mean of the measurements at the same PPFD in all response curves ($n=4$). (These response curves were measured in Autumn 2001).

Sunlit leaves had higher transpiration than shade leaves in both species, and on average slightly higher in *P. latifolia* than in *Q. ilex* (Fig. 9). In the different seasons there were no significant differences between control and drought plants, but when all seasons were considered, sunlit leaves of *P. latifolia* showed higher E rates in control plots than in drought ones during midday ($p=0.005$).

WUE was slightly higher in sunlit leaves than in shade ones, and higher in *Q. ilex* than in *P. latifolia* (3.77 and $2.02 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, respectively for the overall studied period). In sunlit leaves during the morning, this difference between species was

statistically significant ($p=0.044$). In sunlit leaves and during midday, drought treatment exerted an opposite effect on the WUE of the two species in some dates such as January 2001 ($p=0.040$), when *Q. ilex* showed higher WUE in control plots whereas *P. latifolia* experimented higher WUE in drought plots (data not shown).

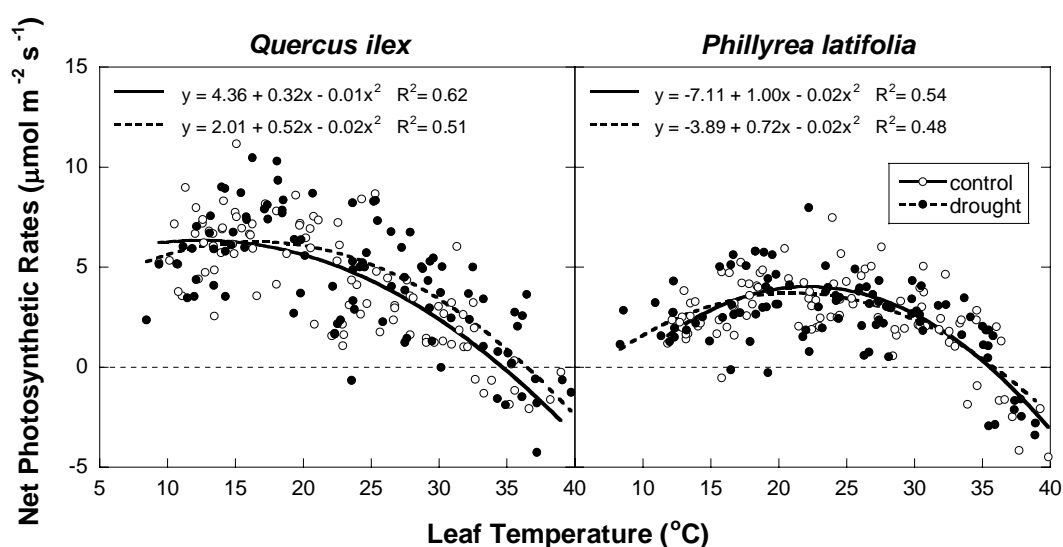


Figure 6. Relationships between net photosynthetic rates and leaf temperature. All measurements were made in sunlit leaves of *Quercus ilex* and *Phillyrea latifolia* and correspond to both morning and midday values of the overall experimental period ($n=192$).

Discussion.

The maximum photochemical efficiency of PSII (F_v/F_m) exhibited lower values in the colder seasons in agreement with several recent studies of Mediterranean plants (Larcher 2000, Oliveira & Peñuelas 2000, 2001). The results were also in agreement with other reports showing higher photosynthetic activity of *Q. ilex* than *P. latifolia* during winter (Tretiach 1993) and of *P. latifolia* than *Q. ilex* during summer drought (Tretiach 1993, Peñuelas et al. 1998). Drought treatment strongly decreased F_v/F_m values of *P. latifolia* during winter 2000, when drought stress coincided with cold stress, while in *Q. ilex*, drought also decreased F_v/F_m values more than one occasion during the study period. These differences indicated a higher resistance of *P. latifolia* to drought. ETR values were

higher in *Q. ilex* than in *P. latifolia*, as a result of its higher actual photochemical efficiencies of PSII ($\Delta F/F_m'$). In sunlit leaves, ETR values were higher during spring, when water availability was higher, in agreement with previous literature results (Valladares & Pearcy 2001).

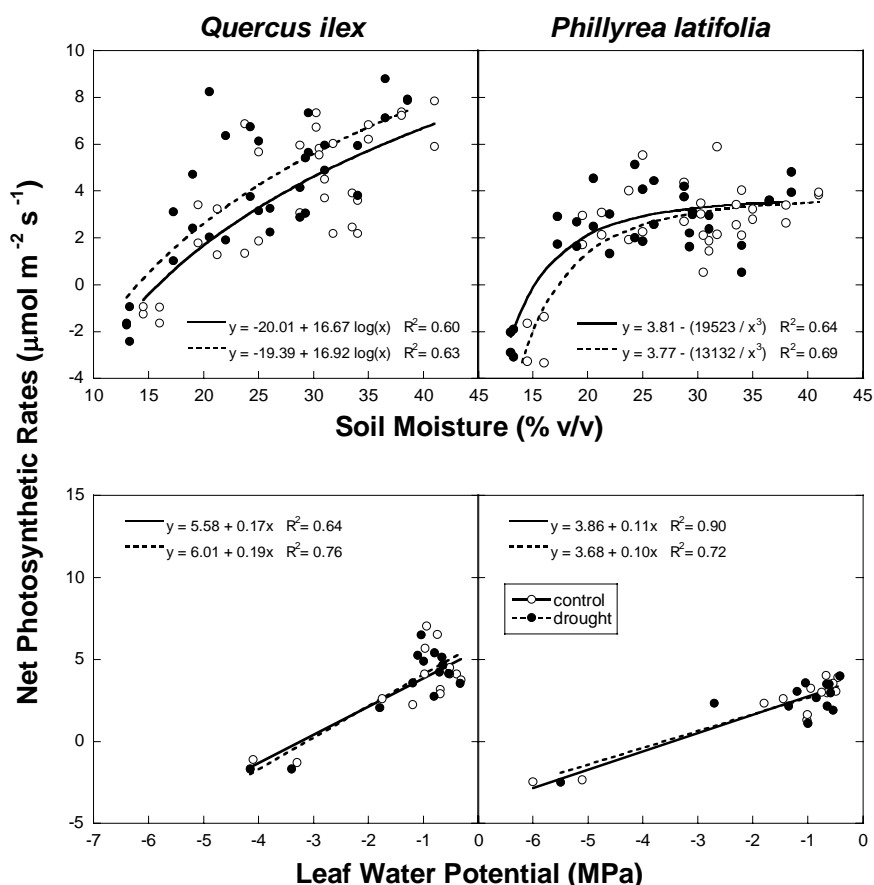


Figure 7. Relationships between net photosynthetic rates and soil moisture and leaf water potential. All measurements were made in sunlit leaves of *Quercus ilex* and *Phillyrea latifolia* and correspond to both morning and midday values of the overall experimental period (n=64 in soil moisture relationships and n=28 in leaf water potential relationships).

In both species A rates were higher in the morning than in the midday, especially in summer, when a strong stomatal closure occurred (Mooney et al. 1975, Tenhunen et al. 1980, Lange et al. 1982, Martínez-Ferri et al. 2000, Llusia & Peñuelas 2000). During late summer 1999 soil moisture was very low. Under the severe drought conditions of that summer none of the two species was able to reach a positive carbon gain, even in the morning. Higher photorespiration rates as a consequence of drought (Wingler et al. 1999)

or high temperatures (Peñuelas & Llusà 2002) might have greatly contributed to such negative net photosynthetic rates. Transpiration rates also decreased in the midday of the hotter seasons, but WUE was similar in morning and in midday because stomatal closure reduced proportionally both A and E rates. During most of the experimental period, *P. latifolia* experienced lower A rates, higher E rates and consequently lower WUE values than *Q. ilex*. However, during the summer drought *P. latifolia* reached similar, or even higher A rates and WUE than *Q. ilex* in agreement with previous results of Peñuelas et al. (1998) and Filella et al. (1998). On the other hand, only *P. latifolia* was able to reduce significantly its E rates (about 33%) and to increase its WUE (about 56%) in sunlit leaves during midday under drought treatment, precisely when water availability was the lowest.

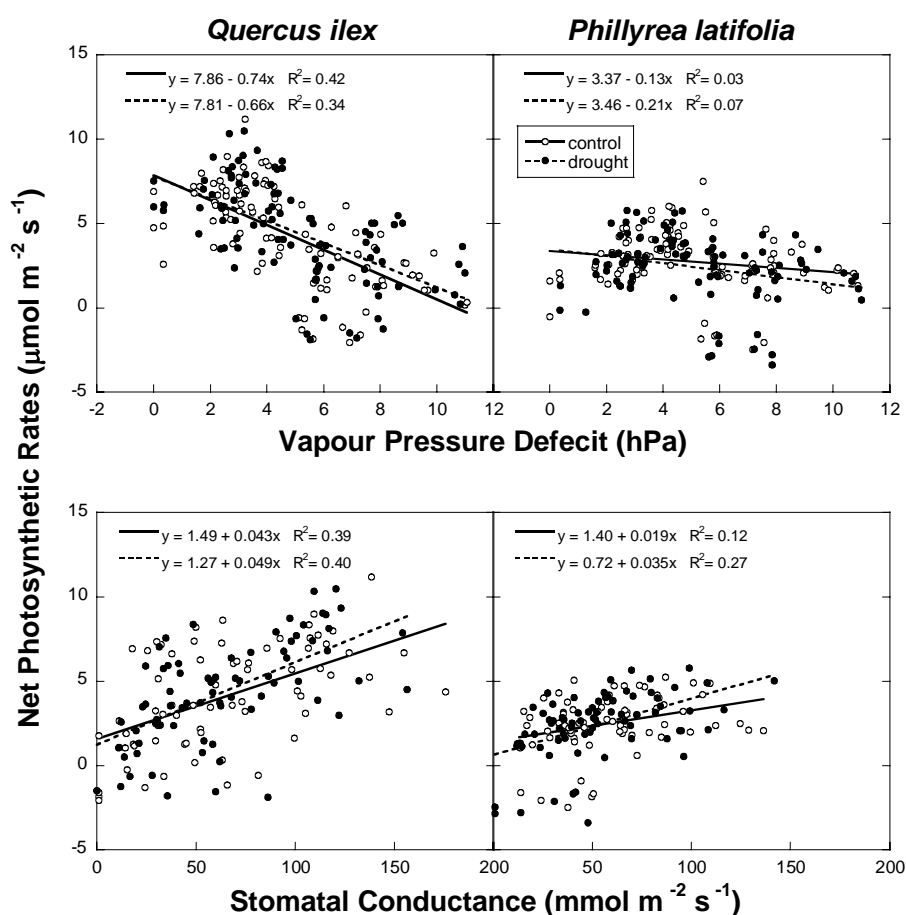


Figure 8. Relationships between net photosynthetic rates and vapour pressure deficit (VPD) and stomatal conductance. All measurements were made in sunlit leaves of *Quercus ilex* and *Phillyrea latifolia* and correspond to both morning and midday values of the overall experimental period (n=192 in vapour pressure deficit relationships and n=146 in stomatal conductance relationships).

Both species developed different physiological responses to the drought treatment. *Q. ilex* showed an increase in the temperature of maximum A rates and in the temperature at which A rates reached values near 0, while *P. latifolia* incremented the slope in A-gs relationship. In drought plots, both species tended to present higher net photosynthetic rates for a same soil humidity than in control plots, indicating a quick and incipient acclimation to continuous lower water availability. However, *Q. ilex* increased A rates with soil moisture whereas *P. latifolia* reached A rates saturation at low soil moisture of 17% (v/v). *Q. ilex* showed a greater water availability dependence and a greater water availability response than *P. latifolia*. In the same way, *Q. ilex* showed higher slopes in the relationships between A rates and leaf water potential, VPD, and stomatal conductance than *P. latifolia*.

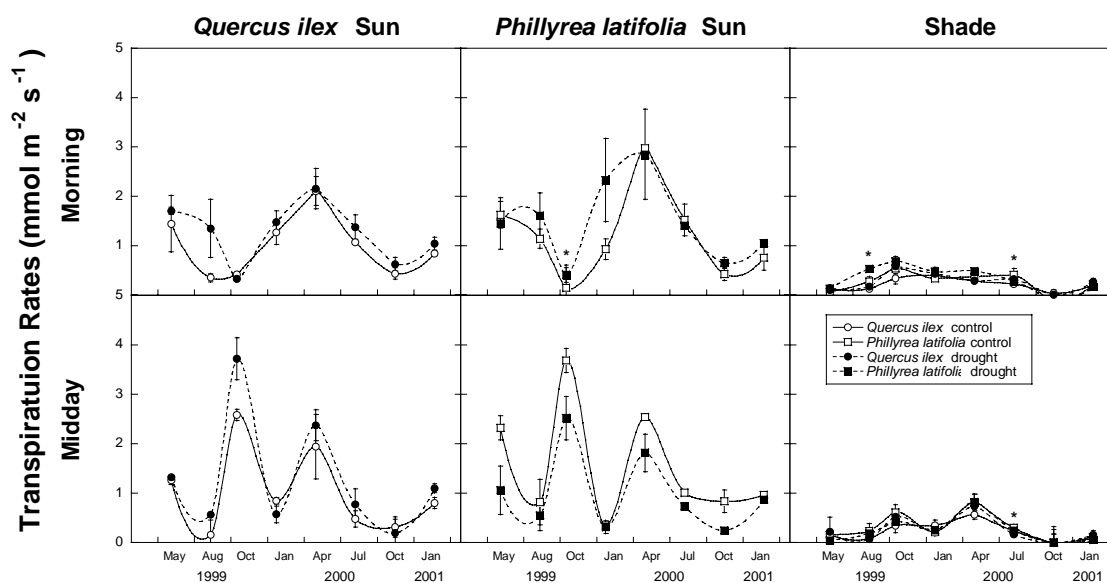


Figure 9. Seasonal course of transpiration rates during the morning and midday in sunlit and shade leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. Error bars indicate standard error of the mean (n=4 plots and 1 measurement per plot). * p<0.1, ** p<0.05, *** p<0.01 statistical significance of the difference between drought and control treatment (ANOVA) for the signaled species.

P. latifolia exhibited lower A rates in the CO₂ and PPFD response curves conducted in the sunlit leaves of the drought plots than in the control ones. These response curves were conducted in late autumn, when temperatures are cold and *P. latifolia* shows a great photoinhibition, especially under drought treatment. *Q. ilex* in the drought plots did not experience either A or E reduction in the response curves, and seemed to conserve less

water than *P. latifolia*. Surprisingly, in PPFDF curves, A rates of shade leaves were slightly higher in drought plots than in control ones in both species. Other studies showed, on the contrary greater A decrease in shade plants than in sunlit plants during a drought period (Valladares & Pearcy 2002). We studied sunlit and shade leaves in the same plant, and it seemed that shade leaves, under drought conditions, experimented an increase in A rates to compensate the A decrease of sunlit leaves. In natural conditions shade leaves received very low radiation and their A rates are low in both control and drought plots, so carbon gain at whole plant level seems to be more dependent on sunlit leaves than on shade ones.

In the marked seasonality of Mediterranean climate conditions, drought has strongly influenced evolution and plant life (Pereira & Chaves 1995). Predicted water stress in the Mediterranean Basin (IPCC 2001) may be associated with physiological and phenological responses in plant species (Peñuelas & Filella 2001, Peñuelas et al. 2002), but different species can develop different responses to these climatic changes. It is likely that more drought- and heat-resistant species such as *P. latifolia* will tolerate the increase of temperature and dry conditions better than more mesic less cold-sensitive species such as *Q. ilex*. These physiological responses could be followed by changes in carbon acquisition of Mediterranean forests (Reichstein et al. 2002), and in a long term, by changes in species distribution conferred by the different capacity of different species to survive under the new climatic constraints.

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Chapter 3.

Phenological patterns and reproductive effort of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* growing under a field experimental drought.

Romà Ogaya & Josep Peñuelas

Abstract.

A holm oak forest located in Prades mountains (NE Spain) was submitted to an experimental drought to elucidate its phenological responses. Soil water availability was reduced about 15% by plastic strips and funnels that partially excluded rain throughfall and by ditch exclusion of water runoff. We monitored eight phenological events: leaf shedding, leaf flushing; flower bud formation, flowering, flower senescence, fruit growth, fruit maturation, and seed dispersal in the three dominant species, *Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo* once a week from winter 1999 to winter 2001. We also collected litterfall in circular baskets randomly distributed on the ground every fifteen days from winter 1999 to winter 2001 and every two months from winter 2001 to winter 2003, and we estimated mushroom production per plot every week. *A. unedo* showed higher proportion of individuals flowering and fruiting than *Q. ilex* and *P. latifolia*. *A. unedo* was also the most sensitive species to water availability since drought treatment delayed its phenophases whereas it did not significantly affect the timing of the other two species phenophases. The flower and fruit production was also greater in *A. unedo* than in the two other species, but inter-annual variability was high and no significant drought effect was detected in any of these three species. Stem litterfall was greater in drought plots than in control plots during the overall studied period. On the other hand, drought treatment did not delay mushroom appearance, but drought plots had on average lower mushroom abundance than control plots. In a drier environment, as predicted for Mediterranean areas by global circulation models in a near future, drought-resistant species such as *P. latifolia* could present greater ability to produce reproductive structures than less resistant species such as *Q. ilex* or *A. unedo*. This different response among species could produce changes in seedling recruitment and resprouting ability, and in the longer term, in species distribution.

Keywords: *Arbutus unedo*, climate change, drought, flowering, fruiting, litterfall, Mediterranean trees, mushroom, *Phillyrea latifolia*, *Quercus ilex*, phenology.

Introduction.

Mediterranean climate is characterized by a pronounced seasonality. Rainfall is concentrated in spring and autumn, the lowest temperatures are present in winter and the highest ones in summer (Mitrakos 1980), when drought period occurs due to the low precipitation and the high temperatures (Di Castri & Mooney 1973, Terradas & Savé 1992, Pereira & Chaves 1995). Avoiding winter cold and summer drought, several Mediterranean plants flower in spring, at the same time that they experience an intense vegetative growth, and some others flower in autumn (Orshan 1989, de Lillis & Fontanella 1992, Castro-Díez & Montserrat-Martí 1998, Picó & Retana 2001). The development of fruits starts few time after flowering, but the duration of fruiting is very variable depending on the species. Evergreen Mediterranean species usually experienced a period of intense leaf shedding coinciding with formation of new leaves (Orshan 1989, de Lillis & Fontanella 1992, Castro-Díez & Montserrat-Martí 1998).

Holm oak (*Quercus 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 *Quercus ilex* (Lloret & Siscart 1995, Peñuelas et al. 1998, 2000, Ogaya & Peñuelas 2003). *Arbutus unedo* L. is another tall shrub typical from holm oak forest that shows higher drought-sensitivity than the two other species, at least in some Mediterranean areas (Ogaya et al. 2003). *Q. ilex* and *P. latifolia* flower during spring, at the same season of a peak in vegetative growth (*Q. ilex* usually also experiences a second vegetative growth peak in autumn). In contrast, *A. unedo* flowers in autumn and usually only shows a peak in vegetative growth during spring (de Lillis & Fontanella 1992, Castro-Díez & Montserrat-Martí 1998).

In the Mediterranean regions, a reduction in water availability is expected in the near future by the predicted increases of temperatures, and the consequent increases of evapotranspiration rates (IPCC 2001), which are predicted to be greater than those already occurred in the XX century (Piñol et al. 1998, Peñuelas et al. 2002). Climatic environmental changes could be followed by changes in timing of some phenological events with likely important ecological consequences for example through the interactions with other species (Fitter et al. 1995, Peñuelas & Filella 2001). Changes in water availability could also produce indirect effects on plant development and phenology, through the effects on ecological processes strongly dependent on climatic constrains such

as soil organic matter decomposition and soil respiration rates (Hanson et al. 2003) or mushroom productivity (Pilz & Molina 2002). The study of changes in phenology is essential to improve predictions about the seedling recruitment of dominant trees, and future changes in their distribution. We aimed to study the effects of an experimental drought on the phenology of these co-occurring Mediterranean woody species with different performances under low water availabilities, to gain further knowledge on the drought effects on the biology of these species and on its near future performance and distribution.

Material and methods.

Study site.

The study was conducted in a holm oak forest at Prades Mountains in Southern Catalonia (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. The average annual temperature is 12°C and the annual rainfall 658 mm. Summer drought is pronounced approximately from mid-June to mid-September.

This holm oak forest is very dense (16616 trees ha⁻¹) and it is dominated by *Quercus ilex* L. (8633 trees ha⁻¹), *Phillyrea latifolia* L. (3600 trees ha⁻¹) and *Arbutus unedo* L. (2200 trees ha⁻¹) 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 (*Sorbus torminalis* (L.) Crantz and *Acer monspessulanum* L.).

Experimental design.

Eight 15 x 10 m plots were delimited at the same altitude along the slope. Half the plots received the drought treatment and the other half were considered control plots. The drought treatment consisted of partial rain exclusion by suspending PVC strips and funnels

at a height of 0.5-0.8 m above the soil. 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. Water intercepted by strips, funnels, and ditches was conducted outside the plots, below the bottom edge of the plots.

An automatic meteorological station installed between the plots monitored temperature, photosynthetic active radiation, air humidity, and precipitation each half-hour. Soil moisture was measured every two weeks throughout the experiment by time domain reflectometry (Tektronix 1502C, Beaverton, Oregon, USA) (Gray & Spies 1995). Three stainless steel cylindrical rods, 25 cm long, were permanently fully driven into the soil at four randomly selected places in each plot. The time domain reflectometer was connected to the ends of the rods in each measurement.

Phenological measurements.

The monitoring of the timing of the phenological events was based in Orshan's phenomorphological method (Orshan 1989). We monitored the presence or absence of eight phenological events: leaf shedding, leaf flushing (period of growth of leaves until full expansion); flower bud formation, flowering (flowers in anthesis), flower senescence (flowers brown or abscising), fruit growth (growth of fruits until full expansion), fruit maturation (fruit maturation after full expansion), and seed dispersal (fruit shedding) in the three dominant species (*Arbutus unedo*, *Phillyrea latifolia*, and *Quercus ilex*), once a week from winter 1999 to winter 2001. The intensity of each phenological event in each plot was visually determined as the percentage of plants showing the phenological event in the plot.

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 winter 2003. Litterfall dry weight was measured after drying it in a oven at 70°C until constant weight.

To estimate the proportion of leaf, flower and fruit litter relative to total remaining leaf biomass and to total aboveground tree biomass of each species, the latter were estimated by allometric relationships with the stem diameter at 50 cm height measured in

Q. ilex and *P. latifolia* growing in the area of study (outside the plots). To estimate *Arbutus unedo* biomass we used the allometric relationship calculated few years ago on the same area by Lledó (1990) (Table 1). The stem diameter was measured each winter in all *Q. ilex*, *P. latifolia* and *A. unedo* trees growing in the plots.

Table 1. Allometric relationships between stem diameter at 50 cm (D50), and leaf biomass (LB) and aboveground biomass (AB) in *Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo*. (*Arbutus unedo* relationships were taken from Lledó 1990).

Species	Allometric relationship	r	n	P-Value
<i>Q.ilex</i>	$\ln LB = 3.481 + 1.695 \ln D50$	0.907	12	<0.0001
<i>P.latifolia</i>	$\ln LB = 1.433 + 2.426 \ln D50$	0.812	13	<0.0001
<i>A.unedo</i>	$\ln LB = 1.887 + 2.157 \ln D50$	0.951	10	<0.0001
<i>Q.ilex</i>	$\ln AB = 4.900 + 2.277 \ln D50$	0.981	12	<0.0001
<i>P.latifolia</i>	$\ln AB = 4.251 + 2.463 \ln D50$	0.974	13	<0.0001
<i>A.unedo</i>	$\ln AB = 3.830 + 2.563 \ln D50$	0.989	10	<0.0001

The abundance of mushrooms in each plot was visually estimated once a week from winter 1999 to winter 2001, by establishing three categories: less than 0.5 kg of fresh weight, more than 0.5 kg and less than 1 kg of fresh weight, and more than 1 kg of fresh weight per plot.

Statistical analyses.

For each date, analyses of variance (ANOVA) were conducted with the percentage of plants presenting each phenological event in each plot as dependent variable, and with species and treatment application as independent factors. Data of percentage of plants presenting each phenological event 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 survival curves with the time of phenological events start as a survival time. We thereafter used the Log-Rank test to assess treatment differences.

Repeated measures ANOVAs were conducted with the shoot litter, flower litter, fruits litter, and leaf litter expressed as percentage of the total aboveground biomass or 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 litter was transformed to $\arcsin(p)^{1/2}$ to reach the normality assumptions of the ANOVA.

Other repeated measures ANOVA was conducted with the maximum mushroom biomass in each plot and season as dependent variable, and with treatment application as independent factors. Data of categories of mushroom biomass were transformed to $\arcsin(p)^{1/2}$ to reach the normality assumptions of the ANOVA. We also used Kaplan-Meier non-parametric method for the computation of survival curves with the start of mushroom growing season as a survival time. We thereafter used the Log-Rank test to assess treatment differences.

All analyses were performed with the Statview software package (Abacus Concepts Inc., 1998) and the Statistica software package (StatSoft Inc., 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 (12.39 °C) than in the year 1999 (12.19 °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 (about 15% v/v) were reached in summer, as a consequence of summer drought, and maximum values (about 35% v/v) in spring and autumn, coinciding with heavy rainfall periods. Differences in soil moisture between control and drought plots were significant on most of the samplings throughout the two years (Fig. 1). Control plots had on average 16% higher soil moisture than drought plots in 2000 and 13% in 1999.

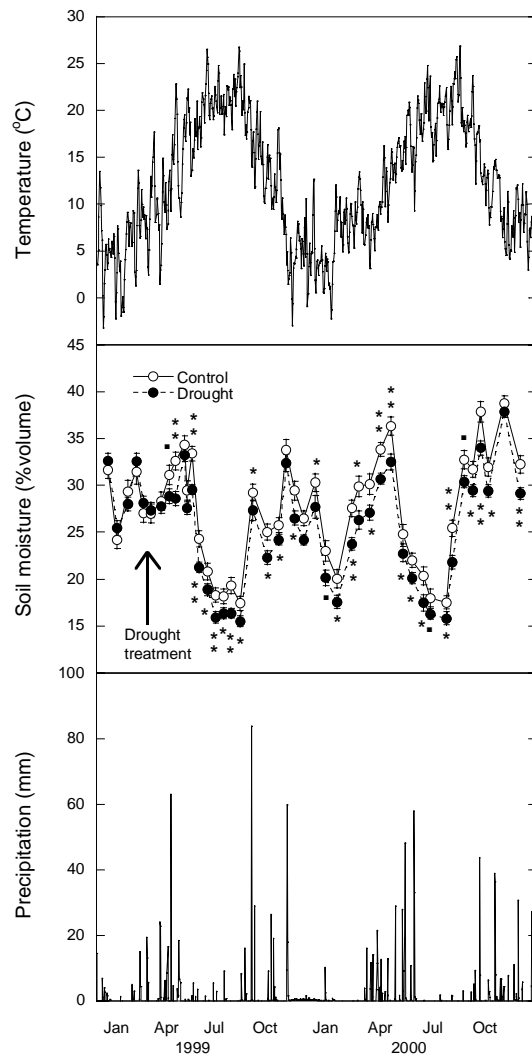


Figure 1. Seasonal course of daily mean temperature, soil moisture (0.25 m depth), and precipitation at the study site. Vertical bars in the soil moisture panel indicate standard errors of the mean ($n=4$ plot averages of 4 TDR measurements per plot). One square indicates ($p<0.1$) significant differences between the two treatments, one asterisk ($p<0.05$), and two asterisks ($p<0.01$). The arrow indicates the drought treatment start.

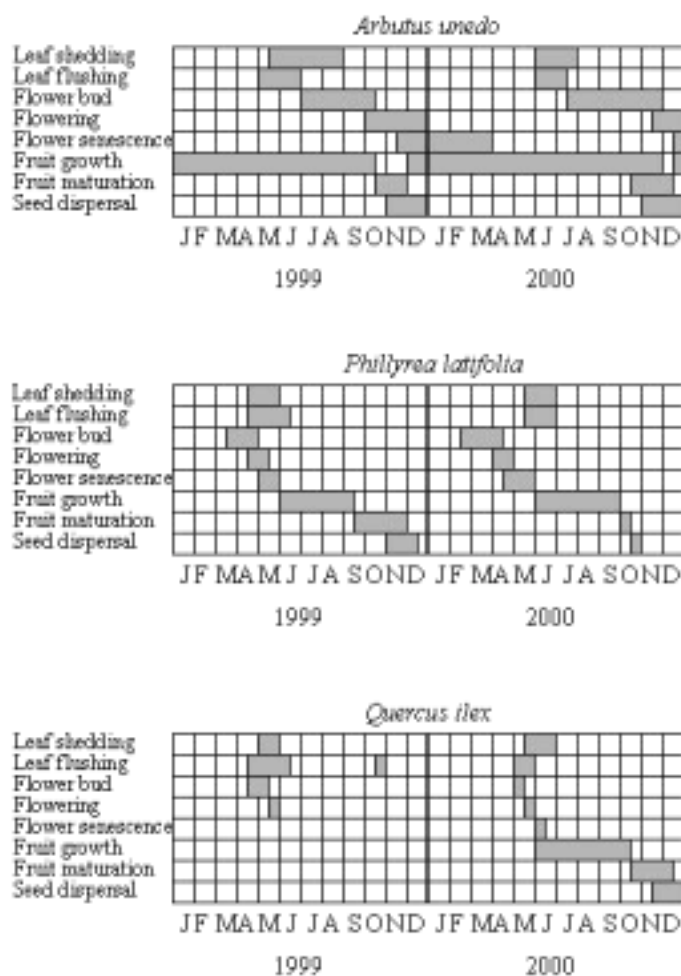


Figure 2. Phenological diagrams of *Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo* during the studied period. Grey bars indicate the duration of each phenophase in the control plots.

Phenological measurements.

The phenological pattern obtained in the experimental period was the typical from the three studied species (Fig. 2). In the area studied, *Q. ilex* leaf flushing took place in spring and in autumn 1999, but these autumn new leaves were damaged by freezing temperatures. This species flowered just after leaf flushing, on the new shoots. *P. latifolia* flowered in early spring on one-year old shoots, and later flushed its new leaves. Both species developed their fruits during summer and fruit maturation took place in the following autumn. *A. unedo* developed the new leaves in spring, and after that, flowered on new shoots during the next autumn. Fruit growth started just after flowering (in late autumn) and lasted one year. Fruit maturation took place the autumn of the following year.

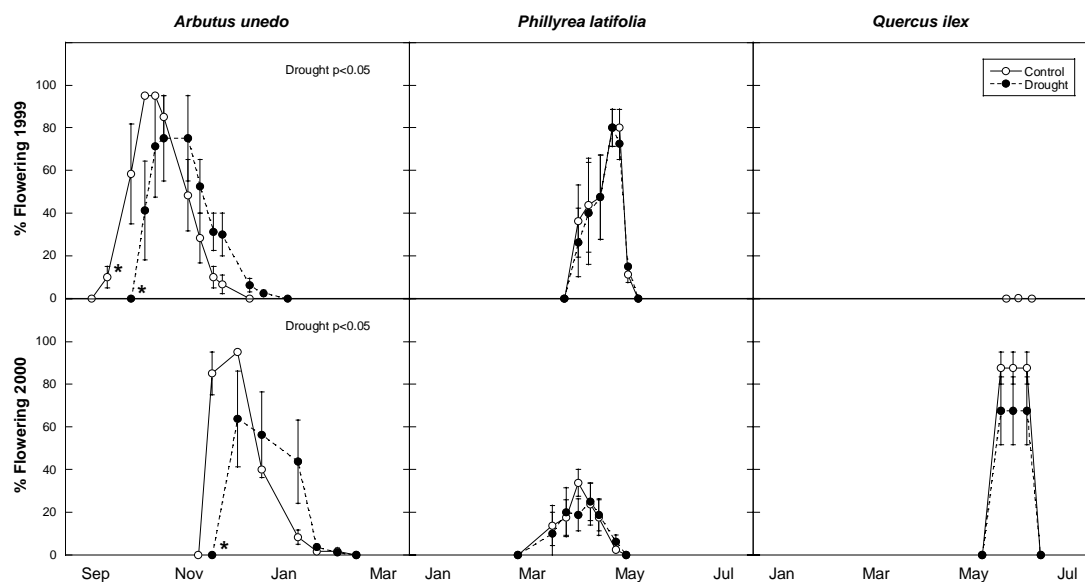


Figure 3. Percentage of *Q. ilex*, *P. latifolia* and *A. unedo* trees flowering during 1999 and 2000. Vertical bars indicate standard error of the mean (n=4 plots). One asterisk indicates (p<0.05) significant differences between the two treatments for each sampling date (ANOVA). The significance for the overall treatment effect was assessed with a Log-Rank test.

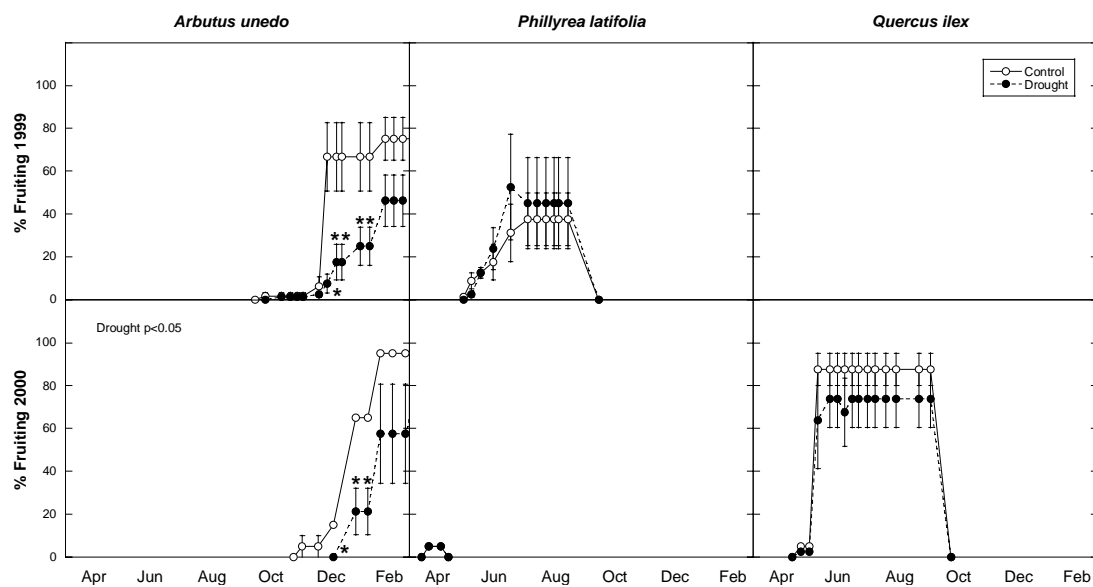


Figure 4. Percentage of *Q. ilex*, *P. latifolia* and *A. unedo* trees developing fruits during 1999 and 2000. Vertical bars indicate standard error of the mean (n=4 plots). One asterisk indicates (p<0.05) significant differences between the two treatments for each sampling date (ANOVA). The significance for the overall treatment effect was assessed with a Log-Rank test.

Q. ilex did not show important inter-annual variations in timing of phenological

events, but in 1999 fruit production was negligible. In the other two species, leaf flushing (and also flowering in *A. unedo*) was delayed in 2000 relative to 1999 (Fig. 2). In contrast, flowering in *P. latifolia* occurred earlier in 2000 than in 1999.

Drought treatment delayed *A. unedo* flowering both in 1999 and 2000 ($p < 0.05$), and also fruit growth in year 2000 ($p < 0.05$) (Fig. 3, 4). On the other hand, *A. unedo* from drought plots reached lower percentages of fruiting trees than those of control plots in both years ($p < 0.05$) (Fig. 4). *Q. ilex* and *P. latifolia* did not show any delay in flowering neither any change in fruit growth (Fig. 3, 4).

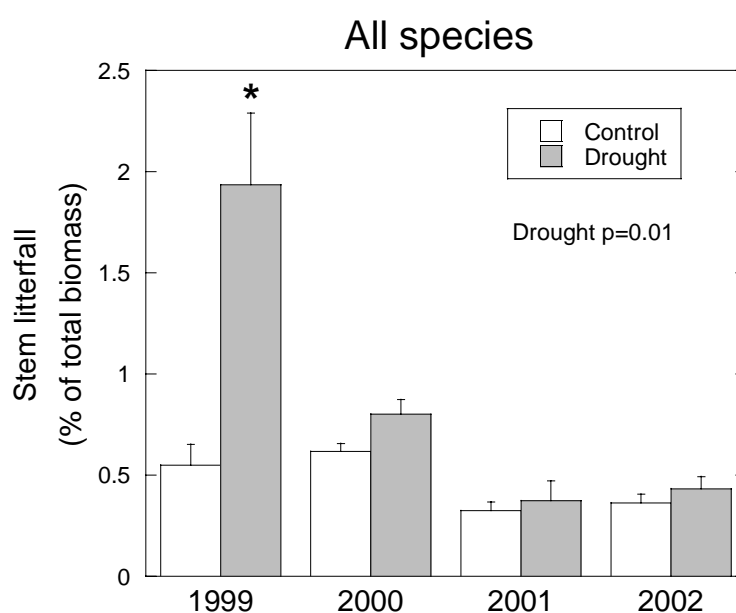


Figure 5. Percentage of shoot litterfall relative to the total aboveground biomass in each plot. Vertical bars indicate standard error of the mean ($n=4$ plots). One asterisk indicates ($p < 0.05$) significant differences between the two treatments for each sampling date (ANOVA), and for the overall treatment effect (repeated measures ANOVA).

Shoot litterfall was higher in drought plots than in control ones during the overall studied period ($p=0.01$) and especially in 1999 (Fig. 5). The amount of flower and fruit production, and also leaf shedding, showed a high inter-annual variability (Fig. 3, 4). Maybe because of that, drought effect was not significant despite the lower fall of flowers and fruits observed in *A. unedo*, and the higher leaf litterfall observed in *P. latifolia*, in some years under drought conditions (Fig. 6). The relative amount of leaf, flower and fruit fall was higher in *A. unedo* than in the two other species ($p < 0.05$), and higher in *P. latifolia*

than in *Q. ilex* (Fig. 6).

Drought treatment did not delay mushroom appearance (data not shown), but mushroom production was on average higher in control plots than in drought ones ($p=0.02$) (Fig. 7).

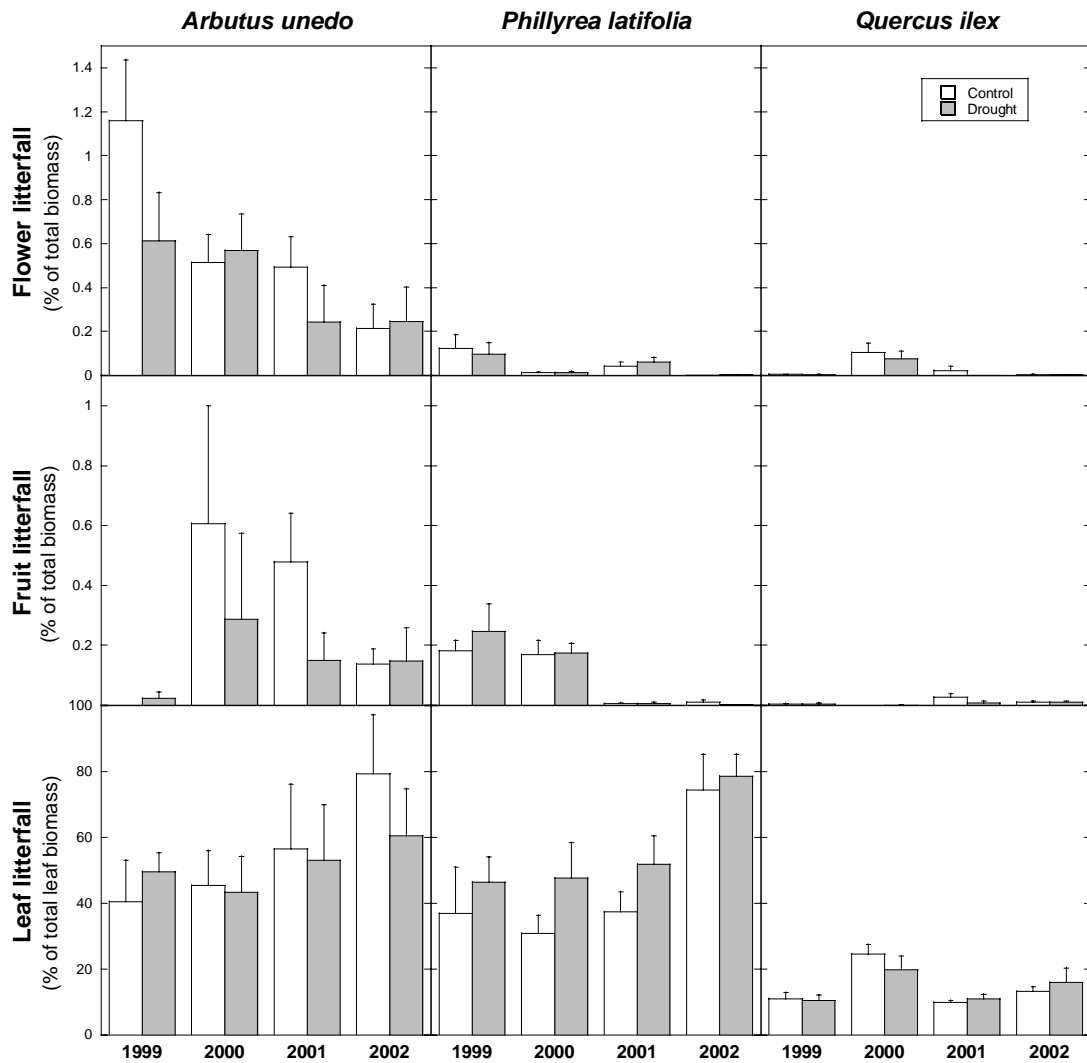


Figure 6. Percentage of flower and fruit litter relative to the total aboveground biomass, and percentage of leaf litter relative to the total biomass of remaining leaves, in each studied species. Vertical bars indicate standard error of the mean (n=4 plots).

Discussion.

In the Mediterranean regions, the occurrence of phenological events is widely correlated with air temperature (García-Mozo et al. 2002, Spano et al. 1999), and also with water availability (Kramer et al. 2000, Pardos et al. 2003). In our study, flowering in *P. latifolia* seemed to be dependent on late winter temperatures, but leaf flushing in *A. unedo* and *P. latifolia*, and flowering in *A. unedo*, seemed to be more dependent on water availability because their occurrence in years 1999 and 2000 coincided with spring rainfall periods. The timing of leaf flushing and flowering in *Q. ilex* was less affected by the climatic constraints, but fruit production in *Q. ilex* could be related to water availability since lower water availability during the first stages of fruit development in year 1999 coincided with very low fruit production.

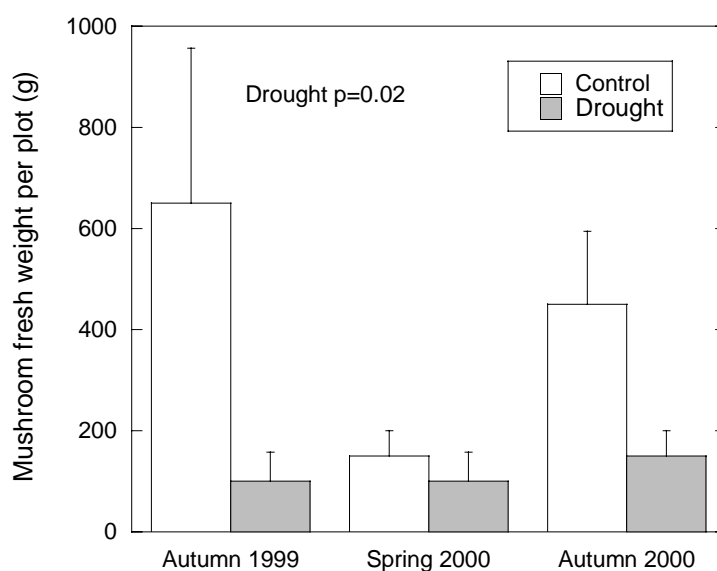


Figure 7. Estimated fresh weight of the overall mushroom biomass produced in each plot. Vertical bars indicate standard error of the mean (n=4 plots).

Flowering is mainly associated with low vegetative growth (Fitter 1986, Bazzaz et al. 1987) and with resource competition between reproductive and vegetative structures (Jonasson et al. 1997). *Q. ilex* and *P. latifolia* developed new leaves and flowers during the same season, but *A. unedo* did not, avoiding competition by resources between the synthesis of reproductive and vegetative structures.

Drought treatment produced a delay in flowering and fruit development only in *A. unedo*, precisely the species showing the highest proportion of individuals developing these phenological events. Other study conducted in the same experimental field area revealed higher stem radial growth rates in *A. unedo* than in *Q. ilex* and *P. latifolia*, and stronger decreases on radial growth of *A. unedo* in response to this experimental decrease in water availability (Ogaya et al. 2003). All these results together show a higher drought sensitivity of *A. unedo* than *Q. ilex* or *P. latifolia*.

Higher stem litter in the drought plots could indicate higher drying rates in current-year shoots. Under drought conditions, trees seem unable to maintain the same number of thin shoots than trees in control plots. On the other hand, higher mortality rates were observed in trees growing in the drought plots than in the control ones (Ogaya et al. 2003), so greater shoot litter from dead trees can be expected in these drought plots.

Low water availability is associated with a reduction in reproductive effort in some Mediterranean plants (Aronson et al. 1993). *A. unedo* presented a higher reproductive effort than the two other species studied, and it was again the species most affected by drought treatment. Despite of a great inter-annual variability, our drought treatment seemed to reduce the production of reproductive structures in *A. unedo*, which will likely reduce recruitment. The recruitment of young plants is a key factor determining the future species distribution. However, *Q. ilex* (the species showing the lowest reproductive effort) and *A. unedo* have a strong capacity to produce sprouts. These sprouts obtain water and nutrient resources from a root system well developed by a previously established individual, showing higher growth rates and better water status than mature plants (Castell et al. 1994). Seedlings only obtain water and nutrient resources from roots developed by themselves, and *P. latifolia* recruitment is ensured by germinating seedlings (Herrera et al. 1994). High drought resistance in *Q. ilex* sprouts, and an important seedling mortality in *P. latifolia* seedlings were observed in the area studied (Lloret et al. submitted). In spite of this, the performance of adult trees is still crucial for the long-term presence of any species. An immediate effect is through the mentioned resprouts nurturing in species such as *Q. ilex* or *A. unedo*, that can not be conducted properly after repeated drought episodes emptying the plant reserves and decreasing the plant capacities to access to water and nutrient sources.

Drought treatment exerted also an important effect on other ecological parameters such as the total amount of mushroom production, and in consequence, the organic matter decomposition rates and the biogeochemical cycling of these ecosystems. These processes will further affect the plants vegetative and reproductive performance.

The results here presented show that the decrease in water availability expected for the next decades in the Mediterranean areas (IPCC 2001) could produce changes on the timing of phenological events and on the reproductive effort of some species. It is likely that not all the species will be affected equally; the less drought-resistant ones could decrease their flower and fruit production, decreasing their seedlings establishment. In a long-term, if the decrease in water availability persists, the distribution of drought-resistant species could be favored in detriment of the less drought-resistant species.

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Chapter 4.

Leaf morphology, longevity, and demography of two coexisting woody species in a holm oak forest submitted to an experimental drought.

Romà Ogaya & Josep Peñuelas

Abstract.

Foliar morphology, longevity, and demography were examined in *Quercus ilex* and *Phillyrea latifolia* growing in a holm oak forest in Prades mountains (NE Spain). Four plots (10 x 15m) of this forest were submitted to an experimental drought during three years. Soil water availability was reduced about 15% by plastic strips and funnels that partially excluded rain throughfall and by ditch exclusion of water runoff. Other four plots with no treatment (control plots) were also monitored. Leaf area, thickness and LMA (leaf mass per area ratio) were measured in sun and shade leaves of both species each one of the seasons of three consecutive years. Leaf longevity, the mean number of current-year shoots produced per previous-year shoot (S_n/S_{n-1}), the mean number of current-year leaves per previous-year shoot (L_n/S_{n-1}), and the percentage of previous-year shoots that developed new ones (% developed) 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 *Quercus ilex* than in *Phillyrea latifolia*, but leaf density was higher in the latter species. Drought treatment reduced ca. 17% the leaf area of *Q. ilex* and ca. 2.5% the leaf thickness and the LMA of both species. LMA was more correlated with leaf thickness than with leaf density. Leaf shedding was greater in *Phillyrea latifolia* than in *Quercus ilex* during overall the studied period. Drought treatment produced a slight advancement in leaf shedding in *Phillyrea latifolia*, and a decrease of 32% in S_n/S_{n-1} , 41% in L_n/S_{n-1} , and 26% in percentage of developed shoots in *Quercus ilex*. Both changes produced lower total plant leaf area of these evergreen species under drought conditions, being the effect stronger in the less drought-resistant species *Quercus ilex*, which, under increasingly drier conditions, might thus lose its current competitive advantage in these Mediterranean holm oak forests.

Keywords: drought, leaf longevity, leaf mass per area, *Phillyrea latifolia*, *Quercus ilex*.

Introduction.

Many Mediterranean plants are evergreen and have leaves with great longevity, thick cuticle, high leaf mass per area ratio (LMA), low nutrient content, high concentration of structural materials, and low photosynthetic rates (Turner 1994a). It has been observed that thickening and high concentration of structural materials give these leaves a high LMA (Cowling & Campbell 1983, Turner 1994b). Several studies have shown an increment of LMA under drought conditions (Salleo & Lo Gullo 1990, Wookey et al. 1995, Carter et al 1997, Yin 2002), and in Mediterranean species, low LMA values during the wettest periods have often been observed (Gratani 1996, Harley et al. 1987, Kyparisis & Manetas 1993).

Other studies have shown similar leaf structures in nutrient-poor soils, where long-lived leaves with high concentrations of structural material are favored for their higher nutrient retention and lower maintaining costs (Turner 1994a, Aerts 1995). This leaf structure has also been often related with herbivorous and general defense in front of stress (Chabot & Hicks 1982, Turner 1994a, Aerts 1995). In fact, although this particular leaf structure (sclerophylly) was once mainly considered an adaptative response to low water availability occurring in Mediterranean environments during summer drought, these sclerophyllous leaf traits were present earlier than the Mediterranean climate appeared in the early Pliocene (Herrera 1992). Therefore, it seems that this leaf structure is not linked to any particular environmental stress. In fact, it confers to the leaf a considerable capacity to resist a wide range of environmental conditions (Turner 1994a).

On the other hand, together with water availability, irradiance has also an important effect on leaf morphology (Yun & Taylor 1986) because like at lower moisture availabilities, at higher irradiances leaves tend to be smaller, with higher LMA, density and thickness (Chabot & Chabot 1977, Sobrado & Medina 1980, Witkowski & Lamont 1991, Groom & Lamont 1997). Higher LMA can be produced by increases in leaf density, in leaf thickness or both. Recent studies showed an important decrease in leaf thickness under low irradiance and an important increase in leaf density under low soil water availability (Witkowski & Lamont 1991, Groom & Lamont 1997, Niinemets 2001).

Leaf longevity is positively correlated with LMA (Chabot & Chabot 1977, Sobrado & Medina 1980, Turner 1994a, Niinemets 2001, Yin 2002), and negatively correlated with assimilation rate per unit dry weight of leaf (Körner 1989, Reich et al. 1992, Eamus & Prichard 1998). 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 semi deciduous plants with lower LMA values, shorter lifespan, and greater CO₂ uptake rates are more frequent (Chabot & Hicks 1982, Harley et al. 1987, Jonasson 1989, Kikuzawa 1991, Kyparissis & Manetas 1993, Werner et al. 1999).

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 (IPCC 2001), which are predicted to be greater than those already occurred in the XX century (Piñol et al. 1998, Peñuelas et al. 2002). Among the different species, different physiological responses are expected under the new climatic conditions. It is likely that more drought tolerant species will be favored in detriment of more mesic ones. Holm oak (*Quercus 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 *Quercus ilex* (Lloret & Siscart 1995, Peñuelas et al. 1998; 2000, Ogaya & Peñuelas 2003). 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 different performances under low water availabilities. We aimed 1) to gain a better understanding of these Mediterranean species leaf biology, 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. The average annual temperature is 12°C and the annual rainfall 658 mm. Summer drought is pronounced and usually lasts for 3 months.

This holm oak forest is very dense and it is dominated by *Quercus ilex* L. and *Phillyrea latifolia* L. with abundant presence of other evergreen species well adapted to

drought conditions (*Arbutus unedo* L., *Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L.), and occasional individuals of deciduous species (*Sorbus torminalis* (L.) Crantz and *Acer monspessulanum* L.).

Experimental design.

Eight 15 x 10 m plots were delimited at the same altitude along the slope. Half the plots received the drought treatment and the other half were used as control plots. The drought treatment consisted of partial rain exclusion by suspending PVC strips and funnels at a height of 0.5-0.8 m above the soil. 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. Water intercepted by strips, funnels, and ditches was conducted outside the plots, below the bottom edge of the plots. Drought treatment was started in March 1999.

Temperature, photosynthetic active radiation, air humidity, 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, Oregon, USA) (Zegelin et al. 1989). Three stainless steel cylindrical rods, 25 cm long, were permanently fully driven into the soil at four randomly selected places in each plot. The time domain reflectometer was connected to the ends of the rods in each measurement.

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. Leaf area of collected leaves was measured in a Li-Cor 3100 Area Meter (Li-Cor Inc., Nebraska, USA), leaf thickness was measured using a Palmer micrometer, and leaf dry weight was measured by weighting the leaves after they had reached constant weight in an oven at 70°C. Leaf per mass area ratio (LMA) was calculated by dividing leaf dry mass by leaf area, and leaf density was calculated by dividing leaf weight by leaf volume (leaf area x 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). On the other hand, 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 from summer 1998 to summer 2002.

Mean number of current-year shoots produced per previous-year shoot (S_n/S_{n-1}), mean number of current-year leaves per previous-year shoot (L_n/S_{n-1}), and the percentage of previous-year shoots that developed new shoots in (% developed) 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 weighted.

To establish the proportion of fallen leaves relative to total leaf biomass, the latter was estimated by allometric relationships between tree leaf biomass (Y) and stem diameter (x) measured in *Q. ilex* ($Y=3.48+1.70x$; $R^2=0.91$) and *P. latifolia* ($Y=1.43+2.43x$; $R^2=0.81$) growing in the area of study (outside the plots). The stem diameter at 50 cm height was measured in all *Q. ilex* and *P. latifolia* trees growing in the plots.

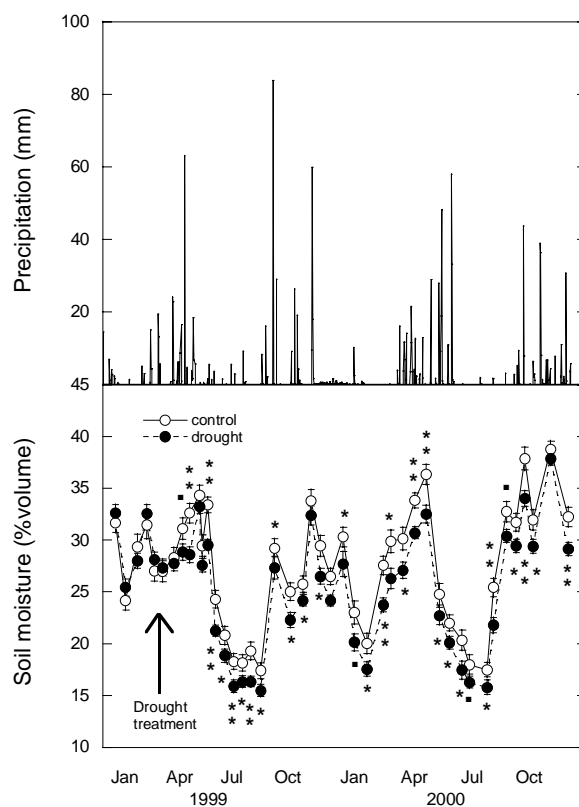


Figure 1. Seasonal course of soil moisture (0.25 m depth), and monthly values of precipitation at the Prades study site. Vertical bars indicate standard errors of the mean ($n=4$ plot average of 4 TDR measurements per plot). One square indicates ($p<0.1$) significant differences between the two treatments, one asterisk ($p<0.05$), and two asterisks ($p<0.01$).

Statistical analyses.

Analyses of variance (ANOVA) were 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 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. ANCOVAs were conducted with sun and shade leaves separately.

Other ANOVAs were 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 in each leaf cohort. We thereafter used the Log-Rank test to assess treatment differences.

Other 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 Inc., 1998) and the Statistica software package (StatSoft Inc., 2001).

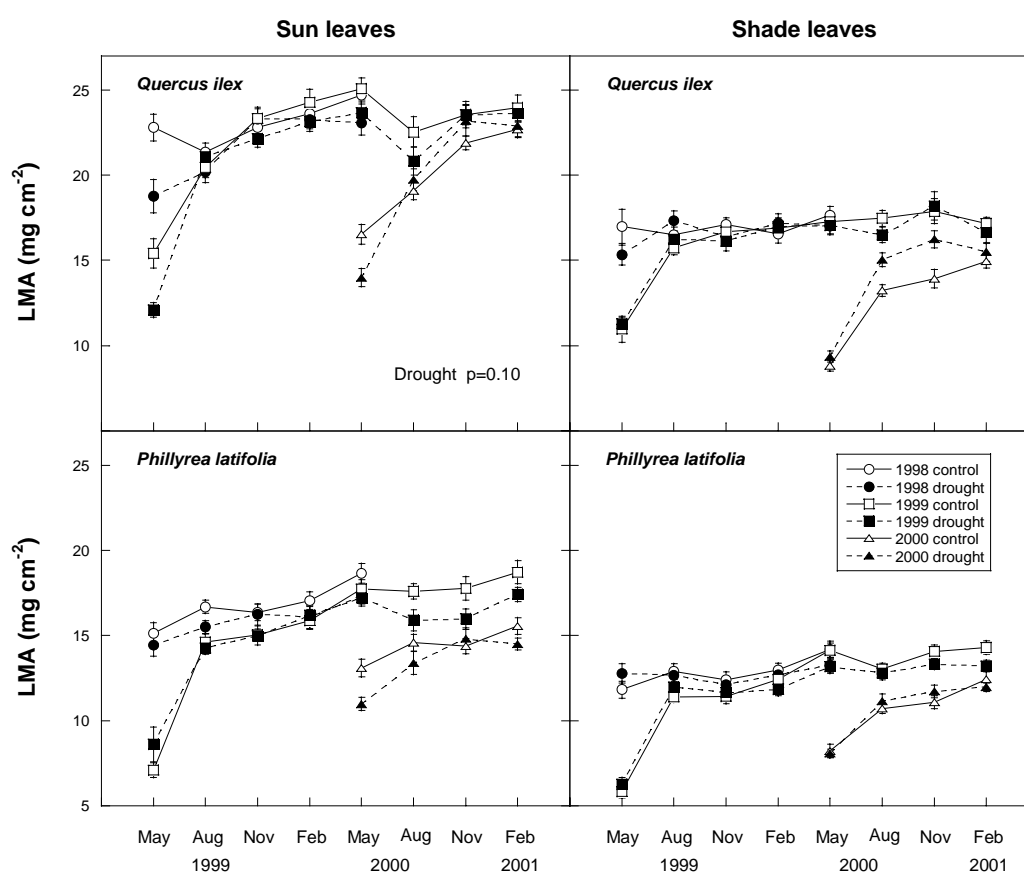


Figure 2. Seasonal course of leaf mass per area ratio (LMA) in leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. Error bars indicate standard error of the mean ($n=4$ plot averages of 5 measurements per plot). When significant (ANOVA), drought treatment effect is depicted in the panels.

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 (12.39 °C) than in the year 1999 (12.19 °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).

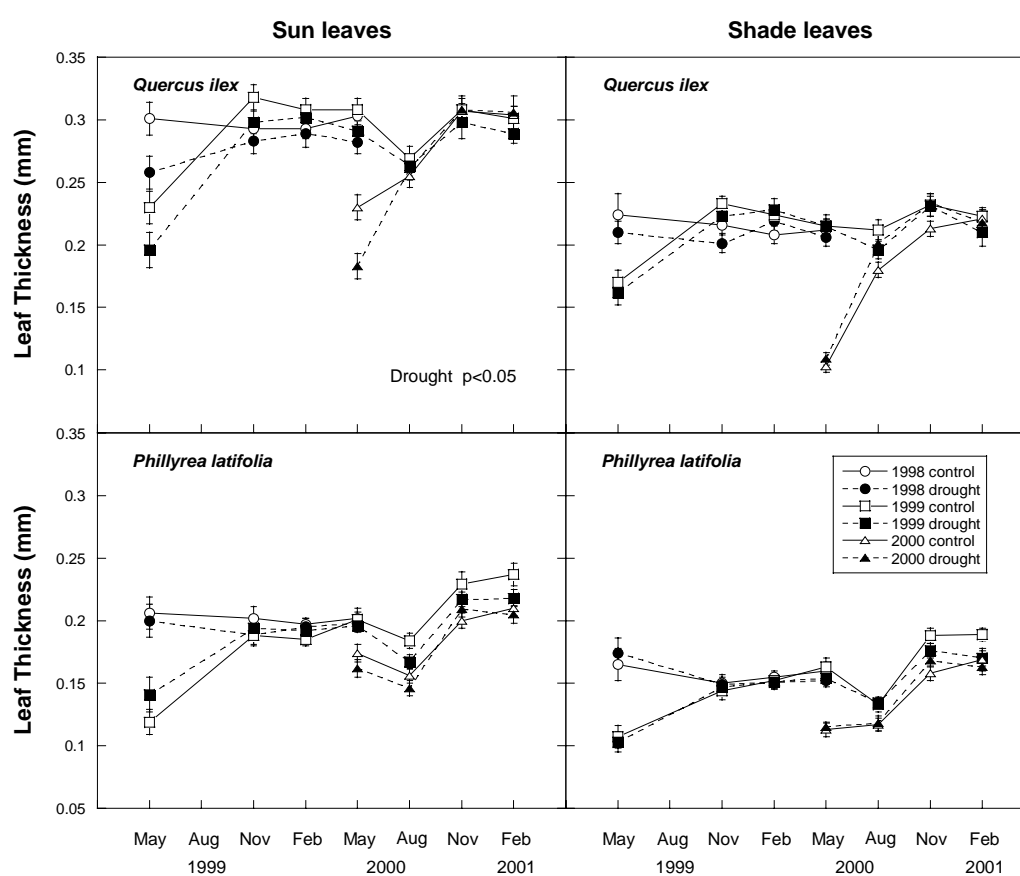


Figure 3. Seasonal course of leaf thickness in leaves of *Quercus ilex* and *Phillyrea latifolia* during the experimental period. Error bars indicate standard error of the mean (n=4 plot averages of 5 measurements per plot). When significant (ANOVA), drought treatment effect is depicted in the panels.

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 (about

15% v/v) were reached in summer, as a consequence of summer drought, and maximum values (about 35% v/v) in spring and autumn, coinciding with heavy rainfall periods. Differences in soil moisture between control and drought plots were significant on most of the samplings throughout the two years (Fig. 1). Control plots had on average 16% higher soil moisture than drought plots in 2000 and 13% in 1999.

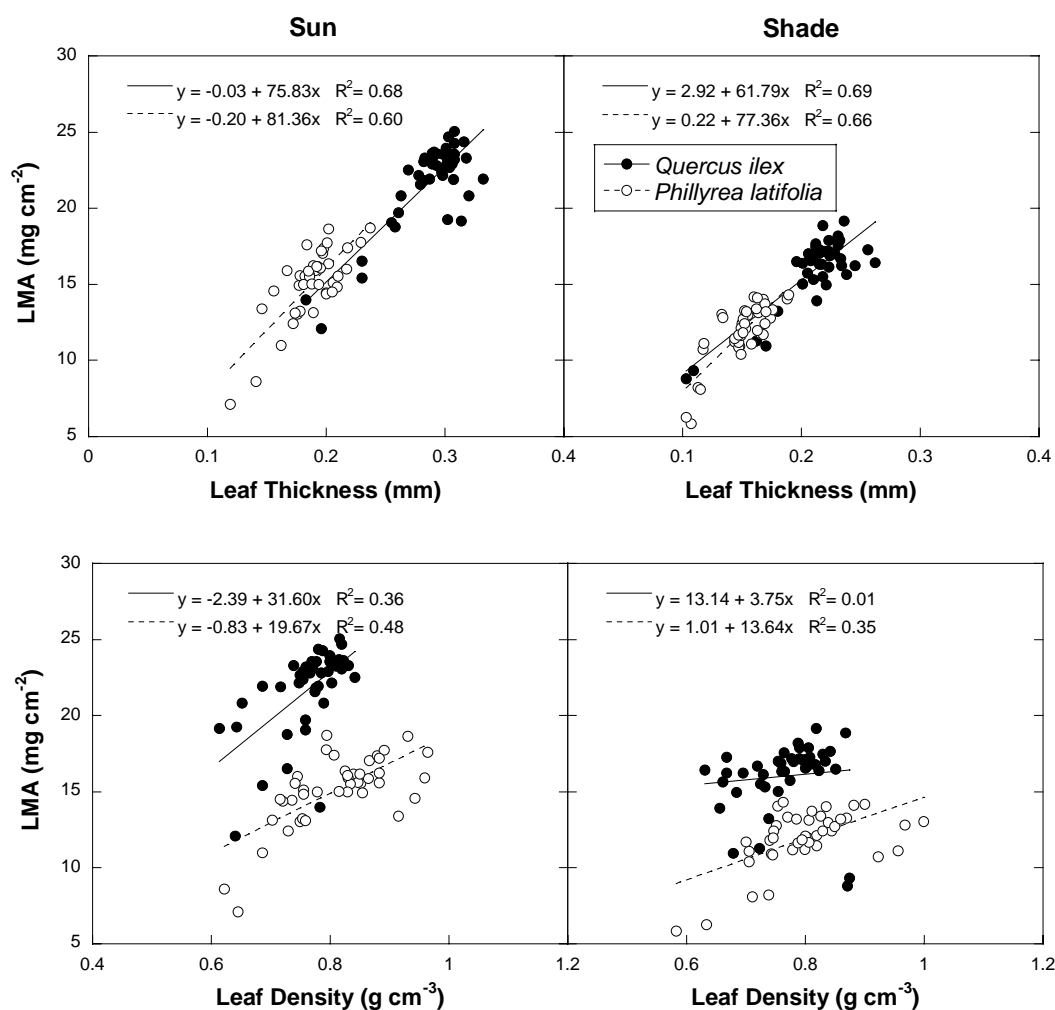


Figure 4. Relationships between leaf mass per area ratio and leaf thickness, and leaf density in sun and shade leaves of *Quercus ilex* and *Phillyrea latifolia*. Each point is the mean of the measurements conducted for each leaf cohort in a plot (n=5). Different points correspond to the different dates.

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 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 and thickness (Fig. 2 and Fig. 3), and lower area than shade ones ($p < 0.001$) (data not shown), 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 ($p < 0.01$, ANOVA) and leaf thickness ($p < 0.05$, ANOVA) than leaves from drought plots in both species and in both years, and 19% higher leaf area only in *Q. ilex* ($p < 0.001$) (Fig. 2 and Fig. 3). In 2000 leaf cohort, leaf area was slightly higher in leaves from control plots than in leaves from drought ones ($p = 0.081$) (data not shown), and LMA and leaf thickness were slightly higher in sun leaves from control plots than in sun leaves from drought plots (3.6%, $p = 0.068$, and 2.9%, $p = 0.062$ for LMA and thickness, respectively) (Fig. 2 and Fig. 3). However, shade *Q. ilex* leaves of 2000 cohort showed slightly higher LMA and leaf thickness in drought 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 ones, especially for shade leaves of *Q. ilex* (Fig. 4). 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 drought plots for any of these two types of relationships.

Leaf longevity.

Shade leaves lasted longer than sun leaves ($p < 0.001$), and *Q. ilex* experimented on average, similar percentage of remaining leaves than *P. latifolia*. In the 1998 cohort, the percentage of remaining leaves differed in control and drought plots depending on the species. *P. latifolia* showed higher percentage of remaining leaves in control plots than in drought plots ($p < 0.001$), whereas *Q. ilex* showed slightly higher values in drought plots than in control ones, 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).

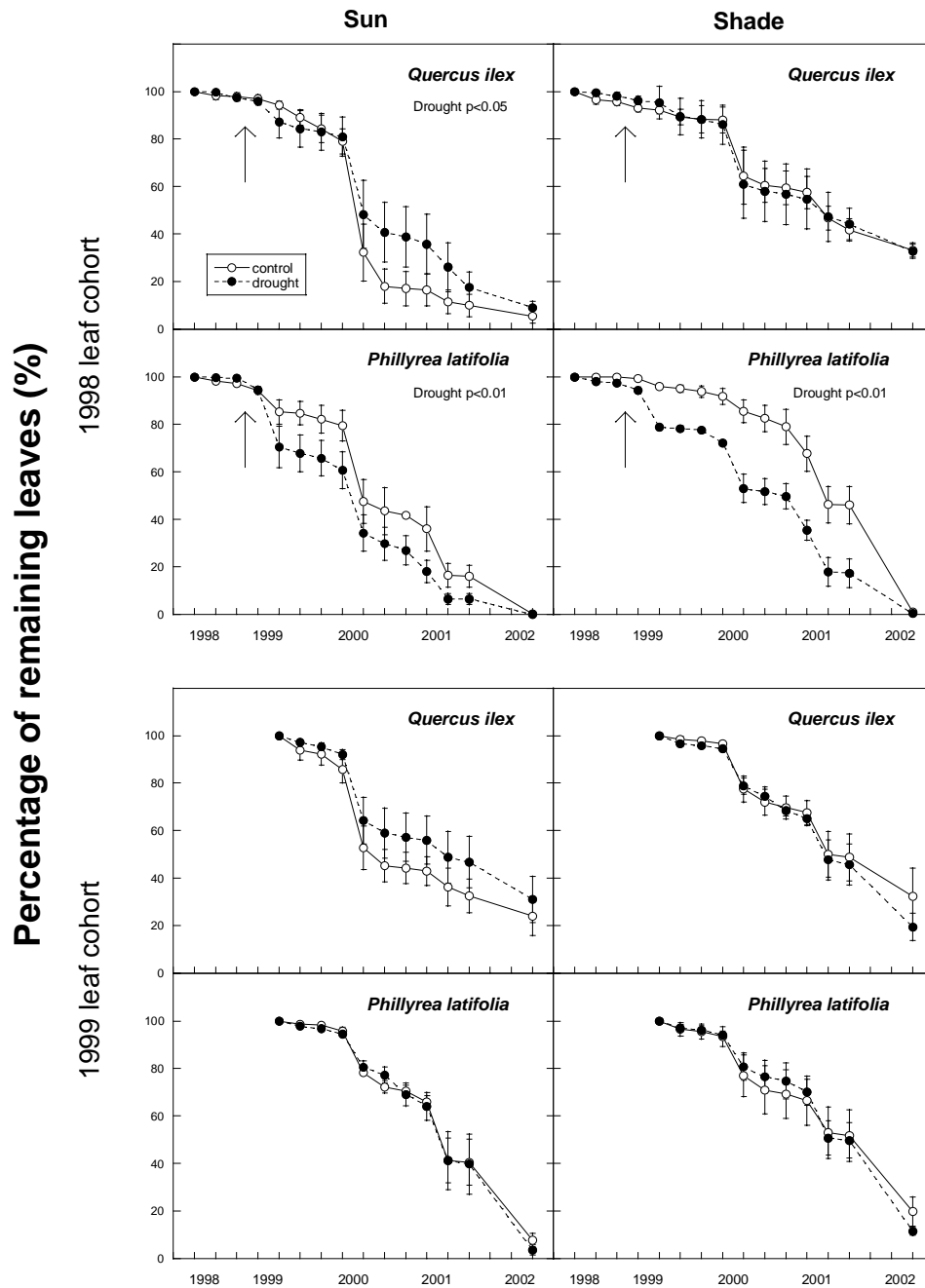


Figure 5. Seasonal course of the percentage of remaining sun and shade leaves of *Quercus ilex* and *Phillyrea latifolia* flushed in 1998 and 1999. Error bars indicate standard error of the mean ($n=4$ plot averages of 10-31 shoots). Significance of drought treatment were tested with Log-Rank tests between Kaplan-Meier survival curves with the time of mortality of all leaves in each shoot computed as a survival time.

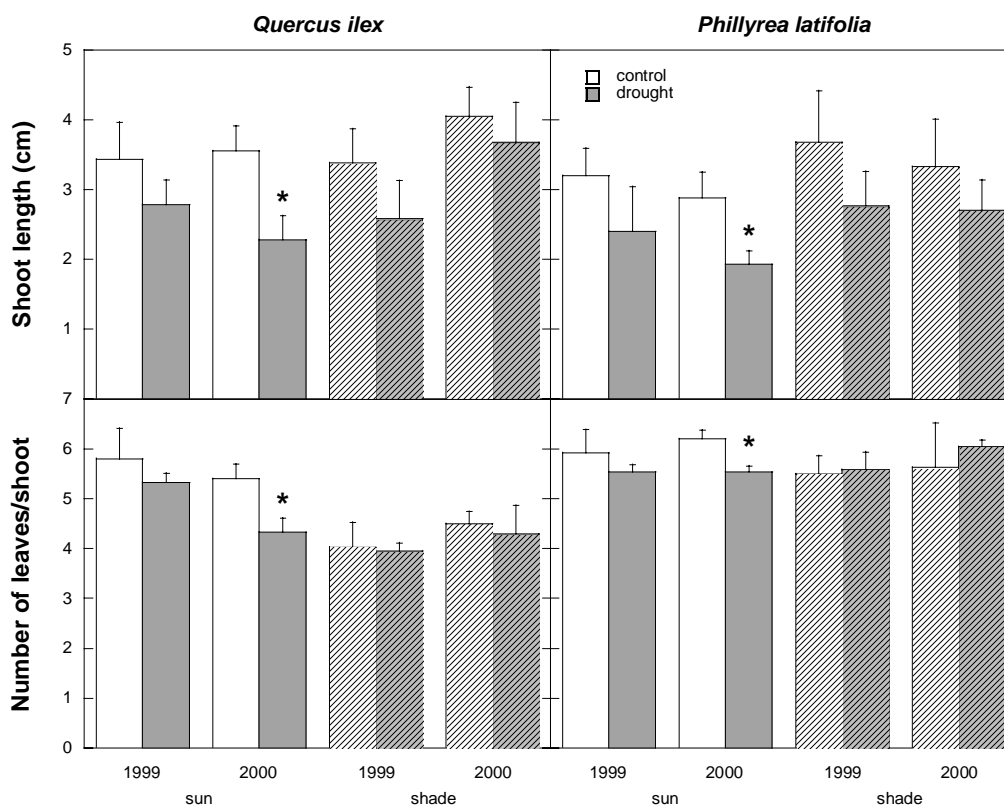


Figure 6. Shoot length and number of leaves per shoot in sun and shade *Quercus ilex* and *Phillyrea latifolia* shoots flushed in 1999 and 2000. One asterisk indicates ($p < 0.05$) significant differences between the two treatments. Error bars indicate standard error of the mean ($n=4$ plot averages of 10-31 shoots).

Shade current-year shoots tended to be longer than sun current-year shoots ($p=0.064$), but no differences were established between the two species (Fig. 6). On the other hand, shoot length was higher in control plots than in drought ones during the two years, 1999 and 2000 (21.4% and 25.2% in *Q.ilex* and *P. latifolia*, respectively; $p=0.002$). The number of leaves per shoot was higher in *P. latifolia* than in *Q. ilex* ($p < 0.001$), and in *Q. ilex*, the number of leaves per shoot was higher in sun shoots than in shade ones ($p=0.008$) (Fig. 6). When only sun shoots were considered, control plots tended to have a greater number of leaves per shoot than drought plots (13.7% and 8.8% in *Q.ilex* and *P. latifolia*, respectively; $p=0.084$).

Mean number of current-year shoots produced per previous-year shoot (S_n/S_{n-1}), and mean number of current-year leaves per previous-year shoot (L_n/S_{n-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). S_n/S_{n-1} and L_n/S_{n-1} were higher in *P. latifolia* than in *Q. ilex*

during 1999 ($p=0.01$ for the two variables). On the contrary, S_n/S_{n-1} and L_n/S_{n-1} were higher in *Q. ilex* in the year 2000 ($p<0.001$ for the two variables). In this year 2000 these variables were also higher in control plots than in drought ones ($p=0.019$ and $p=0.005$ for S_n/S_{n-1} and L_n/S_{n-1} , respectively) (Figure 7).

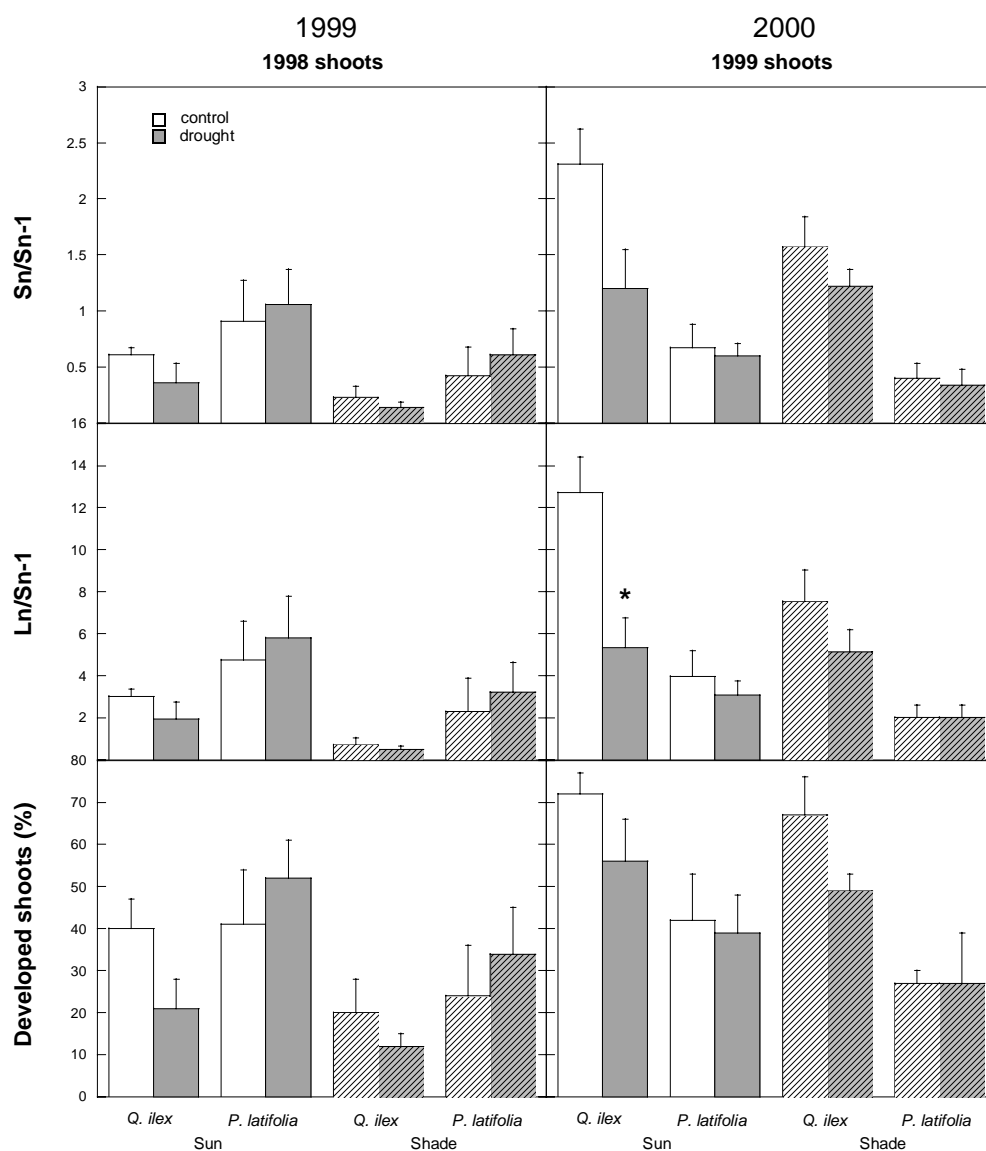


Figure 7. Mean number of current-year shoots produced per previous-year shoot (S_n/S_{n-1}), mean number of current-year leaves produced per previous-year shoot (L_n/S_{n-1}), and percentage of developed shoots on previous-year shoots (% developed) in sun and shade *Quercus ilex* and *Phillyrea latifolia* shoots. Shoots flushed in 1998 were measured in 1999 and shoots flushed in 1999 were measured in 2000. One asterisk indicates ($p<0.05$) significant differences between the two treatments. Error bars indicate standard error of the mean ($n=4$ plot averages of 10-31 shoots).

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 this percentage of developed shoots. Only in *Q. ilex* there was a trend to lower percentage of developed shoots in drought plots than in control ones ($p=0.074$).

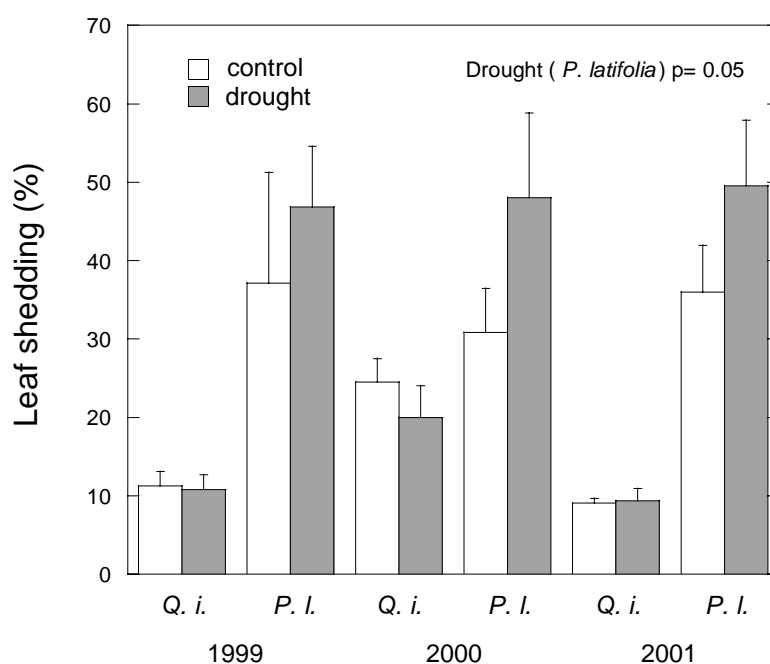


Figure 8. Percentage of annual leaf shedding of *Quercus ilex* and *Phillyrea latifolia* referred to their total amount of leaves in each plot. Error bars indicate standard error of the mean ($n=4$ plots).

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 in all cases, but *P. latifolia* only showed this trend in the upper canopy during 2000 year, whereas in the lower canopy, shade leaves tended to increase under this drought treatment (Fig. 9).

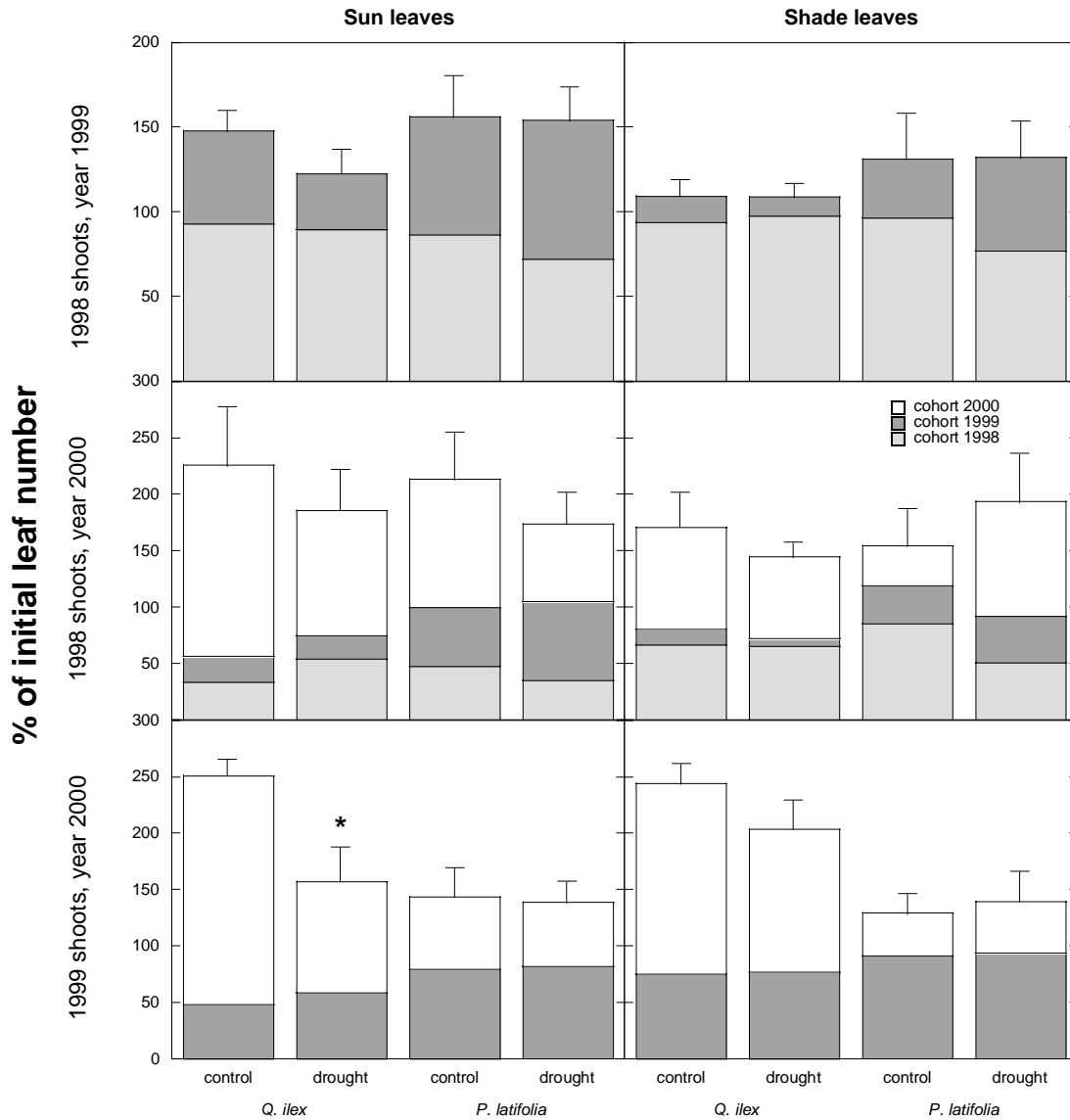


Figure 9. Leaf demography (%) respecting the initial number of leaves in shoots marked in 1998 and 1999, and measured in years 1999 and 2000. One asterisk indicates ($p < 0.05$) significant differences between the two treatments. Error bars indicate standard error of the mean ($n=4$ plot averages of 10-31 shoots).

Discussion.

LMA showed a high increment during the first stage of leaf development in spring, and low increment rates during the rest of leaf growth in both studied species, *Quercus ilex* and *Phillyrea latifolia*, similarly to previously reported for *Q. ilex* (Gratani 1996). LMA and leaf thickness experienced a slight decrease during summer, when leaf usually close stomata and respiration rates may be even greater than photosynthetic rates (Ogaya & Peñuelas 2003) and therefore leaves consume their reserves.

In both species, sun leaves presented higher LMA and thickness, and lower area than shade ones showing a typical trait of all floras and in particular of the Mediterranean flora (Sala et al. 1994), because higher light radiation received by sun leaves generates higher photosynthetic rates, and small and thick leaves show great carbon gain profits over transpirational losses (Givnish 1979, Turner 1994a). High LMA was often 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 drought plots than in control ones. Decrease in LMA can be produced by decrease in leaf thickness, leaf density, or both (Witkowski & Lamont 1991). Leaf thickness was also lower in *P. latifolia* than in *Q. ilex*, in shade leaves than in sun leaves, and in drought plots than in control ones, but leaf density showed similar values in the two species, canopy position, and treatment application. There was a great 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. For LMA-density the relationships were weaker. The capacity to resist water stress of leaves with high LMA is usually attained, in a great part, thanks to a thick cuticle and high cell wall content (Witkowski & Lamont 1991), which implies an increment in leaf density that was not found in this work. The lower LMA values in the drought plots of our study are likely due to lower photosynthetic rates (Ogaya & Peñuelas 2003), and lower carbon allocation to leaves. These decreases in LMA values in the drought 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 & Kull 1998), and only the most drought-sensitive species *Q. ilex* experienced a decrease in leaf area by drought. Many temperate species experience an

increase in LMA when water availability decreases (Salleo & 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). However, there is instead a decrease in LMA values and lifespan in a gradient from mediterranean forests to more xeric vegetation (Chabot & Hicks 1982, Harley et al. 1987, Jonasson 1989, Kikuzawa 1991, Kyparissis & Manetas 1993, Werner et al. 1999). Water availability in the drought plots of the studied forest seems to be insufficient to generate higher LMA in the leaves of plants growing there.

Many studies have described a decrease in the overall plant leaf area in Mediterranean plants when seasonal drought increases (Poole & Miller 1981, Rambal & Leterme 1987). In the year 2000, the percentage of remaining leaves of the 1998 leaf cohort of *P. latifolia* was slightly higher in control plots than in drought plots, a result that is further supported by the higher leaf litterfall in drought plots. In contrast, the less drought-resistant species *Q. ilex* experienced similar percentage of remaining leaves in both treatment applications, or even higher percentage in drought plots when only sun leaves were considered. However, this shorter residence time of control leaves was widely compensated by higher values of S_n/S_{n-1} , L_n/S_{n-1} and percentage of developed shoots in control plots than in drought ones during both 1999 and 2000. *Q. ilex* reached higher leaf flushing and litterfall rates in the wetter 2000 year, whereas *P. latifolia* experienced a more conservative behavior, less dependent on water availability. In *Q. ilex*, the lower sum of leaves from 1999 and 2000 cohorts under drought treatment indicated a smaller increase in the overall plant leaf area, being these differences higher in 2000 than in 1999 (year with lower rainfall and with the first two months without treatment application). Moreover, in *Q. ilex* the proportion of young leaves, with higher photosynthetic capacity, and with higher carbon sequestration rates (Niinemets & Lukjanova 2003), was higher in control plots than in drought ones.

Both species presented slightly shorter current-year shoots in drought plots, very likely as a consequence of the lower photosynthetic rates measured in these plots (Ogaya & Peñuelas 2003) and the consequent lower carbon sequestration. The decrease in the total amount of plant leaf area in drought plots may be also partly responsible for lower carbon

sequestration under drought conditions as shown by the lower biomass accumulation previously described (Ogaya et al. 2003).

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. 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 (Gracia et al. 2001). Lower water availability and higher temperatures could thus have a synergic effect decreasing leaf longevity.

Lower overall leaf area would 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 conditions have already been observed in this experiment, being the decrease larger in *Q. ilex* than in the more drought-tolerant *P. latifolia* (Ogaya et al. 2003).

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Chapter 5.

Effect of drought on diameter increment of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* in a holm oak forest of NE Spain.

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Abstract.

The present study was carried out to elucidate the drought growth responses of *Quercus ilex* L., *Phillyrea latifolia* L., *Arbutus unedo* L., and other accompanying woody species of the Mediterranean holm oak forest. We submitted holm oak forest stands in Prades mountains (NE Spain) to a two-year experimental drought. We reduced soil water availability about 15% by plastic strips and funnels that partially excluded rain throughfall and by ditch interception of water run-off. Mean stem diameter increment showed a great variation depending on the species. *A. unedo* had larger growth rates than *Q. ilex* and *P. latifolia*., but it was also the species that experienced the highest growth reduction in the drought plots (77%), suggesting a higher drought sensitivity than *Q. ilex* (55%) and *P. latifolia* (no drought effect). The growth reduction was specially marked in the larger trees. Aboveground stand biomass increment, estimated from stem diameter by allometric relationships, was 1.9 Mg ha⁻¹ year⁻¹ in the control plots. The 15% reduction in the upper soil moisture produced 42% reduction in this biomass increment. In the drier conditions predicted in this Mediterranean area in the frame of climate change, an important reduction of growth rates can be hence expected, accompanied by a gain of dominance of drought tolerant species such as *P. latifolia* in detriment of more mesic species such as *Q. ilex*.

Keywords: *Arbutus unedo*, drought, Mediterranean trees, *Phillyrea latifolia*, *Quercus ilex*, stem diameter increment.

Introduction.

Summer drought is considered the most important factor determining plant growth and distribution in Mediterranean regions (Mooney 1982). Mediterranean species, mainly evergreen sclerophyllous plants, have several mechanisms to survive under these drought conditions, but their growth rates are low compared to those of temperate forests (Margaris & Mooney 1981). Water availability in Mediterranean regions is likely to be reduced in the near future by the increase of temperatures, and consequently the increase of evapotranspiration rates, and also by likely reductions of precipitation (IPCC 2001).

Stem diameter increments strongly affected by climatic constraints such as precipitation and temperature (Fritts 1976, Orwig & Abrams 1997). This great influence of water availability on stem radial growth has also been observed in the Mediterranean regions (Zhang & Romane 1991, Mayor & Rodà 1994, Oliveira et al. 1994, Caritat et al. 1996, 2000, Boreux et al. 1998, Borghetti et al. 1998, Costa et al. 2001). Water scarcity affects plant growth in several ways. There are some direct effects on photosynthetic capacity by stomatal closure, and there are also indirect effects because nutrient diffusion by roots is under soil water control (Chapin 1980). Thus, many works in Mediterranean areas have shown a fertilization effect on radial growth only under well-watered conditions (Cartan-Son et al., 1992; Mayor et al., 1994).

Holm oak (*Quercus ilex* L.) is a drought tolerant species widely distributed in the Mediterranean basin. There are some species associated with the holm oak forest, such as *Phillyrea latifolia* L., which are even more adapted to drought conditions (Lloret & Siscart 1995, Peñuelas et al. 1998, 2000, 2001). These associated species such as *Arbutus unedo* L. and *Phillyrea latifolia* L. are distributed in the holm oak forest, in boundary zones more limiting for *Q. ilex* or in gaps of holm oak forests (Panaiotis et al. 1995).

If climate becomes drier in Mediterranean regions, it will likely have some consequences on vegetation development and distribution. Responses to climate change may determine individual species ability to persist on its current habitats. Thus accompanying species like the more drought-adapted *P. latifolia* might gain dominance

relative to *Q. ilex* in the driest sites of its distribution.

We aimed to study the effects of drier conditions, experimentally produced by rain and runoff exclusion, on the growth of these three evergreen Mediterranean species and also on the growth of accompanying species of a Mediterranean holm oak forest in Prades mountains (NE Spain). Our hypothesis was that the effect simulated in this experiment would reduced less the growth of the most drought-adapted species, a priori *P. latifolia*. We finally discuss the possible implications of drought effects on community structure and productivity .

Material and Methods.

Study site.

The study was carried out in a holm oak forest growing at Prades Mountains in North-Eastern Spain (41° 13' N, 0° 55' E), on a south-facing slope (25% slope). The soil is a stony xerochrept on a bedrock of metamorphic sandstone, and its depth ranges between 35 and 90 cm. The average annual temperature is 12°C and the average annual rainfall 658 mm. Summer drought is pronounced and usually lasts for 3 months.

Two types of stands can be distinguished in this forest depending on their height. One type has a taller canopy, about 8 or 10 m high, and is dominated by *Quercus ilex* with abundant presence of other evergreen species (*Phillyrea latifolia* and *Arbutus unedo* L.), and occasional individuals of deciduous species (*Sorbus torminalis* (L.) Crantz and *Acer monspessulanum* L.). The other type of stand has a lower, more arbustive canopy, about 3 or 4 m high. These arbustive stands are dominated by *Quercus ilex* and *Phillyrea latifolia* with an important presence of other species well adapted to drought conditions (*Arbutus unedo*, *Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L.). Tree density, is greater in the lower stands than in the higher stands, whereas mean stem diameter (at 50

cm) and aboveground biomass are greater in the higher stands than in the lower stands. No significant differences are observed in the basal area of the two types of stands (Table 1).

Table 1. Stand structure of high and low stands of the holm oak forest studied. P-Values of the one way ANOVA for type of stand are also depicted (n=4 plots).

	High stands	Low stands	P-Value
Stem density (stems ha ⁻¹)	13466 ± 907	19766 ± 1313	0.031
Mean diameter at 50 cm (cm)	5.66 ± 0.27	4.26 ± 0.18	0.022
Basal area (m ² ha ⁻¹)	47.15 ± 2.18	36.22 ± 5.77	ns
Aboveground biomass (Mg ha ⁻¹)	142.31 ± 10.49	87.97 ± 16.91	0.082

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 (12.39 °C) than in the year 1999 (12.19 °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. 1D).

Soil moisture showed great fluctuations during this period but was higher in 2000 than in 1999 (Fig. 1A) in agreement with meteorological data. Minimum values (about 15% v/v) were reached in summer, as a consequence of summer drought, and maximum values (about 35% v/v) in spring and autumn, coinciding with heavy rainfall periods. Differences in soil moisture between control and drought plots were significant on most of the samplings throughout the two years (Fig. 1A). Control plots had on average 15% higher soil moisture than drought plots.

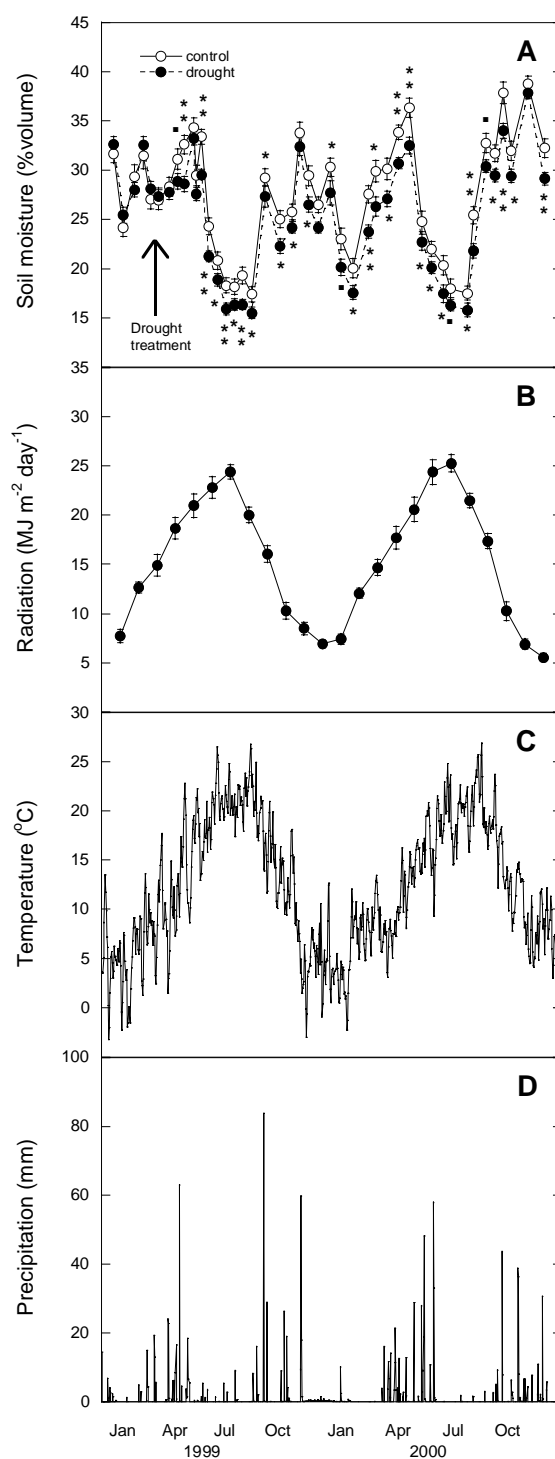


Figure 1. Seasonal course of soil moisture (0.25 m depth) (A), and monthly values of radiation (B), daily mean temperature (C) and precipitation (D) at the study site. Vertical bars in A indicate standard errors of the mean ($n=4$ plot average of 4TDR measurements per plot). One square indicates ($p<0.1$) significant differences between the two treatments, one asterisk ($p<0.05$), and two asterisks ($p<0.01$). Vertical bars in B indicate standard error of the mean ($n=28-31$).

Experimental design and measurements.

Eight 15 x 10 m plots were delimited at the same altitude along the slope, four plots in each type of stand (high and low stands). Half the plots of each type of stand received the drought treatment and the other half were control plots. The drought treatment consisted of partial rain exclusion by suspending PVC strips and funnels at a height of 0.5-0.8 m above the soil. Strips and funnels covered approximately the 30% of the total plot surface. 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. The water intercepted by strips, funnels, and ditches was conducted outside the plots, below their bottom edge.

Temperature, photosynthetic active radiation, air humidity, and precipitation were monitored each half-hour by an automatic meteorological station installed in a forest gap between the plots. Soil moisture was measured every two weeks throughout the experiment by time domain reflectometry (Tektronix 1502C, Beaverton, Oregon, USA) (Zegelin et al. 1989). Three stainless steel cylindrical rods, 25 cm long, were fully driven into the soil at four randomly selected places in each plot. The time domain reflectometer was connected to the ends of the rods to determine the soil moisture.

Drought treatment was applied from March 1999 to March 2001. Just before treatment application all living stems of all the species with a diameter of more than 2 cm at 50 cm height were tagged and their circumference was measured at 50 cm height with a metric tape. A line was painted on the exact point of the stem where circumference had been measured. In March 2000 and March 2001 the circumferences of the stems were measured again to calculate the stem annual diameter increment.

Allometric relationships between tree aboveground biomass and stem diameter were calculated for *Q. ilex* and *P. latifolia* in the area studied (outside the plots). Leaf and stem biomass were measured by weighing the plant material after it had reached constant weight in the oven at 70°C. These allometric relationships (Table 2) were thereafter used to estimate the aboveground standing biomass of the two species in the area studied. To

estimate *Arbutus unedo* biomass we used the allometric relationship (Table 2) calculated few years ago on the same area by Lledó (1990).

Table 2. Allometric relationships between aboveground biomass (AB) and stem diameter at 50 cm (D50) in *Quercus ilex*, *Phillyrea latifolia* and *Arbutus unedo*. (*Arbutus unedo* relationships were taken from Lledó 1990).

Species	Allometric relationship	r	n	P-Value
<i>Q.ilex</i>	$\ln AB = 4.900 + 2.277 \ln D50$	0.981	12	<0.0001
<i>P.latifolia</i>	$\ln AB = 4.251 + 2.463 \ln D50$	0.974	13	<0.0001
<i>A.unedo</i>	$\ln AB = 3.830 + 2.563 \ln D50$	0.989	10	<0.0001

Statistical analyses.

The effects of species, treatment, and canopy position (overstory trees with their canopies at the top of the forest or understory trees with their canopies beneath the dominant ones) on the diameter increment were tested by analysis of variance (ANOVA). The mean of the diameter increment (or biomass) during the two years in each plot was used as the dependent variable. Since in a previous ANOVA it was found that stand height had no significant effect or significant interaction with treatment or species on diameter increment, this factor was not considered in the ANOVA. Additional ANOVAs were conducted for each one of the four diametrical classes in which we classified the trees (2-5 cm, 5-10 cm, 10-15 cm, <15 cm). To test the effect of experimental factors on tree mortality, another ANOVA was conducted with the percentage of tree mortality in each plot as a dependent variable, and treatment and stand height as independent factors. For this ANOVA, percentages of mortality (p) were transformed to $\sin^{-1} p^{1/2}$ to meet the normality assumptions of the ANOVA. All analyses were performed with the Superanova and Statview software packages (Abacus Concepts Inc.).

Results.

Stem growth.

Mean stem diameter increment showed a great variation depending on the species (Fig. 2, Table 3). Stem growth was higher in *A. unedo* than in *Q. ilex* and *P. latifolia* ($p < 0.0001$), and higher in *P. latifolia* than in *Q. ilex* ($p = 0.014$) (Fig. 2). Growth rates of *Arbutus unedo* were 49% higher in the wetter 2000 than in 1999. However, *P. latifolia* had 18% higher growth rates in the drier 1999, and *Q. ilex* showed similar rates in the two years (Fig. 2). The stem growth was 5% higher in 2000 than in 1999 when considering the overall species.

When considering the overall species, the increase of stem diameter was 37% higher in control plots than in drought plots ($p = 0.023$). On the other hand, drought decreased diameter increment of *A. unedo* and *Q. ilex* (77% and 55% respectively), but not the diameter increment of *P. latifolia* (Fig. 3).

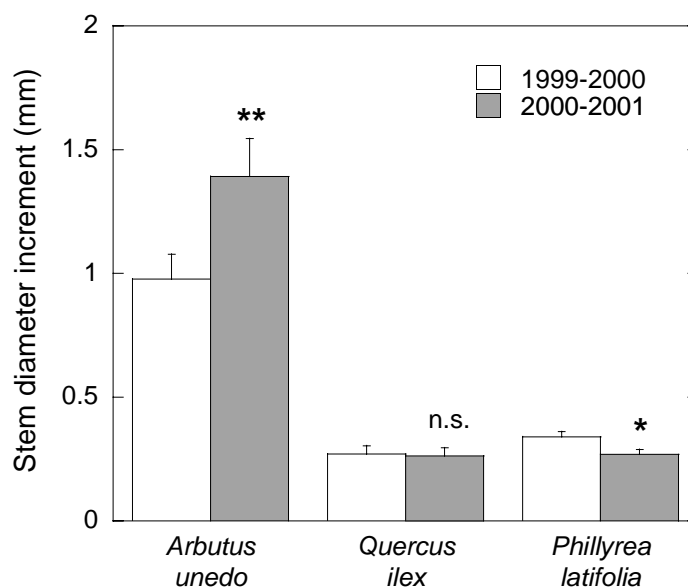


Figure 2. Annual stem diameter increment of the dominant species in the Prades Mediterranean forest during two consecutive years (1999 and 2000). Only control plots are considered. Vertical bars indicate standard errors. *Arbutus unedo* ($n = 85$), *Quercus ilex* ($n = 371$), and *Phillyrea latifolia* ($n = 452$). One asterisk indicates $p < 0.05$ significant difference between the two years, and two asterisks $p < 0.01$.

The stem diameter increment was higher in bigger trees as tested by grouping them in four diametrical classes ($p < 0.0001$) (Fig. 4). However, the annual diameter increment relative to the initial diameter was similar in all diametrical classes when considering the overall species. It was greater for the smallest diametrical class in *A. unedo* ($p < 0.05$) and, instead, greater for the largest diametrical class in *Q. ilex* ($p < 0.05$). Drought affected the stem diameter increment in the overall diametrical classes excepting the smaller one (Fig. 4), *A. unedo* was the only species where drought reduced stem diameter increment of the smaller trees. On the other hand, in *P. latifolia* no significant growth reduction was found in any diametrical class (Fig. 4).

Table 3. ANOVA with the mean of the annual stem diameter increment in the two years in each plot as the dependent variable and species, treatment, and canopy position (overstory or understory) as independent factors (in this analysis *A. unedo*, *Q. ilex* and *P. latifolia* were the considered species).

	D.F.	S.S.	M.S.	F-Value	P-Value
Species	2	1.516	0.758	17.431	<0.0001
Treatment	1	1.266	1.266	29.124	<0.0001
Spe. * Treat.	2	0.944	0.472	10.856	0.0003
Canopy Position	1	0.909	0.909	20.909	<0.0001
Spe. * Can.	2	0.125	0.063	1.441	0.2527
Treat. * Can.	1	0.071	0.071	1.626	0.2121
Spe. * Treat. * Can.	2	0.034	0.017	0.392	0.6789
Residual	30	1.304	0.043		

Overstory vs. Understory trees.

As expected overall overstory trees had higher diameter increment than understory trees in absolute increment (0.44 ± 0.02 vs. 0.14 ± 0.02 mm year⁻¹) and relative increment (0.82 ± 0.03 vs. 0.51 ± 0.05 % of initial diameter) ($p < 0.0001$) (Table 3). Overstory trees of *A. unedo* and *P. latifolia* grew 38% and 37% more than understory trees of the same species.

In *Q. ilex* this difference was even stronger. Understory *Q. ilex* trees did not seem to grow ($-0.07 \pm 0.02 \text{ mm year}^{-1}$) whereas overstory *Q. ilex* trees grew $0.36 \pm 0.03 \text{ mm year}^{-1}$. Drought treatment had a highly significant effect ($p < 0.0001$) on both overstory and understory and in all three dominant species. There was also a significant treatment and species interaction because in *P. latifolia* stem growth was not reduced by drought (Table 3), whereas in *A. unedo* drought reduced about 80% stem diameter increment in both overstory and understory, and in *Q. ilex* drought reduced 53% overstory and 94% understory stem diameter increment.

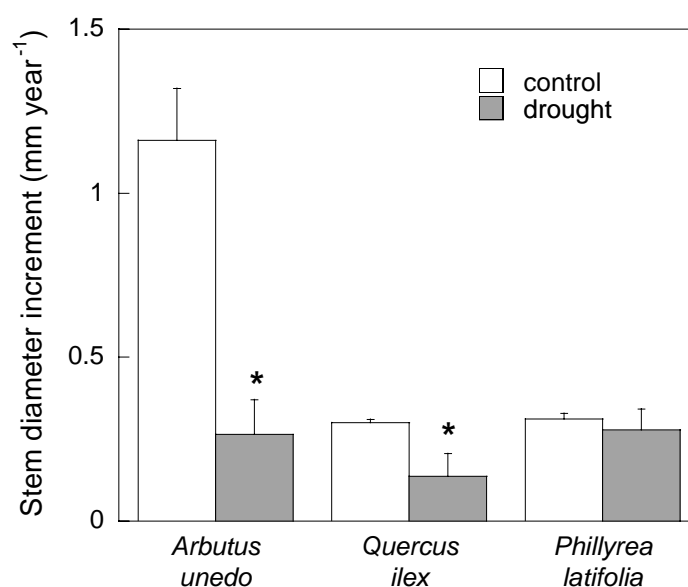


Figure 3. Annual stem diameter increment of *A. unedo*, *Quercus ilex* and *Phillyrea latifolia* in control and drought plots during two consecutive years (1999 and 2000). Vertical bars indicate standard errors (n=4 plot averages of 15-221 individuals each one), and the asterisk indicates $p < 0.05$.

Allometric estimations of aboveground biomass.

Allometric relationships between aboveground biomass and stem diameter were highly significant (Table 2). They provided a good non-destructive method to estimate aboveground biomass in our study area. Our allometric relationships were quite similar to other allometric relationships reported in a nearby area (Lledó, 1990). Aboveground standing biomass derived from these allometric relationships increased in control plots 1.9

Mg ha⁻¹ year⁻¹ and in drought plots 1.1 Mg ha⁻¹ year⁻¹ (Table 4). On the other hand, these biomass data also showed the gradient of drought effect: *A. unedo* > *Q. ilex* > *P. latifolia*.

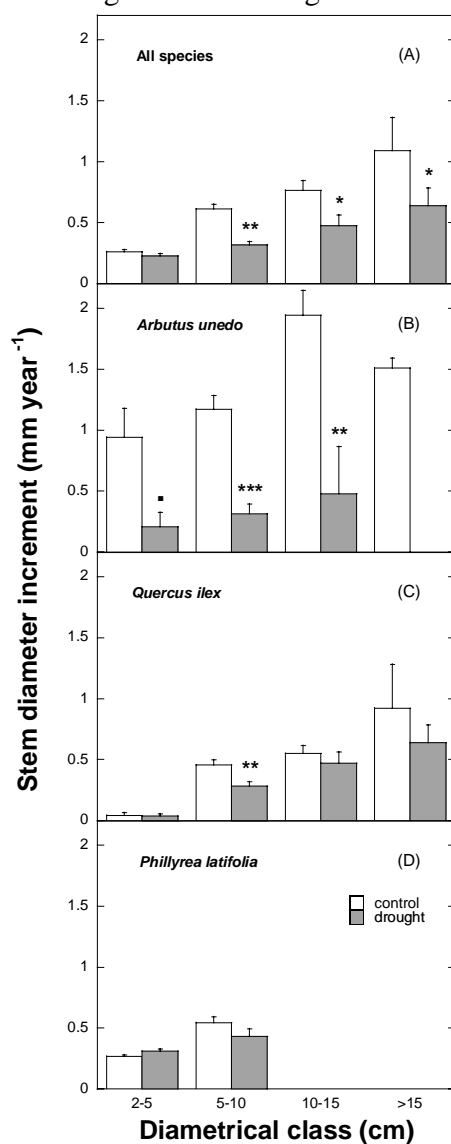


Figure 4. Stem diameter increment versus stem diameter classes in control and drought treatments. Four diametrical classes were established: 2-5 cm, 5-10 cm, 10-15 cm, and more than 15 cm. Vertical bars indicate standard errors. Data for overall species (n=653-695 for 2-5 cm, n=237-261 for 5-10 cm, n=54-72 for 10-15 cm, and n=7-13 for more than 15 cm) (A), for *Arbutus unedo* (n=21-32 for 2-5 cm, n=28-42 for 5-10 cm, n=5-9 for 10-15 cm, and n=2 for more than 15 cm) (B), for *Quercus ilex* (n=171-234 for 2-5 cm, n=149-151 for 5-10 cm, n=48-62 for 10-15 cm, and n=5-13 for more than 15 cm) (C), and for *Phillyrea latifolia* (n=388-405 for 2-5 cm, and n=49-65 for 5-10 cm) (D). One square indicates (p<0.1) significant difference between the two treatments, one asterisk (p<0.05), two asterisks (p<0.01), and three asterisks (p<0.001).

Table 4. Increase of aboveground species biomass in the control and drought plots (average of 1999 and 2000). Units are expressed relative to the total aboveground biomass of each species in

each plot ($\text{Mg Mg}^{-1} \text{ year}^{-1}$), and as total aboveground species biomass increase in the whole control and drought plots ($\text{Mg ha}^{-1} \text{ year}^{-1}$). Different letters indicate significantly different values ($p < 0.05$) (bold a, $p < 0.1$).

Species	$(\text{Mg Mg}^{-1} \text{ year}^{-1})$		$(\text{Mg ha}^{-1} \text{ year}^{-1})$	
	Control	Drought	Control	Drought
<i>Q. ilex</i>	0.011±0.001a	0.003±0.006a	0.971± 0.317a	0.693± 0.329a
<i>P. latifolia</i>	0.021±0.001a	0.019±0.004a	0.327± 0.164a	0.317± 0.148a
<i>A. unedo</i>	0.042±0.003a	0.009±0.004b	0.519± 0.257a	0.060± 0.048a
Other	0.033±0.011a	0.019±0.009a	0.091± 0.084a	0.041± 0.019a
Total	0.016±0.002a	0.009±0.001b	1.908± 0.306a	1.110± 0.255a

Mortality.

The overall mortality rate averaged 2.5 % in these two years. Most 84% of the dead trees belonged to the smaller tree sizes (smaller than 5 cm diameter). Mortality in *Q. ilex* was higher than in *A. unedo* and *P. latifolia* ($p=0.005$ and $p=0.002$, respectively), whereas no significant differences were detected between *A. unedo* and *P. latifolia* (Fig. 5). Mortality in *Q. ilex* trees was significantly ($p=0.05$) higher in drought plots (15%) than in control plots (5%), when only considering the low forest stands.

Discussion.

Stem growth.

The stem diameter increments were small in comparison with those measured in other holm oak forests receiving higher rainfall (Ducrey & Toth 1992, Ibáñez et al. 1999), but similar to the rates previously measured in the same area (Mayor & Rodà 1994, Albeza 2000). These small growth rates in our study were very likely due to the strong soil wa

deficit during summer months and to the elevated stem density (Ibáñez et al. 1999).

Stem diameter increment was slightly higher in 2000 than in 1999 in agreement with the second year of the study (2000) being slightly wetter and warmer than the first year (1999) (Fig. 1). The year 2000 had a shorter summer drought and greater soil moistures, and therefore greater water availability. In general all the species presented higher growth rates in 2000, but *P. latifolia* grew more in 1999 despite of the drier conditions of this year. Differences in the interannual stem diameter increment between coexisting Mediterranean woody species have also been reported previously (Tognetti et al. 2000).

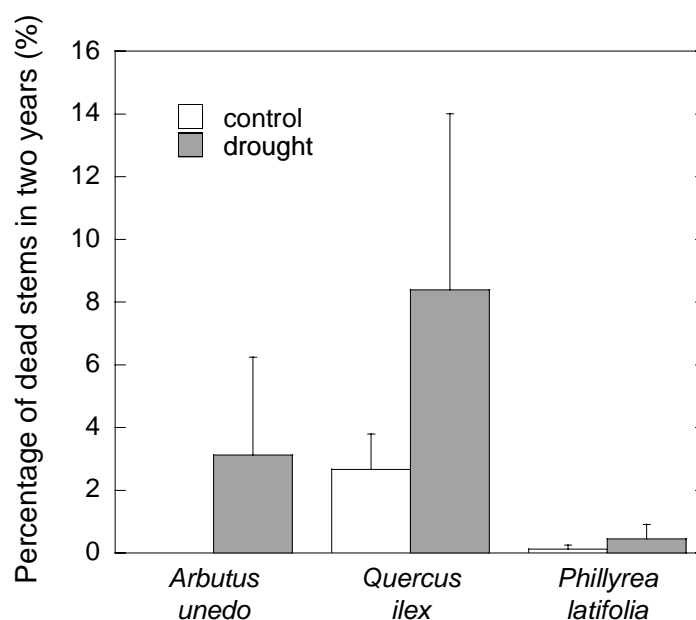


Figure 5. Stem mortality rates in *Arbutus unedo*, *Quercus ilex*, and *Phillyrea latifolia* trees in the control and drought plots. Vertical bars indicate standard errors (n=4 plot averages of 15-221 individuals monitored per plot).

A. unedo grew faster than *Q. ilex* and *P. latifolia*, but drought treatment produced a great reduction in *A. unedo* stem growth. As a result, despite of its higher growth rates, *A. unedo* did not seem more tolerant than *Q. ilex* or *P. latifolia* to the additional experimental drought we generated. However, similar photosynthetic rates in *A. unedo* and *Q. ilex* with higher stomatal conductances and transpiration rates in *Q. ilex* have been reported during summer drought (Castell et al. 1994) indicating thus higher water use efficiencies in *A.*

unedo. Moreover, *A. unedo* stem growth in drought treatments was still slightly larger than *Q. ilex* stem growth (Fig. 3), and *A. unedo* mortality was smaller than *Q. ilex* mortality (Fig. 5). *Q. ilex* had also lower stem diameter increment than the more drought tolerant species *P. latifolia*, a species which has been observed to present higher photosynthetic rates and water use efficiencies (Peñuelas et al. 1998) and lower sap flux reduction (Martínez-Vilalta et al. 2003) than *Q. ilex* during summer drought. Therefore, an species gradient of growth responses to drought can be established from the most sensitive *Q. ilex* (smaller growth and greater mortality in the drought treatments) to the less sensitive *P. latifolia*. However mortality data showed greater mortality in *Q. ilex* (Fig. 5).

Overstory vs. Understory trees.

Both absolute and relative stem diameter increment were larger (214 and 61 %, respectively) in overstory than in understory trees. The drought treatment exerted a strong effect on larger trees, probably because the photosynthetic rates of larger overstory trees are more dependent on water availability, while photosynthetic rates of understory trees are more limited by light incidence. Difference in stem radial growth between overstory and understory trees was larger in *Q. ilex* than in *A. unedo* or *P. latifolia*. Since *P. latifolia* had higher water use efficiency during summer drought (Peñuelas et al. 1998) and lower xylem embolism vulnerability than *Q. ilex* and *A. unedo* (Martínez-Vilalta et al. 2003), overstory and understory trees of *P. latifolia* were less affected by low water availability than the other two species.

Aboveground biomass.

Aboveground biomass increment (1.9 Mg ha^{-1}) was small, but similar rates have been reported in the same area (Ibáñez et al. 1999). Drought treatment produced a strong (72 %) reduction on overall biomass increment. *Q. ilex* and *A. unedo* reduced their biomass increment in drought plots but *P. latifolia*, species which is more drought resistant did not

show this pattern. It seems thus that under drought conditions *P. latifolia* was more capable of obtaining water resources or of avoiding water loss than *Q. ilex*. The lack of drought effect in *P. latifolia* was especially evident in the low forest stands, where drought conditions were more limiting to the other species.

Mortality.

Mortality rates were higher in *Q. ilex* than in *A. unedo* or *P. latifolia* showing once more a lower drought resistance in *Q. ilex*. Drought treatment increased stem mortality in *Q. ilex* trees of the low forest stands, probably because there the conditions were more limiting to *Q. ilex* development. These results supported the hypothesis of possible changes in dominance and distribution of these species in a drier environment and agree with the differential mortality of these species after severe drought events (Peñuelas et al. 2000, Martínez-Vilalta et al. 2003).

Climate change and shifts in species dominance and carbon sink.

These results are relevant when considering the increasingly warmer (and more arid since precipitation does not increase) conditions of the Mediterranean zones (Piñol et al. 1998, IPCC 2001, Peñuelas & Filella 2001, Peñuelas et al. 2002). Our results have shown an important reduction in growth rates and higher mortality rates under dry conditions, as also found in similar experiments in saplings of other regions such as North-American temperate areas (Hanson et al. 2001). However, there was a high variability in growth reduction in the different species, depending on their capacity to cope with low water availability. Climate change may thus decrease tree growth, and lead to a decline of forests or to a decline of particular species within forests (Kirschbaum 2000). In the Mediterranean areas drought-tolerant tree species could be favored in detriment of more mesic ones, with consequent changes in species dominance and distribution. In this study we have found low growth rates in the more mesic species, especially the scarce deciduous

species (data not shown) and *Q. ilex*, while more drought-resistant species such as *P. latifolia* showed no reductions on its growth rates under drought treatment. These changes in dominance are, however, difficult to predict because they involve many factors. For example, the response to drought of early stages of these species could be very different from that of mature plants. *A. unedo* and *Q. ilex* trees have a strong capacity to produce sprouts with higher growth rates and better water status during summer drought than mature plants (Castell et al. 1994), while *P. latifolia* recruitment is ensured by seedlings (Herrera et al. 1994). The young plants recruitment is crucial to establish future species distribution, and in this area it has been observed a high drought resistance of *Q. ilex* sprouts and an important seedling mortality of *P. latifolia* under low water availability (Lloret et al. submitted). In any case, though, the ability of adults to survive poses a limit on the long-term presence of any species in a determined area.

Terrestrial forests play an important role as a sink of carbon emissions, so changes in their growth rates will imply changes in carbon sequestration by forests. The 19% decrease in precipitation and the 0.2 °C colder conditions in the year 1999 (relative to 2000) was accompanied by a 4.5% decrease in the aboveground biomass growth, i.e. net aboveground primary productivity (0.84 Mg C ha⁻¹ year⁻¹ in 1999 and 0.88 Mg C ha⁻¹ year⁻¹ in 2000). However, the 15% further and continuous decrease in soil water availability of the drought treatment produced a 42% decrease in the above ground biomass growth (0.86 Mg C ha⁻¹ year⁻¹ in control plots and 0.50 Mg C ha⁻¹ year⁻¹ in drought plots), and consequently in the carbon sink capacity of these Mediterranean forests, which already grow little in the current conditions compared to other Mediterranean areas with higher water availability or to nearby temperate forests (for example, Northern Spain temperate forests accumulate 1.46 Mg C ha⁻¹ year⁻¹; Rodríguez Murillo 1997).

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Resum dels resultats i conclusions.

Activitat fotosintètica.

Quercus ilex i *Phillyrea latifolia* han mostrat diferents sensibilitats als diversos factors climàtics que condicionen l'activitat fotosintètica. *Q. ilex* ha mostrat una major tolerància a

les baixes temperatures que *P. latifolia* (Tretiach et al. 1997), però *P. latifolia* ha estat més resistent a la sequera i les altes temperatures de l'estiu (Filella et al. 1998, Peñuelas et al. 1998).

La fluorescència de la clorofil·la ha estat particularment sensible a les baixes temperatures hivernals (Larcher 2000, Oliveira & Peñuelas 2000). En aquests períodes, quan el tractament de sequera experimental ha coincidit amb baixes temperatures, s'ha pogut constatar una disminució de l'eficiència fotoquímica causada pel tractament, especialment acusada en *P. latifolia*.

Les taxes fotosintètiques en canvi, han estat molt més condicionades per la disponibilitat d'aigua (Lange et al. 1982, Tenhunen et al. 1990, Peñuelas et al. 1998) assolint valors molt baixos, i fins i tot un balanç negatiu de carboni, durant els períodes més secs de l'estiu.

En les dues espècies durant les hores centrals del dia, les condicions de sequera experimental van fer disminuir l'eficiència fotoquímica, i la taxa fotosintètica neta, però només *P. latifolia* va ser capaç de reduir la transpiració (33%) i incrementar l'eficiència en l'ús de l'aigua (56%). També s'ha constatat en aquestes dues espècies una tendència a assolir una taxa fotosintètica més elevada, per a una mateixa humitat del sòl, en condicions de sequera experimental que en condicions control.

Fenologia i esforç reproductiu.

A les zones de clima mediterrani, l'aparició dels diversos estadis fenològics va molt correlacionada amb la temperatura (Spano et al. 1999, Peñuelas i Filella 2001, García-Mozo et al. 2002) i amb la disponibilitat hídrica (Kramer et al. 2000, Pardos et al. 2003). En aquest treball la floració ha mostrat una forta dependència de la temperatura en *P. latifolia* i de la disponibilitat d'aigua en *A. unedo*. La disponibilitat d'aigua també sembla haver estat el més determinant en la brotada de noves fulles en ambdues espècies. *Q. ilex* en canvi, ha experimentat una menor dependència de les condicions ambientals.

A. unedo ha estat l'espècie que ha produït més quantitat de flors i fruits en proporció a la seva biomassa, però alhora ha estat l'espècie més sensible a les condicions

climàtiques, o si més no, a la disponibilitat d'aigua, ja que l'arribada d'aquestes fenofases s'ha endarrerit (entre una i dues setmanes) en resposta a la sequera experimental. La quantitat proporcional de flors i fruits ha tingut una gran variabilitat interanual, sent difícil establir-hi l'efecte d'altres factors, però hi ha una tendència cap a una producció més baixa en condicions de sequera en *A. unedo*, i cap a una major caiguda de fulles en *P. Latifolia*.

Una menor disponibilitat d'aigua no només té efectes directes sobre la fenologia i l'esforç reproductiu de les plantes, sinó també sobre els dels altres organismes i per tant sobre el funcionament global de l'ecosistema, exercint així un efecte indirecte addicional sobre la vegetació. Per exemple, la quantitat de bolets produïts a les parcel·les sequera ha estat significativament inferior al de les parcel·les control, la qual cosa segurament està relacionada amb una menor capacitat de descomposició de la virosta.

Morfologia i demografia foliar.

Una característica típica de la vegetació mediterrània és la presència de fulles esclerofil·les. Aquestes fulles són típicament perennes, de llarga longevitat, cutícula gruixuda, elevada relació massa/àrea foliar (LMA), baix contingut en nutrients, elevada concentració de materials estructurals i baixes taxes fotosintètiques (Turner 1994). Les fulles de *P. latifolia* tenen una àrea i un gruix inferior a les de *Q. ilex*, i sota condicions de sequera experimental ha disminuït el gruix (2.5%) en les dues espècies i l'àrea (17%) en *Q. ilex*, possiblement per una menor expansió cel·lular durant el període de desenvolupament foliar en condicions de dèficit hídric (Hsiao et al. 1985, Niinemets & Kull 1998). La LMA és menor en *P. latifolia* que en *Q. ilex*, i també ha disminuït en condicions de sequera (2.5%), tot i que *P. latifolia* ha resultat ser més tolerant a les condicions de sequera que *Q. ilex*, i que diversos autors han atribuït una elevada LMA a una adaptació a condicions de baixa disponibilitat d'aigua (Harley et al. 1987, Salleo & Lo Gullo 1990, Wookey et al. 1995, Gratani 1996), i a més existeix un gradient en el que la LMA augmenta des de els boscos temperats fins als mediterranis (Niinemets 2001). Però també és cert que des dels boscos mediterranis cap a ambients més xèrics la LMA decreix i la vegetació tendeix al caducifolisme estival (Chabot & Hicks 1982, Kikuzawa 1991). La zona on s'ha

desenvolupat l'experiment ja té una baixa disponibilitat hídrica i una disminució en l'aport d'aigua pot comportar una disminució de la LMA.

Els efectes del tractament de sequera en la longevitat foliar han estat diferents depenent de l'espècie. La sequera ha disminuït la longevitat foliar en *P. latifolia*, i en canvi, l'ha augmentat en *Q. Ilex*, però en tots els casos, la producció de noves fulles és menor, de manera que l'àrea foliar de tota la planta disminueix en condicions de sequera.

Creixement diametral.

Les diferents espècies han tingut unes taxes de creixement diametral i una sensibilitat al tractament de sequera molt variades. *A. unedo* ha experimentat un creixement diametral més elevat que les altres dues espècies, però la forta reducció del creixement diametral (77%) experimentada sota condicions de dèficit hídric mostra una major sensibilitat a la sequera que la experimentada per *Q. ilex* (55%). En canvi *P. latifolia* ha estat la espècie més tolerant al tractament experimental, sent l'única que no ha mostrat una disminució de les seves taxes de creixement en condicions de sequera. Tanmateix, la mortalitat d'individus ha estat sensiblement més elevada en *Q. ilex* que en *P. latifolia*.

El creixement de l'arbre (estimat aquí a partir del creixement diametral del tronc) resulta del balanç de carboni entre les taxes fotosintètiques i les de respiració. La demografia foliar juga un paper molt important en aquest balanç de carboni, doncs la quantitat de carboni atmosfèric fixat per un arbre dependrà en gran part de la quantitat total de fulles que presenti. En el present treball hem pogut constatar en condicions de sequera, una lleugera disminució de les taxes fotosintètiques de les hores centrals del dia i una menor quantitat de fulles (efecte especialment destacat en *Q. ilex*). Això ha contribuït a una menor capacitat de segrestar carboni atmosfèric en les plantes que han viscut en condicions de sequera (sobretot en *Q. ilex*) tal i com hem pogut constatar en les taxes de creixement diametral i el corresponent increment de biomassa, ja que el 15% de reducció en la humitat de les capes superiors del sòl s'ha traduït en un 42% de reducció de l'acumulació de nova biomassa del bosc de Prades.

Conclusions generals.

- Una disminució en la disponibilitat hídrica d'aproximadament el 15% ha comportat una disminució de les taxes fotosintètiques durant les hores centrals del dia, i una disminució de l'eficiència fotoquímica durant els períodes més freds de l'any.
- També ha comportat la reducció de la quantitat total de fulles i la formació de fulles amb una àrea menor i menys gruixudes.
- Tot això s'ha traduït en una menor capacitat per segrestar carboni atmosfèric i acumular nova biomassa (1.9Mg ha^{-1} i 1.1Mg ha^{-1} a les parcel·les control i sequera respectivament).
- L'aparició dels diferents estadis fenològics han estat alterats, especialment en *A. unedo*, cosa que pot afectar les relacions tròfiques amb altres espècies de l'ecosistema. L'esforç reproductiu també s'ha vist alterat: la producció de flors i fruits ha disminuït en *Q. ilex* i sobretot en *A. unedo* en condicions de sequera, però l'elevada variabilitat interanual fa difícil trobar-hi diferències significatives.
- Tots aquests efectes no han estat, i per tant és d'esperar que no ho siguin en el futur immediat, iguals per a totes les espècies. Les tres espècies aquí estudiades, les dominants al bosc de Prades, han mostrat una notable diversitat de sensibilitats a la lleugera disminució de la disponibilitat d'aigua del nostre experiment. A llarg terme, hi podria haver canvis en la distribució d'aquestes espècies resultant-ne afavorides les més tolerants a la sequera com *P. latifolia* en detriment de les més sensibles com *Q. ilex*.

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