

## CHAPTER 6

### Sap flow of three co-occurring Mediterranean species under varying atmospheric and soil water conditions

Jordi Martínez-Vilalta, Marta Mangirón, Romà Ogaya, Miquel Sauret,  
Lydia Serrano, Josep Peñuelas & Josep Piñol

*submitted*



(Bill Watterson, *Calvin & Hobbes*)

### Resum

En aquest treball s'estudien els patrons estacionals d'ús de l'aigua en tres espècies que conviuen en un alzinar del nord-est d'Espanya. Les tres espècies estudiades (*Quercus ilex* L., *Phillyrea latifolia* L. i *Arbutus unedo* L.) constitueixen 99% de l'àrea basal de l'alzinar. El període d'estudi va incloure les estacions seques dels anys 1999 i 2000. L'ús de l'aigua es va estimar mitjançant sensors de flux de saba de tipus Granier. També es van mesurar les variables meteorològiques estàndard, el contingut d'aigua del sòl, i els potencials hídrics a les fulles. Els fluxos de saba diaris ( $J_s$ ) van estar molt influïts per les variables ambientals. En general,  $J_s$  es correlacionava sobretot amb el dèficit de pressió de vapor de l'atmosfera durant la primavera, mentre que durant l'estiu el dèficit hídric del sòl explicava la major part de la variància de  $J_s$ . La reducció mitjana de  $J_s$  entre el començament de l'estiu i el pic de l'eixut fou del 60% per *A. unedo*, del 48% per *P. latifolia* i del 80% per *Q. ilex*. Aquest estudi va formar part també d'un experiment de simulació de sequera. *A. unedo* va respondre a la reducció experimental de la disponibilitat d'aigua reduint el flux de saba durant l'estiu. Aquesta espècie també va modificar l'ús de l'aigua entre anys segons els diferents patrons de precipitació que hi van tenir lloc. La transpiració estimada a nivell de parcel·la durant l'any 1999, que fou relativament humit, va ser el 98% de la precipitació neta, corroborant que la comunitat estudiada estava limitada per la disponibilitat hídrica. Els nostres resultats, combinats amb investigacions prèvies realitzades a la mateixa zona, perfilen estratègies molt diferents per fer front a l'eixut en cadascuna de les tres espècies estudiades. *A. unedo* i *Q. ilex* van requerir un control estomàtic molt estricte per evitar potencials hídrics excessivament baixos que podien causar nivells perillosos d'embolisme. D'altra banda, la seva poca vulnerabilitat a l'embolisme va permetre a *P. latifolia* tenir un control estomàtic més lax i mantenir la transpiració a potencials hídrics més baixos que les altres dues espècies. *Q. ilex* va patir una reducció del flux de saba a llarg terme que relacionem amb els elevats nivells d'embolisme predits per aquesta espècie. En conjunt, els nostres resultats suggereixen que *Q. ilex* i, en menor grau, *A. unedo*, podrien estar al seu límit per fer front a l'eixut en diverses zones de la Mediterrània, en concordança amb els greus efectes que la sequera de 1994 va tenir en moltes poblacions de *Q. ilex* del nord-est d'Espanya. Aquests resultats són discutits en relació amb els possibles impactes que el canvi climàtic tindrà en els boscos actualment dominats per *Q. ilex*.

### Abstract

We studied the seasonal patterns of water use of three woody species co-occurring in a Holm oak forest in NE Spain. The three species studied (*Quercus ilex* L., *Phillyrea latifolia* L. and *Arbutus unedo* L.) constitute 99% of the total basal area of the forest. The study period included the dry seasons of years 1999 and 2000. Water use was estimated by means of Granier-type sap flux sensors. Standard meteorological variables, soil water content, and leaf water potentials were also monitored. Daily sap flux ( $J_s$ ) was strongly influenced by environmental variables. In general,  $J_s$  was mainly correlated with atmospheric water pressure deficit during the spring (wet period), while during the summer (dry period) soil moisture deficit explained most of the variance in  $J_s$ . The average reduction in  $J_s$  between early summer and the peak of the drought was 60% for *A. unedo*, 48% for *P. latifolia*, and 80% for *Q. ilex*. This study was also part of a drought simulation experiment. *A. unedo* responded to the experimental decrease in water availability reducing sap flux during the summer. This species also modified its water use between years according to their different seasonal patterns of precipitation. Estimated stand transpiration during the "relatively wet" 1999 year was 98% of net rainfall, further supporting that the community was limited by water availability. Our results, combined with previous studies in the same area, outlined very different strategies to cope with water shortage for the three species. *A. unedo* and *Q. ilex* needed a strong stomatal control over water loss to avoid low water potentials that could cause dangerous levels of xylem embolism. On the other hand, low vulnerability to xylem embolism allowed *P. latifolia* to have a less strict stomatal regulation and maintain transpiration at lower water potentials than the other two species. *Q. ilex* showed a long-term reduction of sap flux which we relate to the high levels of embolism predicted for this species. Our results suggest that *Q. ilex* and, to a lesser extent, *A. unedo*, may be at their limit to cope with water stress in many Mediterranean areas, in agreement with the acute impact that the 1994 drought had on *Q. ilex* populations in NE Spain. These results are discussed in relation to the possible impacts that climate change will have on *Q. ilex*-dominated forests.

## Introduction

Climate in the Mediterranean basin is characterized by an acute summer drought which, according to most climatic scenarios, will intensify as a result of climate change (IPCC 2001). In this context, a proper understanding of the effects of water shortage is required if predictions of the impact of climate change on Mediterranean vegetation are to be made (Borghetti *et al.* 1998). The long-term measurement of water use of co-occurring species in relation to environmental variables provides a powerful approach to study the response of plants to water availability, particularly when the species studied show contrasting adaptations to drought (*e.g.*, Pataki *et al.* 2000). Since plant responses are likely to be influenced by different environmental variables depending on the temporal scale considered (Pataki *et al.* 1998), it is important that data are gathered at sufficiently high temporal resolution. This requirement is fulfilled by sap flow studies because they provide estimates of transpiration with a temporal resolution of less than one day (Granier *et al.* 1996).

Evergreen forests dominated by Holm oak (*Quercus ilex* L.) are one of the most important vegetation types in the Mediterranean basin. In Spain, for example, Holm oak forests constitute 25% of the total forested area (Terradas 1999). *Arbutus unedo* L. and *Phillyrea latifolia* L. co-occur in Holm oak forests throughout the Mediterranean, typically forming a lower tree layer under the *Q. ilex* canopy. Despite the three species are evergreen and have a similar distribution (Bolós & Vigo 1990-1995), previous studies have shown that they differ markedly in water

relations and resistance to drought. In contrast to the other two species *P. latifolia* appears to exhibit little stomatal control over water loss and is able to maintain physiological activity under very low leaf water potentials (Tretiach 1993, Castell *et al.* 1994, de Lillis & Mirgone 1994, Peñuelas *et al.* 1998). Within a site *P. latifolia* tends to occupy the driest areas whereas *A. unedo* is usually restricted to the most humid microenvironments. However, recent drought episodes in E Spain, particularly the one in summer 1994, have shown that *Q. ilex* is closer to its limit to cope with water stress than the other two species: up to 80% of *Q. ilex* individuals dried and 15% died in some areas as a result of the 1994 drought (Lloret & Siscart 1995, Peñuelas *et al.* 1998, Peñuelas *et al.* 2000). On account of these results, it has been hypothesized that *Q. ilex* may be substituted by *P. latifolia* in the most water-limited areas of the Mediterranean if climate gets drier (Peñuelas *et al.* 1998, Peñuelas *et al.* 2000, Chapter 7).

A recent study (Chapter 3) has shown that *A. unedo*, *P. latifolia* and *Q. ilex* have contrasting hydraulic properties, which partially explain the observed differences in water relations and drought resistance. In particular, *P. latifolia* is much more resistant to xylem embolism than the other two species, while *A. unedo* is slightly more resistant than *Q. ilex*. In the present study, we used daily sap flow measurements to evaluate seasonal patterns of water use in these species, with the aim of integrating previous knowledge on their water relations and understand the underlying mechanisms that explain their contrasting responses to seasonal drought. It was hypothesized that: (1) according to the

previous considerations, the reduction of sap flux during summer drought would follow a pattern of *Q. ilex* > *A. unedo* > *P. latifolia*; (2) there would be an irreversible reduction of sap flux associated with the embolism levels experienced during the drought, which would be apparent in the wet period immediately after the summer drought; and (3) as a result of water being a limiting resource in the community studied, water use would respond to the differences in seasonal water availability between years, and to an experimental reduction of water availability.

## Material and methods

### Study site

The site was located in a forested area in the Prades Mountains, NE Spain (41° 13' N, 0° 55' E). The climate is Mediterranean, with a mean annual rainfall of 537 mm (1981-1995) and moderately warm temperatures (10.0°C mean at Prades, 1000 m asl). Additional information about the study area can be found in Hereter & Sánchez (1999). Experimental plots were located at the south-facing upper slopes of the Torners valley (*ca.* 40% slope). In that area, the substrate is fractured schist, and soils are *xerochrepts* with a clay-loam texture. Soil depth is 65 ±3 cm. The forest was dominated by *Q. ilex*, *P. latifolia* and *A. unedo*, which constitute 99% of total basal area in the two study plots (Table1). Canopy height was 5-7 m. This community is known to be limited by water availability (Rodà *et al.* 1999). The study period included the dry seasons of years 1999 and 2000 (Figure 1).

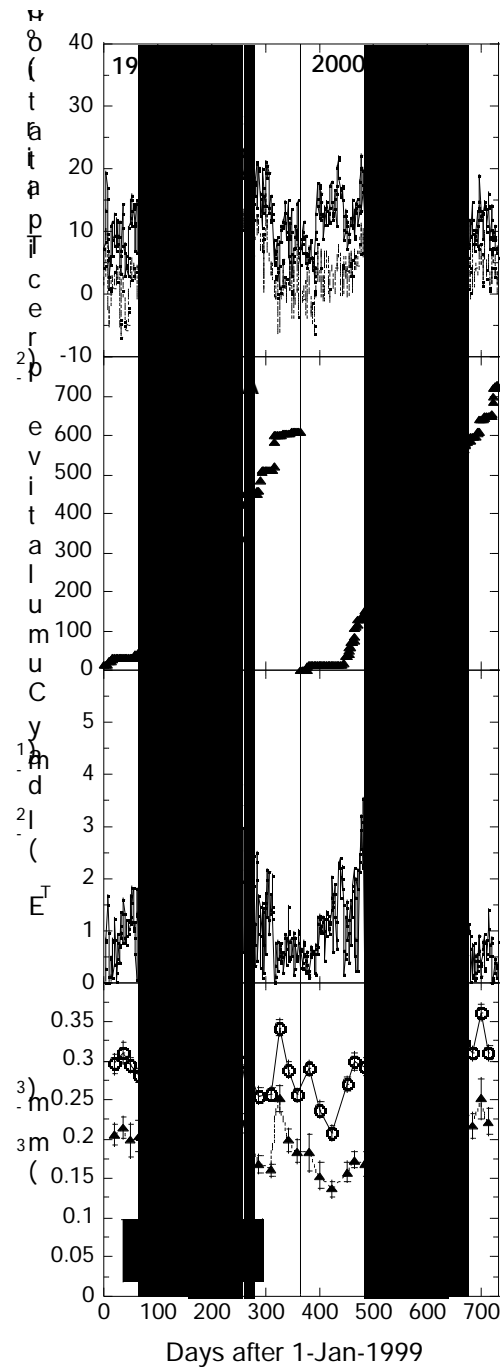


Figure 1. Daily values of maximum and minimum air temperature ( $T_{air}$ ), cumulative precipitation, potential evapotranspiration ( $E_T$ ) and volumetric soil water content ( $\theta_v$ ) at two different depths during the years 1999 and 2000 at the study site. Shaded areas indicate the different periods in which the study was divided (Sp: spring, Su: summer, A: autumn). Error bars (only for  $\theta_v$ ) represent standard errors.

**Table 1.** Tree density (stems ha<sup>-1</sup>) and basal area (m<sup>2</sup> ha<sup>-1</sup>) at the two plots studied. Only adult individuals with diameter at 0.5 m >2 cm were counted.

Species	Control plot		Treatment plot	
	Density (stems ha <sup>-1</sup> )	Basal area (m <sup>2</sup> ha <sup>-1</sup> )	Density (stems ha <sup>-1</sup> )	Basal area (m <sup>2</sup> ha <sup>-1</sup> )
<i>Quercus ilex</i> L.	9467	33.87	7733	32.95
<i>Phillyrea latifolia</i> L.	3667	3.46	3533	3.13
<i>Arbutus unedo</i> L.	2200	5.40	2133	7.90
<i>Calicotome spinosa</i> (L.) Link	133	0.06	0	0
<i>Juniperus oxycedrus</i> L.	133	0.06	0	0
<i>Rhamnus alaternus</i> L.	67	0.03	0	0
<i>Sorbus torminalis</i> (L.) Crantz	0	0	200	0.31
<i>Sorbus domestica</i> L.	0	0	133	0.15
Total	15667	42.88	13733	44.44

### *Design of the experiment*

This study was part of a drought simulation experiment. Under the drought treatment, water availability to trees was restricted from March 1999 till the end of the study period using plastic sheets and collectors suspended 50-100 cm above the soil, and 1-m deep trenches on the upper sides of the plots to exclude lateral water flow. The plastic structures were made with transparent PVC and covered 30% of the surface area of the plots. The effectiveness of plastic sheets in excluding throughfall was tested in one of the plots by placing three groups of paired TDR sensors and monitoring soil water content over all the study period. Within each group, one sensor was under a plastic sheet and the other in an adjacent, uncovered position. Water content was, on average, 3 % units lower under the sheets than in uncovered positions. Although the effect was not significant when integrated over all the study period ( $p=0.15$ , two-way ANOVA with position and sampling date as repeated measurement

factors), it tended to be significant after rain events ( $p<0.05$ , paired t-tests). The whole experiment included eight 15x10 m plots (four control and four droughted). However, sap flow sensors were installed only in two of the plots (one assigned to each treatment), and we focus only on those in this study. Within each plot, 2-3 dominant trees per species were monitored for sap flow. All sensors were installed between November 1998 and February 1999, except the ones on *A. unedo* individuals in the droughted plot, which were installed in October 1999. Measured individuals ranged from 4.1 to 14.5 cm in diameter.

The composition of the plant community was very similar at the two plots studied (Table 1). PAR intercepted by the canopy was measured with a ceptometer in the two plots. The results supported that there was no between-plots difference in initial leaf area index (LAI) nor in the dynamics of LAI during the study period (data not shown).

### *Atmospheric and soil moisture measurements*

Sensors to measure temperature and relative humidity (model 50Y, Campbell Scientific Inc., Logan, UT, USA), solar radiation (Campbell SP1110 pyranometer) and wind speed (Campbell A100R switching anemometer) were located on a tower, at approximately crown height. The tower and a standard rain gauge (Campbell ARG100 tipping bucket rain gauge; 1.5 m height) were situated in a clearing within 40 m of all measured trees. All data were stored on a datalogger (Campbell CR10X) which sampled at 5 s intervals and averaged every 30 min continuously. Hourly evapotranspiration ( $E_T$ ) was calculated using the Penman-Monteith equation (Monteith & Unsworth 1990), assuming a constant canopy resistance of  $70 \text{ s m}^{-1}$ . Soil moisture deficit (SMD) was calculated as the difference between cumulative rainfall and cumulative  $E_T$ , with  $\text{SMD} < 0$  set to zero. It was assumed that on 1-Jan-1999, after 77 mm of precipitation on the previous week,  $\text{SMD} = 0$ . SMD was calculated using  $E_T$  instead of actual estimates of canopy transpiration because our sap flux measurements were too fragmentary to be used throughout the two years studied. Soil moisture was measured every 2-3 weeks throughout the study period using time domain reflectometry (TDR). Three-rodged steel sensors were located randomly throughout the study plots, and volumetric water content ( ) at 0-25 cm (8 sensors per plot) and 0-40 cm (2 sensors per plot) was estimated using a cable tester (1502B, Tektronix, Beaverton, OR, USA).

### *Leaf water potential*

Leaf water potentials were measured seasonally throughout the study period with a pressure bomb (PMS Instruments, Corvallis, OR, USA) (Scholander *et al.* 1965). Each sampling date shoot tips from the trees monitored with sap flow sensors or adjacent individuals were measured at predawn (0200-0400 h, solar time) and at midday (1100-1300 h). *A. unedo* individuals were only measured during the year 2000.

### *Sap flux*

Stem flux was monitored with 20-mm-long, constant heat flow gauges constructed after Granier (Granier 1985, 1987). The Granier-type sensors consisted of two stainless steel probes with copper-constantan thermocouple junctions inserted halfway along each probe. At each tree, two probes were inserted radially into the stem at breast height. The vertical separation between the probes was approximately 15 cm. The upper probe was electrically heated with a constant power source. The temperature difference between the two probes was recorded continuously, and was used to obtain the sap flux ( $J_s$ , in  $\text{dm}^3 \text{ dm}^{-2} \text{ h}^{-1}$ ) by means of the equation derived empirically by Granier (1985, 1987):

$$J_s = 4.28 \frac{T_{\max} - T}{T}^{1.23} \quad (\text{eq. 1})$$

where  $T$  is the temperature difference between the heated and unheated probes, and  $T_{\max}$  is the temperature difference

under zero flow conditions. The daily maximum temperature difference was determined from the stored data and used as an estimate of  $T_{\max} - T_{\min}$ .  $T_{\max} - T_{\min}$  was nearly constant during the period studied (the slope of the relationship between  $T_{\max}$ , measured as the electromotive force produced by the thermocouples, and time, was between  $-1 \times 10^{-4}$  and  $+1 \times 10^{-4}$  mV day<sup>-1</sup>), suggesting that no substantial variation in the thermal properties of the surrounding wood occurred during the period of operability of the sensors. The sensors and the trunk were insulated with glass wool to minimize ambient temperature gradients. All sensors were oriented to the north to avoid azimuthal effects (Oliveras & Llorens 2001). Sap flow data were sampled at 5 s intervals and stored with the same datalogger used for microclimate measurements.

An increment coring tool was used to determine the thickness of active sapwood in all measured trees at the end of the study. The depth of active sapwood was determined visually by the wood translucency in *A. unedo* and *P. latifolia*, and with the aid of a stereoscope in the case of *Q. ilex*. Sapwood depth ranged between 18.5 and 36.3 mm. In cases where sapwood depth was less than the 20-mm sensor length, the measured temperature difference was corrected according to Clearwater *et al.* (1999). Since the three species studied are diffuse-porous, when sapwood depth exceeded sensor length  $J_S$  was assumed uniform over the entire sapwood depth. The area of active sapwood ( $A_{sw}$ ) was obtained averaging the thickness of sapwood at both ends of the core. The allometric relationships obtained in the same

study area by R. Ogaya (unpublished results) for *P. latifolia* and *Q. ilex*, and Lledó (1990) for *A. unedo* were used to calculate sap flux per unit of leaf area.

Sap flux estimates for the year 1999 were scaled to the stand level using only data from the control plot. The following formulae was used:

$$E_C = \sum_{i=1}^3 A_{swi} : A_{plot} \langle J_S \rangle_i \quad (\text{eq. 2})$$

where  $E_C$  is stand transpiration considering only the contribution of *A. unedo*, *P. latifolia* and *Q. ilex* individuals with diameter at 0.5 m  $>2$  cm,  $A_{swi} : A_{plot}$  is the sapwood-to-ground area ratio of species  $i$ , and  $\langle J_S \rangle_i$  is the mean sap flux of species  $i$ . Since sapwood depth was independent of tree size in the three species studied (2.9  $\pm$  3 cm in *A. unedo*, 2.2  $\pm$  2 cm in *P. latifolia* and 2.8  $\pm$  2 cm in *Q. ilex*) we estimated sapwood area of each individual in the plot considering that sapwood depth was equal to the mean of the species or, if the radius of the tree was smaller than the species mean, equal to the radius of the tree. The days when sap flow data was missing, the regressions between  $J_S$  and environmental variables (water pressure deficit or soil moisture deficit) were used to calculate  $\langle J_S \rangle_i$ . In the case of *Q. ilex*, only one individual was measured throughout the summer period because of sensor malfunction in other trees, and the regression between its  $J_S$  and  $\langle J_S \rangle_{Q.ilex}$  in spring and autumn ( $r^2=0.93$ ) was used to calculate  $\langle J_S \rangle_{Q.ilex}$  during the summer.

### **Data analysis**

Since we were interested in the drought response of the studied species, we only considered sap flux data measured between the early growing season (spring) and the period immediately after the summer drought (until mid autumn). Diurnal values of sap flux were summed to daily values to avoid the complications introduced by stem capacitance (Oren & Pataki 2001). After a preliminary analysis of data, daily values of sap flux were divided into three periods: (1) *spring*, from the first spring day with  $\theta_{0-40} > 0.2$  to the beginning of summer drought (first day with  $\theta_{0-40} < 0.15$ ), (2) *summer*, from the beginning of summer drought to the first autumn rains, and (3) *autumn*, from those first rains to the first freezing episode. If not otherwise stated we refer to those defined periods and not to calendar seasons throughout the article. Soil water content was nearly identical between the spring ( $\theta_{0-40} = 0.20-0.21\%$ ) and autumn periods ( $\theta_{0-40} = 0.19-0.22\%$ ) for both years. Differences in the response of daily sap flux to environmental variables were tested among species, treatments, years or periods within years by means of F tests comparing the MS of the curvilinear regression with and without segregating data according to the variable under consideration (Potvin *et al.* 1990). When comparing species, periods or years only individuals situated in the control plot were used. Whenever the treatment effect was clearly non significant ( $p > 0.15$ ) data was pooled per species. Comparisons were carried out only if there were at least two replicates within each level of the factor under consideration. Soil water content and leaf water potentials were compared among species and periods with a two way ANOVA

with repeated measurements. All statistical analyses were conducted with SPSS (v. 10.0.6, SPSS Inc., Chicago, IL, USA) and Statistica software (v. 5.95, StatSoft Inc., Tulsa, OK, USA).

## **Results**

### ***Seasonal water relations and treatment effects***

The dynamics of soil water content showed the typical pattern, with lowest values during the summer months of both years, coinciding with the periods with highest temperatures and potential evapotranspirations (Figure 1). As apparent from the rainfall pattern and the dynamics of soil water (Figure 1), summer drought was shorter but more acute in 2000 than in 1999. Water content was, on average, 9 % units higher at the first 25 cm of soil than at the uppermost 40 cm (Figure 1d). Considering all the experimental plots, there was a significant reduction in water content at the first 25 cm of soil associated with the drought treatment ( $p=0.03$ ; three-way, nested ANOVA), but no difference was found between the two plots with sap flow sensors ( $p=0.28$ ). This was because the plot that was randomly assigned to the drought treatment was originally more humid than the control one. Despite the treatment caused the treated plot to be consistently drier than the control (Figure 2, maximum difference of *ca.* 15%), the magnitude of the effect was not enough to cause a significant difference between the plots. The change in the average difference in soil water content between the two study plots (3.2 % units at 0-25 cm and 2.0 % units at 0-40 cm) was consistent with the reduction of



2.8 (0-25 cm) and 4.1 (0-40) % units observed when considering all the plots.

There was no significant difference in water potentials between treatments for *P. latifolia* or *Q. ilex* ( $p > 0.17$  for predawns and  $p > 0.13$  for middays) and, thus, the values were pooled per species in the subsequent analysis. In contrast, *A. unedo* predawn water potentials were significantly lower in the dry plot ( $p = 0.02$ ) during the spring/summer 2000 (the only period in which this species was measured). No treatment effect was observed in midday water potentials nor in the difference between midday and predawn values for *A. unedo*. Among species, predawn water potentials were significantly lower in *Q. ilex* than in *A. unedo* ( $p < 0.01$ ), and in *P. latifolia* than in *Q. ilex* ( $p < 0.01$ ). They remained  $> -1.2$  MPa except during the summer months of both years, when they reached minimum values ranging between  $-2.7$  MPa (*A. unedo* control) and  $-6.2$  MPa (*P. latifolia*) (Figure 3). Midday values were also lowest in *P. latifolia* ( $p < 0.01$ ), but the difference was not significant between *A. unedo* and *Q. ilex* ( $p = 0.09$ ). The difference between midday and predawn water potentials decreased significantly during the summer period for the three species ( $p < 0.01$ ). The reduction was largest in *Q. ilex* ( $p < 0.05$ ), reaching a value of approximately 0 ( $< 0.1$  MPa) at the peak of the 2000 drought. For *Q. ilex* and *P. latifolia*, water potentials tended to be slightly lower in 2000 than in 1999 during the summer period (Figure 3).

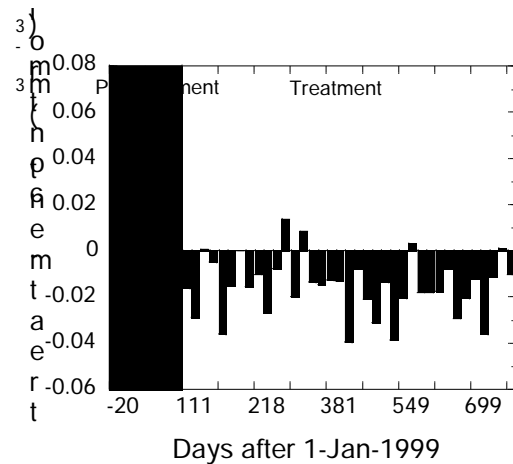


Figure 2. Difference in volumetric soil water content ( $\Delta\theta_v$ ) between the droughted and the control plot. The period before the beginning of the treatment is shaded.

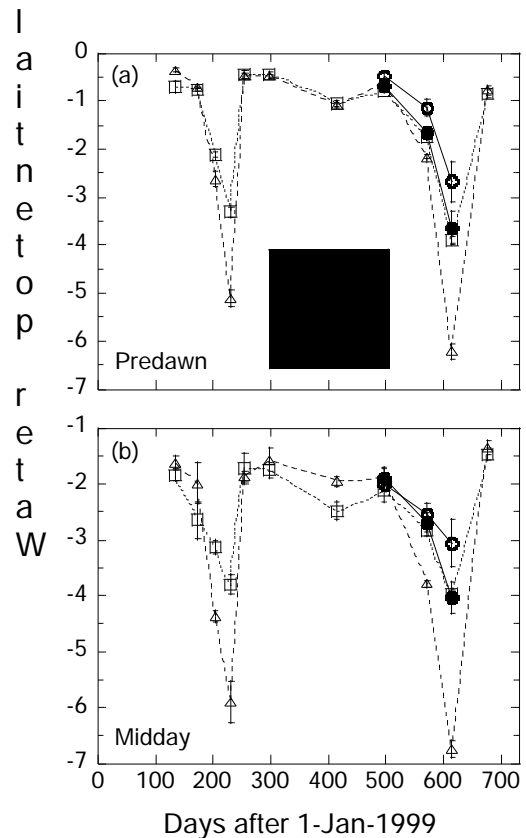


Figure 3. Seasonal patterns of predawn (a) and midday (b) leaf water potentials during the years 1999 and 2000. *A. unedo* individuals (Au) were measured only in 2000, and are segregated between treatments (dr: drought, ct: control). *P. latifolia* (PI) and *Q. ilex* (Qi) are not segregated since no difference was found between treatments. Error bars represent standard errors.

### *Sap flux in relation to environmental variables*

Daily sap flux ( $J_S$ ) was strongly influenced by environmental variables. For both years and all species, mean daytime water pressure deficit ( $D$ ) was the variable that showed the strongest correlation with  $J_S$  during the *spring* and *autumn* periods, whereas during the *summer*, soil moisture deficit (SMD) was the variable that explained a larger part of the variance in  $J_S$ . Differences among species in the response of  $J_S$  to environmental variables were tested only with 1999 data, because sample size was  $<2$  both for *P. latifolia* and *Q. ilex* for most of the 2000 study period due to malfunction of the sap flow sensors. The response of  $J_S$  to  $D$  in spring 1999 was different among species, with *Q. ilex* reaching higher maximum values of  $J_S$  ( $p < 0.01$ , Figure 4a). The ratio between sapwood area and leaf area ( $A_{sw}:A_l$ ) was much lower in *Q. ilex* ( $0.47 \times 10^{-3} \pm 0.07 \times 10^{-3}$ ) than in the other two species:  $1.07 \times 10^{-3} \pm 0.05 \times 10^{-3}$  in *A. unedo* and  $1.14 \times 10^{-3} \pm 0.03 \times 10^{-3}$  in *P. latifolia*. As a result, the differences in spring sap flux were less pronounced when  $J_S$  was expressed per unit of leaf area (Figure 5). The slope of the relationship between  $J_S$  and  $D$  decreased with  $D$  in the three species, but the effect was less marked in *P. latifolia* (Figure 4a).

During the summer period, a continuous increase in SMD was apparent resulting in a reduction in  $J_S$  in all species despite  $D$  and  $E_T$  being higher than in spring (Figures 4b and 1c). The response of  $J_S$  to SMD was significantly different between *A. unedo* and *P. latifolia* ( $p < 0.01$ ) because of the initially higher  $J_S$  values in *P. latifolia*. At the end of

the summer, sap flux tended to be higher in *P. latifolia* than in the two other species (Figure 5). The average decline in  $J_S$  between early summer and the peak of the drought (SMD=319 mm at the end of the summer period) was 60% for *A. unedo*, 48% for *P. latifolia* and 80% for *Q. ilex*. During the autumn period the three species increased  $J_S$  in response to the higher water availability. The relationship between  $J_S$  and  $D$  during autumn was similar among species ( $p > 0.2$ ), and only in the case of *Q. ilex* it was different to the spring relationship ( $p < 0.01$ ). For this species there was a nearly constant reduction of ca. 37% between spring and autumn (Figure 6).

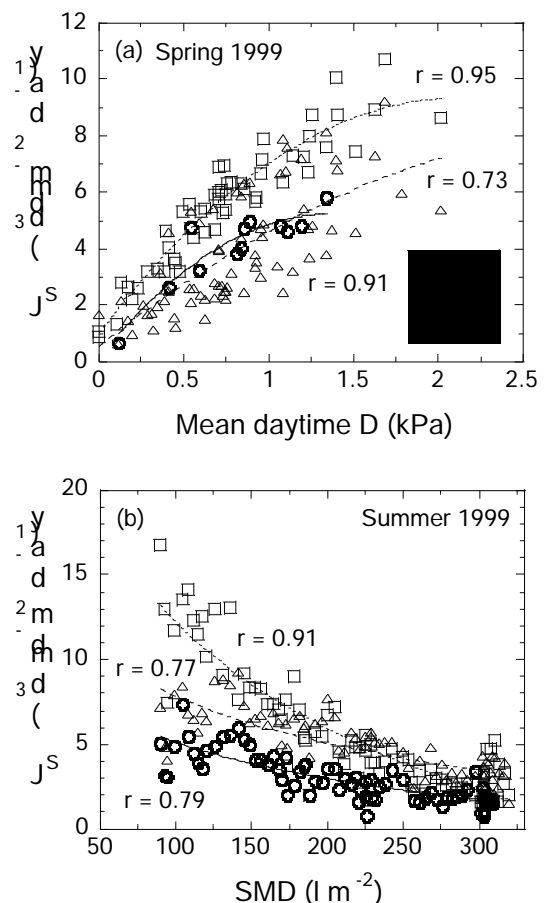


Figure 4. (a) Daily sap flux per unit of sapwood area ( $J_S$ ) in relation to mean daytime water pressure deficit ( $D$ ) in the spring of 1999, and (b)  $J_S$  in relation to soil moisture deficit (SMD) in the summer of the same year. The values are species means except for *Q. ilex* during the summer period, when one single individual was measured. Regression curves (quadratic in (a) and logarithmic in (b)) are shown for each species. Abbreviations as in Figure 3.

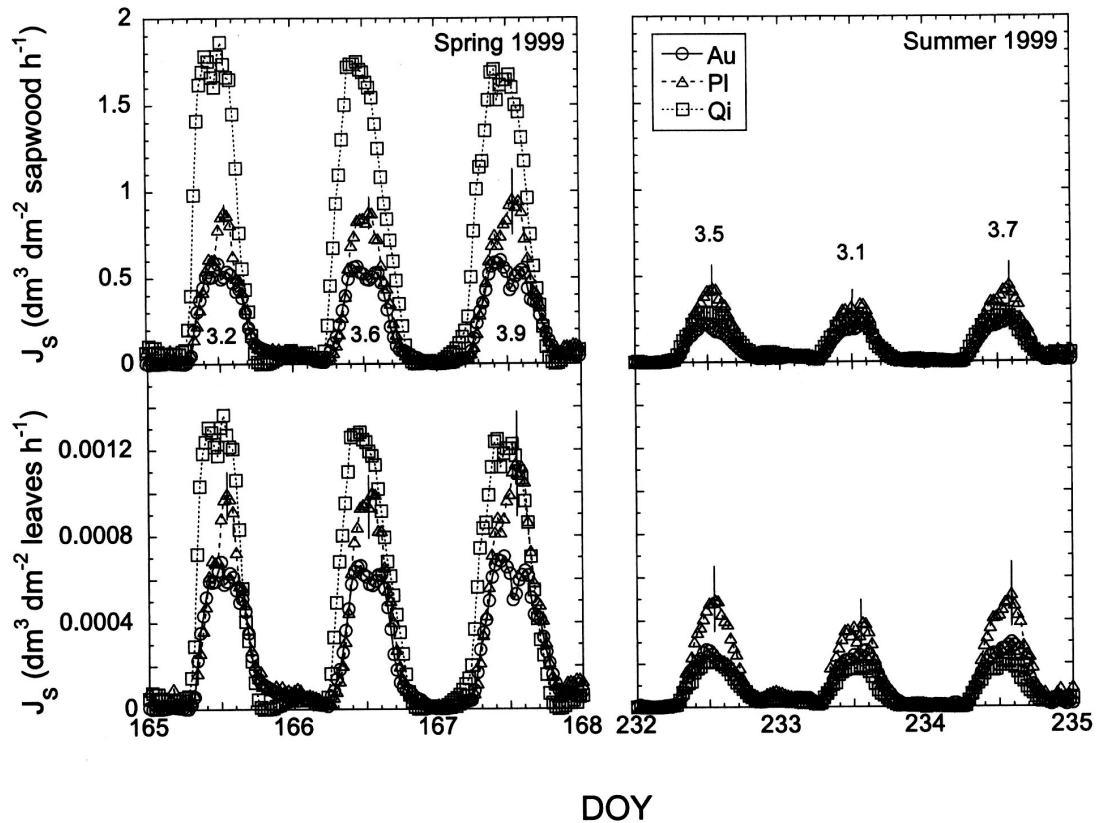


Figure 5. Diurnal sap flux ( $J_s$ ) per unit of sapwood area (upper panels) and per unit of leaf area (lower panels) on two three-day periods in the year 1999, one during the spring before the onset of drought (left panels) and the other at the end of the summer drought (right panels). The values are species means, except for *Q. ilex*. The vertical bars represent the daily maximum standard error for any species. The numbers indicate daily potential evapotranspiration in  $l\ m^{-2}$ . Abbreviations as in Figure 3.

The effects of the drought treatment were tested only in *A. unedo*, which was the only species that showed lower water potentials under the treatment, and the year 2000. The relationship between  $D$  and  $J_s$  was indistinguishable between plots both in spring and autumn ( $p > 0.6$ ). However, a treatment effect was apparent during the summer (Figure 7), in which the individuals of the dry plot reduced  $J_s$  to values *ca.* 55% of those in the control plot at the end of the season. The average reduction of  $J_s$  during the summer period was 22%. Year effects were also tested using the control individuals of *A.*

*unedo*.  $J_s$  was markedly lower in spring 1999 than in 2000 for any value of  $D$  ( $p = 0.02$ , Figure 8a). This difference disappeared in the autumn period ( $p = 0.12$ ). The relationship between  $J_s$  and SMD during the summer was different between years because of the higher  $J_s$  in the spring of 2000, but the slope was similar for both years (Figure 8b).

### Stand transpiration

Estimated stand transpiration ( $E_c$ ) during the period studied in 1999 was 294 mm, 56% of

the 524 mm calculated using the Penman-Monteith equation. There was a strong seasonal pattern in the relationship between stand transpiration and potential evapotranspiration:  $E_C$  was 76% of  $E_T$  in the spring, decreased to 41% during the summer, and increased again to 63% in autumn. For the same periods, precipitation was 184, 49 and 113 mm, respectively.  $E_C$  represented 85% of total precipitation during the study period. Considering a 13% of interception losses (Bellot *et al.* 1999, data from the same study area),  $E_C$  constituted 98% of net precipitation. *Q. ilex* was, by far, the main contributor to stand transpiration, accounting for 81% of total transpiration in the control plot.

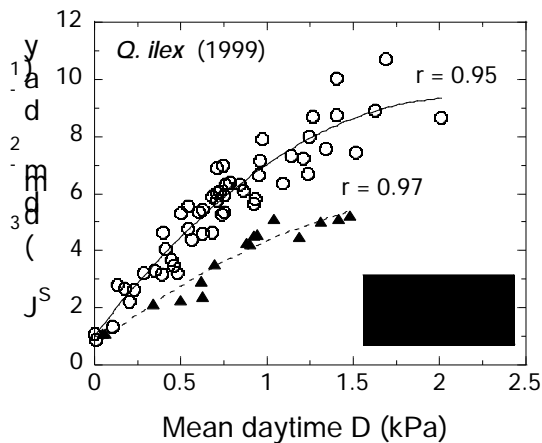


Figure 6. Daily sap flux per unit of sapwood area ( $J_s$ ) in relation to mean daytime water pressure deficit ( $D$ ) for *Q. ilex* in the spring and autumn of 1999. Quadratic regression curves are shown for both periods.

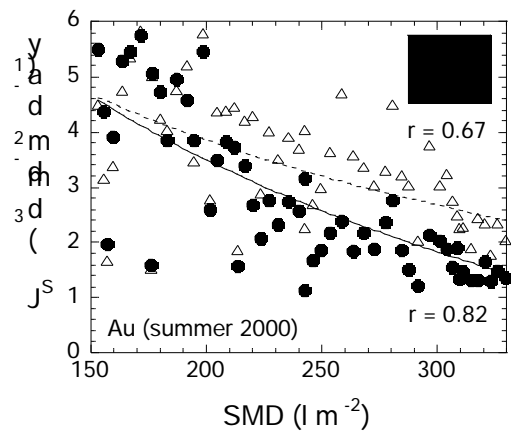


Figure 7. Daily sap flux per unit of sapwood area ( $J_s$ ) in relation to soil moisture deficit (SMD) for *A. unedo* in the summer of 2000. A logarithmic regression curve is depicted for each treatment. Abbreviations as in Figure 3.

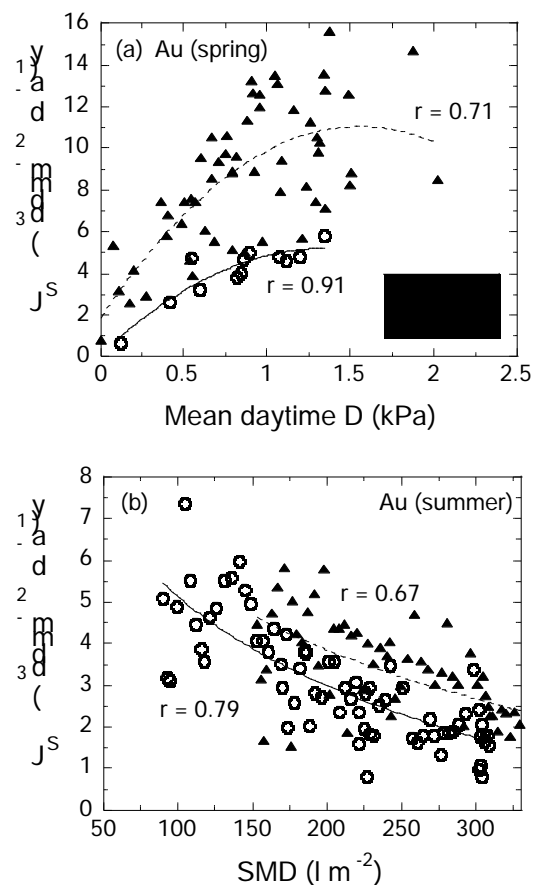


Figure 8. (a) Daily sap flux per unit of sapwood area ( $J_s$ ) in relation to mean daytime water pressure deficit ( $D$ ) for *A. unedo* in the spring, and (b)  $J_s$  in relation to soil moisture deficit (SMD) for the same species in the summer. Regression curves (quadratic in (a) and logarithmic in (b)) are shown for each year. Abbreviations as in Figure 3.

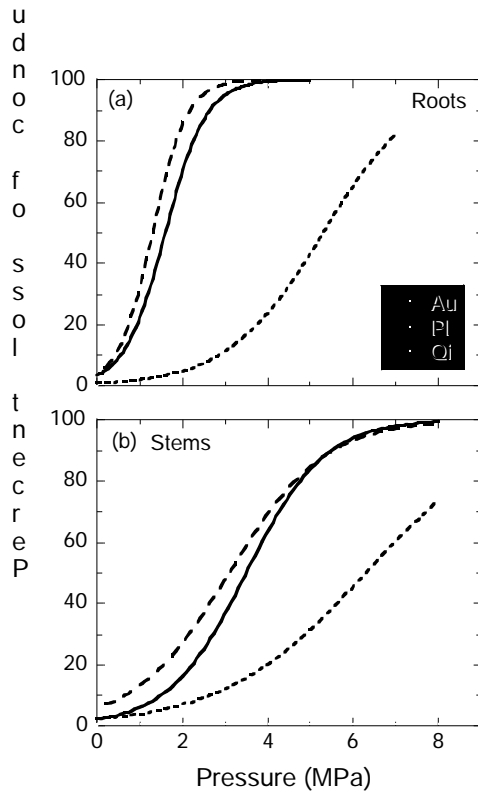


Figure 9. Vulnerability curves of roots (a) and stems (b) of the three studied species, showing the percent loss of conductivity due to embolism at increasing xylem pressures (corresponding to  $-$ water potentials). Data is from Chapter 3. Abbreviations as in Figure 3.

## Discussion

Measured sap flows provided reasonable estimates of stand transpiration, comparable to values reported previously for the same study area using different methods: Sala & Tenhunen (1996) obtained that canopy transpiration represented 87% of 1989 total precipitation using a coupled photosynthesis-transpiration model; in the same area, Piñol (1990) used the hydrological balance method to estimate that canopy transpiration accounted for 81% of total precipitation during the 1981-88. Both values compare favourably with our 85% estimate. The fact that stand transpiration represented 98% of net precipitation during the period studied in the "relatively wet" 1999 year, indicates to what

extent the forest studied is limited by water. The three species studied showed similar patterns of seasonal water use, with acute reductions in sap flux during the summer drought and increases after the first autumn rains. However, the absolute rates of water use, the responses of sap flux to environmental variables and the seasonal dynamics of water potentials varied by species, suggesting that the mechanisms underlying the different responses to drought were also species dependant.

In all three species, the shape of the relationship between  $D$  and  $J_s$  was parabolic with substantial reductions in  $J_s$  at high  $D$  when all measurements from spring to autumn were considered (data not shown). However, this apparent response was an artefact produced by seasonal changes in soil moisture, for the period with highest  $D$  coincided with the lowest soil water contents (Figure 1). Within the summer period, the effect of water availability was dominant and SMD accounted for a higher proportion of the variance of data than  $D$  (Figure 4b). When the analysis was restricted to the spring period, only *A. unedo* during the year 2000 showed a slight reduction of  $J_s$  at high  $D$  (Figure 8a). Less than linear increases in  $J_s$  with  $D$  during periods of moist bulk soil (e.g., our spring period) have been related to quick reductions in soil water content near roots under high transpiration rates (Pataki *et al.* 1998, 2000). Both *A. unedo* and *Q. ilex* are known to close stomata at relatively high water potentials (Castell *et al.* 1994, Sala & Tenhunen 1994, de Lillis & Mirgone 1994, Peñuelas *et al.* 1998). In cases of high vulnerability to xylem embolism (e.g., *A. unedo* and *Q. ilex*, Figure 9), a strict stomatal control is probably

required even under the relatively wet spring conditions to avoid the development of substantial xylem embolism (Sperry 2000). Oren *et al.* (1999) have shown that a decline in hydraulic conductance with increasing D could explain the reduction in transpiration at high D, such as the one reported here for *A. unedo* (Figure 8a). The higher maximum  $J_S$  in *Q. ilex* than in the other two species is not surprising since this species is known to have much higher maximum hydraulic conductivities both per unit of wood surface and per unit of leaf area (Chapter 3).

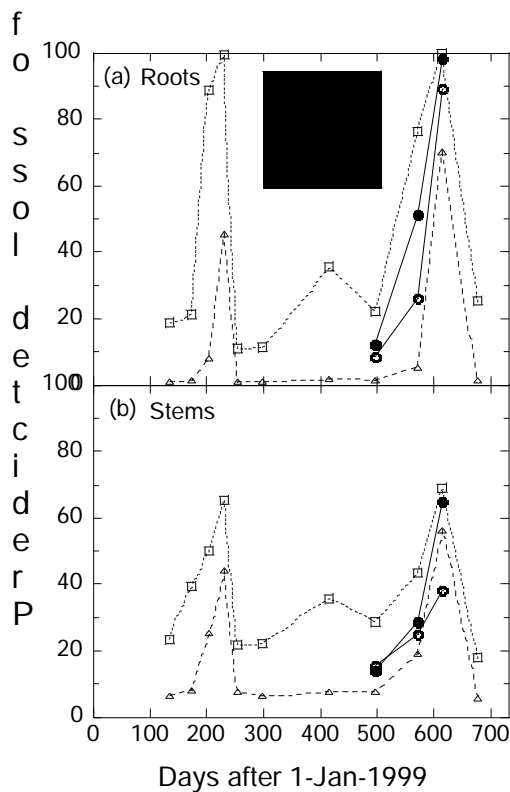


Figure 10. Seasonal patterns of predicted percent loss of conductivity due to xylem embolism for roots (a) and stems (b) of the three studied species. Data was obtained combining water potentials (Figure 3) and vulnerability curves (Figure 9). Predawn water potentials were used for roots and midday values for stems. Perfect reversibility of embolisms is assumed. Abbreviations as in Figure 3.

The acute reductions in  $J_S$  observed during the summer period are probably due to a combination of increased stomatal control over transpiration and substantial losses of conductivity in the hydraulic continuum from soil to leaves. An estimate of native levels of xylem embolism (Figure 10) can be obtained by combining water potential measurements (Figure 3) with vulnerability curves (Figure 9). *Q. ilex* experiences much higher levels of drought-induced xylem embolism than *P. latifolia* and, if it is assumed that the pattern observed in 2000 holds for 1999, also than *A. unedo*. This is in agreement with the larger decline in  $J_S$  that we found in the only *Q. ilex* individual that was measured during the summer (Figure 4b), and with the incomplete recovery observed in this species after the summer period (Figure 6). Two mechanisms may explain this long-term reduction in  $J_S$ . Since xylem vessels are much wider in *Q. ilex* than in the other two species, refilling of previously embolized vessels is probably slower in this species (Yang & Tyree 1992). Alternatively, the extremely high embolism predicted for *Q. ilex* roots (Figure 10) may have caused a substantial drought-induced mortality in this tissue, resulting in a change in the water relations of whole trees. Sala & Tenhunen (1996) also found seasonal variations in the stomatal response of *Q. ilex* as a result of water stress in the same study area.

Our results agreed with the hypothesis that the effects of drought on transpiration would be highest in *Q. ilex* and lowest in *P. latifolia*. The apparently higher resistance to drought of *A. unedo* than *Q. ilex* in this study is probably due to a combination of slightly lower vulnerability to xylem embolism and

more effective stomatal control over water loss. The fact that the difference between midday and predawn water potentials approached zero in *Q. ilex* during the summer period (particularly in the year 2000) is a good illustration of the acute effects of water stress in this species. It is interesting to note that this extremely low water potential difference (0 MPa in two of the individuals monitored) occurred while  $J_S$  in the same individuals was clearly positive, implying very high soil-to-leaf hydraulic conductivities. Since xylem embolism was also predicted to increase during the summer, this apparent paradox suggests that stored water (*e.g.*, in the xylem) can suppose a substantial contribution to transpiration in *Q. ilex* during dry periods. The low resistance to drought of *Q. ilex* seems surprising because this species is one of the most widely distributed trees in the Mediterranean basin. However, it is in agreement with previous ecophysiological (Lo Gullo & Salleo 1993, Peñuelas *et al.* 1998) and modelling studies (Chapter 7). The success of this species is probably due to: (1) high transport efficiency of the xylem, which allows *Q. ilex* to support the same leaf area with half the area of sapwood needed by the two other species studied; and (2) high transpiration (Figures 4 and 5) and, presumably, assimilation rates per unit of leaf area when water is not limiting.

In *A. unedo*, the response of  $J_S$  to environmental variables varied between years and as a result of the drought treatment (Figures 7 and 8). The higher sap flux during the spring of 2000 is probably due to between-years differences in the distribution of precipitation (Figure 1). Cumulative precipitation to the beginning of the summer

period was 289 mm in 1999 and 393 in 2000 and, what is probably more important, in 2000 high rainfall and soil humidity coincided with a period of high evaporative demand (Figure 1). Regarding the treatment effect, our results generally agreed with other throughfall manipulation experiments (Borghetti *et al.* 1998, Hanson *et al.* 2001 and references therein). The treatment caused a maximum decrease in the water content of surface soil of approximately 15%. This effect was associated with a 36% reduction in predawn water potentials (Figure 3), a 10 and 71% increase in predicted xylem embolism in roots and stems, respectively (Figure 10), and a 45% reduction in  $J_S$  at the peak of the 2000 drought (Figure 7). *A. unedo* was the only of the species studied for which a treatment effect in growth was observed the year 2000 (R. Ogaya, unpublished results). In agreement with our results, the reduction in growth in the dry plot was substantial (66%), and similar to the decline observed when considered all the plots studied in the water exclusion experiment (Ogaya *et al.*, unpublished results). *A. unedo* was also the only species which modified the carbon isotopic composition of leaves in response to the treatment, with higher integrated WUE in the dry plot (M. Mangirón, unpublished results). Unfortunately we did not have enough sample size to test the effect of the treatment on sap flux in the other two species. However, the fact that their water potentials and growth were unaffected suggests that the impact, if any, was minor. *A. unedo* is normally considered to be a shallow rooted species (*e.g.*, Castell *et al.* 1994), in contrast to *Q. ilex* and, possibly, *P. latifolia*. Differences in rooting depth may be the cause of the higher sensitivity of *A. unedo* to an

experimental reduction of water content in surface soil. However, the high predawn water potentials in *A. unedo* are difficult to fit into this interpretation.

In summary, the three species studied presented very different strategies to deal with low water availability, as has been shown for other co-occurring Mediterranean species (e.g., Nardini *et al.* 1999). Low vulnerability to xylem embolism allowed *P. latifolia* to have a less strict stomatal regulation and maintain transpiration at lower water potentials than the other two species. On the other hand, *A. unedo* and *Q. ilex* needed a strong stomatal control over water loss to avoid low water potentials that could cause dangerous levels of xylem embolism ("runaway embolism", Tyree & Sperry 1988). Under extremely dry conditions, stomatal control was not enough for these species to escape high losses of hydraulic conductivity due to embolism, particularly in *Q. ilex*. Since predawn water potentials can be considered as an environmental constraint with small room for physiological modification (at least over the short term), *Q. ilex* and, to a lesser extent, *A. unedo*, seem to be at their limit to cope with water stress. This result agrees with the acute impact that the 1994 drought had on *Q. ilex* (Lloret & Siscart 1995, Peñuelas *et al.* 2000), and with previous ecophysiological studies (see above). Considering that our study site is representative of many Mediterranean areas, it is likely that in the context of increased aridity predicted by climate change models (IPCC 2001) and already observed in NE Spain (Piñol *et al.* 1998), important changes in the composition of plant communities will occur. Although acclimation to water stress or to increased CO<sub>2</sub> concentration could

ameliorate the consequences of decreased water availability (Gebre *et al.* 1998, Tognetti *et al.* 1998, Osborne *et al.* 2000), it is unlikely that this acclimation would be enough to compensate for the predicted increase in the frequency and intensity of extreme droughts.

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