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Between-tree variations in leaf $\delta^{13}C$ of *Quercus pubescens* and *Quercus ilex* among Mediterranean habitats with different water availability

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Abstract In this study, sun leaf carbon isotope composition ($\delta^{13}C$) of two co-occurring woody Mediterranean species (*Quercus pubescens* Willd., a deciduous oak, and *Q. ilex* L., an evergreen one) was investigated on four sites with different water availability. The total range of $\delta^{13}C$ values was 4.4 and 3.1‰ for *Q. pubescens* and *Q. ilex* respectively. The intra-site variability was about 3‰. Total mean per species was equal. There were significant differences among sites, but at each site means of $\delta^{13}C$ were not significantly different between species. A simple physiological model predicts no difference in intrinsic water-use efficiency (WUE_i) between evergreen and deciduous oaks. The relationship between site means of $\delta^{13}C$ and water parameters suggests that there is a leaf functional adjustment with respect to available water resource. No correlation was found between $\delta^{13}C$ and the contents of any mass-based biochemical constituent. Nevertheless there was a significant correlation between $\delta^{13}C$ and leaf mass per area of *Q. ilex*. For both species, there is also a positive correlation between leaf $\delta^{13}C$ and individual crown area, i.e. a structural characteristic at tree level. Causal relations between $\delta^{13}C$ and plant-environment interactions are discussed.

Key words Mediterranean oaks · Carbon isotope composition · Water availability · Foliar chemical constituents · Tree structure

Introduction

Theory predicts that the values of leaf $\delta^{13}C$ are positively related, via the ratio between the concentration of CO_2 in the leaf and in the air (Farquhar et al. 1982), to the intrinsic water use efficiency (WUE_i) defined as the ratio between photosynthesis per unit leaf area and stomatal conductance (for model description see Farquhar et al. 1989). The naturally occurring plant tissue $\delta^{13}C$ is more and more used in ecological research as an indicator of WUE_i. One of its uses aims at comparing plant behaviour and at distinguishing functional types either within a species between juvenile and reproductive stages (Donovan and Ehleringer 1991), between male and female individuals (Dawson and Ehleringer 1993), for example, or between species such as species with different habit (annuals versus perennials: Smedley et al. 1991; trees versus herbs: Handley et al. 1994). Plants with different leaf life-span (evergreen versus deciduous) are also compared and are generally found to differ. In conifer species, Gower and Richards (1990) and Valentini et al. (1994) showed that, at several sites, a deciduous species, the larch, had a lower leaf $\delta^{13}C$ than the coexisting evergreen species. In a tropical dry forest, Mooney et al. (1989) also showed that evergreen species had higher $\delta^{13}C$ than deciduous species. The same result was found by Marshall and Zhang (1994) on the whole shrubs and trees along an altitudinal transect of the Rocky Mountains.

C_3 plant isotopic composition may vary (Mooney et al. 1989), typically between −22 and −32‰ (Troughton 1979). Variation, up to 5.5‰, has been found among different habitats, in plants of the same genus (Körner et al. 1988; Read and Farquhar 1991) or of the same species (Leavitt and Long 1986; Friend et al. 1989). Within a site, variability also exists between co-occurring species (Jaindl et al. 1993; Kolb and Davis 1994) and within species (Meinzer et al. 1992a; Ehleringer 1993). In a first step, comparisons of $\delta^{13}C$ values of whole-leaf tissue should take into account differences in

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chemical composition, as there are large differences between $\delta^{13}C$ of various molecules. Indeed, proteins tend to have less negative $\delta^{13}C$ value than cellulose, while lipids have more negative $\delta^{13}C$ value (up to 10‰) than whole-leaf carbon (Ziegler 1979; O'Leary 1981). To our knowledge, studies that examine $\delta^{13}C$ variation with leaf biochemical composition are rare (Medina et al. 1994). Next, natural $\delta^{13}C$ variations can give interesting information in ecological studies if they can be understood in terms of plant response to environmental factors. Much field variation has been related to environmental factors such as water availability (Ehleringer and Cooper 1988; Meinzer et al. 1992b; Stewart et al. 1995) or irradiance level (Mulkey 1986; Ehleringer et al. 1986; Zimmerman and Ehleringer 1990). It would be interesting to relate $\delta^{13}C$ value to total plant growth, but it is rather problematic especially for mature trees. To improve the understanding of the ecological meaning of $\delta^{13}C$ signatures, it may be advisable to examine them with other indicators of plant function such as morphological attributes (Meinzer et al. 1992b; Kohorn et al. 1994).

We studied two co-occurring Mediterranean congeneric tree species differing in leaf life-span and leaf structure: *Quercus pubescens* Willd., a deciduous oak, and *Q. ilex* L., an evergreen one with smaller and thicker leaves. Both oaks are found in southern France. They cooccur at many sites but *Q. ilex* is found in more xeric sites than *Q. pubescens* (Timbal 1975; Lepart 1984). Our first objective is to compare the leaf carbon isotope composition between both species. Recognizing that others have observed differences between deciduous and evergreen species, we tested the generality of the hypothesis that evergreen species would have higher leaf carbon isotope composition (and presumably higher water use efficiency) than co-occurring deciduous species. Our second objective was to investigate specific plasticity of the leaf carbon isotope composition within and between field sites. Sampling collection has been made in Southern France, in four sites differing by rainfall and plant water status. One hypothesis is that both species have a higher foliar $\delta^{13}C$ in sites where the water availability is lower. As Mediterranean woodland ecosystems are very heterogeneous, we could expect large inter-tree variation in leaf $\delta^{13}C$. The third objective is to interpret $\delta^{13}C$ variation in conjunction with other plant characteristics either at leaf or at individual levels. We examine the relationships between $\delta^{13}C$ and leaf structural parameters (leaf mass per area, chemical constituent contents) and structure of individuals within the ecosystem (e.g. height, crown area, crowding index).

Materials and methods

Study sites

Four study sites, in which *Q. pubescens* and *Q. ilex* co-occur, were chosen within a 60-km radius around Montpellier (Southern France, 43°35′N, 3°58′E). They were labelled Béziers, La Cadière, Saint Martin and Vailhan. Mean annual precipitation and mean annual maximum and minimum temperatures were obtained from the records of the nearest meteorological sites (maximum distance: 10 km), for the periods 1954–1993 for rainfall and 1962–1993 for temperatures. Water availability was assessed in several ways: (1) from the mean annual precipitation, (2) from rainfall during the September 1992 to May 1993, and (3) from the time-course of predawn water potential during the growing season. Period from September to May was chosen because it corresponds to the fall and spring rainfalls that recharge the soil for the next growing season. The four sites have similar mean annual maximum and minimum temperatures but contrasting pluviometric conditions (Table 1). The mean annual precipitation over 40 years at La Cadière is 1.7 times greater than at Béziers. The differences between precipitation from September 1992 and May 1993 are less important. Penman estimates of annual potential evapotranspiration (PET) for the four sites were all within 5% of the regional value (*c.* 1000 mm a^{-1}); hence PET was not retained as a causal factor to explain intra-site $\delta^{13}C$ variation (see Comstock and Ehleringer 1992).

Vegetation description

Mediterranean forest ecosystems are very heterogeneous with individuals differing in diameter, number of resprouts, and number and distance of neighbours. At the four sites, trees generally have re-sprouted trunks, each of them 30–50 years old. On 1993, leaf growth on *Q. pubescens* started at the end of March and the beginning of April. For *Q. ilex*, it started about 3 weeks later. At each site, ten co-dominant or dominant trees of *Q. ilex* and *Q. pubescens* were randomly selected from a 40 × 40 m quadrat. For each tree, the height, the stem number of the stump, the stool basal area and the crown area were measured. The crown area was obtained by measuring the largest crown diameter and its perpendicular one and considering the crown as an ellipse. To describe the local neighbourhood interference around each target tree, a crowding index was calculated as in Takahashi (1996) from log transformation of the Weiner's index (Weiner 1984):

$$W = \log\left(\sum_{i=1}^{n} s_i \Big/ d_i^2\right)$$

where n is the total number of neighbours, d_i is the distance (m) from the target tree to the i th neighbour and s_i is the size of the i th

Table 1 Some environmental characteristics of the four study sites of La Cadière, Vailhan, Saint Martin, and Béziers (*Pm* mean annual precipitation, *SD* standard deviation; *Psept92–may93* precipitation from September 1992 to May 1993, *Tmin*, *Tmax* mean annual minimum and maximum air temperature)

	La Cadière	Vailhan	Saint Martin	Béziers
Altitude (m)	250	186	250	25
Pm ± SD (mm)	1134 ± 347	728 ± 244	1129 ± 377	663 ± 205
Psept92–may93 (mm)	961	739	879	689
Tmin (°C)	8.5	8.1	7.1	9.4
Tmax (°C)	19.8	18.0	17.9	19.1
Formation	Mid-dense woodland	Dense forest	Open woodland	Open woodland

Table 2 Structural parameters [height (m), stool basal area (cm^2), stem number of the stump, crown area (m^2) and crowding index] of the sampled individuals in the four sites for *Quercus ilex* and *Q. pubescens* ($n = 10$ except for *Q. pubescens* at Béziers and Vailhan where $n = 9$). The standard deviation is indicated in parentheses. Mean values within a row are significantly different at $P < 0.05$ if followed by different letters

Parameters	Species	Sites			
		La Cadière	Vailhan	Saint Martin	Béziers
Height	*Q. pubescens*	7.4 (1.5) b	8.3 (2.3) b	5.8 (1.5) a	5.3 (1.0) a
	Q. ilex	5.9 (1.2) b	8.1 (1.3) c	5.0 (0.9) ab	4.1 (0.8) a
Stool basal area	*Q. pubescens*	555 (361) a	438 (321) a	401 (342) a	505 (261) a
	Q. ilex	460 (355) a	195 (60) a	528 (345) a	535 (325) a
Stem number	Q. pubescens	2.4 (1.7) b	1.7 (1.0) a	2.3 (1.2) b	2.3 (1.9) b
	Q. ilex	4 (3.0) ab	2.2 (0.9) a	6.9 (4.2) b	5.5 (3.3) ab
Crown area	*Q. pubescens*	29.4 (17.3) a	23.5 (11.0) a	20.9 (11.9) a	27.8 (13.6) a
	Q. ilex	15.9 (10.0) ab	9.9 (10.3) a	23.4 (17.6) bc	28.8 (12.9) c
Crowding index	*Q. pubescens*	1.91 (0.48) a	2.56 (0.23) b	1.94 (0.51) a	1.68 (0.31) a
	Q. ilex	2.05 (0.36) a	2.39 (0.25) b	2.16 (0.35) ab	1.97 (0.44) a

neighbour. In this study, the size of the neighbour (s_i) is represented by its basal area at breast height (cm^2). The neighbour was defined as any woody plant taller than 2 m and within 15 m of the target tree. All the structural parameters of the individuals are summarized in Table 2.

Leaf sampling

To minimize microenvironmental effects, isotopic measurements were all conducted on sun-exposed leaves in the upper part of the canopy. All leaves collected grew in spring 1993. For each site, three trees per species (among the ten selected trees) were sampled four times during 1993 (twice in spring, in July and in September). For *Q. ilex*, the trees were also sampled twice in 1994 (in January and September). On October to November 1993 for *Q. pubescens* and in January 1994 for *Q. ilex*, leaf samples were collected at each site from the ten trees per species (only nine individuals of *Q. pubescens* at Béziers). For *Q. pubescens*, the autumn samples were taken before the leaves became brown.The samples consisted of 60 leaves per tree. The evolution of $\delta^{13}C$ values during leaf life span was followed on three trees per species and per site.

Predawn water potential

Three of these trees per species at each site were used for water potential measurements. Seasonal changes in predawn water potential were measured from July 1993 to October 1993, using a pressure chamber (PMS, Corvallis, Or., USA). In summer, measurements were made at approximately 2-week intervals. Values for the start of summer (6 and 7 July 1993) were used to estimate the plant water constraint at the end of the leaf development, and the minimum values recorded over the whole summer period were used to estimate the maximum water constraint endured by leaves that had become mature.

Leaf area and mass per area

For each tree, the mean leaf area and leaf mass per area (LMA) were determined on a sub-sample of 20 leaves per individual. The area was measured using a leaf area meter (Delta-T Image Analysis System, Delta-T Devices Ltd., UK). The dry mass was determined after drying for 48 h in an oven at 60°C.

Biochemical determinations

After drying, all leaves from each tree was milled (Cyclotec 1093 Sample Mill, Tecator, Höganäs, Sweden). The samples were next scanned with a near-infrared reflectance spectrophotometer (NIRSystems 6500). Each sample was packed into a sample cell with a quartz-glass sample. Two reflectance measurements of monochromatic light were made from 400 to 2500 nm to produce an average spectrum with 1050 data points at 2-nm intervals over this range. The band-pass used was 10 nm and the wavelength accuracy 0.5 nm. Reflectance (R) was converted to absorbance (A) using the following equation:

$$A = \log(I/R)$$

The spectral and wet chemical database used to build the calibration equations comprised leaves of *Quercus* spp collected by us throughout all the French Mediterranean area and includes part of the database of Meuret et al. (1993). The concentrations of nitrogen, cellulose, lignin and total lipids in these calibration set samples were determined using wet chemistry methods. Nitrogen content was determined on 284 samples with a Perkin Elmer elemental analyser (PE 2400 CHN) and carbon fraction (cellulose, lignin) was determined on 184 samples using the Fibertec procedure (Van Soest and Robertson 1985). The lipid content was determined on 50 samples by weighing the residue extracted by a chloroform-methanol mixture (see Allen 1989).

Calibration equations between spectral and chemical data were conducted using the ISI software system (Shenk and Westerhaus 1991a). Stepwise regression calibrations and partial least squares (PLS) calibrations were developed and compared. PLS differs from stepwise wavelength searches in that it uses all the information in the spectrum to determine any component concentration, a fundamental advantage over single wavelength applications (Shenk and Westerhaus 1991b). As emphasized by Joffre et al. (1992) and Bolster et al. (1996), the full-spectrum calibration method (PLS regression) performed better than stepwise regression on dry ground foliage or litter samples. The final calibration equations allowed the percentage content of biochemical constituents of the leaves to be determined from the spectra, using partial least squares regression, with a standard error of prediction of 0.13% for nitrogen, 0.97% for cellulose, 1.33% for lignin and 1.52% for lipids.

Carbon isotope composition

A subsample of leaf tissue from each tree was more finely ground with a vibro grinder (Type MM 2000 Retsch, Haan, Germany) and sent to the CNRS Central Analytical Department at Vernaison (France) to measure the ^{13}C isotopic composition. The carbon isotope ratios were determined on CO_2, after combustion of leaf tissue, in an isotope ratio mass spectrometer (delta S, Finnigan MAT, San Jose, Calif., USA). The ^{13}C content is expressed in delta notation as $\delta^{13}C$ (‰) relative to the Pee Dee Belemnite (PDB) standard. The sample precision of the analysis was ± 0.3 ‰. The $\delta^{13}C$ of atmospheric CO_2 was not measured. Since samples were taken from the top of the canopy at more than 4 metres height, it was unlikely that the $\delta^{13}C$ of CO_2 originating from the soil contribute substantially to the $\delta^{13}C$ of the air surrounding the leaves.

Statistical analysis

Analysis were computed with the Statistical Analysis System Version 6.03 statistical package (SAS 1988). Sites and species effects were tested by analysis of variance (ANOVA) and repeted measures ANOVA using the General Linear Models procedure. When a factor was significant, means were compared with Tukey tests. Relationships among characters were tested with Pearson's product moment correlation coefficients.

Results

Plant water constraint

The predawn water potentials for *Q. ilex* were always slightly lower than those of *Q. pubescens* (Fig. 1). The intensity of the plant water constraint did differ between species and between sites (two-way repeated measures ANOVA, site × species: $F_{\mathrm{site}} = 7.02$, $P < 0.01$, $F_{\mathrm{species}} = 4.76, P < 0.05$, $F_{\mathrm{site \times species}} = 1.04, P > 0.05$). At the start of summer, trees of both species had significantly lower potentials at Béziers, with those of *Q. pubescens* already reaching −1 MPa (Table 3). At the end of summer (in August), when water availability is the lowest, water constraint was much more pronounced at Saint Martin with values of predawn water potential of less than −2.5 MPa (Table 3). Vailhan was the most mesic site. The rankings of plant water constraint between sites changed between the start and the end of summer but they were the same for both species.

$\delta^{13}C$

The pattern of $\delta^{13}C$ time-variation showed that there is no more variation from July, i.e., when leaves are mature (Fig. 2). For *Q. pubescens*, there was only a slight decline in fall. For *Q. ilex*, there is no variation between values from summer 1993 and those from winter 1994. The overall mean value for all sites was identical for both species at −27.4‰. *Q. ilex* did however have a wider range of variation with minimum and maximum values of leaf $\delta^{13}C$ of −29.1 and −24.7 respectively, compared to −28.8 and −25.7‰ for *Q. pubescens* (Fig. 3). The values of $\delta^{13}C$ varied at each site, the maximum range within one site being 3‰ and 3.2‰ for *Q. pubescens* at Saint Martin and *Q. ilex* at Vailhan, respectively. There was no effect of species on the $\delta^{13}C$ values, but the means differed significantly between sites (Table 4). For both species, the sites were ranked as follows in increasing mean values order of $\delta^{13}C$: La Cadière, Vailhan, Saint Martin, Béziers (Table 5a, b).

Relationship between $\delta^{13}C$ and water conditions

Béziers, which was the site with the lowest mean annual precipitation and with the lowest rainfall value between September 1992 and May 1993, had the highest mean value of $\delta^{13}C$ (Table 5a, b), whereas site La Cadière, which had the highest annual rainfall, had the lowest mean $\delta^{13}C$ value. However, Saint Martin, which had a similar mean annual precipitation to that of La Cadière, had a significantly higher mean $\delta^{13}C$ than that of La Cadière for both species. The trees at Saint Martin, which had a lower water potential than others at the end of the summer, did not have a higher mean $\delta^{13}C$ value. It

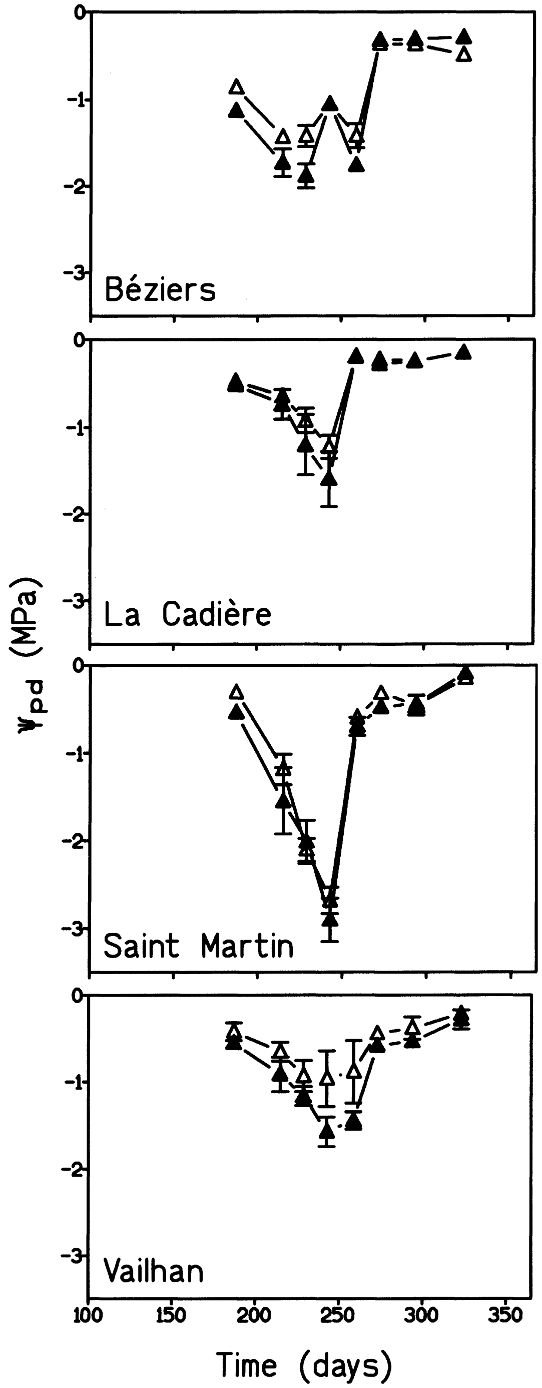

Fig. 1 Predawn water potential evolution of *Quercus pubescens* (*open symbols*) and *Q. ilex* (*solid symbols*) at the four study sites (La Cadière, Vailhan, Saint Martin, Béziers) during 1993

Table 3 Predawn water potential (MPa) of *Q. pubescens* and *Q. ilex* in early (6,7 July) and late summer (15 August for Béziers and 30,31 August for the other sites) 1993 in the four field sites. Data are means ± 1 SE ($n = 3$). Values within a row are significantly different at $P < 0.05$ if followed by different letters

		La Cadière	Vailhan	Saint Martin	Béziers
Early summer	*Q. pubescens*	−0.48 ± 0.03 a	−0.42 ± 0.10 a	−0.32 ± 0.03 a	−0.86 ± 0.06 b
	Q. ilex	−0.52 ± 0.08 a	−0.55 ± 0.05 a	−0.54 ± 0.06 a	−1.13 ± 0.09 b
Late summer	*Q. pubescens*	−1.22 ± 0.13 a	−0.96 ± 0.32 a	−2.68 ± 0.35 b	−1.43 ± 0.08 a
	Q. ilex	−1.59 ± 0.32 a	−1.57 ± 0.17 a	−2.90 ± 0.25 b	−1.88 ± 0.14 a

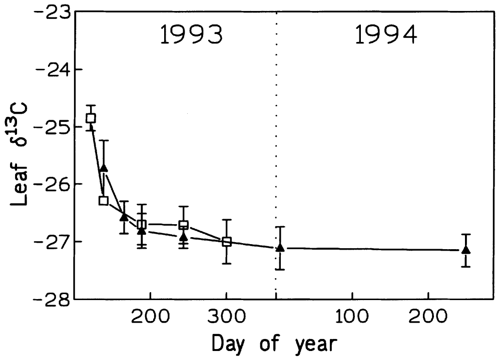

Fig. 2 Time variation of leaf $\delta^{13}C$ on *Q. pubescens* (*open symbols*) and *Q. ilex* (*solid symbols*) (individuals ($n = 12$; 3 trees per site and species, mean ± SE)

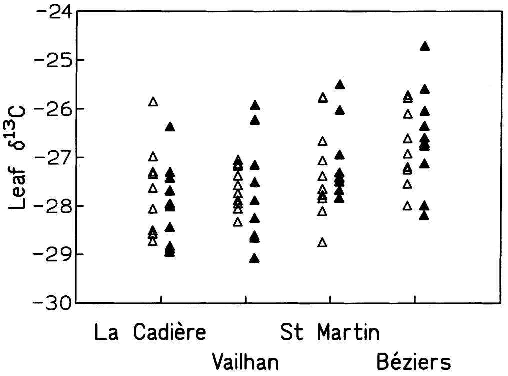

Fig. 3 Sun leaf $\delta^{13}C$ variations of ten *Q. pubescens* (*open symbols*) and *Q. ilex* (*solid symbols*) individuals, sampled in October-November 1993 and January 1994, respectively at four field sites (La Cadière, Vailhan, Saint Martin, Béziers)

Table 4 Two-way ANOVA table for $\delta^{13}C$ in mature sun-exposed leaves sampled in ten trees per species (*Q. pubescens* and *Q. ilex*) growing at four Mediterranean study sites

Source	*df*	SS	MS	*F*	*P*
Species	1	0.00685	0.00685	0.01	0.926
Sites	3	16.833	5.611	7.13	0.0003
Interaction	3	0.697	0.229	0.29	0.832
Error	71	55.898	0.787		

was at the site where the trees had the lowest predawn water potentials at the start of summer (Béziers) that the $\delta^{13}C$ were highest. Nevertheless the difference with Saint Martin was not significant for *Q. pubescens*.

Correlations between $\delta^{13}C$ and leaf structure variables

The correlations between $\delta^{13}C$ and leaf mass per area (LMA) were tested for both species (Table 6). Only the correlation coefficient for *Q. ilex* was significant (Fig. 4). The mean LMA values differed significantly between sites, but the rankings depended on species and were not the same as those for mean $\delta^{13}C$ values. For the mean values of each site there was therefore no correlation between LMA and $\delta^{13}C$, as there was for individual trees for *Q. ilex*. All four sites combined, there was no correlation for both species between leaf $\delta^{13}C$ and the mean leaf area of individual trees. The correlations between these variables for individual sites was only significant for *Q. pubescens* at Béziers ($r^2 = 0.78, n = 9, P < 0.01$). For *Q. pubescens*, the smaller mean leaf area is at Béziers, and the greater one at La Cadière the most mesic site.

Correlations between $\delta^{13}C$ and chemical composition

Foliar chemical composition are shown in Table 5a, b. There was hardly any variation in mean nitrogen and lipid contents between the four sites. The Béziers site was distinct from other sites in terms of its lower lignin contents, but these differences were only significant for *Q. ilex*. For both species, there was no significant correlation between $\delta^{13}C$ and the contents of any chemical constituent whether we consider site means or individual values. For *Q. ilex*, for which there was a significant correlation between LMA and $\delta^{13}C$, the conversion of the mass-based content into area-based content made the correlations significant for all constituents except lignin (Table 6), but the correlation coefficients were not higher than for the relation between $\delta^{13}C$ and LMA.

Correlations between $\delta^{13}C$ and tree structure variables

Considering individuals from the four sites all together, for both species, there is no correlation between leaf $\delta^{13}C$ and tree height or stool basal area (Table 6). For each oak species, there is a positive correlation between $\delta^{13}C$ and the crown area. For *Quercus ilex*, there is also a significant correlation between $\delta^{13}C$ and the stem number of the stump (positive correlation) and for *Quercus pubescens*, between $\delta^{13}C$ and the crowding index (negative correlation).

Table 5 Mean $\delta^{13}C$ (‰), leaf area (A, cm^2), leaf mass per area (LMA, $g.m^{-2}$), nitrogen, cellulose, lignine, and lipids mass-based content (%) in leaves of ten trees of **a** *Q. pubescens* and **b** *Q. ilex* sampled from four sites, in October-November 1993 and January 1994. Means in the same row with different superscripts are significantly different ($P < 0.05$). The SD is given in parentheses. The sample number is 10 except for biochemical constituents of *Q. pubescens* where $n = 3$

a	Sites			
	La Cadière	Vailhan	Saint Martin	Béziers
$\delta^{13}C$	−27.8 (0.92) a	−27.6 (0.44) ab	−27.3 (0.98) b	−26.8 (0.80) b
A	17.2 (1.5) b	14.4 (4.9) ab	14.8 (3.2) ab	13.0 (2.6) a
LMA	105 (11) b	86 (15) a	97 (9) ab	102 (8) b
Nitrogen	1.81 (0.57) a	1.80 (0.12) a	1.82 (0.25) a	2.05 (0.18) a
Cellulose	19.3 (1.33) a	19.0 (0.80) a	18.8 (1.12) a	19.2 (0.57) a
Lignin	12.0 (1.16) a	13.3 (0.60) a	13.4 (2.60) a	11.3 (2.43) a
Lipids	16.2 (1.27) a	18.9 (2.02) a	16.5 (3.06) a	18.4 (0.50) a

b	Sites			
	La Cadière	Vailhan	Saint Martin	Béziers
$\delta^{13}C$	−28.0 (0.83) a	−27.8 (1.12) ab	−27.2 (0.79) bc	−26.6 (1.04) c
A	3.9 (0.6) a	4.3 (0.8) a	3.5 (0.7) a	3.1 (0.7) a
LMA	186 (16) a	180 (21) a	206 (9) b	206 (16) b
Nitrogen	1.48 (0.24) a	1.71 (0.16) a	1.58 (0.18) a	1.72 (0.19) a
Cellulose	22.9 (0.77) b	20.7 (1.08) ab	22.2 (1.38) b	20.9 (1.14) a
Lignin	18.4 (2.30) b	18.0 (1.87) ab	19.2 (1.35) b	14.7 (2.01) a
Lipids	17.8 (1.07) a	17.6 (0.89) a	17.4 (1.56) a	18.3 (1.36) a

Table 6 Correlation coefficients for *Q. pubescens* and *Q. ilex* between leaf mass per area, foliar chemical constituent content (nitrogen, cellulose, lignine, lipid, % on mass basis, values in brackets indicate correlation with the area basis value), structural tree parameters and foliar $\delta^{13}C$ with all sampled trees ($n = 38$ for *Q. pubescens* and $n = 40$ for *Q. ilex*)

Parameters	*Quercus pubescens*	*Quercus ilex*
LMA	NS	0.50***
Nitrogen	NS (NS)	NS (0.46**)
Cellulose	NS (NS)	NS (0.46**)
Lignin	NS (NS)	NS (NS)
Lipid	NS (NS)	NS (0.36*)
Height	NS	NS
Stool basal area	NS	NS
Stem number	NS	0.40**
Crown area	0.37*	0.48**
Crowding index	−0.40*	NS

NS not significant, $^{*}P < 0.05$; $^{**}P < 0.01$

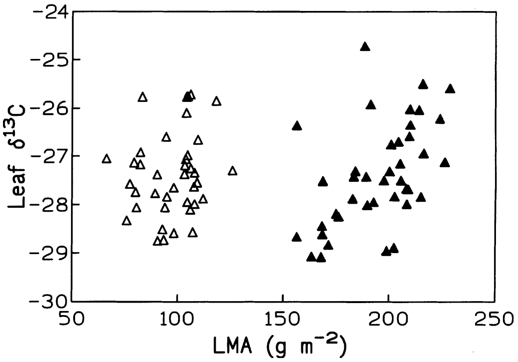

Fig. 4 Patterns of variation of foliar $\delta^{13}C$ with leaf mass per area (LMA) of *Q. pubescens* (*open symbols*) and *Q. ilex* (*solid symbols*). Each point represents an individual sampled at one of four Mediterranean sites ($n = 40$). Correlation coefficients are 0.17 (ns) and 0.50 ($P < 0.001$) respectively for *Q. pubescens* ($n = 39$) and *Q. ilex* ($n = 40$)

Discussion

As the values of leaf $\delta^{13}C$ are stable from July, when leaves have ended their growth, the samples done in fall for *Q. pubescens* and in winter for *Q. ilex* should give results similar to those from an earlier collection. In all the following discussion, we will interpret the spatial variability from the results obtained on the 40 individuals sampled in October for *Q. pubescens* and in January for *Q. ilex*. The results conflict with our initial hypothesis of a difference between evergreen and deciduous species. Read and Farquhar (1991) have also demonstrated very similar carbon isotopic discriminations between evergreen and deciduous species of the genus *Nothofagus* growing in the same region. Ehleringer and Cooper (1988) did not find any correlation between $\delta^{13}C$ and leaf longevity in desert plants. Nevertheless, by comparing *Q. pubescens* and *Q. ilex* trees growing at the same site, Valentini et al. (1992, 1995) showed that the evergreen species had lower $\delta^{13}C$, the difference being about 1‰. They associated this difference to the pattern of soil water uptake during the summer drought period. They suggest that *Q. ilex* uses soil water only from the surface layers, whereas *Q. pubescens* uses deep soil water, a more reliable source. By assuming the uniformity of the isotope composition of CO_2 in the air between the four sites, the identity of $\delta^{13}C$ between species at each site suggests that *Q. pubescens* and *Q. ilex* function with the same leaf intrinsic water use efficiency (Farquhar et al. 1989) for any given soil water availability. This does not fit the result of Epron and Dreyer (1990), who, by measuring gas exchanges on saplings under controlled conditions, recorded a higher WUE_i in *Q. ilex* than in *Q. pubescens* under water-limited conditions.

For both species, our data showed that maximum differences in $\delta^{13}C$ values between sites and within a site were greater than 1‰. Such variations are higher than spectrometer precision and sample preparation error and can be interpreted in ecological studies (O'Leary 1988). For both species, the ranges of $\delta^{13}C$ values between and within sites were greater than 3‰. These ranges fit with those already obtained in the field for several species. Ranges from 1‰ to 5‰ within a species have been observed both at different sites and microsites (Ehleringer and Cooper 1988; Friend et al. 1989; Mooney et al. 1989) or at the same site (Leavitt and Long 1986; Meinzer et al. 1992a; Ehleringer 1993; Kohorn et al. 1994). The absence of any correlation between leaf $\delta^{13}C$ and the mass-based cellulose, lignin and lipid contents per unit dry mass, despite their differences in $\delta^{13}C$ at the molecular scale (O'Leary 1981), shows that relative proportions of the chemical constituents do not determine the isotope composition of leaf tissue. Medina et al. (1994) also showed that differences in $\delta^{13}C$ between shade and sun leaves could not be explained by variations in lipid content in *Ananas comosus*. As chemical composition is not responsible for $\delta^{13}C$ variations, we will interpret $\delta^{13}C$ in relation with climate and structural data. We will examine at first inter-site variations, then intra-site variations. We will discuss about relationships between morphological features and $\delta^{13}C$ values.

The greater mean value of foliar $\delta^{13}C$ is found at Béziers, the site with the lower rainfall, whereas the smaller mean value is at La Cadière, the most mesic site. This result suggests an adjustment of leaf gas exchange at the leaf level with respect to the water availability. An increase of $\delta^{13}C$ have been often recorded with decreasing soil moisture content either in controlled conditions (Condon et al. 1992; Meinzer et al. 1992b) or in the field (Ehleringer and Cooper 1988; Morecroft and Woodward 1990; Jaindl et al. 1993; Osório and Pereira 1994). In Mediterranean-type climate, rainfall amounts are not a good indicator of the plant water status at a site (Rambal 1984). Saint Martin, which has a high annual precipitation, showed the greater intensity of plant water constraint at the end of the summer (because of a very shallow soil), but its trees did not have the greatest $\delta^{13}C$. For *Q. pubescens* and *Q. ilex*, it has been shown that leaf $\delta^{13}C$ value was determined during leaf growth, i.e. in spring (Damesin 1996). During leaf development, assimilated carbon was being used to build leaf tissue and $\delta^{13}C$ of mature leaves is likely a signature of leaf functioning during its growth (Hansen and Steig 1993). The summer constraint could therefore only have an indirect effect on $\delta^{13}C$ value, and it is more interesting to look at the impact of spring weather conditions on $\delta^{13}C$ values than summer ones. For both species, the $\delta^{13}C$ values were higher at the site Béziers where the leaf water potentials were lowest at the start of summer. During the same period, predawn water potential was similar between sites for each species. It can't explain the lowest mean of $\delta^{13}C$ at La Cadière.

Although there were inter-site differences, there were also important $\delta^{13}C$ intra-site variations. Variations in $\delta^{13}C$ can be associated with morphological adjustments. For *Q. ilex*, the $\delta^{13}C$ values were correlated with LMA for all individuals values pooled. As was suggested by Vitousek et al. (1990), it is possible that, a higher LMA leads to greater internal resistance contributing to increasing the $\delta^{13}C$ by lowering the concentration of CO_2 at carboxylation sites. Difference between sub-stomatal CO_2 concentration and that in the chloroplasts of *Q. ilex* was more than 160 $\mu l \cdot l^{-1}$ (Di Marco et al. 1990) suggesting an important contribution of internal resistance to the CO_2 diffusion. It would be interesting to study whether this difference varies with LMA within a single species. Positive covariations have frequently been observed between $\delta^{13}C$ and LMA (Schleser et al. 1989; Collet et al. 1993; Zhang et al. 1993; Osório and Pereira 1994). Nevertheless, since LMA and $\delta^{13}C$ are both implicated at the stage of leaf growth, there is not necessarily any causal relation between these two variables, each of which integrates leaf functioning in response to its environment. Knowledge of the cause and effect relations between these two variables would be needed to determine what information could be drawn from them in terms of plant performance.

For both species, leaf $\delta^{13}C$ was positively correlated to the crown area. This observation suggests that there is interdependence of characteristics at different scales. How to explain this correlation? When the crown area is larger, the total tree transpiration is supposed to be greater. Indeed, in the Vailhan site, Teixeira (1996) observed on five *Q. ilex* that sap flow over the season and stem diameter were not related. Similar results have been found by Doley and Grieve (1966), Hatton and Vertessy (1989) and Kelliher et al. (1992). Hatton and Vertessy (1989) observed daily differences in sap flow density of up to 70% between two neighbours *Pinus radiata* even though these two trees were only 3 m apart, occupied almost the same area of ground and had similar stem diameters and heights. Doley and Grieve (1966) working on 13 *Eucalyptus marginata* of similar size observed that "water consumption could not be closely related with their diameters, heights or crown exposure although observation of the crowns suggested that the amount of leaf material may have had an important influence". This finding was in accordance with the pioneer results of Ladefoged (1963). He found in northern European mixed deciduous forests that crown shape had a marked influence on transpiration. In recent works, Le Goff et al. (1995) and Sala et al. (1996) found a linear relationship between cumulated water consumption and tree leaf area in dense canopies. For *Q. pubescens* and *Q. ilex*, the larger plant water loss imposed by a greater total leaf area was compensated by a higher leaf water use efficiency. For each individual there is probably a trade-off between tree and leaf characteristics. Plants controlled their water loss by adjusting both crown size and leaf functioning. For *Q. pubescens*, it is surprising to find a negative correlation between leaf $\delta^{13}C$ and the crowding index. As the crowding index is used to quantify the competition, we could have expected a higher $\delta^{13}C$ when the competition is higher. But when competition is high, having a low water use efficiency in spring (i.e. during leaf growth) can be an advantage: it can permit to use water before the others extract it. The different result for *Q. ilex* (no correlation betwen $\delta^{13}C$ and crowding index) suggest a different response to competition which can be realized at the canopy scale by an adjustment of leaf area index for example. As shown by Zhang et al. (1996) who studied correlations between tree growth traits and carbon isotope discrimination on coniferous species, the interaction between component of water use efficiency and tree morphology in a competing vegetation is rather complex and the mechanisms underlying growth variation differ among species.

To improve understanding of inter-tree variability it should be interesting to observe tree phenology. Apart plant water constraint, some other environment factors, such as light, leaf-to-air vapour pressure deficit and temperature can lead to variations in $\delta^{13}C$ (O'Leary 1981). Du Merle (1983) observed leaf emergence extending over 32 days for *Q. pubescens* and 61 days for *Q. ilex*. The resulting variable environmental conditions during leaf growth can lead to individual differences in $\delta^{13}C$. The data presented here do not permit us to say to what extent the observed variability in $\delta^{13}C$ values is due to phenotypic functional plasticity in leaves in response to the environment, or to genotypic differences, but there was a significant correlation between $\delta^{13}C$ values of two successive years (data not shown). This suggests a genetic component in the leaf $\delta^{13}C$ values, as for *Picea mariana* (Flanagan and Johnsen 1995). Kohorn et al. (1994) also attribute the wide variability in isotopic composition observed in the field for a desert shrub in part to intra-population genotypic diversity.

In conclusion, isotopic compositions show that on average the leaves of *Q. pubescens* and *Q. ilex* function theoretically with the same WUE_i, despite their different life-spans and structures. Between-site variations in $\delta^{13}C$ suggest that in both species there is a functional adjustment with respect to available water resource. Within any one site, variations between individuals of the same species show that a great number of individuals have to be sampled to characterize a population. These variations can be interpreted as shifts in leaf intrinsic water use efficiency allowing the trees to regulate their transpiration and control the water constraint of the whole plant.

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