

## Carbon isotope discrimination and wood anatomy variations in mixed stands of *Quercus robur* and *Quercus petraea*

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### ABSTRACT

The two most common oak species in western Europe, *Quercus robur* and *Quercus petraea*, display different ecological behaviours, particularly with respect to their responses to drought. The ecophysiological basis of this niche difference is not understood well. Here we test the hypothesis that these two species present distinct water use efficiencies (WUEs), using the carbon isotope discrimination approach. Leaves and 13 dated ring sequences were sampled in 10 pairs of adult trees growing side by side. Carbon isotope composition was measured on cellulose extracts. In addition, relationships between carbon isotope discrimination and wood anatomy were assessed at the tree level. *Quercus robur* displayed a 1.0‰ larger isotopic discrimination than *Q. petraea*, and therefore a lower intrinsic WUE (–13%). This interspecific difference of isotopic discrimination was quite stable with time and independent of tree radial growth and climate fluctuations. A strong positive correlation was observed between average tree values of earlywood vessel surface area and <sup>13</sup>C isotopic discrimination. This correlation was even higher with <sup>13</sup>C of the 1976 dry year ( $r = 0.86$ ). These observations led to the hypothesis that hydraulic properties of xylem could exert a constraint on leaf gas exchange, resulting in a larger WUE for individuals with smaller vessel cross-section area.

**Key-words:** Interspecific variability; oak; tree ring; vessel area; water use efficiency.

### INTRODUCTION

*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. are two of the most important sympatric broad-leaved forest tree species in western Europe. These two species display a high genetic proximity but different ecological behaviours. Difficulties have been encountered in detecting genetic markers of the interspecific differentiation (Petit, Wagner & Kremer 1993), although a few distinctive genomic regions were observed recently (Bodénès *et al.* 1997). Nevertheless, leaf morphology (Dupouey & Badeau 1993) and wood anatomy (Feuillat *et al.* 1997) clearly differentiate the

two species. Oaks are affected recurrently by important decline episodes; severe cases were recorded in France at the end of the 1970s and the beginning of the 1990s. Drought is now considered to be the primary cause of many of these decline episodes (Landmann *et al.* 1993). In addition, *Q. robur* was often more prone to decline than *Q. petraea* (Becker & Lévy 1982; Landmann *et al.* 1993). Ecological surveys showed that *Q. robur* was distributed in soils with larger water and nutrient availabilities than *Q. petraea* (Lévy, Becker & Duhamel 1992; Bréda *et al.* 1993). Despite these differences recorded *in situ*, most of the comparative ecophysiological studies conducted with seedlings under controlled conditions resulted in only small differences between the two species. Vivin, Aussenac & Lévy (1993) observed a higher death rate in *Q. robur* saplings than in *Q. petraea* saplings subjected to a very severe water stress, whereas gas exchange (net CO<sub>2</sub> assimilation and transpiration) decreased similarly in the two species with decreasing soil water availability. Experiments with adult trees in a stand in north-eastern France revealed few distinctive functional features. The drought-induced decline of stomatal conductance and total transpiration was very close in the two species (Bréda *et al.* 1993; Epron & Dreyer 1993). Nevertheless, a slightly higher vulnerability to water-stress-induced xylem embolism (Cochard *et al.* 1992; Tyree & Cochard 1996), a lower intrinsic water use efficiency (WUE) (Epron & Dreyer 1993) and a shallower root system (Bréda *et al.* 1995) were found in *Q. robur* as compared with *Q. petraea*. Our knowledge of the ecophysiological differences between these two oak species is still not sufficient to understand clearly the observed differences in their ecological niches.

In the present study, we tested whether carbon isotope discrimination ( $\Delta$ ), a functional marker of time-integrated WUE, differed between the two species. Isotope discrimination has been used widely as a screening tool for interspecific differences among forest tree species (Guehl *et al.* 1998; Bonal *et al.* 2000). Because trees, like other plants, have to transpire water in order to fix carbon, genotypes that use water more efficiently could have a competitive advantage over other ones. Water use efficiency is defined as the ratio of plant biomass production to plant transpiration (transpiration efficiency,  $WUE_T$ ), or, at leaf level, as the ratio of net carbon dioxide assimilation to transpiration ( $WUE_i$ ). Carbon isotope discrimination has been shown to

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be negatively correlated with  $WUE_t$  and  $WUE_i$  (reviewed by Farquhar, Ehleringer & Hubick 1989). The isotope discrimination recorded in plant tissue therefore provides a time- and plant-integrated estimate of WUE.

Wood anatomy differs significantly between *Q. robur* and *Q. petraea*. Earlywood width, as well as the number of vessel rows and total vessel surface area in the earlywood, is significantly larger in *Q. robur* than in *Q. petraea*, independently of the total year ring width. Conversely, the relative area of fibre zones is significantly higher in *Q. petraea* than in *Q. robur* (Feuillat *et al.* 1997). The potential consequences of such anatomical differences on hydraulic properties may be detectable in the carbon isotope signal recorded in plant tissues. A close correlation between hydraulic properties and  $\Delta$  has been hypothesized several times but rarely observed (Waring & Silvester 1994; Ryan & Yoder 1997; Bonal *et al.* 2000). The underlying idea is that the levels of resistance to water transfer in the trees may exert a strong constraint on stomatal conductance and therefore WUE and  $\Delta$  (Jones & Sutherland 1991).

We had two primary objectives in the present study. Firstly, to determine whether significant differences in WUE, assessed from  $\Delta$  measurements, existed between the two *Quercus* species in mixed stands. To accomplish this, we compared the isotopic composition of leaves and wood cellulose extracted from a range of annual rings in several pairs of trees, each pair including a tree of each species. Secondly, to test if interspecific and between-tree differences in  $\Delta$  could be related to differences in anatomical properties of the wood, especially vessel characteristics.

## MATERIALS AND METHODS

### Tree sampling and growth analysis

Fourteen mixed plots of *Q. robur* and *Q. petraea* were selected in the forest of Cîteaux (Feuillat *et al.* 1997), 25 km from Dijon (eastern France). These stands came from natural regeneration and have been managed as coppice with standards, with hornbeam (*Carpinus betulus* L.) growing under a dominant canopy of oaks. Soils belonged to the luvisol-redoxysol type, with an intermittently waterlogged layer at a depth of 30 cm, and were developed on loams. Slopes were nearly horizontal. Extractable soil water was high (above 200 mm) and mean annual rainfall close to 700 mm. In each plot, two adjacent dominant trees from each of the species were selected. These 56 individuals were ascribed to one or the other of the two species based on a leaf morphology analysis (Dupouey & Badeau 1993). Moreover, molecular analyses were performed on 47 of the 56 trees identified previously, with random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers. Five RAPD primers and one AFLP-PEC (primer enzyme combination) were selected, because they allowed the amplification of fragments that presented significant frequency differences between the two species, following the protocols detailed in Bodénès *et al.* (1997) for the RAPD technique and in Ger-

ber *et al.* (2000) for the AFLP technique. Finally, the molecular analysis provided nine RAPD and two AFLP markers.

Girth, tree height, relative crown length and horizontal crown projection area were recorded for each tree. Based on one core per tree, annual ring width were measured to the nearest 1/100 mm with a semiautomated device and afterwards cross-dated according to the procedure of Becker (1989).

Four of the 14 plots were discarded because of abrupt radial growth changes, which suggested important variations of the former dominance status of trees. In each of the 10 remaining plots, one tree of each species was chosen (trees with the most symmetrical crown selected by visual estimation). The 20 trees selected displayed mean ages of 138 and 151 years for *Q. petraea* and *Q. robur*, respectively. This difference was not significant ( $P = 0.09$ ). According to Leavitt & Long (1984), and in order to increase the amount of wood available for the isotopic analysis, eight cores were sampled (in four opposite directions) per tree at 1.30 m during early spring 1999 and dried in ambient air.

### Stable carbon isotope analysis

For the study of interspecific differences in isotopic discrimination, two timescales were considered: (i) a year-by-year analysis centred on a short time period, including years with different climatic conditions (and diverse annual ring widths); (ii) an analysis of two contrasting periods of the stand history. For the year-by-year analysis, 11 rings (earlywood plus latewood) were cut individually from each of the eight cores per tree. They corresponded to the years 1965–69 and 1975–80, which were periods of high and low growth, respectively. For the larger timescale analysis, two ring sequences were extracted, corresponding to the time periods 1907–1918 (young stage of the stand development) and 1981–1990 (mature adult stand), respectively. For each year or time period, the eight rings or ring sequences from a single tree were composited and ground. To avoid artefacts due to radial translocation of carbon (Tans, De Jong & Mook 1978), and because wood is composed of various metabolites that undergo different isotopic fractionations (Benner *et al.* 1987), isotopic analyses were performed on holocellulose extracted from wood as described by Leavitt & Danzer (1993).

In addition, 100 top-crown, sun-exposed leaves per tree were collected during the beginning of August 1999. All leaves from each tree were mixed, so that the carbon isotope composition was analysed on one sample per tree.

Stable carbon isotope abundance of wood cellulose and leaf tissues were measured with a continuous flow elemental analyser (Carlo Erba Analyser-NA1500; CE Instruments, Rodano, Italy) interfaced with an isotope mass spectrometer (Finnigan Mat; Delta S, Bremen, Germany). Stable carbon isotope ratio was expressed as the  $^{13}\text{C} : ^{12}\text{C}$  ratio relative to Pee Dee Belemnite. The precision of spectrometric analysis (standard deviation in  $\delta$ ) of a laboratory standard (ground needles of *Pinus pinaster*) was 0.19‰ ( $n = 32$ ).

Carbon isotope discrimination against air ( $\Delta$ ) has the advantage of taking into account the time course of atmospheric  $^{13}\text{C}$  composition ( $\delta_a$ ). The relationship between  $\Delta$  and  $\delta$  is as follows:

$$\Delta = \frac{\delta_a - \delta_p}{1 - \delta_p},$$

where  $\delta_p$  refers to the isotopic composition of the photosynthesis products (wood cellulose or leaf material in this case). At the beginning of the century,  $\delta_a$  was about  $-6.7\text{‰}$ , compared with about  $-8\text{‰}$  in 1990. Data for  $\delta_a$  were estimated as indicated in Bert, Leavitt & Dupouey (1997).

### Wood anatomy measurements

Anatomical data collected in a previous study by Feuillat *et al.* (1997) were used for the present comparison between wood anatomy and carbon isotope discrimination. The methodology is briefly resumed as follows. One core was taken per tree; juvenile and sapwood zones were discarded; four  $\sim 2\text{-mm}$ -wide rings were selected per core for image analysis. These rings were equally distributed along the core to represent different age classes. Thus, the rings used for isotopic and anatomical measurements were independent within a tree. X-ray photographs were prepared following the classical method of Polge, Lemoine & Deret (1977). The x-ray films were digitized using a charged coupled device (CCD) camera mounted on binoculars and coupled with a microcomputer. Each selected ring was analysed using semiautomated software developed under the Visilog<sup>®</sup> image analysis system (NOESIS, Les Ulis, France) (Anonymous 1992). Image thresholding and drawing of the different wood tissue boundaries were conducted manually in a  $3.5\text{-mm}$ -wide window along the tangential direction. Size and proportion of tissues (earlywood and latewood vessels, fibres, parenchyma) were characterized. The total number of earlywood vessels, and the number of rows they formed, were counted. For each vessel, the following measurements were taken: lumen area and tangential, maximum, minimum and mean lumen diameters. Lumen area was cumulated over the earlywood vessel population of a given ring, giving the earlywood vessel surface area (EVSA). Measurements from the four selected rings per tree were finally averaged.

### Data analysis

Data were analysed using the SAS statistical package (SAS Institute Inc., Cary, NC, USA) (SAS 1988). One *Q. petraea* outlier value of earlywood vessel surface area was discarded from the calculations. Pearson and Spearman (rank) correlation coefficients and simple and multiple linear regressions were used as exploratory tools. A correspondence analysis (CA) was performed on molecular data. Because of missing values, nine of the 47 trees could not be included in the CA. Leaf morphological variables were analysed by principal component analysis. For the study of

interspecific variation, an analysis of variance was carried out. The following hierarchical model was used:

$$\Delta_{ijk} = a + b_i + c_{j(i)} + d_k + e_{ijk}, \quad (\text{model 1})$$

where  $\Delta_{ijk}$  = measured values of  $\Delta$  for tree  $j$ , within species  $i$ , at year or time sequence  $k$ ,  $a$  = constant,  $b_i$  = effect of species  $i$ ,  $c_{j(i)}$  = effect of tree  $j$  within species  $i$ ,  $d_k$  = effect of year or time sequence  $k$  and  $e_{ijk}$  = error term.

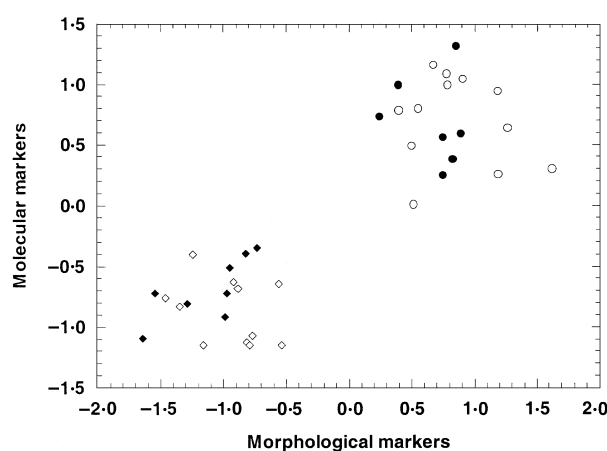
## RESULTS

### Interspecific variability of isotopic discrimination

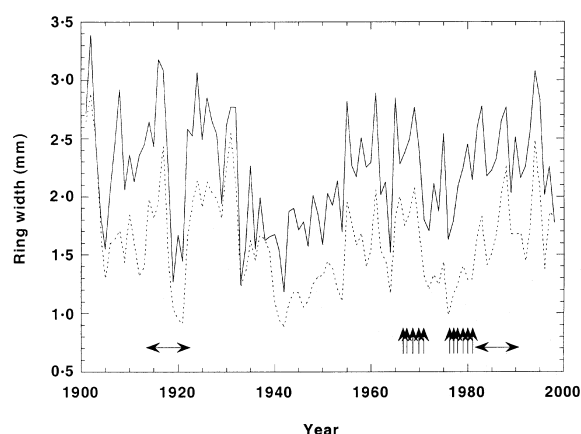
Both morphological and molecular markers provided a clear distinction between the two species (Fig. 1). Radial growth in the two species over the last century is shown in Fig. 2. *Quercus petraea* has displayed larger annual ring widths than *Q. robur* since the beginning of the century (27% more on the average).

Variations of  $\Delta$  with time period and species are shown in Fig. 3a. There was a clear difference in  $\Delta$  between the two species in all rings or ring sequences measured. Mean  $\Delta_{\text{wood}}$  fluctuated between  $16\text{‰}$  and  $17.2\text{‰}$  for *Q. petraea* and between  $17.1\text{‰}$  and  $18\text{‰}$  for *Q. robur*. For the two species, the lowest value was observed in the ring of the dry year (1976) and the highest in that of the period 1908–17.

Over the whole century, there was a significant tendency for  $\Delta$  to decline from  $17.2\text{‰}$  to  $16.3\text{‰}$  and from  $18.0\text{‰}$  to  $17.4\text{‰}$  for *Q. petraea* and *Q. robur*, respectively. The negative long-term trend in  $\Delta_{\text{wood}}$  was steeper for *Q. petraea* than for *Q. robur* [slopes  $-0.014\text{‰ year}^{-1}$  ( $P = 0.006$ ) and  $-0.010\text{‰ year}^{-1}$  ( $P = 0.01$ ), respectively]. Mean  $\Delta_{\text{wood}}$  was  $16.6\text{‰}$  and  $17.5\text{‰}$ , and mean  $\Delta_{\text{leaf}}$  was  $17.7\text{‰}$  and  $18.9\text{‰}$  for *Q. petraea* and *Q. robur*, respectively. Interspecific differences in isotopic discrimination ( $\Delta_{Q.robur} - \Delta_{Q.petraea}$ ) varied between  $0.8\text{‰}$  and  $1.2\text{‰}$ , with a mean value of  $1.0\text{‰}$  (Fig.



**Figure 1.** Multivariate analyses of morphological and molecular markers of 38 oaks in the Cîteaux forest (circles = *Q. petraea*; diamonds = *Q. robur*). Closed symbols correspond to trees that were retained for the carbon isotope measurements.



**Figure 2.** Average ring width displayed during the 20th century by 20 co-occurring *Q. robur* (dotted line) and *Q. petraea* (solid line) trees. Arrows indicate the individual rings and ring sequences that were selected for carbon isotope measurements.

3b). The apparent tendency of this interspecific difference to increase with time was not statistically significant. Results of a multiple linear regression including monthly temperature and rainfall revealed no significant relationship between the interspecific difference of  $\Delta$  and these climatic data.

The analysis of variance (model 1, Table 1) showed that species, tree within species and time period had significant effects on  $\Delta_{\text{wood}}$ . This model explained 87% of total  $\Delta_{\text{wood}}$  variance. The partition of sum of squares showed that interspecific variation represented 23% of the total variation, whereas 68% was due to intraspecific variability and only 9% due to interannual variations. A one-factor analysis of variance of  $\Delta$  in the leaves showed that the species effect was still significant, although lower than in wood cellulose ( $F = 5.0$ ,  $P = 0.04$ ).

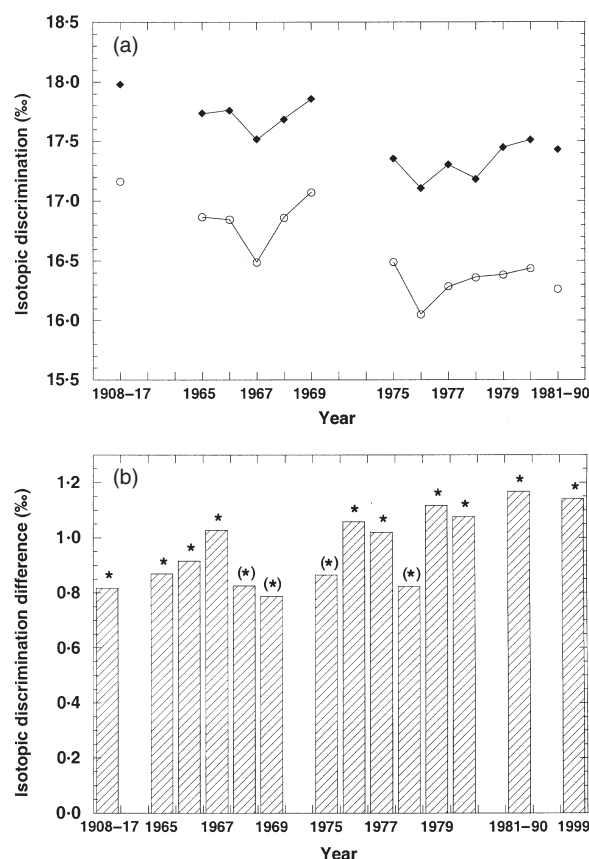
A positive correlation was found between mean annual  $\Delta_{\text{wood}}$  and mean ring width ( $r = 0.74$ ,  $P = 0.004$  and  $r = 0.85$ ,  $P = 0.0002$  for *Q. petraea* and *Q. robur*, respectively). The species regression lines were parallel but had significantly different intercepts ( $P = 0.03$ ) (Fig. 4).

**Table 1.** Analysis of variance of the effects of species, tree within species and time period on carbon isotope discrimination in the wood cellulose

| Source of variation | d.f. | SS    | F-value  |
|---------------------|------|-------|----------|
| Species             | 1    | 57.7  | 353.7*** |
| Tree (species)      | 18   | 166.4 | 56.6***  |
| Time period         | 12   | 21.3  | 10.9***  |
| Model               | 31   | 245.8 | 48.6***  |

d.f., degrees of freedom; SS, sum of squares.

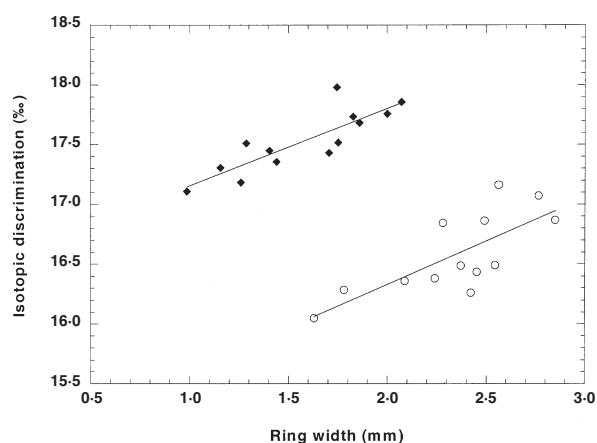
(\*) =  $P < 0.1$ ; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ , and  $P > 0.1$  otherwise.



**Figure 3.** (a): Carbon isotope discrimination ( $\Delta_{\text{wood}}$ ) recorded in the cellulose of annual rings in the trunk of the two oak species ( $\circ = Q. petraea$ ;  $\bullet = Q. robur$ ) as a function of the year of formation of the annual rings. Each point is the mean of 10  $\Delta_{\text{wood}}$  values, corresponding to 10 selected trees by species. (b): Interspecific differences ( $Q. robur - Q. petraea$ ) of isotopic discrimination as a function of time. For 1999, the represented difference was recorded in the leaf carbon ( $\Delta_{\text{leaf}}$ ).  $P$ -values of the  $t$ -test of comparison of means per species are as follows: (\*) =  $P < 0.1$ ; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ , and  $P > 0.1$  otherwise.

### Between-tree variability of isotopic discrimination and relationship with wood anatomy

As shown in Table 1, the largest variability in isotopic discrimination occurred at the intraspecific level, between trees: the average standard deviation of  $\Delta_{\text{wood}}$  over the 10 trees and the 13 time periods was 1.0‰ for *Q. petraea* and 0.8‰ for *Q. robur*. Standard deviations of  $\Delta_{\text{leaf}}$  were 1.0 and 1.2‰ for *Q. petraea* and *Q. robur*, respectively. Respective ranges of  $\Delta$  measured in wood and leaves were 3.2‰ and 3.1‰ in *Q. petraea* and 2.4‰ and 3.5‰ in *Q. robur*. However, we observed that the ranking of the trees according to their  $\Delta_{\text{wood}}$  values remained very similar over all time periods. Rank correlations displayed an average value of 0.82 ( $P < 0.004$ ), with no decrease when the interval between considered time periods increased.



**Figure 4.** Relationship between average values of  $\Delta_{\text{wood}}$  and ring width from each time period ( $\circ = Q. \text{petraea}$ ,  $\blacklozenge = Q. \text{robur}$ ). Each point is the mean of 10  $\Delta_{\text{wood}}$  values, corresponding to the 10 selected trees per species.

In order to explore this between-tree variability, relationships between  $\Delta$  and dendrometrical and wood anatomical parameters were assessed. No significant relationship was found between mean isotopic discrimination per tree and any dendrometrical parameter (height, crown basis height, relative crown length, girth, horizontal crown projection area and age), globally or by species (Table 2a).

In contrast, a strong correlation was observed between average  $\Delta_{\text{wood}}$  per tree and average EVSA ( $r = 0.79$ ,  $P = 0.0001$ ) (Table 2b). *Quercus robur* displayed significantly larger values of isotopic discrimination (lower intrinsic WUE) and EVSA. A correlation calculation was made for each time period between EVSA (which was constant whatever the considered time period) and individual values of  $\Delta$ . The best correlation was observed for 1976 (Fig. 5), the driest year studied (233 mm of precipitation from May to September compared with an average century value of 320 mm). Whatever the time period, correlations were larger for *Q. robur* than for *Q. petraea*. EVSA was better linked with earlywood vessels number ( $r = 0.67$ ,  $P = 0.002$ ) than with average surface area of individual vessels ( $r = 0.43$ ,  $P = 0.06$ ). Correlations between average  $\Delta_{\text{wood}}$  and earlywood vessels number or average surface area of individual vessels remained low ( $r = 0.48$ ,  $P = 0.004$  and  $r = 0.44$ ,  $P = 0.06$ , respectively). Furthermore, significant correlations were observed between the average  $\Delta_{\text{wood}}$  and the ratio of earlywood width to total ring width ( $r = 0.55$ ,  $P = 0.01$ ), a parameter that is linked with EVSA and is easier to measure.

EVSA has been shown to be the most discriminating feature in the interspecific analysis of wood anatomy (Feuillat *et al.* 1997). Interestingly, there were no significant differences of intercept and slope between the *Q. robur* and *Q. petraea* regressions of  $\Delta_{\text{wood}}$  upon EVSA, neither for the 1976 data nor for average  $\Delta$ . This means that between-

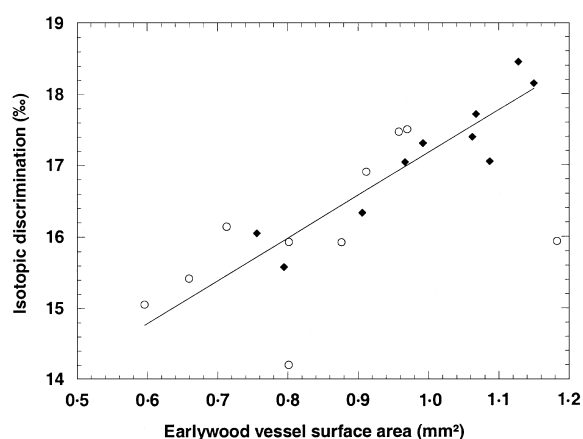
**Table 2.** Pearson correlation coefficients between mean isotopic discrimination per tree and dendrometrical parameters (a) and between mean or 1976 isotopic discrimination and anatomical features (b)

| (a)                              | Mean $\Delta_{\text{wood}}$         |                                       |                                                              |
|----------------------------------|-------------------------------------|---------------------------------------|--------------------------------------------------------------|
|                                  | <i>Q. robur</i><br>( <i>n</i> = 10) | <i>Q. petraea</i><br>( <i>n</i> = 10) | <i>Q. robur</i> and<br><i>Q. petraea</i><br>( <i>n</i> = 20) |
| Total height                     | -0.20                               | -0.35                                 | -0.36                                                        |
| Crown base height                | -0.41                               | -0.03                                 | -0.06                                                        |
| Relative crown length            | -0.30                               | -0.08                                 | -0.10                                                        |
| Girth                            | -0.52                               | -0.01                                 | -0.25                                                        |
| Crown horizontal projection area | -0.49                               | -0.12                                 | -0.36                                                        |
| Age                              | -0.42                               | 0.13                                  | 0.07                                                         |

| (b)                                          | Mean $\Delta_{\text{wood}}$         |                                       |                                                              | $\Delta_{1976}$                     |                                       |                                                              |
|----------------------------------------------|-------------------------------------|---------------------------------------|--------------------------------------------------------------|-------------------------------------|---------------------------------------|--------------------------------------------------------------|
|                                              | <i>Q. robur</i><br>( <i>n</i> = 10) | <i>Q. petraea</i><br>( <i>n</i> = 10) | <i>Q. robur</i> and<br><i>Q. petraea</i><br>( <i>n</i> = 20) | <i>Q. robur</i><br>( <i>n</i> = 10) | <i>Q. petraea</i><br>( <i>n</i> = 10) | <i>Q. robur</i> and<br><i>Q. petraea</i><br>( <i>n</i> = 20) |
| Mean ring width                              | 0.23                                | -0.16                                 | -0.32                                                        | 0.39                                | 0.16                                  | -0.12                                                        |
| 1976 ring width                              | 0.40                                | 0.042                                 | -0.18                                                        | 0.51                                | 0.26                                  | -0.05                                                        |
|                                              | ( <i>n</i> = 10)                    | ( <i>n</i> = 9)                       | ( <i>n</i> = 19)                                             | ( <i>n</i> = 10)                    | ( <i>n</i> = 9)                       | ( <i>n</i> = 19)                                             |
| Earlywood vessel surface area                | 0.84**                              | 0.62(*)                               | 0.79***                                                      | 0.92***                             | 0.72*                                 | 0.86***                                                      |
| Earlywood vessels number                     | 0.28                                | 0.32                                  | 0.48*                                                        | 0.41                                | 0.26                                  | 0.51*                                                        |
| Average surface area of individual vessels   | 0.64*                               | 0.42                                  | 0.44(*)                                                      | 0.60(*)                             | 0.56                                  | 0.51*                                                        |
| Ratio of earlywood width to total ring width | 0.55                                | 0.28                                  | 0.55*                                                        | 0.62*                               | 0.36                                  | 0.62**                                                       |

(\*) =  $P < 0.1$ ; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ , and  $P > 0.1$  otherwise.



**Figure 5.** Relationship between isotopic discrimination during 1976 and average tree earlywood vessel surface area ( $r = 0.79$ ,  $P = 0.0001$ ) ( $\circ = Q. petraea$ ,  $\blacklozenge = Q. robur$ ).

tree differences in earlywood vessel surface area were sufficient to account for species differences of isotopic discrimination.

## DISCUSSION

### Interspecific variability in isotopic discrimination and WUE

Our results highlight the occurrence of interspecific differences in isotopic discrimination (in both wood cellulose and leaves) among adult trees from the two oak species, *Q. petraea* and *Q. robur*, growing in mixed stands. According to the Francey–Farquhar model (Francey & Farquhar 1982), it may be assumed that *Q. robur* displayed a lower intrinsic WUE than *Q. petraea* (average 13%). This difference is in agreement with measurements of instantaneous intrinsic WUE (ratio of net carbon assimilation rate to stomatal conductance for water vapour) recorded by Epron & Dreyer (1993) in a natural stand (average difference of 15% in a sample of four trees from each species). Two main sources of variation – one related to genotype and the other to environment – may be invoked to explain this interspecific variability in isotopic discrimination.

The observed interspecific differences in  $\Delta_{\text{wood}}$ , and thus intrinsic WUE, had already appeared at the beginning of stand life (a century ago) and were kept almost constant with time. Both species displayed a decreasing  $\Delta$  trend during the 20th century. This long-term trend in  $\Delta$  has already been identified in several other species (Bert *et al.* 1997, Duquesnay *et al.* 1998). It could be because of the tree or stand age effects (changes of microenvironmental conditions during stand maturation and physiological changes related to structural development of the trees with ageing) or global environmental changes, such as the increase in atmospheric  $\text{CO}_2$  concentration or nitrogen deposition. In our study, the effects of tree age and date were confounded, so that the separation between these two types of causes was not possible.

Besides the significant species effects, a large intraspecific variability was also observed. Intra-site variability of  $\Delta$  is known to be large and ranges of values observed in each species (2.4‰ and 3.2‰ in *Q. robur* and *Q. petraea*, respectively) were of the same order of magnitude as those proposed by Leavitt & Long (1986) (2–3‰). The relative ranking among individual trees concerning  $\Delta_{\text{wood}}$  was maintained throughout successive years, as found in numerous studies (Donovan & Ehleringer 1994; Flanagan & Johnsen 1995; Goulden 1996; Damesin, Rambal & Joffre 1998). This suggests strong genetic control over  $\Delta$ .

Moreover, the homogeneity of environmental conditions observed in the sampled stands, as well as the lack of relationship between individual  $\Delta_{\text{wood}}$  and any dendrometrical parameter, strengthens the hypothesis of a genetic control over the observed inter- and intraspecific differences of  $\Delta$ . High heritabilities have been recorded for carbon isotope discrimination ( $h^2 = 0.6\text{--}0.9$ , Farquhar *et al.* 1989). Nevertheless, it is not possible here to strictly dismiss the hypothesis that stable individual differences in  $\Delta$  could be partly due to microsite differences (Battaglia & Williams 1996).

In mixed stands, *Q. petraea* commonly displays a larger radial growth than *Q. robur* (Lévy *et al.* 1992). However, no significant correlation was observed between the average individual  $\Delta$  and the average individual ring width (Table 2b). Thus, if a higher WUE provides trees with some competitive advantage, this is due to processes not recorded in radial growth, such as the ability to survive strong drought events or the amount of carbon stored after the cessation of radial growth.

### Relationship between isotopic discrimination and hydraulic properties of trees

The strong relationship observed in this study between  $\Delta_{\text{wood}}$  and EVSA may be an index for the influence of the hydraulic properties of trees on carbon isotope discrimination. This relationship was exactly the same for *Q. robur* and *Q. petraea*, and thus seems to be of general applicability among trees of the white oak complex.

According to Poiseuille's law, xylem hydraulic conductivity is a function of conduit dimensions, and thus a function of EVSA. Interindividual variations in  $\Delta$ , and in intrinsic WUE, are due to differences in stomatal conductance that may be linked with differences in xylem hydraulic conductivity. Different mechanisms directly coupling stomatal conductance to root-to-leaf conductance have been proposed (Sperry 2000; Nardini & Salleo 2000). An evolutionary trade-off between hydraulic conductivity and vulnerability to xylem embolism may also be the reason for an indirect link between stomatal and xylem conductance (Tyree, Davis & Cochard 1994). The better correlation observed between  $\Delta$  (and WUE) and EVSA for the year 1976, which was affected by a long severe drought, suggests a causal link between these two variables because the degree of constraint imposed by hydraulic properties on stomatal conductance is expected to increase during water shortage.

Nevertheless, the alternative hypothesis that the relationship between isotopic discrimination and EVSA is not directly causal but due to a common controlling factor cannot be discarded definitely. It is often assumed that resistance to water transport along the soil-to-leaf pathway is higher at the intervessel junctions (because of low conductance of pit membranes) or at branch junction constrictions. Consequently, vessel diameter is not always the most limiting factor controlling the total resistance to water transfer. Thus, the relationship we observed between  $\Delta$  and EVSA could be the indirect consequence of a more global correlation between hydraulic conductances of the different components of the total hydraulic system.

## CONCLUSIONS

The present study revealed a lower  $\Delta$  in *Q. petraea* than in *Q. robur*. This can be interpreted as the result of a higher intrinsic WUE. The strong relationship recorded between  $\Delta$  and the earlywood vessel surface area supports the hypothesis of interspecific differences in hydraulic conductance related to the respective species' ecological optimum. Under natural conditions, *Q. robur* preferentially colonizes wet bottom lands whereas *Q. petraea* prefers slopes and the tops of hills. Even small environmental differences in soil water regime may strongly select plant genotypes with adapted strategies for water transport reflected in hydraulic architecture. *Quercus robur* appears as a species maximizing water transfer capacity in the xylem, whereas *Q. petraea* seems to favour the avoidance of drought-induced embolism. This view takes into account the lower vulnerability to drought-induced embolism observed in *Q. petraea* and the larger growth rates of *Q. robur* observed with seedlings or adult trees growing in pure and well-watered stands.

In most mixed stands, like the forest of Cîteaux, habitats are more favourable to *Q. petraea* but *Q. robur* has been introduced or maintained by forestry management. This may in turn lead to the observed higher sensitivity of *Q. robur* to drought-induced declines.

## ACKNOWLEDGMENTS

S.P. was supported by a PhD grant from Institut National de la Recherche Agronomique and from the Regional Council of Lorraine. This study was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme (PATHOAK), CT97-3926 ('Long-term dynamics of oak ecosystems: assessment of the role of root pathogens and environmental constraints as interacting decline inducing factors'). It in no way represents the views of the Commission or its services or their future policy in this area. We gratefully acknowledge the technical assistance of Patrick Behr, Christian Kieffer and Sébastien Diette during field work and measurements. We thank Claude Bréchet for advice in mass-spectrometric analysis, Jean-Marc Guehl for

helpful discussions and Christopher Swanston for correcting the English grammar of the manuscript.

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Received 15 December 2000; received in revised form 28 April 2001; accepted for publication 28 April 2001