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Summary Seedlings of two sympatric oak species, *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl., were grown in common garden conditions to test for potential interspecific differences in intrinsic water-use efficiency (WUE). Intrinsic water-use efficiency was estimated based on carbon isotope composition of shoots ($\delta^{13}C$) and on gas exchange measurements (ratio of net CO_2 assimilation rate to stomatal conductance (A/g_{sw})). In addition, genotype × environment interactions were tested by subjecting the seedlings to four irradiance treatments (8, 18, 48 and 100% of incident solar irradiance) imposed by neutral shading nets, and, in the 100% irradiance treatment, two watering regimes. In all treatments, initial growth of *Q. robur* was faster than that of *Q. petraea*. In both species, there was a tight correlation between $\delta^{13}C$ and A/g_{sw}. Intrinsic water-use efficiency increased with increasing irradiance (almost doubling from 8 to 100% irradiance), and this effect paralleled the increase in *A* with increasing irradiance. In full sun, WUE of *Q. petraea* seedlings was 10–15% higher than in *Q. robur* seedlings, with the difference attributable to a difference between the species in g_{sw}. The interspecific difference in WUE was maintained during drought, despite the appreciable increase in WUE and decrease in growth imposed by drought. No interspecific differences in WUE were observed at low irradiances, suggesting a strong genotype × environment interaction for WUE. These findings confirm the existence of interspecific genetic differences in WUE, but also show that there is large intraspecific variability and plasticity in WUE. The initially greater height and biomass increments in *Q. robur* seedlings illustrate the ability of this species to out-compete *Q. petraea* in the early stages of forest regeneration. For adult trees growing in closed canopies, the high WUE of *Q. petraea* may contribute significantly to its survival during dry years, whereas the low WUE of *Q. robur* may account for the frequently observed declines in adult trees of this species following drought.

Keywords: carbon isotope composition, gas exchange, interspecific variability, light, Quercus petraea, Quercus robur.

Introduction

Quercus robur L. and *Quercus petraea* (Matt.) Liebl. are important, sympatric, broad-leaved forest tree species in western Europe. These species display a high genetic proximity with only a few distinctive genomic regions separating one from the other (Bodénès et al. 1997). It has often been observed that *Q. robur* exhibits severe declines after drought events, whereas *Q. petraea* is much less affected. Soil water deficit is a major inducing factor in many of these decline episodes (Landmann et al. 1993). Such declines were particularly obvious in France after the drought events of 1976 (Becker and Lévy 1982) and during the 1980s. In addition to these observations, ecological surveys show that *Q. robur* is more frequent on soils with high water and nutrient availability than *Q. petraea* (Lévy et al. 1992). However, *Q. robur* may colonize habitats beyond its ecological niche, for instance on soils of low mineral fertility, high acidity and low water availability. This occurs as a result of management practices and because the species exhibits pioneering ecological characteristics. In unsuitable habitats, *Q. robur* frequently displays lower radial growth than co-occurring *Q. petraea* (Becker and Lévy 1982, 1990).

Numerous studies have focused on possible ecophysiological traits underlying the difference in drought tolerance between *Q. robur* and *Q. petraea*. Under natural conditions, adult *Q. robur* trees display a slightly higher vulnerability to water-stress-induced xylem embolism (Cochard et al. 1992, Tyree and Cochard 1996), a shallower root system (Bréda et al. 1993) and a lower intrinsic water-use efficiency (WUE; estimated as the ratio of net CO_2 assimilation rate to stomatal conductance) (Epron and Dreyer 1993) than adult *Q. petraea* trees. However, it is difficult to draw firm conclusions from these studies because many were based on local observations and short-term measurements made on a limited number of trees.

Unlike the ratio of net carbon dioxide assimilation to stomatal conductance for water vapor (A/g_{sw}), which provides an instantaneous measure of WUE, carbon isotope composition ($\delta^{13}C$) provides a time-integrated estimate of WUE (Farquhar et al. 1989). Recently, Ponton et al. (2001) reported that $\delta^{13}C$

of wood cellulose of the two species growing in mixed adult stands differed, indicating a 13% lower WUE for *Q. robur* compared with *Q. petraea*. This difference was stable with time, being already visible during the early stages of tree development, and occurring during both wet and dry years. Although a genetic basis for such in situ differences is highly probable, an interacting effect of micro-environmental variation could not be excluded. Therefore, we tested whether similar interspecific differences in WUE could be detected between seedlings of the two species growing under controlled and homogeneous conditions.

Reduced soil water availability results in increased $\delta^{13}C$, i.e., increased WUE (Farquhar et al. 1989, Picon et al. 1996, Brugnoli et al. 1998, Roupsard et al. 1998). Because the two oak species differ in drought tolerance, we also tested whether the potential interspecific difference in WUE was affected by varying soil water availability. In addition to water availability, variability in irradiance is a major factor governing seedling growth in forest ecosystems. *Quercus robur* is more heliophilic than *Q. petraea* during the early stages of development. It is also well known that $\delta^{13}C$ increases with irradiance (Farquhar et al. 1989, Broadmeadow and Griffiths 1993); e.g., in vertical transects from lower-canopy to upper-canopy leaves. However, the increase in $\delta^{13}C$ from lower-canopy leaves to upper-canopy leaves may be a result of the combined effects of changes in $\delta^{13}C$ of source CO_2, which is usually lower in the lower canopy because of respiratory recycling below canopies (Schleser and Jayasekera 1985, Berry et al. 1997, Hanba et al. 1997), and of changes in WUE (Farquhar et al. 1989, Zimmerman and Ehleringer 1990). It is possible that changes in WUE occur in response to decreased vapor pressure deficit (VPD) or as a result of the direct effects of a change in irradiance. In an experiment with banana (*Musa* sp.), large decreases in A/g_{sw} and $\delta^{13}C$ were observed when incident irradiance was reduced experimentally with almost no change in VPD, indicating that the effect could be attributed largely to reduced photosynthetic capacity (Israeli et al. 1996).

Our first objective was to determine whether the interspecific differences in WUE observed in adult trees (Ponton et al. 2001) are genetically determined, i.e., species specific, and whether they are expressed in seedling phenotypes. Our second objective was to evaluate the impact of gradients in light availability on oak $\delta^{13}C$ and to compare light-induced variations in WUE between the two species. We assessed WUE by means of both gas exchange measurements (A/g_{sw}) and carbon isotope composition ($\delta^{13}C$). The gas exchange measurements were used to infer whether inter- and intraspecific differences in WUE were a result of stomatal control or changes in photosynthetic capacity.

Materials and methods

In autumn 1997, acorns were collected under individual trees of *Q. petraea* and *Q. robur* in two adjacent pure stands of each species growing in the forest of Perseigne, France (48°24′21″ N, 0°19′33″ W). Adult trees were identified based on acorn

peduncle size and characteristic leaf traits (Dupouey and Badeau 1993). Acorns were heat treated to destroy fungi (Delatour et al. 1980) and treated with the fungicide, Rodiazan®, then stored at –1 °C. After weighing, acorns were sown during May 1998, in 10-dm³ plastic containers filled with a 2:1 (v/v) sand:peat mixture. The pots were placed in an experimental nursery (Champenoux, Nancy, France). The seedlings were automatically irrigated twice a day and fertilized twice during summer (Nutricote®, Chisso-Asahi, Tokyo, Japan, N:P:K, 13:13:13 + trace elements, 40 g per pot).

The containers were distributed among three shelters built with polyethylene nets incorporating aluminum strips. Different mesh sizes provided transmitted irradiances corresponding to 8 (deep shade), 18 (medium shade) and 48% (light shade) of external global radiation. The control treatment (100%; full sun) was installed in a net-free area. A mobile weather station recorded the mean climate in the different shelters during Year 1 (2 weeks in each shelter), and permanently installed sensors (thermistors and Vaisala hygrometers (Vaisala, Helsinki, Finland)) provided records of temperature and relative humidity in Year 2. The shelter transmittance resulted in global radiation and photosynthetic photon flux densities close to the target values. Air temperature was only slightly modified by the shelters (data not shown). During 1999, the shelter temperature exceeded the lowest (below 15 °C) and highest (above 25 °C) external temperatures by 1 °C at most. The deviation was greater in the deep shade shelter than in the other treatments. In the 15–25 °C range, there was no visible deviation from external temperatures. The VPD in the shelters was usually slightly lower than in full light, and the difference varied from 80 to 160 Pa over the range 0 to 2500 Pa. No significant difference in VPD was detected among the three shelters.

From July 5, 1999 to the end of the experiment, the seedlings growing in full sun were divided into two sets of 20 individuals (10 *Q. petraea* and 10 *Q. robur*). A moderate drought was applied to one group of seedlings, whereas the other group was well watered. Three or four times a week, all containers were weighed and water added to maintain soil water content at field capacity for the control treatment, and at 30% of field capacity for the water-stress treatment. To avoid uncontrolled water supply by rain, a polyethylene roof was erected above the seedlings.

The experiment was continued for two growing seasons (from seed germination until the end of summer 1999). Growth and $\delta^{13}C$ were measured at the end of each season, on 10 seedlings per species and treatment. Gas exchange measurements were performed during the second year on the seedlings that had been selected for growth and isotopic composition measurements.

Gas exchange measurements and water relations

Net CO_2 assimilation rate (A) and stomatal conductance (g_{sw}) to water vapor were monitored weekly between June 2 and September 2, 1999, with a portable photosynthesis system (LI-6200, Li-Cor, Lincoln, NE) with a 4-dm³ assimilation

chamber, on 10 saplings per species in each of the five treatments. Measurements were performed on one sun-exposed leaf per tree under various, but stable, conditions of sunshine. Leaves were selected on the last fully expanded growth flush, and the measurement leaf was changed after each new growth flush. At the end of the experiment, seedlings had developed two or three flushes, depending on the irradiance treatment. Drought stress began after full expansion of the second growth flush. Gas exchange parameters were calculated on a leaf area basis. Leaf area was estimated a posteriori with an area meter (Delta T Devices, Cambridge, U.K.).

Volumetric soil water content was assessed several times during the drought experiment by time domain reflectometry (Trase system, Soil Moisture Equipment, Santa Barbara, CA). Predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potentials were measured with a pressure chamber (PMS Instrument, Corvallis, OR) on September 8, 1999 (end of the drought experiment) on one leaf per seedling.

Growth measurements

At the end of the first year (summer 1998), 10 seedlings were harvested per treatment and species. Height, stem basal diameter and total leaf area (LI-3000A and LI-3100, Li-Cor) were measured. Roots were washed, and leaves, stems and roots were oven dried at 60 °C for 72 h. Dry biomass was estimated per compartment (roots, stems, leaves from the successive growth flushes). Mean leaf mass per area (LMA) was calculated for each flush and seedling.

During the second year, height and diameter were measured on 100 saplings at the beginning and at the end of the growth season. At the end of the experiment (September 1999), all seedlings were harvested, and biomass was estimated as at the end of the first year.

Carbon isotope composition measurement

Before sowing, $\delta^{13}C$ was measured in the cotyledons of 10 acorns per species. At the end of the first year (summer 1998), all leaves from each single flush were pooled for each of the 10 selected seedlings per species and treatment. Stable carbon isotope composition was measured in leaves ($\delta^{13}C_{flush1}$ and $\delta^{13}C_{flush2}$) and stem ($\delta^{13}C_{stem}$). The same measurements were performed on all 100 remaining seedlings at the end of the second year (summer 1999). In addition, to analyze the effect of drought on stem isotopic composition, $\delta^{13}C$ of third flush stems (flush developed during the drought) was measured. Dry material was finely ground and a 1-mg sample was analyzed. Stable carbon isotope composition was measured with a continuous-flow elemental analyzer (Carlo Erba Analyzer-NA1500, Rodano, Italy) interfaced with an isotope mass spectrometer (Finnigan Mat, Delta S, Bremen, Germany). Stable carbon isotope ratio was expressed as the $^{13}C/^{12}C$ ratio relative to the Pee Dee Belemnite standard ($\delta^{13}C$; ‰). The precision of spectrometric analysis (standard deviation of δ) of a laboratory standard (ground needles of *Pinus pinaster* Ait.) was 0.19‰ ($n = 32$). Leaf nitrogen concentration on an area basis was calculated from the mass-based nitrogen concentration

(measured with the elemental analyzer) and LMA.

An integrated $\delta^{13}C$ value of the whole shoot was calculated as the mean of the leaf and stem compartment values weighted by their respective biomass.

Data analysis

The experiment was analyzed as a full factorial design with two main factors: species (two levels) and irradiance regime (four levels), with 10 replicate saplings per treatment. The effect of drought was analyzed separately, as a two factorial design (species and irrigation regime), with 10 replicates. The following model (Model 1) was applied in both cases:

$$Y_{ijk} = a + b_i + c_j + (bc)_{ij} + e_{ijk}, \qquad \text{(Model 1)}$$

where Y_{ijk} = measured values for seedling k, within species j, under treatment i; a = constant; b_i = effect of light or drought; c_j = effect of species j; $(bc)_{ij}$ = interaction between effects of treatment and species; and e_{ijk} = error term.

Data are presented as means ± standard deviation, with $n = 10$. Data were analyzed with the SAS statistical package (1988; SAS Institute, Cary, NC). The relationship between variables was estimated by Pearson's correlation coefficients. At the intra-treatment level, correlation analysis was performed on the residuals of Model 1 in order to take the whole sampling into account. Bonferroni tests were used for comparisons of means.

Results

Growth

Acorn biomass was significantly ($P < 0.001$) larger in *Q. robur* (3.2 ± 0.8 g) than in *Q. petraea* (1.6 ± 0.4 g). At the end of the first year of growth (1998), seedlings had accumulated around 2 to 8 g total biomass, and significant interspecific differences in biomass were detected between light treatments ($P < 0.05$), with higher values for *Q. robur* than for *Q. petraea* in all treatments (Figure 1). The effect of irradiance varied with species. Total biomass of *Q. robur* seedlings increased with increasing irradiance up to 48% of incident irradiance, then slightly decreased in full sun, whereas total biomass of *Q. petraea* did not vary significantly among irradiance treatments during the first growing season. The irradiance regime also influenced seedling height, root/shoot ratio, total leaf area and leaf mass to area ratio (LMA) in both species ($P < 0.001$). Across all treatments and both species, there was a significant positive correlation between acorn and seedling biomass $r^2 = 0.64$, $P < 0.001$). This correlation was lower for *Q. robur* alone $r^2 = 0.50$, $P < 0.001$) and not significant for *Q. petraea* $r^2 = 0.27$, $P > 0.05$). In full sun, a significant correlation between acorn weight and seedling biomass $r^2 = 0.90$, $P < 0.001$), height $r^2 = 0.91$, $P < 0.001$), basal stem diameter $r^2 = 0.88$, $P < 0.001$), total leaf area $r^2 = 0.87$, $P < 0.001$) and root/shoot ratio $r^2 = -0.70$, $P < 0.05$) was detected in *Q. robur* seedlings. These high correlations suggest a maternal effect on seedling growth during the first year.
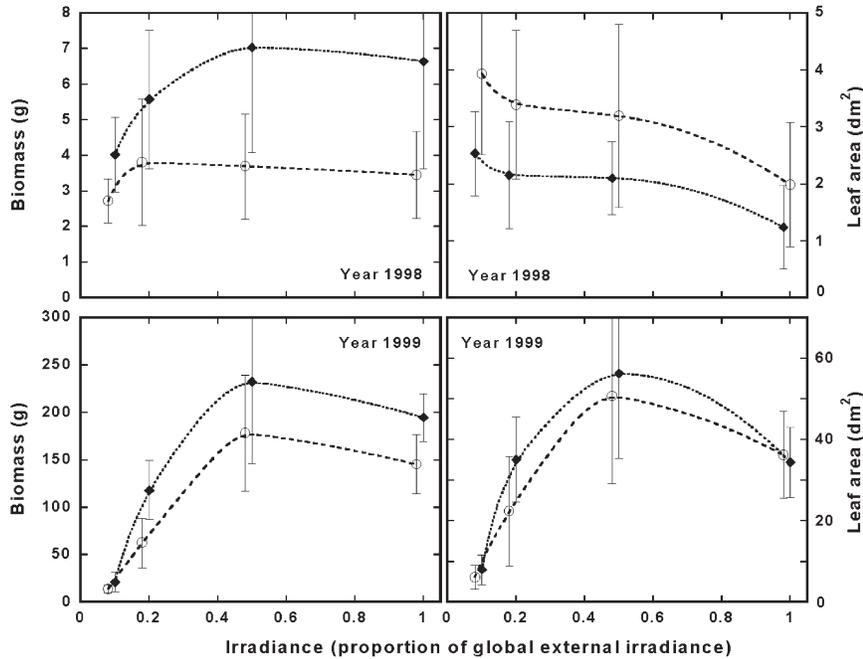
Figure 1. Total biomass and leaf area of two oak species (○: *Q. petraea*; ◆: *Q. robur*) measured at the end of the first and second year of growth as a function of irradiance regime expressed as fraction of external solar irradiance. Values are means ± SD.

Biomass increment was 3–20 times larger during the second year than during the first year, depending on irradiance regime (Figure 1). Again, *Q. robur* had a larger biomass than *Q. petraea* (30 to 100% larger depending on irradiance). In both species, biomass increment increased significantly with increasing irradiance from 8 to 48% of incident irradiance (Figure 1), but decreased slightly as incident irradiation increased from 48 to 100%. Final height, stem diameter and total leaf area paralleled the response of biomass to irradiance (Table 1), except for *Q. robur* height, which was maximum in the treatment providing 18% of incident irradiance. There was an increasing trend in LMA with both irradiance and growth flush. Root/shoot ratio was unaffected by irradiance (data not shown). Leaf N concentration per area (N_a) was similar in the two species and increased significantly ($P < 0.01$) with irradiance. Overall, irradiance effects on growth parameters were larger and interspecific differences were smaller during the second year than during the first year of growth.

Drought was moderate but constant throughout the summer (not shown). Drought significantly ($P < 0.001$) reduced pre-dawn leaf water potential (Ψ_{pd}) from −0.20 to −0.51 MPa in *Q. petraea*, and from −0.28 to −0.55 MPa in *Q. robur*, but had no effect on midday leaf water potential (Ψ_{md}). No interspecific difference was observed in leaf water potential. After

Table 1. Mean height, leaf mass to area ratio (LMA) and leaf nitrogen concentration per unit leaf area (N_a) at the end of the first and second year of growth as a function of irradiance regime (expressed as fraction of external solar irradiance). Values are means ± SD, $n = 10$. Different letters within an irradiance regime and a year indicate significant interspecific differences.

	Species	8%	18%	48%	100%
Year 1998					
Height (cm)	*Q. petraea*	27.1 ± 3.1 a	22.7 ± 7.4 a	16.1 ± 4.4 a	11.2 ± 2.8 a
	Q. robur	31.2 ± 7.5 a	30.2 ± 6.4 b	27.7 ± 9.7 b	19.0 ± 5.7 b
LMA (g m^{-2})	*Q. petraea*	48.7 ± 5.5 b	60.1 ± 5.9 b	67.2 ± 6.8 a	89.6 ± 11.8 a
	Q. robur	38.7 ± 2.6 a	54.8 ± 5.9 a	61.0 ± 7.7 a	87.0 ± 8.8 a
N_a (g m^{-2})	*Q. petraea*	1.7 ± 0.3 a	2.1 ± 0.2 a	2.2 ± 0.4 a	2.6 ± 0.6 a
	Q. robur	1.5 ± 0.2 a	2.0 ± 0.2 a	2.3 ± 0.4 a	2.7 ± 0.5 a
Year 1999					
Height (cm)	*Q. petraea*	40.8 ± 13.9 a	76.8 ± 28.7 a	130.4 ± 47.7 a	53.2 ± 14.5 a
	Q. robur	72.1 ± 18.7 b	155.2 ± 57.3 b	114.8 ± 26.7 a	62.6 ± 12.1 a
LMA (g m^{-2})	*Q. petraea*	39.9 ± 15.7 a	59.8 ± 7.0 b	63.8 ± 10.4 a	55.7 ± 33.2 a
	Q. robur	41.3 ± 6.6 a	49.5 ± 12.3 a	58.7 ± 1.7 a	69.3 ± 38.4 a
N_a (g m^{-2})	*Q. petraea*	1.6 ± 0.4 a	2.0 ± 0.4 a	1.8 ± 0.4 a	2.3 ± 0.6 a
	Q. robur	1.5 ± 0.2 a	1.9 ± 0.4 a	1.8 ± 0.2 a	2.7 ± 0.3 a

each biweekly watering, soil volumetric water content reached about 30 and 15% in the control and water-stress treatments, respectively. It decreased to 19 and 10% when measured before the next watering.

Growth was affected more severely by drought in *Q. petraea* than in *Q. robur*, although no significant interaction between species and drought was detected. Drought limited biomass increment (38 and 50% decrease with respect to controls in *Q. robur* and *Q. petraea*, respectively, $P < 0.001$), total leaf area (18 and 55%, $P < 0.001$) and diameter ($P < 0.001$), but did not modify the root/shoot ratio. Height of water-stressed seedlings was less than that of controls in *Q. petraea* only ($P < 0.05$).

Stable carbon isotope ($\delta^{13}C$)

Significant differences ($P < 0.001$) in $\delta^{13}C$ of acorns were detected between *Q. petraea* ($-24.2 \pm 1.1‰$) and *Q. robur* ($-25.7 \pm 1.2‰$, Figure 2). During the first year of growth, $\delta^{13}C$ of shoots increased significantly with irradiance in both species ($P < 0.001$, Figure 2). However, the increase was larger for *Q. petraea* (2.5‰) than for *Q. robur* (1.8‰), indicating a 51 and 38% increase in WUE with increasing irradiance for *Q. petraea* and *Q. robur*, respectively (Farquhar et al. 1989). Values of $\delta^{13}C$ followed the same pattern in stems and leaves of the two growth flushes, but were higher in leaves from the second flush than in leaves from the first flush, and lower in stems (not shown). The interspecific difference in $\delta^{13}C$ was significant in shoots ($P < 0.001$) and stems ($P < 0.001$), but not in leaves (Table 2). However, within each treatment, the interspecific difference in $\delta^{13}C$ was significant only in the 18% of incident irradiance treatment ($P < 0.05$) and in full sun ($P < 0.01$) for $\delta^{13}C_{shoot}$.

During the second year (summer 1999), most of the seedlings produced three flushes. The effect of irradiance on $\delta^{13}C_{shoot}$ followed the same pattern as during the first year, but was more marked (4.5 and 3.8‰ difference between the deep shade and full sun treatments for *Q. petraea* and *Q. robur*, respectively, yielding 86 and 72% greater WUE; Figure 2). This increase was a result of lower values in the treatment providing 8% of incident irradiance and higher values in full sun during the second year. Model 1 revealed no significant difference between species in any plant organ (Table 2). When each

treatment was analyzed separately (Figure 2), significant interspecific differences in $\delta^{13}C_{shoot}$ and $\delta^{13}C_{leaf}$ were observed in the 18% of incident irradiance treatment ($P < 0.05$ and $P < 0.01$, respectively) and in full sun ($P < 0.05$ and $P < 0.01$, respectively). This difference was significant in $\delta^{13}C_{stem}$ ($P < 0.01$) only in full sun. The interspecific difference in $\delta^{13}C_{shoot}$ corresponded to a 7% higher WUE in *Q. petraea* than in *Q. robur* in full sun, and to a lower WUE in the treatment providing 18% of incident irradiance.

In both species, water-stressed seedlings displayed significantly higher $\delta^{13}C_{shoot}$ than controls (1.0 and 0.8‰ for *Q. petraea* and *Q. robur*, respectively, i.e., 10 and 9% higher WUE; Figure 3). Under drought conditions, the interspecific difference in $\delta^{13}C$ remained significant ($P < 0.01$) in stems, leaves and shoot (Table 3). This difference slightly increased $\delta^{13}C$ in the shoot (0.1‰) and leaves (0.3‰), but had no effect on $\delta^{13}C$ in stems, compared with control seedlings.

Leaf gas exchange

Irradiance affected net carbon assimilation rate (A), stomatal conductance (g_{sw}) and instantaneous WUE (A/g_{sw}), with an interspecific difference only in A (Table 4). Water supply af-

Table 2. Analysis of variance of effects of intensities (four intensities), species (*Q. robur* and *Q. petraea*) and the irradiance × species (Irrad. × Spec.) interaction on $\delta^{13}C$ of stems, leaves (Flush 1, 2 and 3) and whole shoot after 1 and 2 years of growth. Values correspond to the *F*-value. The *P*-values are denoted as: (*) = $P < 0.1$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; and nonsignificant otherwise.

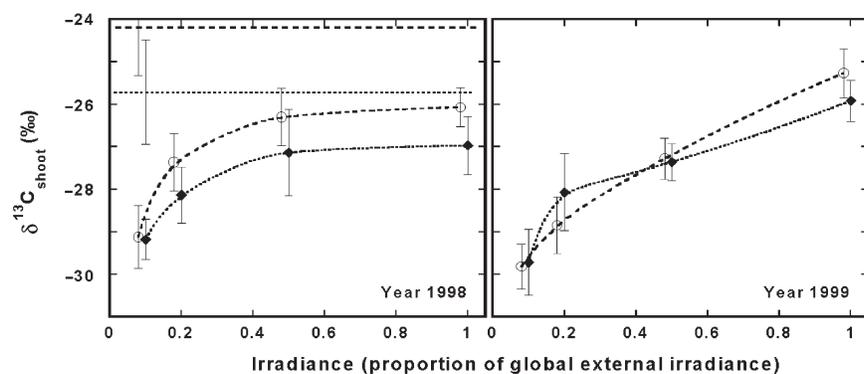|	Leaves (Flush 1)	Leaves (Flush 2)	Leaves (Flush 3)	Stems	Shoot
Year 1998					
Irradiance	6.4***	42.1***	–	35.2***	52.0***
Species	1.3	3.4(*)	–	10.6**	14.4***
Irrad. × Spec.	1.4	0.9	–	2.7(*)	1.4
Model	3.5**	18.3***	–	17.5***	23.9***
Year 1999					
Irradiance	71.1***	98.6***	108.1***	164.1***	161.2***
Species	0.7	1.4	1.4	1.3	0.07
Irrad. × Spec.	2.7(*)	6.2***	5.4**	4.5**	4.2**
Model	32.0***	45.8***	48.8***	72.4***	71.2***

Figure 2. Variation in $\delta^{13}C$ in shoots of seedlings of the two oak species measured at the end of the first and second years of growth as a function of irradiance regime. Values are means ± SD, $n = 10$. Horizontal lines correspond to the mean $\delta^{13}C$ of 10 acorns per species (long dashes: *Q. petraea*; short dashes: *Q. robur*).

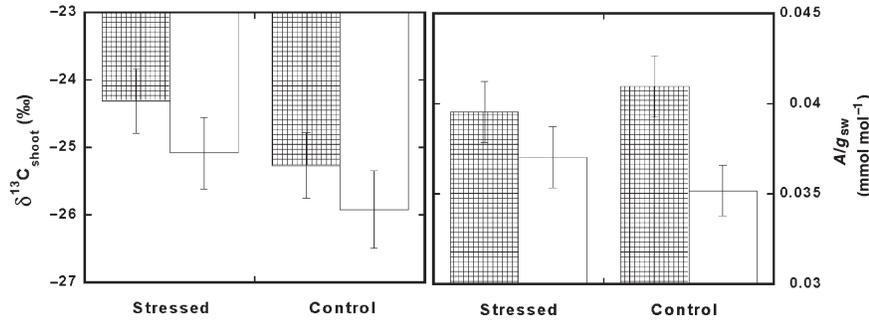Figure 3. Mean $\delta^{13}C$ (measured in shoots at the end of the second year of growth) and A/g_{sw} of water-stressed and control seedlings from the two oak species. Values are means ± SD (cross-hatched columns: *Q. petraea*, blank columns: *Q. robur*).

fected A and g_{sw} but not A/g_{sw}, whereas interspecific differences were detected for g_{sw} and A/g_{sw}.

As expected, A increased almost four times as irradiance increased from 8 to 100% of full sun ($P < 0.001$, Figure 4). The increase in A was larger for *Q. robur* than for *Q. petraea*. In the latter species, a plateau was reached at 18% of incident solar irradiance. Stomatal conductance (g_{sw}) paralleled A in *Q. robur*. In *Q. petraea*, g_{sw} increased as incident irradiance increased from 8 to 18% but remained stable as incident irradiance increased from 18 to 100%. Instantaneous water-use efficiency (A/g_{sw}) increased with increasing irradiance in *Q. petraea* (43%, $P < 0.001$), and in *Q. robur* (27%, $P < 0.05$), but displayed a plateau at incident irradiances above 48% in *Q. robur* (data not shown). Interspecific differences in A ($P < 0.05$), g_{sw} ($P < 0.001$) and A/g_{sw} ($P < 0.001$) were observed only in full sun. *Quercus robur* displayed higher A and g_{sw} than *Q. petraea* (18 and 34%, respectively), and 11% lower A/g_{sw} values. At the intra-treatment level under control conditions, A/g_{sw} was strongly correlated with g_{sw} (linear relationship with $1/g_{sw}$: $n = 80$, $r = 0.77$, $P < 0.001$) but not with A. However, the inter-treatment variations in A/g_{sw} were mainly a result of variations in A, because positive correlations were observed between treatment mean A/g_{sw} and both A ($n = 4$, $r = 0.93$) and g_{sw} ($n = 4$, $r = 0.79$).

Relationships between $\delta^{13}C$ and A/g_{sw} were recorded on the leaves of the last growth flush, and averaged for each seedling (12 repeated individual measurements per leaf). A tight correlation was observed between A/g_{sw} and $\delta^{13}C_{shoot}$ ($n = 40$, $r = 0.87$, $P < 0.001$ for *Q. petraea* and $n = 40$, $r = 0.82$, $P < 0.001$ for *Q. robur*; Figure 5). Correlations of the same order of magnitude were observed with $\delta^{13}C_{leaf}$ of the last developed flush instead of $\delta^{13}C_{shoot}$. A large fraction of the observed variability was related to variability in A (Table 4). When taking into account only the intra-treatment variability under control conditions, $\delta^{13}C_{shoot}$ was significantly correlated with both A/g_{sw} ($n = 80$, $r = 0.42$, $P < 0.001$) and g_{sw} ($n = 80$, $r = -0.49$, $P < 0.001$), but not with A.

Water-stressed seedlings of both species displayed significantly lower values of A and g_{sw} than controls ($P < 0.001$, not shown). The effect of water stress on gas exchange was more pronounced for *Q. robur* than for *Q. petraea*, but did not significantly change A/g_{sw}. Under drought conditions, there was no difference in A, g_{sw} and A/g_{sw} between the two *Quercus* species. No significant correlation was observed between $\delta^{13}C$ and gas exchange in water-stressed seedlings.

Discussion

Interspecific differences in $\delta^{13}C$ and WUE in Q. petraea and Q. robur seedlings in full sun

In agreement with observations in adult oaks (Ponton et al. 2001), seedlings of *Q. petraea* consistently displayed a higher $\delta^{13}C$ when grown in full sun than did seedlings of *Q. robur*. This difference translated into a 7 to 10% larger WUE in *Q. petraea* than in *Q. robur* during the second year of growth,

Table 3. Analysis of variance of the effects of water supply, species, and water supply × species (Water × Spec.) interaction on $\delta^{13}C$ of leaves (Flush 1, 2 and 3), stems and shoot at the end of the second year of growth. Values correspond to the F-value. The P-values are denoted as: (*) = $P < 0.1$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; and nonsignificant otherwise.

Year 1999	Leaves (Flush 1)	Leaves (Flush 2)	Leaves (Flush 3)	Stems	Shoot
Water supply	4.0(*)	9.3**	15.2***	39.6***	29.3***
Species	20.5***	19.1***	9.9**	24.8***	18.5***
Water × Spec.	0.6	0.1	0.0	0.0	0.1
Model	8.4***	9.5***	8.4***	22.1***	16.4***

Table 4. Analysis of variance of the effects of irradiance (a) or drought (b), species and their interaction on net carbon assimilation (A), stomatal conductance to water vapor (g_{sw}) and instantaneous WUE (A/g_{sw}) measured during the second year of growth. Values correspond to the F-value. The P-values are denoted as: (*) = $P < 0.1$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; and nonsignificant otherwise.

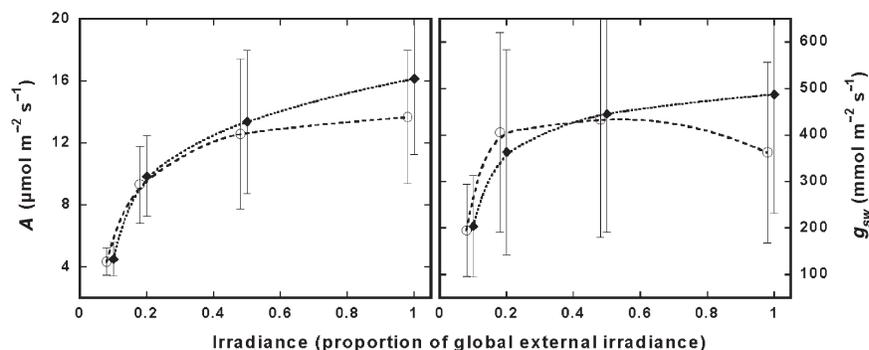| Source of variation	A	g_{sw}	A/g_{sw}
a. Irradiance	268.6***	45.0***	9.6***
Species	12.4***	2.9	0.0
Irradiance × Species	3.4*	4.6**	2.1
Model	118.6***	21.8***	5.1***
b. Water supply	41.5***	42.9***	0.1
Species	2.5	10.5***	11.3**
Water supply × Species	2.9	5.2*	1.8
Model	15.7***	19.8***	4.5**

Figure 4. Net CO_2 assimilation rate and stomatal conductance of two oak species as a function of irradiance regime. Values are means ± SD; $n = 10$ individuals and 12 repeated measurements.

which is close to the 13% difference estimated in adult trees (Ponton et al. 2001). However, it contrasts with the lack of interspecific difference reported in other seedling studies (Thomas and Gausling 2000). Likewise, gas exchange measurements on seedlings grown in lysimeters (Vivin et al. 1993) revealed no difference between species. Our gas-exchange-based estimates of WUE resulted in a 11–14% interspecific difference, similar to the 15% found in a sample of four adult trees from each species (Epron and Dreyer 1993).

When data from all irradiance treatments were analyzed, $\delta^{13}C$ was found to be closely correlated with A/g_{sw}. This tight correlation obtained during the second year of growth validates the use of $\delta^{13}C$ as a time-integrated estimate of WUE. Such validation is usually difficult to obtain because of discrepancies in time integration among the two variables (instantaneous for A/g_w and integrated over the growth season for $\delta^{13}C$). Similar validations have been found when comparing continuous online measurements of $\delta^{13}C$ and gas exchange (Farquhar et al. 1989, von Caemmerer and Evans 1991), or when using soluble carbohydrate $\delta^{13}C$, which integrates dis-

crimination over short timescales (Brugnoli et al. 1988, 1998, Picon et al. 1997). In addition, we demonstrated that, within an irradiance treatment, i.e., at the inter-plant and interspecific level, the variability in $\delta^{13}C$ was largely related to variation in g_{sw} and not to A, which is similar to findings in tropical tree species (Bonal et al. 2000).

The interspecific differences in $\delta^{13}C$ recorded during the second year of growth were probably largely a result of discrimination during CO_2 assimilation. Similar data recorded after the first year of growth have to be analyzed with more caution. Indeed, the large interspecific difference in $\delta^{13}C$ of acorns, and the small difference in biomass between acorns and seedlings suggests a maternal effect resulting from carbon translocation from acorns to seedlings.

The interspecific difference in WUE was related to a difference in g_{sw}, because $Q.\ petraea$ was found to operate at smaller g_{sw} than $Q.\ robur$. Although we did not establish why $Q.\ petraea$ operates at low g_{sw} in full sun, the hydraulic constraints may be larger in $Q.\ petraea$ than in $Q.\ robur$ (Ponton et al. 2001).
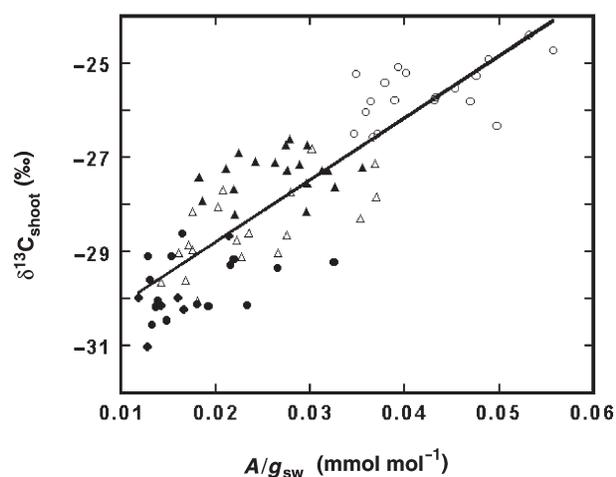

Figure 5. Relationship between individual $\delta^{13}C_{shoot}$ and mean instantaneous water-use efficiency (A/g_{sw}, 12 repeated measurements per leaf, last developed growth flush) in seedlings of $Q.\ petraea$ and $Q.\ robur$ grown in four irradiance regimes (●: 8%, △: 18%, ▲: 48%, ○: 100%). The linear regression between A/g_{sw} and $\delta^{13}C_{shoot}$ was similar for the two species.

Effect of irradiance on $\delta^{13}C$ and WUE

Several authors have shown that decreasing irradiance immediately results in lower leaf $\delta^{13}C$ (von Caemmerer and Evans 1991). Similar effects of irradiance have been observed in time-integrated discrimination studies under field conditions (Broadmeadow and Griffiths 1993, Collet et al. 1996, Koesmaryono et al. 1998, Carelli et al. 1999) and in forest stands (Zimmerman and Ehleringer 1990, Berry et al. 1997, Hanba et al. 1997). In banana plants grown at several irradiances, leaf $\delta^{13}C$ decreased by 2.5‰ as external global irradiance decreased from 100 to 30% (Israeli et al. 1996). In our study, increasing incident irradiance from 8 to 100% resulted in a large range of $\delta^{13}C$ values (4.5‰ in $Q.\ petraea$ and 3.8‰ in $Q.\ robur$ in shoots harvested at the end of the second year of growth). A large range of A/g_{sw} values in response to increasing irradiance was also found in both species. However, the irradiance treatments had a smaller effect on A/g_{sw} than on $\delta^{13}C$, probably because shoot $\delta^{13}C$ values are integrated over a larger timescale than leaf $\delta^{13}C$, and over an even larger timescale than instantaneous A/g_{sw} values. Beside temporal effects, spatial effects could also account for some of the difference

between $\delta^{13}C$ and A/g_{sw}, because gas exchange was only measured on sun-exposed leaves whereas $\delta^{13}C$ integrated the functioning of the whole photosynthetic area, including the contribution of shaded leaves.

Because ambient conditions, including air temperature, VPD and atmospheric CO_2 concentrations, were similar in all treatments, variations in $\delta^{13}C$ among treatments were probably caused by the irradiance treatments. Carbon assimilation more than tripled as incident irradiance increased from 8 to 100%, whereas g_{sw} only doubled. Thus, variations in WUE along the irradiance gradient were caused mainly by variations in A, as observed in *Coffea* sp. (Carelli et al. 1999), tropical shrubs (Jackson et al. 1993) and banana (Israeli et al. 1996). Observations made on tropical orchids (*Catasetum viridiflavum* Hook) revealed no change in mass-based leaf N concentration, and led to the conclusion that irradiance influences $\delta^{13}C$ predominantly by increasing stomatal limitation to photosynthesis (Zimmerman and Ehleringer 1990). In our study, increasing leaf N concentrations (area based) with increasing irradiance indicate that irradiance-induced variations in WUE were a result of changes in photosynthetic capacity (data not shown). Lower WUE in shaded plants than in sun-exposed plants is consistent with the concept that maximizing carbon gain is a more important constraint on survival under conditions of low light than the maintenance of high WUE through restriction of gas exchange.

Genotype × environment interactions on $\delta^{13}C$ and WUE

Time-integrated WUE estimated by $\delta^{13}C$ measurements was increased by water stress, as the theory predicted and as already observed in *Quercus* species (Guehl et al. 1994, Picon et al. 1996). However, these results contrast with reports by Thomas and Gausling (2000) and Thomas (2000) that mild drought and severe drought, respectively, have no effect on WUE of *Q. petraea* seedlings. In our study, the interspecific difference in $\delta^{13}C$ was maintained and even slightly increased during drought, whereas interspecific differences in A, g_{sw} or A/g_{sw} were not detected. Moreover, drought markedly decreased A and g_{sw}, but in the same proportion so that A/g_{sw} was the same in control and water-stressed seedlings. One reason for the discrepancy between WUE estimated as A/g_{sw} and $\delta^{13}C$ during drought could be related to the timing of the gas exchange measurements. During drought, a large fraction of carbon is assimilated early in the day when stomata are open, whereas gas exchange measurements were made during the afternoon, when stomata were already closed. Therefore, the measured values of A/g_{sw} are probably a poor reflection of the values generally prevailing during drought. We conclude that there was no strong environment × genotype interaction on $\delta^{13}C$ in response to drought.

The interspecific difference in WUE observed in full sun was not detected under shade conditions. Abrams (1994) also observed that genotypic variations in ecophysiological characteristics were not expressed in all phenotypes, e.g., shade plants. The most likely explanation is that, at low irradiances, the constraints imposed on carbon assimilation by stomata are released, allowing maximum carbon gain despite lowered photosynthetic capacity. This explanation implies that, at low irradiances, Q. *robur* and Q. *petraea* have similar values of g_{sw} and do not express the potential, genetically controlled, interspecific difference. Our measurements of gas exchange support this view, because A and g_{sw} were similar in the two species in the shade treatments.

Conclusion

Quercus petraea seedlings had 10–15% higher WUE than *Q. robur* seedlings in full sun, despite growth under common conditions. This result supports the hypothesis, previously elaborated from studies of adult trees, that the interspecific difference in WUE has a genetic basis. The interspecific difference was maintained in seedlings subjected to drought in the full sun treatment, but disappeared at low irradiances, when carbon gain is probably maximized regardless of water use. It is unlikely that this small interspecific difference in WUE provides a competitive advantage to *Q. petraea* seedlings under natural conditions, given that the initial growth of *Q. robur* seedlings is far more rapid under all conditions. However, the interspecific difference in WUE, which is observed in both seedlings and adult trees, likely has a significant impact on survival in adult stands, where it has been observed that *Q. robur* frequently declines following drought, whereas *Q. petraea* is much less affected. Thus, low WUE can be regarded as a characteristic of pioneering species. We conclude that, in these two sympatric oak species, there is an inverse relationship between initial growth rate and long-term survival in drought-prone habitats. *Quercus robur* tends to maximize growth and exhibits low adult tree survival following drought, whereas the slow growth exhibited by *Q. petraea* is associated with the long-term survival of adult trees in drought-prone environments.

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