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A new attempt at discrimination between *Quercus petraea* and *Quercus robur* based on wood anatomy

François Feuillat, Jean-Luc Dupouey, Delphine Sciama, and René Keller

Abstract: The interspecific variability of wood anatomy between the two major oak species *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. is still largely unknown. However, anatomy strongly influences the technological properties of wood and the ecophysiological functioning of trees. Moreover, identification of oak wood species is a long-standing challenge and important for many purposes. In the Cîteaux Forest (Burgundy), 58 oaks from 14 mixed stands were sampled for wood anatomy characterization. Image analyses of four ring radiographies per tree were carried out. Shape, size, and proportion of tissues (earlywood vessels, fiber, parenchyma, and latewood vessels) were characterized, taking into account cambial age and ring-width effects. Taxonomic status of the trees was assessed by foliar morphology analysis. Significant differences in numerous anatomical features appeared between the two species. The surface proportion of earlywood vessels (the proportion of total ring surface area taken up by these vessels), the number of earlywood vessels, and the size and surface proportion of fiber zones were among the largest. The diameter and shape of earlywood vessels did not differ between the two species. A bivariate discriminant function reached a 78% rate of success for species recognition. However, a much clearer foliar morphology gap existed between the two species. At the intraspecific level, linear correlations between anatomical and morphological variables were significant for a few variables only. Nevertheless, for each species, trees displaying an atypical wood anatomy were also less morphologically representative. Technological and ecophysiological implications of these results are discussed. In mixed stands, hybridization introgression between the two species could explain the large anatomical overlap. Thus, anatomical discrimination between the two oak species may be easier in pure stands. Finally, a practical identification key is given.

Résumé : La variabilité inter-spécifique de l'anatomie du bois des deux principaux chênes de la forêt européenne (*Quercus robur* L. et *Quercus petraea* (Matt.) Liebl.) est encore paradoxalement peu connue. Pourtant, l'anatomie conditionne pour une large part les propriétés technologiques du bois et le fonctionnement écophysologique des arbres. La possibilité d'identifier l'espèce à partir d'échantillons de bois offrirait aussi une large gamme d'applications. Quatorze peuplements mélangés de chênes pédonculé et sessile de la forêt de Cîteaux (Bourgogne) ont été échantillonnés (58 chênes au total). Par analyse d'images radiographiques de carottes de sondage, on a caractérisé la forme, la taille et la proportion des différents tissus (gros vaisseaux, fibres, parenchyme et petits vaisseaux) de quatre cerne par arbre, en contrôlant les effets âge et largeur de cerne. Le statut taxonomique des arbres a été précisé par analyse de la morphologie foliaire. Nous mettons en évidence, entre autres, des différences très significatives entre espèces pour le nombre et la proportion surfacique des gros vaisseaux dans le cerne, ainsi que pour la taille et la proportion surfacique des plages de fibre. Par contre, le diamètre et la forme des gros vaisseaux ne diffèrent pas. Une analyse discriminante incluant deux variables (proportion surfacique des gros vaisseaux et des fibres dans le cerne) permet un taux de reconnaissance de l'espèce de 78%. La morphologie foliaire sépare cependant beaucoup plus clairement les deux espèces. Au niveau intra-spécifique, les corrélations linéaires entre variables anatomiques et morphologiques sont rarement significatives. Toutefois, pour chacune des deux espèces, les arbres qui présentent une anatomie atypique sont aussi morphologiquement les moins caractéristiques. Les conséquences écophysologiques et technologiques de la variabilité anatomique observée sont discutées. Dans ces peuplements mélangés, l'hybridation-introgression pourrait expliquer le fort recouvrement des valeurs des variables anatomiques entre les deux espèces. Si tel est le cas, la discrimination anatomique des deux espèces de chênes pourrait s'avérer meilleure en peuplements purs. Une clef pratique d'identification est donnée.

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Introduction

Quercus robur L. (pedunculate oak) and *Quercus petraea* (Matt.) Liebl. (sessile oak) are the two major oak species of European forests (27% of the area of European broad-leaved forests). They are seldom distinguished during management operations, and never after felling. However, separation of *Q. robur* and *Q. petraea* wood could be very valuable for reasons outlined below.

Small but statistically significant differences in physical and chemical wood properties between the two species have been reported (Nepveu 1995). Wood density and shrinkage are lower in *Q. robur* (Deret-Varcin 1983; Nepveu 1984; Lévy

et al. 1992). Heartwood formation is quicker, thus leading to a thinner sapwood zone (Deret-Varcin 1983; Lévy et al. 1992), and wood colour is slightly different (Klumpers et al. 1993). Such differences have a significant effect on mechanical properties and thus the industrial use of the wood. Moreover, differences in wood extracts (Masson et al. 1995; Mosedale and Savill 1996) and porosity (Feuillat et al. 1993) between species have recently been found. This is important for oak selection before stave wood manufacturing in cooperage. Thus, the wood industry could benefit from a better identification of logs at the species level.

Xylem anatomy has direct implications on tree ecophysiology. The hydraulic functioning of trees depends largely on anatomical features (Zimmermann 1983). The vulnerability of oaks to cavitation (Cochard and Tyree 1990) and shake (Savill 1986) increases with increasing vessel size. However, little information exists about the differences in anatomical characteristics between *Q. petraea* and *Q. robur*.

Finally, our current inability to identify samples at the species level is a serious limitation on paleoecological and historical studies based on subfossil oaks, charcoal, or macrosamples found in peat bogs, rivers, or buildings (Fletcher 1978). The two species are known to have different ecological requirements concerning water and nutrient availability (Lévy et al. 1992), as well as different abilities to compete in opened areas. Thus, any information at the species level should be of great interest for such paleoecological or historical studies.

The genetic structure of this complex is not entirely clear yet (see Kremer et al. 1993, for a recent synthesis). Significant interspecific fertilization rates obtained in artificial hybridization (Steinhoff 1993) are probably responsible for the large morphological variability observed within the two species. However, numerous studies have shown that among adult trees, the two species can be clearly distinguished when multivariate approaches are used (Dupouey and Badeau 1993; Aas 1993) and that there are very few individuals which cannot be ascribed to one species or the other, even in mixed stands. Besides morphological characteristics, molecular markers are now employed for taxonomic studies: isoenzymes, chloroplast DNA polymorphism (Kremer et al. 1991; Petit et al. 1993), and nuclear DNA polymorphism. Very few studies focused on the interspecific variability of wood anatomy characteristics.

A review of previously published differences between the wood anatomy of the two species is given in Table 1. Despite the findings of Hartig (1901, as cited in Huber et al. 1941), that the two species were inseparable based on their wood anatomy, Huber et al. (1941) identified four main features allowing a correct classification of individuals from both species (Table 1): number of rows of earlywood vessels; earlywood vessel shape; layout of latewood vessels; and ratio of the earlywood vessel zone to total ring width. Walker (1978) recognized three other main diagnostic characteristics, the two first already mentioned by Huber et al. (1941): gradualness of the transition between early- and late-wood; degree of forking of the latewood "flames" (areas of small vessels); and whether earlywood vessels are crowded. Deret-Varcin (1983) added the total number of earlywood vessels per ring.

Anatomical variations observed within both species were large, and reliable species identification was not possible with any one anatomical characteristic taken alone. Huber et al. (1941)

and Walker (1978) built empirical keys for identification based on several features.

Besides these taxonomic differences, the same authors and others (Von Courtois et al. 1964; Gasson 1987; Huber 1993) recognized a strong influence of cambial age (age of the tree ring counted from the pith) or ring width upon numerous anatomical characteristics. Narrow or juvenile rings were generally considered unsuitable for species identification.

Our main goal was to construct a multivariate discriminant function for oak species identification based on wood anatomy. The following questions were addressed: What are the discriminant anatomical features? Does an appropriate combination of these features allow a reliable wood species identification? Is there any relationship between inter- and intra-specific variability of anatomical and morphological characteristics?

To answer these questions, we analyzed wood anatomical and leaf morphological variability together in mixed *Q. robur* – *Q. petraea* stands. We tested a large number of anatomical features using an automated image analysis system. Ring width and age effects were taken into account during sampling and analyses.

Material and methods

Trees and wood sampling

In the old ecclesiastical forest of Cîteaux, in Burgundy (eastern-central part of France), 14 mixed stands of *Q. robur* and *Q. petraea* were selected within a very limited range of environmental variations, using the site classification of Bailly (1995). Environmental homogeneity was monitored by vegetation and soil studies. Stands were previously managed as self-grown coppice with standards with hornbeam (*Carpinus betulus* L.) developed under a dominant canopy of oaks and a few beeches. Soils were acidic, developed on loams, with a layer of temporary waterlogging at a depth of 30 cm. Slopes were nearly horizontal.

In each plot, two trees from each species were selected from the dominant standards, within the diameter class 55–60 cm. Two additional trees from the same forest were included in our wood anatomy sample (58 trees in total). Species were roughly determined in the forest using morphological attributes (bark structure, branch architecture, peduncle length). One 5 mm diameter core was extracted at breast height for wood anatomy analysis. Twenty leaves were collected using a shotgun from the upper part of the crown of a subset of 53 trees for subsequent taxonomic characterization.

Wood anatomy measurements

Juvenile and sapwood zones of the wood were discarded by extracting a 120 mm long sample from each core, starting at the sapwood–heartwood limit and extending inwards. X-ray photographs were prepared following the classical methodology of Polge et al. (1977). Optimum conditions were obtained with 2 mm thick wood cross sections, irradiated at 2.5 m, 9.5 kV, 10 mA for 16 h with a fine-grain film.

Four rings per core were selected for image analysis (232 rings in total). These rings were equally distributed along the core (to represent all age-classes), and as far as possible were chosen close to 2 mm wide and with a sharp X-ray image. Cambial age was counted from the pith for each chosen ring.

The X-ray films were digitized using a CCD camera mounted on binoculars (512 × 512 pixels of ca. 7 × 7 µm each), and coupled with a microcomputer. Each selected ring was analyzed using semiautomated software developed under the Visilog® image analysis system (Anonymous 1992). Image thresholding and delimitation of the different wood tissue boundaries were conducted manually in a 3.5 mm width window along the tangential direction.

Table 1. Review of previously published differences in wood anatomy between *Quercus petraea* and *Quercus robur*.

Anatomical feature	<i>Quercus robur</i>		<i>Quercus petraea</i>	References ^a
No. of earlywood vessel rows				
In rings over 2 mm wide	4 or more		1 or 2, in rare cases as many as 3	1
In rings over 2 mm and beyond year 5	3 or more		1 or 2, in rare cases as many as 3	3
Earlywood vessel shape (tangential/radial diameter)	Clearly oval (0.75)		Approximately circular (0.83)	1
	Clearly oval or crowded and distorted		Approximately circular, uncrowded	3
		No clear differences		2
No. of earlywood vessels (mean per ring; 5-mm tangential width)	24	Statistically different	17	4
Cross-sectional area of individual earlywood vessels	Generally larger			1
		Not statistically different		4, 6
Ratio of the earlywood vessel width to the total ring width (in rings over 2-mm wide)	Over 0.25		Under 0.25	1
	Predominantly over 0.25		Predominantly under 0.25	3
			Generally smaller	2
Appearance of latewood vessel groups (flames)	Clear and wide, particularly in youth; when older, often faded		Narrow and clear	1
	Wide and broadened towards the end of the year; if not, bifurcation frequent		Narrow and straplike; bifurcation, if present, weak	3
Transition from earlywood to latewood (in rings over 2 mm wide)	Predominantly gradual		Predominantly abrupt	1
	Gradual		Abrupt	3
Individual cross-sectional area of latewood vessels	Predominantly decreasing towards the end of the ring		Predominantly relatively uniform	1
Proportion of fiber tracheids	Higher			1
Proportion of fiber tissues (only in narrow rings)			Predominantly higher	2
Proportion of longitudinal parenchyma (only in narrow and medium rings)	Predominantly higher			2
No. of multiseriate rays (per cm) measured on transversal section		Not statistically different		5

^a1, Huber et al. (1941, 52 *Q. robur* and 41 *Q. petraea* from mixed and pure stands); 2, Courtois et al. (1964, 5 *Q. robur* and 6 *Q. petraea* from pure stands); 3, Walker (1978, 9 *Q. robur* and 11 *Q. petraea*); 4, Deret-Varcin (1983, 12 *Q. robur* and 12 *Q. petraea* from a mixed stand); 5, G. Nepveu and F. Huber (personal communication, 30 *Q. robur* and 66 *Q. petraea* from pure stands); 6, Mather et al. (1993, 110 trees in a provenance trial).

From these digitized images, total ring, early- and late-wood width, and surface area were measured (see Fig. 1 and Schweingruber 1990 for a general presentation of oak anatomy and technical terms used). The total number of earlywood vessels, and the number of rows they formed, were counted, then entire vessels only were selected and counted. For each selected vessel, the following measurements were made: lumen area; radial, tangential, maximum, minimum, and mean lumen diameters.

Transitions between early- and late-wood were recorded as gradual or abrupt based on the distribution of vessel sizes along the radial direction.

Each fiber zone was delimited (including both libriform and tracheid fibers) and classified within one of the three following shape groups: triangular, rectangular, or undetermined. The number of fiber zones in each class was counted. The area of each entire fiber surface was measured separately in early- and late-wood. Multiseriate rays were counted and measured for surface area and tangential width. The tangential extent of the wood area studied was too small for multise-

riate rays to be adequately sampled, thus, they were not taken into account in subsequent surface area calculations.

Surface areas of parenchyma in the earlywood were estimated as the difference between total earlywood surface area and previously measured vessels and fiber areas. In fact, this difference includes both parenchyma and vasicentric tracheids, which are indistinguishable on cross section. The cumulative surface area of parenchyma and small vessels in the latewood was calculated as the total latewood surface area minus the fiber area. The shape of these parenchyma and small vessel zones (often referred to as "flames") was assessed using two binary variables: (1) forked or not, (2) narrow and straplike or wide and broadened towards the end of the year.

Foliar morphology measurements

For each tree 10 entire leaves were selected and measured using a semiautomated digitizing tablet coupled with a microcomputer. Twenty-nine variables concerning many aspects of foliar morphology

Fig. 1. X-ray photograph of a tranverse section of *Quercus petraea*.

(size, shape, pilosity of various parts) were measured or calculated. A more extensive description of the methodology is given in Dupouey and Badeau (1993).

Data analysis

Univariate analysis of wood anatomy

Potential discriminant variables were identified using a hierarchical analysis of variance model taking into account the species and tree within species effects. An initial analysis showed that despite our careful selection of rings within a limited range of widths, the variation was still large, between 1.45 and 2.96 mm (average of 2.21 mm over the 232 rings, standard deviation 0.31 mm). Moreover, a small but significant ring width difference between the two species existed in our sample (2.29 mm for *Q. petraea* and 2.13 mm for *Q. robur*). Mean ring cambial age varied between 28 and 165 years but was not different, on average, between the two species (76 years, standard deviation 25 years). Cambial age was correlated neither with ring width nor with surface area ($r = -0.12$ and -0.05 , respectively). Thus, we included ring width or surface area, depending on the type of variable analyzed, and age as covariates in our model:

$$[1] \quad X_{ijk} = a + b_i + c_{j(i)} + w_{ijk} + k_{ijk} + e_{ijk}$$

where

X_{ijk} is anatomical characteristic X measured within tree ring k of tree j from species i

a is a constant

b_i represents the effect of species i

$c_{j(i)}$ is the effect of tree j within species i

w_{ijk} is ring width or surface area

k_{ijk} is cambial age

e_{ijk} is the error term

Species was treated as a fixed variable; trees were treated as a random factor nested within species. Because of a slight imbalance in the sampling scheme, we preferred not to include the stand effect in the model. The species effect was tested using variation among trees as the error term. Initial measurements as well as combinations of these raw variables were tested using this model. To reduce the effect of ring width, several measurements were expressed as proportions of total ring width or surface area, or as proportions of early- or latewood width or surface area. These are latewood percent = latewood width/total ring width (sometimes referred to as texture); vessel shape = tangential diameter/radial diameter; surface proportion of vessels = total surface area of vessels/ring area; surface proportion of fibers = total surface area of fiber zones/ring area.

Differences between the two species for categorical variables were tested using the χ^2 statistic.

Multivariate analysis of wood anatomy

All variables that displayed a significant species effect in the previous analysis of variance were submitted to a principal component analysis, in order to identify groups of correlated anatomical features. This was done at the ring level.

A discriminant function was built in at the tree level, using the following iterative approach: at each step, the discriminating power of all measured or calculated variables was assessed using the F statistic. Variables were eventually corrected for the effect of ring width or area before entering the equation, by using the residuals of a linear regression equation. We avoided instability in our model by discarding variables displaying high correlations with those previously entered. Stability of the results was assessed by cross-validation (SAS Institute Inc. 1988).

Relationships between anatomy and foliar morphology

The relationship between both sets of variables, anatomy and foliar morphology, was assessed at the intraspecific level. Morphological variables were submitted to a principal component analysis. We calculated correlations between basic variables, as well as correlations between these variables and principal component coordinates or between principal components. Because of the large number of comparisons made, only correlations with a significance level over 1% were retained.

Results

Univariate analysis

Surface and width of earlywood and latewood differed significantly between the two species, as well as the latewood percent (Table 2 and Fig. 2).

In the earlywood, the number and total surface area of vessels, as well as the number of vessel rows (Fig. 2) were highly significantly different between the two species. This effect was independent of total ring width and strongly dependent on earlywood width. The average surface area, diameter, or shape of individual vessels did not differ between species, nor did the frequency distribution of vessel surface areas. Only large vessels were proportionally more frequent in the wood of *Q. robur* (6.0% of vessels over 0.1 mm²) than in that of *Q. petraea* (3.5%). However, this difference was not statistically significant. Transition between early- and late-wood was slightly but not significantly more frequently gradual within *Q. robur* rings (63% of observed rings) than within *Q. petraea* rings (52%).

Total and mean surface area of individual fiber zones in rings differed significantly between species, and independently from ring width (Table 2). However, this difference was much less pronounced in the earlywood than in the latewood. In the

Table 2. Analysis of variance of wood anatomy characteristics of *Quercus petraea* and *Quercus robur* in mixed stands of the Cîteaux Forest.

Anatomical features	$F_{[1,56]}$ (species)	$F_{[56,172]}$ (tree)	$F_{[1,172]}$ (ring width or ring area)	$F_{[1,172]}$ (age)	Mean values	
					<i>Q. robur</i> (27 trees)	<i>Q. petraea</i> (31 trees)
Earlywood width (mm)	13.0***	5.9***	28.1***	6.4*	0.8	0.72
Latewood width (mm)	13.0***	5.9***	619.6***	6.4*	1.36	1.49
Latewood percent (%)	14.1***	5.7***	28.4***	4.8*	61	67
No. of earlywood vessel rows	19.4*** ^a	—	—	—	2.4	2.0
No. of earlywood vessels/ring (1-mm tangential width)	15.8***	4.5***	8.0**	7.5**	5.46	4.51
No. of earlywood vessels/ring area (mm ²)	16.2***	4.8***	27.7***	7.4**	2.6	2.2
Surface percent of earlywood vessels in ring	19.0***	5.4***	36.8***	2.8ns	14.4	11.5
Surface percent of fibers in earlywood	4.5*	2.5***	8.6**	0.9ns	7.7	10.1
Surface percent of fibers in latewood	7.5**	3.8***	0.7ns	1.1ns	42	49
Surface percent of fibers in ring	16.2***	4.0***	6.3*	0.3ns	29	36
Mean surface area of fiber zones in latewood (mm ²)	14.1***	2.3***	12.0***	0.3ns	0.47	0.67
Surface percent of parenchyma in latewood ^b	7.5**	3.8***	0.7ns	1.1ns	58	51
Surface percent of parenchyma in ring ^b	7.3**	3.6***	0.4ns	1.3ns	57	52
Appearance of latewood vessel groups (flames)	8.2*** ^a	—	—	—	Wide and broadened towards the end of the ring	Narrow and straplike

Note: Only variables with a significant difference between the two species are listed. *F*, significant at 0.1% (***); 1% (**); and 5% (*); ns, not significantly different at 5%; degrees of freedom are in brackets.

^a χ^2 test (3 df for number of earlywood vessel rows and 1 df for flames).

^bParenchyma is the sum of longitudinal parenchyma and small vessels in the latewood.

Fig. 2. Number of earlywood vessel rows versus latewood percent for *Quercus robur* and *Quercus petraea* trees from the Cîteaux Forest (average of four rings per tree). Within each species, trees with unusual values for these two variables are shown as circles (three *Q. petraea* and one *Q. robur*).

latter case, this difference was partly due to latewood width differences. The shape of latewood fiber zones differed only slightly between the two species: *Q. petraea* displayed slightly more rectangular fiber zones and indeterminate shapes were more abundant for *Q. robur*.

The number and tangential width of multiseriate rays appeared to be identical in the two species.

The surface proportion of cumulated parenchyma and small vessels in the latewood was significantly higher for *Q. robur* (58%) than for *Q. petraea* (51%) (Table 2). No difference appeared in the earlywood. Broadened flames were more frequent in *Q. robur* (58%) than in *Q. petraea* (40%) rings. No differences in the degree of forking of flames were observed.

For those features that distinguished *Q. petraea* and *Q. robur*, tree effects were always highly significant (Table 2). Cambial age effect on wood anatomy was much smaller than ring-width effect. This effect of age was not significant for any fiber characteristic.

Multivariate analysis

Principal component analysis

Only the first three components (of 36) displayed high percentages of explained variance (48, 17, and 13%). Table 3 lists the anatomical features displaying the highest correlations with each of these three components. The first was primarily related to variables describing fibers in the latewood (total or average surface area, either in absolute or relative values). The second could be ascribed mainly to vessel characteristics (number, surface, ...). The third represented a size effect (surface or width of earlywood, latewood, or total ring). It must be mentioned that vessel features were also partly correlated with the first axis. Vessel shape correlated with the third component. Parenchyma characteristics were spread in both first and second components.

Table 3. Principal component analysis of anatomical features (232 rings $\times$ 36 wood characteristics): correlation coefficients between wood characteristics and the first three principal components (only correlations higher than 0.40 are listed).

Anatomical features	First PC	Second PC	Third PC
Surface percent of fibers in ring	0.94	—	—
Surface percent of fibers in latewood	0.83	—	0.47
Surface percent of latewood fibers in ring	0.96	—	—
Mean surface area of fiber zones (mm ²)	0.69	—	—
Total no. of earlywood vessels/ring width (mm)	-0.69	0.51	—
Surface percent of earlywood vessels in ring	-0.69	0.58	—
No. of earlywood vessel rows/ring width (mm)	—	0.50	—
Earlywood width (mm)	-0.61	0.44	0.55
Latewood width (mm)	0.62	—	0.62
Latewood percent	0.78	-0.51	—
Ring width (mm)	—	—	0.91
Earlywood vessel shape	—	—	-0.42

Fig. 3. Total surface area of fiber zones per ring area versus total surface area of earlywood vessels per ring area for *Quercus robur* (white) and *Quercus petraea* (black) trees from the Cîteaux Forest (average of four rings per tree). Circles: see Fig. 2.

Discriminant function

Only two variables could be entered in the discriminant equation: total surface area of fiber zones/ring area and total surface area of earlywood vessels/ring area. The discriminant function was (Fig. 3)

$$[2] F = 12.73(\text{total surface area of fiber zones/ring area}) - 30.05(\text{total surface area of earlywood vessels/ring area}) - 0.04$$

Surface area proportions are expressed between 0 and 1. This equation holds for the average of four rings per tree, each around 2 mm in width. The function gives positive values for *Q. petraea* and negative values for *Q. robur*. Using the same sample as for the calculation of the equation, 78% of the 58 trees were correctly reclassified for both species (76% after cross-validation classification).

Relationship between anatomical and morphological characteristics

Figure 4A shows a very clear morphological separation between *Q. petraea* and *Q. robur* in these mixed stands along the

first axis of the principal component analysis. A comparatively large overlap of the two species appears when using anatomical features (Fig. 4B).

Within *Q. petraea*, the three trees that displayed an unusually high number of vessel rows and low latewood percent (Fig. 2) also appeared morphologically closer to *Q. robur* in the multivariate analysis (Fig. 4A). However, the total correlations, at the intraspecific level, between the position of trees along morphological and anatomical multivariate principal components were not significant.

Few correlations between basic anatomical and morphological variables were significant at the 1% level. For *Q. petraea*, the number of foliar lobes was correlated with the latewood width ($r = 0.48$, $n = 29$). This number of lobes was also correlated, although at the 5% level only, with other anatomical variables discriminating between the two species (fiber zone surface areas, vessel surface areas, ...). For *Q. robur*, leaf width was negatively correlated with earlywood width and positively with latewood percent ($r = -0.56$ and 0.52 , respectively, $n = 24$). For the seven *Q. robur* that were not glabrous, a high correlation between the density of pilosity and surface proportion of fibers appeared ($r = 0.92$). Within the *Q. petraea* group, this latter correlation was significant at the 5% level only ($r = 0.38$).

Discussion

New and old discriminant anatomical features

This study has highlighted a large number of anatomical differences between *Q. robur* and *Q. petraea*, which are independent of ring width variations. Some of these were previously known (number of earlywood vessel rows, latewood percent, abrupt or gradual transition from early- to late-wood, shape of latewood vessel groups). We did not confirm two anatomical features previously given as good discriminating characteristics. Forking degree of latewood vessel groups (Walker 1978) was not different between the two species in our sample. Shape of earlywood vessels (Huber et al. 1941; Walker 1978) was also not confirmed in our study. Von Courtois et al. (1964) and P.S. Savill and R.A. Mather (personal communication in Huber 1993) also found no specific differences for the latter feature.

Fig. 4. Principal components analyses of foliar morphological measurements (A) and wood anatomy characteristics (B) of *Quercus robur* (white) and *Quercus petraea* (black) trees from the Cîteaux Forest. Plane of components 1 and 2. Circles: see Fig. 2.

The main new anatomical features displaying highly significant differences between the two species were related to fiber zone size and area. *Quercus petraea* displayed larger zones, and they occupied a larger part of the ring surface. Consequently, parenchyma and small vessels cover a smaller area. Differences were higher in late- than early-wood.

Differences in the number of earlywood vessel rows were quantified in terms of vessel number and surface area. We observed a significantly larger total surface area of earlywood vessels in *Q. robur*. This difference is due to a higher number of vessels (already observed by Deret-Varcin 1983), but not to different individual vessel sizes, which did not differ between the two species. Mather et al. (1993) also reported the lack of individual vessel area difference.

The absence of differences between multiseriate ray characteristics was not conclusive in our study as a result of a measurement window that was too narrow. However, G. Nepveu and F. Huber (personal communication) also observed a constant number of multiseriate rays (4.3/circumferential cm) from different pure stands of both species.

Species identification using wood anatomy

Although numerous anatomical features had significantly different means between the two oak species, no single variable provided a reliable method for species separation, as has already been observed by Huber et al. (1941) and Walker (1978). We even observed a greater overlap of values between species

than the previous authors for important diagnostic features, such as the number of earlywood vessel rows. We never observed only one vessel row in *Q. robur* rings. But *Q. petraea* rings displayed up to three vessel rows (13% of cases) and in exceptional cases, four (2%).

Unfortunately, the multivariate approach did not produce an unambiguous means of wood recognition, despite a clear gap of foliar morphology between trees of the two species in our sample. Our discriminant function should provide a 78% success rate for species identification, which is no better than previously published methods. However, it uses only two weakly linked variables that can be objectively measured (surface proportion of fiber zones and surface proportion of earlywood vessels), whereas previous keys were based on four or five features, often highly correlated and partly qualitative. We used a computer analysis of X-ray images to build our discriminant function, techniques that are fast but not always available. However, fiber and vessel area proportions can be obtained from classical microscopic analysis of thin cuts. Finally in Fig. 2, we give the scattergram for two variables that are easier to measure, but less reliable: latewood percent and number of earlywood vessel rows. The problems encountered in multivariate-based wood recognition can be related to the high correlations existing between all the anatomical features that we measured.

It must be pointed out that Masson et al. (1995) and Mosedale and Savill (1996) obtained a clear separation between species based on oak lactone contents. However, this result is still based on a limited sample.

Consequences of anatomical differences on wood properties and tree functioning

The different proportions of latewood fibers and earlywood vessels observed for the two oak species explain a large range of wood property variations, especially density and shrinkage. Previous studies (cf. Nepveu et al. 1996) of *Q. petraea* wood tissue densities gave the following estimations: 1200 kg/m³ for fibers, 700 kg/m³ for parenchyma, and 300 kg/m³ for large vessels. Using these values for both species, we calculated an estimated average density from our measurements of tissue proportions (Table 2). We obtained 787 kg/m³ for *Q. robur* and 835 kg/m³ for *Q. petraea*. This difference (6% in relative value) is in agreement with or slightly less than previously measured mean density differences: Deret-Varcin (1983, 9%), Nepveu (1984, 6%), Lévy et al. (1992, 11%). However, the specific wood densities of each tissue have been shown to vary significantly between trees (Nepveu et al. 1996). Thus, it is also probable that these tissue densities, especially for fibers, are higher for *Q. petraea* than for *Q. robur*.

The larger total earlywood vessel surface area, and thus hydraulic conductivity, of *Q. robur* (25% more area than *Q. petraea* for a given ring width, Table 2) could explain its higher water requirements (Lévy et al. 1992) and play a role in differences in vulnerability to cavitation (Cochard et al. 1992).

Genetic basis of anatomical variations

The wood anatomy differences observed between species and between trees are largely genetically based because we worked in mixed stands and controlled age and ring width effects. Thus, these important economic and ecological characteristics could be used for genetic improvement. This genetic control

has already been demonstrated for several anatomical features (see Nepveu 1993 and Savill and Kanowski 1993, for a synthesis).

However, we must point out that ring width (or surface) effects on anatomical properties were frequently very strong (Table 2). Thus, it is essential that any attempt to recognize species should take this effect into account either by a careful selection of constant ring widths or by statistical correction. This is especially relevant to mixed stands, where systematic differences of ring width have been reported in favour of *Q. petraea* oak (Lévy et al. 1992). Unexpectedly, the age seemed to play a minor role in the observed anatomical variations.

Despite the clear foliar morphological gap observed between the two species in our sample, the large overlap for all wood characteristics is perhaps due to hybridization and introgression. This genetic exchange is more likely to occur in mixed stands, as suggested by results from large morphological surveys (Grandjean and Sigaud 1987). Thus, the 78% rate of successful identification we obtained in mixed stands could be higher in pure stands, where populations of the two species are probably more genetically differentiated.

Anatomical characteristics were only weakly linked to morphological ones. However, our sample was probably not adequate for the study of these relationships: there were not enough trees and, more importantly, there were a limited range of site variations. Such a lack of correlation between morphology and other sets of characteristics within the oak complex has also been noted by Bacilieri et al. (1995). The negative link that appeared between leaf size and earlywood width could be ecophysiologically meaningful and deserves more attention. It could be the consequence of a trade-off between allocation to vessels or leaves at bud burst time.

Conclusions

We observed numerous significant anatomical differences between *Q. robur* and *Q. petraea*, which have important ecological and economic implications. Our results provide a reliable basis for wood recognition within the *Q. robur* – *Q. petraea* complex. We propose the following guidelines for the identification of an undetermined oak wood sample:

- (1) If possible, select four rings around 2 mm wide and between 60 and 120 years old.
- (2) Measure the latewood percent and count the number of earlywood vessel rows for each ring, then average.
- (3) Plot the result on Fig. 2. If the point falls at one of the extremities of the cloud of points, the species is determined (two vessel rows or more and latewood percent less than 0.6 for *Q. robur*, two vessel rows or less and latewood percent more than 0.7 for *Q. petraea*); use appearance of latewood vessel groups (flames) to confirm your diagnostic (see Table 2).
- (4) If the average point falls between the two species, or if a more secure identification is needed, then prepare a thin cut or a radiography of the four selected rings.
- (5) Measure fiber surface area and earlywood vessel area under the microscope (on a drawing or a picture of the ring, or by image analysis); calculate, for each ring, the relative proportion from total ring area (not including rays), then average.
- (6) Plot the resulting point on Fig. 3 and compute the discriminant function, using eq. 2. Positive values are expected for

Q. petraea, negative for *Q. robur*. The rate of successful identification should be around 75%.

Other anatomical features could be tested and could further improve this rate of species recognition: cell wall thickness measurements or the study of tangential and radial sections (characteristics of pits). Microdensitometric variations of wood tissues or their chemical characterization seem to be even more promising.

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