Ann. For. Sci. 61 (2004) 73–80
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DOI: 10.1051/forest:2003086

Original article

Leaf morphology as species indicator in seedlings of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl.: modulation by irradiance and growth flush

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(Received 7 February 2002; accepted 17 February 2003)

Abstract – Morphological description of leaves provides the most reliable criteria to discriminate between the two oak species *Quercus petraea* and *Q. robur*. However, most earlier studies only assessed leaf morphology of adult trees, whereas interspecific variations between seedlings were poorly documented. We studied variations of leaf morphology on two-year-old seedlings growing in a common garden experiment and exposed to different irradiance regimes. Morphological measurements were performed on leaves from each growth flush. Large interspecific differences were detected. The discrimination between the two species was slightly better with first flush leaves. Irradiance influenced leaf size, but did not affect the characters usually used to discriminate the two species, like relative petiole length or angle of auricle at the base of the lamina. A discriminant function, based on the two most discriminating traits (lamina pilosity density and angle of auricles at the lamina base), provided less than 0.5% leaf misclassification. It appeared that, contrary to what is generally accepted, species recognition of oak seedlings based on leaf morphology is possible. Moreover, this is true whatever the irradiance regime, and even slightly easier under light shade than in full sun.

morphology / leaf / light / flush / oak

Résumé – La morphologie foliaire comme indicateur spécifique chez les semis de *Quercus robur* L. et *Q. petraea* (Matt.) Liebl.: variation avec l'éclairement et l'unité de croissance. La discrimination taxonomique des espèces *Quercus petraea* et *Q. robur* est essentiellement basée sur l'examen des fructifications, souvent absentes, ou de la morphologie foliaire. Alors que l'ensemble des travaux sur le sujet concerne des arbres adultes, nous avons étudié les variations morphologiques foliaires de jeunes plants de *Q. petraea* et *Q. robur* croissant sous différents régimes d'éclairement. La morphologie des feuilles des différents flushs a été analysée durant la deuxième année de croissance des plants en pépinière. Une analyse factorielle des correspondances portant sur l'ensemble de l'échantillon et intégrant toutes les variables morphologiques aboutit à une discrimination interspécifique nette dans la majorité des cas. La discrimination apparaît cependant meilleure pour les feuilles de la première unité de croissance. Le régime d'éclairement a principalement affecté la taille des feuilles. Les variables usuelles permettant la discrimination morphologique entre *Q. petraea* et *Q. robur* n'ont pas été affectées par les différences d'éclairement. Une fonction discriminante a été construite avec les deux variables les plus discriminantes (densité de pilosité du limbe et angle des oreillettes), aboutissant à moins de 0.5 % d'erreur dans le classement des feuilles. Contrairement à une opinion répandue, la discrimination des espèces de chêne est possible dès le stade jeune plant. De plus, elle ne nécessite pas obligatoirement l'observation des seules feuilles développées en pleine lumière. Elle est même légèrement meilleure sous un léger ombrage.

morphologie / feuille / lumière / unité de croissance / chêne

1. INTRODUCTION

An efficient identification of *Quercus petraea* and *Q. robur* is essential for foresters and scientists because these two interfertile and sympatric oak species display different ecological requirements [16]. Because of the large variability among individuals in morphological features, this identification may be difficult, in particular within mixed stands where the taxonomical status of trees is often uncertain. In the absence of fruits, leaf morphology remains one of the most reliable criteria to discriminate between the two species [1, 7, 9, 12, 13, 15]. Consequently, many studies comparing *Q. petraea* and *Q. robur* use identification criteria based on leaf morphology. In particular, genetic studies aiming to find species-specific molecular markers still rely on morphological characters to define these two species [6, 18, 19].

Most of the studies assessing interspecific variations of leaf morphology were performed on sun leaves of adult trees. Kleinschmit et al. [14] analysed the morphology of offspring from controlled crosses and reported the occurrence of a juvenile leaf morphology, which differed from that of adult individuals. Nevertheless, there is still a need to unequivocally

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identify seedlings from the two species in natural regenerations (i.e., shaded by surrounding adult trees). For sun leaves of adult trees, the best features discriminating between the two species are pilosity development, presence of intercalary veins, length of petiole and angle of the auricles at the lamina base [3, 7, 8]. However, leaf morphology is influenced by shading. Besides the well-known increase of leaf size and decrease of thickness [2] under shade, Blue and Jensen [5] indicated that sun leaves of oak (*Q. velutina, Q. rubra* and *Q. palustris*) had larger and deeper sinuses, a narrower blade and a higher number of veins than shade leaves located at the same level in the crown. Rushton [23] and Baranski [4] considered that, for leaf characters, the typical expression of the genotype of a tree occurs in the most light-exposed part of the crown.

Another source of variation of leaf morphology is related to the polycyclic pattern of growth of oaks that usually build two or three growth units (flushes) during the growing season. Kissling [13] found that leaves of the second flush presented a narrower base, shallower lobes, a reduced pubescence and a shorter petiole than those of the first flush. This is of particular importance as the shape of the lamina base, pilosity and petiole length are among the main criteria used to discriminate the two species.

The objectives of the present study were:

– to check whether the discrimination was still possible among the two species (i) on seedlings, (ii) under different light environments and (iii) with leaves from various growth flushes;

– to examine the variations induced in leaf morphology on seedlings exposed to different irradiance regimes during their development;

– to assess the variations in morphology occurring between flushes and to compare them with light-induced variations;

– to provide a useful tool for a rapid identification of oak seedlings from these two species, under different irradiance levels and at different stages of development.

To answer these questions, seedlings of *Q. petraea* and *Q. robur* were grown under different irradiance regimes during two years. Leaf morphology measurements were performed at the end of the experiment.

Table I. List of descriptors of leaf morphology measured or calculated. Letters in parentheses refer to labelled points in Figure 1.

Primary variables
Dimensional characters
PL: petiole length (A-B, cm)
LL: lamina length (B-D, cm)
PERI: lamina perimeter (cm)
AREA: lamina surface area (cm^2)
LW: maximal lamina width (E-F, cm)
LWL: length of lamina to the largest width (B-G, cm)
MASS: leaf mass (g)
Lobes and veins
NLOB: number of lobes (except the terminal lobe, irrigated by the midrib)
NLUB: number of lobules (lobes irrigated by a third order vein)
LOBL: mean of the length of the six largest lobes (e.g. HI, mm)
LOBT: mean of the thickness of the six largest lobes (e.g. LK, mm)
LOBH: mean of the height of the six largest lobes (e.g. JM, mm)
NIV: number of intercalary veins (veins irrigating a sinus)
AURI: average angle of the two auricles at the lamina base (e.g. A-B-C, °)
AVEIN: mean of the vein angles of the six largest lobes (e.g. K-G-D, °)
ASIN: mean of the sinus angles of the six largest lobes (e.g. I-J-K, °)
ALOB: mean of the angles of the six largest lobes (e.g. J-K-M, °)
Abaxial pubescence
PPD: petiole pilosity density, score from 0 (hairless) to 6 (dense pilosity); grading system from Kissling [13]
MPD: midrib pilosity density
LPD: lamina pilosity density
PPL: hair length on the petiole, graded from 0 (hairless) to 4 (very long)
MPL: hair length on the midrib
LPL: hair length on the lamina
Calculated variables
PRL: relative length of the petiole, PL / (LL+PL) (%)
LRW: relative width of the lamina, LW / LL (%)
LWRL: relative length of lamina at largest width, LWL / LL (%)
ARPE: surface area to perimeter ratio, AREA / PERI
ISOP: isoperimetric deficit, $1 - (4\pi \times \text{AREA} / \text{PERI}^2)$
ELD: elliptic deficit, $(\pi \times \text{LL} \times \text{LW}) / (4 \times \text{AREA})$
IVLOB: number of intercalary veins per lobe, NIV / NLOB (%)
RLIV: relative length of intercalary vein (e.g. NO/NP, %)
PERINL: perimeter to number of lobes ratio, PERI / NLOB (cm)
LUBLOB: number of lobules per lobe, NLUB / NLOB (%)
LMA: leaf mass per area, MASS / AREA ($g \cdot cm^{-2}$)

2. MATERIAL AND METHODS

2.1. Plant material and experimental design

Acorns were collected during autumn 1997 in two adjacent pure stands of *Q. petraea* and *Q. robur* in the forest of Perseigne (48° 24' 21" N, 0° 19' 33" W, Western France). Adult trees were identified based on acorn peduncle size and characteristic leaf traits [8]. The two specific sets of acorns were a composite harvest from around 20 randomly selected trees. Acorns were sown in an experimental nursery (Champenoux, Nancy, N.E. France) during spring 1998. Seedlings were grown in 10 litre plastic containers filled with a sand/peat mixture (2/1, v/v) until autumn 1999. They were automatically irrigated twice a day and fertilised two times during summer (Nutricot®, N/P/K 13/13/13 + trace elements, 4 $g \cdot L_{(substrate)}^{-1}$). Before germination, the containers were distributed to four treatments differing by transmitted irradiance: 8 (deep shade), 18 (medium shade), 48 (light shade) and 100% (full sun) of external global irradiance. Shading was obtained using shelters built with polyethylene nets incorporating aluminium strips. Three different mesh sizes provided the required levels of transmitted irradiance.

Ten seedlings were randomly selected per species during autumn 1999 for subsequent morphological analysis in each of the four irradiance treatments. A detailed description of the experimental design and microclimate is provided in Ponton et al. [21].

2.2. Morphological analysis

At the end of the experiment, seedlings had developed up to four growth flushes. This number of flushes was influenced by growth irradiance, but there was no significant interspecific difference within an irradiance regime. Before leaf senescence, one fully expanded leaf per flush was sampled from between 8 and 10 seedlings per species and irradiance level. Leaves of the fourth flush were discarded because it developed on only 21% individuals of the total sample.

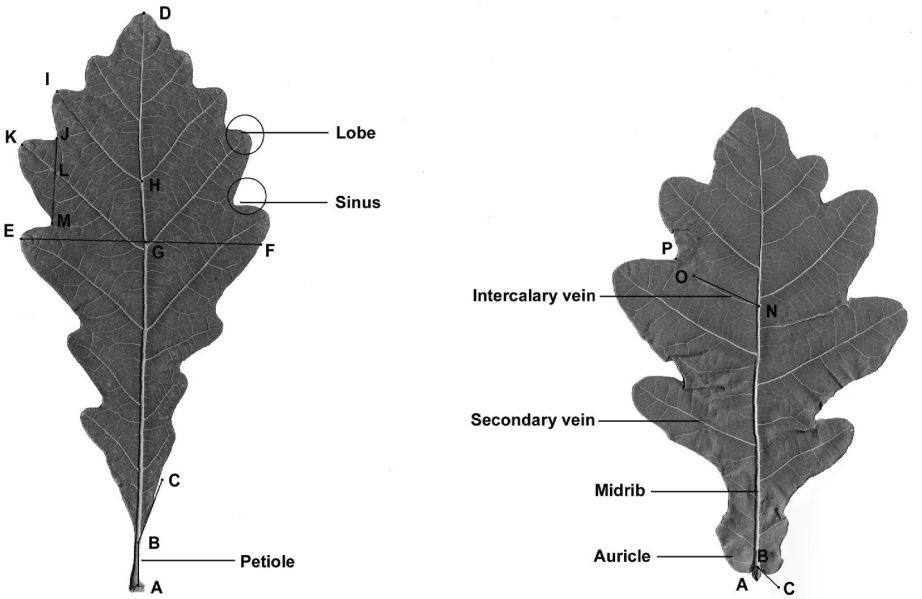

Figure 1. Typical leaves of *Q. robur* and *Q. petraea*. The landmarks used for morphological measurements are indicated.

Leaves of the third flush of *Quercus petraea* grown under 8% irradiance could be collected from five trees only. For each growth flush on the main stem, the largest leaf of the final rosette (top of the growth unit) was harvested. Selected leaves were free of insect attacks or disease symptoms. Finally, 221 leaves were sampled from 80 trees. Measurements were performed with a digitizing tablet interfaced with a computer. The protocol of leaf morphology assessment is described by Dupouey and Badeau [8]. Twenty-three variables were measured and used to derive 11 calculated variables (Tab. I and Fig. 1). These variables concern various aspects of leaf morphology such as size, shape and pilosity. The dry mass of each leaf was measured and the leaf mass to area ratio calculated.

2.3. Statistical analysis

The set of morphological descriptors included quantitative continuous variables as well as quantitative discrete (counted variables) and qualitative variables (describing pubescence). In order to analyse the relationships between these variables, all the quantitative continuous variables were converted into scored variables, each made of five equidistributed groups (equal number of observations in each group). When discrete and qualitative variables contained initially more than five groups, initial groups were combined into five new groups with equal or nearly equal numbers of observations. Then, these 34 variables × 5 classes were analysed by multiple correspondence analysis (MCA). A stepwise discriminant analysis was performed to select the two best variables for species discrimination.

The effects of species, irradiance regime, growth flush and their interactions on continuous morphological variables and on MCA factors were estimated and tested with an analysis of variance (ANOVA). The following linear model was used:

$$Y_{ijkl} = a + b_i + c_j + d_k + (bc)_{ij} + (bd)_{ik} + (cd)_{jk} + e_{ijkl} \qquad \text{(model 1)}$$

with Y_{ijkl}: measured value for flush k of seedling l, within species i, under irradiance regime j, a: overall mean, b_i: effect of species i, c_j: effect of irradiance regime j, d_k: effect of flush k, $(bc)_{ij}$: interaction between effects of species and irradiance regime, $(bd)_{ik}$: interaction between effects of species and flush, $(cd)_{jk}$: interaction between effects of irradiance regime and flush, e_{ijkl}: error term.

Third order interaction between irradiance, species and flush effects was never significant. Thus, it was finally excluded from the model. The effects of species, irradiance regime and growth flush on quantitative discrete (PL, NLOB, NLUB, NIV) and qualitative morphological variables (pubescence related variables) were separately tested using a Chi-square test. Tukey studentized range tests (also called HSD) were used for multiple comparisons of means. Pearson and Spearman (rank) correlation coefficients were used as exploratory tools. Data are presented as means ± standard deviation. Statistical analyses were performed using the SAS software (SAS, version 6.03, Institute Inc., Cary, NC, USA) [24].

3. RESULTS

3.1. Modulation of leaf morphology by irradiance

The effects of irradiance regime on leaf morphology were mainly visible on size related variables (Tabs. II and III), but also on relative length of petiole (PRL) and leaf mass per area (LMA). Irradiance regime was the first source of variation (25% of total variability) for leaf length (LL + PL). Very significant correlations occurred between leaf length and other dimensional traits (LWL, PERI, AREA, LOBL, LW, LOBT… see Tab. IV). Beside these expected correlations, a weaker but still significant correlation was observed with petiole length ($r = 0.58$, $P < 0.001$ for *Q. petraea* and $r = 0.37$, $P < 0.001$ for *Q. robur*) which is usually considered to be one of the most reliable traits to discriminate between the two species. No correlation occurred with relative petiole length. Leaf length was maximum under medium shade (12.5 ± 2.4 cm), decreased in light shade (11.6 ± 2.3 cm) and deep shade (10.3 ± 1.9 cm), and was minimum under full sun (9.1 ± 2.2 cm). The other size variables (MASS, LW, LWL, PERI, SURF, NLOB, LOBL, LOBT, LOBH) displayed the same trend. This pattern of irradiance regime-induced variations was observed on both species, although it was slightly more marked on *Q. robur* (larger magnitude of values from medium shade to full sun).

Table II. Sources of variation (species, irradiance regime, flush and their interactions) for morphological variables and coordinates on the first two axes of MCA, revealed by ANOVA. *P*-values are symbolized as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and non significant otherwise. Variables for which no significant effect was observed are not shown.

Variable	Species	Irradiance	Flush	Species* light	Species* flush	Light* flush
MASS	***	***	***	*		*
LMA	***	***	***			***
LL		***	***		*	
LTOT	**	***	***		*	
LW		***				
LWL	*	***	***		**	
PERI		***	***			
AREA	*	***	***			
PRL	***	***	***	*		*
LRW			***			
ARPE	***	***	*			*
ISOP	***	**	***			***
ELD	***	*	***			
RLIV	***		***		***	
PERINL	***	***	***	**		
LUBLOB			*			
IVLOB	***		***			
AURI	***		**			
LOBL	***	***				
LOBT	***	***	***		*	
LOBH	***	***	**			
AVEIN			**	*		
ASIN	**		*			***
ALOB	***					*
MCAaxis1	***	***	***	*	**	*
MCAaxis2	***	***	***			

Table III. χ^2 test of species, light and flush effects on morphological discrete variables. See Table II for significance of stars.

Variable	Species	Irradiance	Flush
PL	***		**
PPD	***		
PPL	***	*	
MPD	***		
MPL	***	*	*
LPD	***		
LPL	***		***
NLOB	***		
NLUB		**	*
NIV	***		***

Table IV. Significant Pearson's correlation ($P < 0.05$) between leaf length (LL + PL) and others morphological traits ($n = 221$) or coordinates on the first two axis of MCA. See Table II for significance of stars.

Variable	Coefficient of correlation
LL	0.99 ***
LWL	0.89 ***
PERI	0.89 ***
AREA	0.89 ***
LOBL	0.81 ***
MCA axis2	0.79 ***
LW	0.77 ***
LOBT	0.76 ***
MASS	0.74 ***
ARPE	0.71 ***
PERINL	0.58 ***
LOBH	0.52 ***
MCA axis1	–0.44 ***
PL	0.43 ***
LRW	–0.35 ***
NLOB	0.32 ***
ELD	0.26 ***
ISOP	0.22 **
NLUB	0.22 **
LMA	–0.14 *

As expected, leaf mass per area gradually increased with increasing irradiance. Leaves of medium and light shades had a higher degree of dissection of the blade than leaves of deep shade and full sun (ELD, ISOP). These two dissection parameters were weakly related to leaf size ($r = 0.26$, $P < 0.001$ and $r = 0.22$, $P < 0.01$, respectively; Tab. IV).

3.2. Leaf morphology variation among growth units

Flushing was the second source of variation in leaf size (20% and 7% of the total variability of leaf length and surface area, respectively; Tabs. II and III). Leaf size increased with flush rank in both species: average leaf lengths were 9.4 ± 2.0 cm, 11.5 ± 2.4 cm and 12.1 ± 2.4 cm, from the first to the third flush, respectively. Lamina dissection (ELD, ISOP) increased from the first to the third flush, even if the ratio 'number of lobes to perimeter' slightly decreased. The effects of growth flush rank on leaf size and lamina dissection were larger on *Q. petraea* than on *Q. robur* (larger range of values from flush 1 to flush 3). For both species, the number of intercalary veins (NIV) increased from flush 1 to 3 (0.3 ± 0.6 to 2.3 ± 1.6 for *Q. petraea*, 2.5 ± 0.5 to 3.9 ± 1.7 for *Q. robur*). Lamina pubescence (LPL) decreased from flush 1 to 3, in *Q. petraea* only. The angle of lamina base (AURI) was larger in flushes 1 and 2 than in flush 3 in *Q. petraea*. In *Q. robur*, auricles at the lamina base were slightly more developed in flush 3 than in flush 2 (i.e. larger AURI values in flush 2).

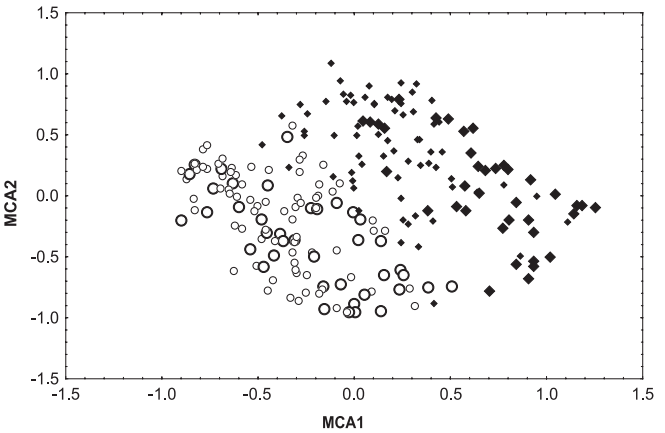

Figure 2. Position of the 221 leaves along the first two factors of a multiple correspondence analysis (MCA). Large symbols: leaves from flush 1, small symbols: leaves from flushes 2 and 3. *Q. petraea*: ○, *Q. robur*: ◆.

3.3. Species differentiation

The three first synthetic variables of MCA explained 6.9%, 6.6% and 4.0% of the total variance, respectively, over a total of 170 classes analysed. The first factor was highly correlated with morphological traits that are usually recognized as species specific (in descending order of correlation: AURI, LPD, MPD, NIV, LPL…). The second factor of MCA correlated with size variables (in descending order of correlation: LOBL, LL, LW, AREA, PERI, LWL…). The third synthetic variable was mainly related to the degree of lamina dissection (in descending order of correlation: ARPE, AREA, ISOP, ELD, ASIN, ALOB…). The plane of the first two axes of MCA showed a clear discrimination between the two species, with very limited overlap (Fig. 2). When considering leaves from the first flush only, a total separation was observed between the two species on this factorial plane. Average values of the main discriminant variables for the two species are given in Table V.

The two first variables selected by stepwise discriminant analysis of leaf morphology were lamina pilosity density (LPD) and average angle of auricles at lamina base (AURI). The discriminant function was (Fig. 3):

$$ID_1 = 2367 - 537 \times LPD - 13 \times AURI.$$

This function provides positive ID_1 values for *Q. robur* and negative ones for *Q. petraea*. According to this function, the percentage of misclassification of leaves was below 0.5% (one misclassified leaf over 221). The whole data set showed a very clear bimodal distribution for ID_1 values (Fig. 4). The degree of discrimination between the two species was separately tested for each flush by a comparison of mean ID_1 values. The discrimination was larger for the leaves from the first flush ($F = 827$, $P < 0.001$), intermediate for the leaves from the second flush ($F = 540$, $P < 0.001$), and lower for the leaves from the third flush ($F = 463$, $P < 0.001$). Tested by the same method within each irradiance regime, the species discrimination was larger in light shade ($F = 723$, $P < 0.001$) than in medium shade ($F = 548$, $P < 0.001$), and lower under full sun ($F = 342$, $P < 0.001$) and deep shade ($F = 339$, $P < 0.001$). The only one misclassified leaf came from the third flush of a *Q. robur* seedling growing under deep shade.

Table V. Minimum, average and maximum values of discriminant or remarkable morphological traits for *Q. petraea* and *Q. robur*. Leaves from all light treatments, but from the first flush only, are taken into account.

Variable	*Q. petraea* ($n = 38$)			*Q. robur* ($n = 40$)		
	Min	Average	Max	Min	Average	Max
LPD	2.0	4.0	5.0	0.0	0.2	2.0
MPD	1.0	2.8	4.0	0.0	0.8	2.0
PPD	0.0	0.9	4.0	0.0	0.0	0.0
LPL	1.0	1.6	3.0	0.0	0.3	3.0
MPL	2.0	3.5	4.0	0.0	2.2	4.0
PPL	0.0	1.3	4.0	0.0	0.0	0.0
AURI (°)	112	143	155	–10	41	113
PL (mm)	2.0	5.1	10.0	0.0	1.6	5.0
LL (mm)	57	88	123	58	92	145
AREA (cm^2)	11	28	53	10	28	70
NIV	0.0	0.3	2.0	0.0	2.5	6.0
NLOB	6.0	12.3	18.0	8.0	10.6	15.0
NLUB	0.0	1.6	10.0	0.0	1.1	4.0
LMA ($g \cdot cm^{-2}$)	0.36	0.68	1.11	0.38	0.65	1.08

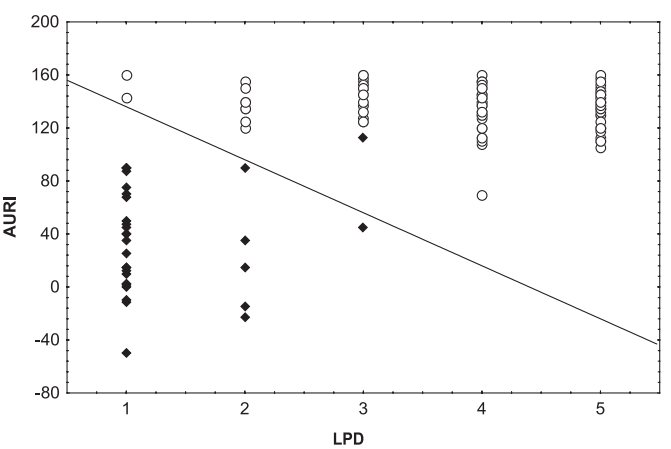

Figure 3. Distribution of the 221 leaves according to the average angle of auricles at the lamina base (AURI) and the lamina pilosity density (LPD). The diagonal straight line is the discriminant line: $2367 - 537 \times LPD - 13 \times AURI = 0$. *Q. petraea*: ○, *Q. robur*: ◆.

We tested the stability across growth flushes of morphological traits which discriminate between the two species. Ranking of the seedlings according to their ID_1 values strongly varied from one flush to the next. Rank correlations were not significant for *Q. robur* ($P > 0.05$) and only significant

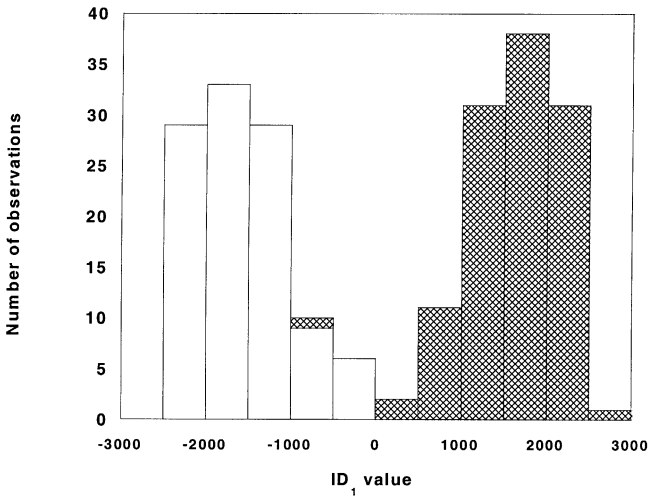

Figure 4. Distribution of the discriminant function values: $ID_1 = 2367 - 537 \times LPD - 13 \times AURI$ for the 221 tested leaves.

between flushes 1 and 2 ($r = 0.35$, $P < 0.05$) and between flushes 2 and 3 ($r = 0.37$, $P < 0.05$) for *Q. petraea*.

To compare our results obtained on seedlings with those obtained on adult trees, another discriminant function was calculated based on petiole length (PL) and number of intercalary veins (NIV), as proposed by Dupouey and Badeau [8] and Kremer et al. [15]:

$$ID_2 = 4178 - 1507 \times PL + 900 \times NIV.$$

Using this function resulted in the misclassification of 13 *Q. robur* and 11 *Q. petraea* leaves (11% of the total number of leaves).

4. DISCUSSION

The multivariate correspondence analysis separated the sampled leaves into 2 groups corresponding to the two oak species, *Q. robur* and *Q. petraea* (Fig. 2). As frequently mentioned, no absolute diagnostic character discriminating between the two species could be detected [1, 3]. However, species discrimination was already very efficient with a combination of only two morphological traits. This situation is in agreement with what has been classically observed on sun leaves collected from first growth flush of adult trees, where a clear bimodal distribution was detected for synthetic discriminant variables [3, 8, 15].

On adult trees, Bacilieri et al. [3] observed 1% misclassification by means of a factorial discriminant analysis computed with 16 morphological characters, and Kremer et al. [15] calculated a discriminant function resulting in 1.6% misclassification with two variables only. This rate was obtained using samples of five leaves per tree and increased up to 5.6% with samples of only one leaf. Based on average angle of auricles at lamina base (AURI) and lamina pilosity density (LPD), our discriminant function (ID_1) revealed only one misclassified leaf (Fig. 3). These two traits appeared to be very discriminant but are not very easy to measure. However, using the more classical petiole length (PL) and number of intercalary veins (NIV), as did Kremer et al. [15], resulted in 11% misclassification in our sample. Thus, these two last traits were not as reliable in the case of young seedlings as for adults.

Even if species identification with ID_1 values was correct in nearly all cases, it appeared that the discrimination between *Q. robur* and *Q. petraea* was better with leaves from the first flush than from the followings. The number of intercalary veins (NIV) was strongly influenced by flush, what could partially explain the mediocre species discrimination based on ID_2 values. Leaves from flushes 2 and 3 differed less from each other than they did from flush 1, as already observed by Masarovicova and Pozgaj [17]. Potter [22] reported that leaves exhibit a larger variability on the second than on the first flush. These observations could be related to the fact that the first growth unit is entirely preformed in the winter bud, whereas the second and third growth units bear both preformed and neoformed segments [10]. Thus, neoformed leaves could be under stronger influence of the environment than preformed ones, the development of the latter being probably more genetically controlled. This hypothesis is reinforced by the observation that oak epicormic shoots, which usually display atypical leaves similar to those found on lammas (second flush) shoots, are also entirely neoformed [11]. Following previous authors [13, 20, 22], we recommend that leaves from both lammas and epicormic shoots should be discarded from samples used for species discrimination.

According to the result of the F-test of species effect on ID_1 under each irradiance regime, individuals growing under intermediate levels of shade displayed the most species-discriminating features. This observation opposes the common practice for adult trees, where shaded leaves are discarded for species identification [5, 8, 20, 23]. This effect of light regime could be partly related to the leaf size, because we observed a link between irradiance level, leaf size and species discrimination: for a given flush, small leaves of *Q. robur* and *Q. petraea*, grown under full sun or deep shade were poorly separated, whereas larger leaves were better identified, as was the case under light and especially medium shade (Fig. 5). Finally, one can note that under natural regeneration conditions, the fraction of global irradiance reaching forest floor is generally between 20 and 40%, which corresponds to the range of irradiance where the specific discrimination was the best in our study.

5. CONCLUSION

Our results showed a clear interspecific discrimination among leaves sampled on oak seedlings exposed to different irradiance regimes and collected on successive growth units. Identification of the species, at the seedling stage, was possible using only two morphological traits, the density of pilosity of the lamina and the average angle of auricles at the lamina base. Consequently, *Q. robur* and *Q. petraea* offspring can be discriminated in experimental or natural conditions, whenever shaded or not by surrounding adult trees, and preferably using leaves from the first flush. Species recognition could probably

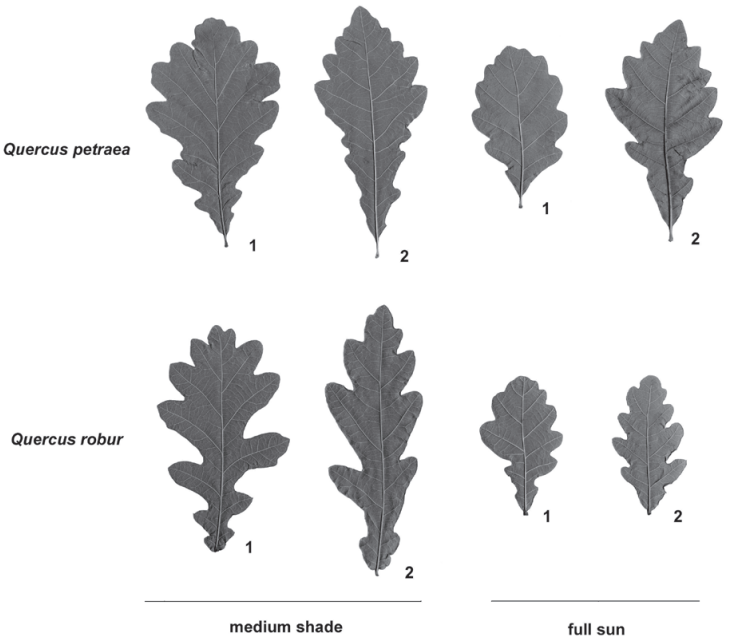

Figure 5. Typical leaves of *Q. robur* and *Q. petraea* grown under full sun or medium shade. Leaves from flushes 1 and 2 were sampled on the same tree.

be even improved using of a discriminant function based on the average measurements of several leaves collected on the same tree.

Acknowledgements: S. Ponton was supported by a PhD grant from the Institut National de la Recherche Agronomique and from the Regional Council of Lorraine. We gratefully acknowledge the technical assistance of Roger Schipfer, Virginie Legroux, Sébastien Diette and Christian Kieffer for morphological analyses, and of Jean-Marie Gioria for seedling cultivation. Financial support from the French initiative "GIP-Ecofor" for experiment installation is gratefully acknowledged. The study was partly carried out with a 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").

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