Eur J Forest Res (2013) 132:61–69
DOI 10.1007/s10342-012-0654-1

ORIGINAL PAPER

Distribution of heartwood extractives in hybrid larches and in their related European and Japanese larch parents: relationship with wood colour parameters

Luc E. Pâques · Maria del Carmen García-Casas · Jean-Paul Charpentier

Received: 7 September 2011 / Revised: 27 April 2012 / Accepted: 7 August 2012 / Published online: 25 August 2012

Abstract Heartwood is the most valuable part of larch timber when either natural durability or aesthetic aspects of the wood are required. Both properties are directly linked to chemical extractives and particularly to phenols. Based on a broad sample of trees (583) from European and Japanese larch and their interspecific hybrid, we investigated the variability in phenolic content, and particularly of two major compounds, Taxifolin (Tax) and Dihydrokaempferol (DHK), and their link with wood colour. At the individual wood sample level, phenolic contents ranged from 6.0 to 55.9 mg eq. Tax/g DW. Taxifolin was the most abundant constituent (range: 1.3–41.7 mg/g DW) compared with DHK (0.5–13.7 mg eq. Tax/g DW). A high variability among taxa, genotypes and individual trees within taxa and within trees was observed. Japanese larch had the highest amount of total phenols and of Taxifolin and European larch the lowest. For DHK, Japanese larch was the poorest compared with European larch. Hybrid larch had both a high content of Taxifolin and of DHK. Variability for colour parameters was on average weaker than for phenolic content but still large enough to show significant differences between taxa. Correlations between colour parameters and extractives were moderate to weak. At the mean genotype level, a good link ($r > 0.51$, $p < 0.001$) was found between a* (red–green axis in CIELAB) and total phenols and Taxifolin contents and between L* (Lightness) and DHK content ($r > 0.54$). The broad variability observed in this study at different levels for phenolics offers opportunities for breeders to genetically improve the quality of larch heartwood, in particular in relation to decay resistance.

Keywords Larch · Heartwood · Extractives · Phenolic compounds · Colour

Communicated by T. Seifert.

L. E. Pâques · M. d. C. García-Casas · J.-P. Charpentier (✉)
INRA, UR 0588, AGPF, Centre de recherche d'Orléans,
2163 avenue de la Pomme de Pin CS 40001 ARDON,
45075 Orleans Cedex 2, France
e-mail: jean-paul.charpentier@orleans.inra.fr

L. E. Pâques
e-mail: luc.paques@orleans.inra.fr

Introduction

Heartwood is the most valuable part of timber for species characterised by a high natural durability or decay resistance, making it suitable for outdoor use. The high natural durability of heartwood and inversely the low durability of sapwood have been demonstrated in several species. In addition, heartwood is also highly appreciated for aesthetic purposes with its rich coloured wood that contrasts with the pale sapwood.

The higher natural durability of heartwood has been linked to extractives which include terpenoids, tropolones, flavonoids, stilbenes and other aromatic compounds (Scheffer and Cowling 1966; Pometti et al. 2010), while colour of wood depends on the chemical compounds interacting with light (Hillis 1987). Therefore, several authors have found an indirect connection between the colour of wood and its natural durability (Moya and Berrocal 2010).

Larch (*Larix* sp.) is one of the most valuable conifers in boreal and temperate forests as well as in mountainous regions where it is either native or introduced in artificial plantations. It is highly appreciated for wood properties including high mechanical strength, attractive reddish

colour and high natural durability. The early and abundant extension of the heartwood is also a typical feature of larch which makes it unique among conifers (Pâques 2001). These properties justify why traditionally larch wood has been used both outdoors (construction of houses and bridges) and indoors (wall panelling and flooring).

However, a large variability in the heartwood properties studied so far in the *Larix* genus has been revealed depending both on genetic (species, genetic origin within species, individual within origin) and on environmental effects (site, silviculture) but also position within the tree and age. This has been documented, for example, for heartwood extension (Pâques 2001), natural durability (Curnel et al. 2008), extractives content (Gierlinger et al. 2004b; Gierlinger and Wimmer 2004), wood colour (Gierlinger et al. 2004a) and modulus of elasticity (Pâques et al. 2009).

In Europe, both European (*Larix decidua*, EL) and Japanese (*Larix kaempferi*, JL) larches are used in reforestation, and because of outstanding growth, plantations with their interspecific hybrid (HL) are expanding, especially in Western Europe. Improvements in heartwood volume and in natural durability are realistic objectives for larch breeders. Indeed, both properties show a large enough genetic variability together with high levels of heritability (for heartwood extent), which are two conditions for an efficient selection (Pâques 2001; Curnel et al. 2008).

However, evaluation of decay resistance by biological testing, as in the standardised method NF EN 113 (1996), is unrealistic in tree breeding programmes where large sets of genotypes and trees must be screened. Cost, time and lack of precision are major impediments to this approach. Indirect non-destructive methodologies less dependant on a biological agent such as physicochemical measures of wood are searched. Attempts to link decay resistance to extractives were successful in several larch species. Windeisen et al. (2002) found correlation coefficients of −0.77 to −0.80 between mass loss (with *Coniophora puteana*) and total extractives content in hybrid larch trees (study on 20 trees only). Gierlinger et al. (2004b) showed that total phenols contents in EL and JL were highly correlated with total extractives and were tightly linked to mass losses: −0.81 with *Coniophora puteana* and −0.83 with *Poria placenta.* In a study based on one JL and one HL tree contrasted for their extractives content, Windeisen and Wegener (2003) identified two major compounds in the acetone/methanol extract, namely Dihydrokaempferol (DHK) and Dihydroquercetin or Taxifolin (Tax).

On the other hand, the reddish colour of larch heartwood, as measured by a* in the CIE parameters, was highly correlated with the phenol content (0.84) and thereby to natural durability (correlation of −0.63 with the mass loss) (Gierlinger et al. 2004a). Thus, a* potentially appeared as a suitable indirect predictor of both phenol content and natural durability, and it has the major advantage of being easily assessed.

Our objective in this study was (a) to investigate differences in phenolic extractives content in the three larch taxa (EL, JL and HL) with a special focus on the two major molecular constituents (Tax and DHK); (b) to study their link with colour parameters; and (c) to verify within a large sample the relative distribution of each compound (Tax and DHK) in heartwood extracts of the three larch species.

Materials and methods

Wood samples

Both European larch (EL) and Japanese larch (JL) trees and their interspecific hybrids (HL) were used in this study. European larch was represented by twelve clones of Sudeten origin and Japanese larch by seven clones of various genetic origins. An average of eleven ramets per clone for EL and five ramets for JL cultivated in two clonal banks located in the Massif Central range (respectively, Lat. 44°54′ N, Long. 2°4′ E, 637 m and 44°36′ N, 1°21′ E, 250 m) were sampled. Interspecific offspring of these parents were also sampled for the study and are part of a progeny test established at Beaumont-du-Lac (West Massif Central range, Lat.45° 44′46″ N, Long. 1°50′19″ E, alt. 540 m) in spring 1985. Forty-three full-sib families were available, and an average of 12 trees per family were sampled.

Diameter increment cores (5 mm diameter) were collected at breast height from these trees. In total, 670 trees were sampled, namely 131 EL, 35 JL and 504 HL trees. Trees were then 25- , 31- and 21-years old, respectively, for EL, JL and HL.

After removal of the sapwood, the heartwood was split into two parts: an inner part close to the pith (in) and an outer part (out) represented by the last five rings of heartwood. They were further analysed separately with the exception of a few samples with too little wood (<250 mg). The in and out wood samples were dried and then ground into a fine powder using a steel ball grinder (Dangoumeau type) to a particle size of 40–60 mesh (approximately 0.2–0.4 mm) and stored in 10-ml glass bottles in dry conditions before analysis.

Heartwood colour determination

Colour was assessed on the dry powder to minimise structural effects of the wood. The reflection spectrum was taken from an 18-mm measuring spot in the 400–700 nm region with a *Microflash 200d* spectrocolorimeter using the CIE standard illuminant D65 (corresponding to daylight) and an observation angle of 10°. The CIELAB colour

parameters: L* (lightness), a* (red–green axis) and b* (yellow–blue axis) were obtained.

Extraction of soluble wood phenolic compounds

Soluble phenolic compounds were extracted twice from 50 mg of dry wood powder in two ml acetone/water (8:2, v/v) containing 10^{-4} mol/l 6-methoxyflavone (*Sigma*) as internal standard. The mixture was sonicated for 1 h then agitated for 1 h before being centrifuged at 18,000*g* for 20 min. A part of pooled supernatants (500 µl) was removed and dried under vacuum using a *Speed-Vac system* (*Savant Instrument, India*). The dry residue was diluted in 250 µl of methanol. All the steps were carried out at 4 °C.

Total polyphenols quantification

Total polyphenols were estimated by an adapted Folin–Ciocalteu method. Twenty microliters of the phenolic extract in methanol was diluted in 80 µl of ultra-pure water, then 500 µl of Folin–Ciocalteu reagent (*Sigma*) diluted 10 times in ultra-pure water and 400 µl of $NaCO_3$ 75 g l^{-1} was successively mixed and incubated for 5 min at 40 °C. Then, 250 µl of this reactive mixture was distributed in triplicate on a 96-well plate. The absorbance at 735 nm was measured spectrophotometrically, and the results are expressed in mg equivalent of Taxifolin per gram dry weight. Calibration is achieved with Taxifolin methanol solutions (0–20 µg ml^{-1}).

Chromatographic separation of wood extract compounds

After centrifugation (10,000*g* for 3 min) of the phenolic extract in methanol to avoid impurities, only 15 µl was injected, separated, characterised and quantified by HPLC on a *32 Karat system* (*Beckman Coulter, France*) using a 250 × 4 mm *LiChrospher* 100 RP-18e column (5 µm) (*Merck, Germany*) stabilised at 40 °C, at a flow rate of 1 ml/min^{-1} with the following linear elution gradient: initial conditions: 30 % solvent B (methanol/acetonitrile, 50:50, v/v) in solvent A (1 % acetic acid in ultra-pure water); 0–6 min: 30 to 55 %; 6–7 min: 55 to 100 % B; 7–12 min: 100 % B; 12–13 min: 100 to 30 % B. Compounds were characterised by their elution time and their UV absorption spectrum (diode array: 230–430 nm). Taxifolin was identified by co-chromatography with a standard (*Sigma*). No DHK commercial standard exists, but it was determined as with a previous study on larch wood (Windeisen and Wegener 2003). Quantitative determination of Tax and DHK was performed at 315 nm with an external calibration (Taxifolin methanol solutions; 10 points from 1 to 15 µg), and the results are expressed in mg per gram of dry weight for Tax and equivalent of Tax per gram dry weight for DHK.

Statistical analysis

A total of 653 out of 670 samples were chemically analysed of which 17 had to be discarded due to their small mass or high resin content. In addition, the inner and outer parts of 70 samples had to be mixed and assessed jointly because they were so small. Therefore, to take full advantage of the largest number of samples (653, *data set 1*), overall sample colour parameters and extractives contents were computed by weighing heartwood subsamples (inner and outer parts) data for dry weight. *Data set 2* included the remaining 583 samples of which heartwood position effects could be tested.

Differences between taxa for overall sample parameters were first tested from *data set 1* using a one-way analysis of variance following the model

$$\mathrm{Yij} = \mu + Ti + \varepsilon\mathrm{ij}, \quad \text{where}$$
$$\mathrm{Ti} = \text{taxa effect and } \varepsilon\mathrm{ij} = \text{residual}.$$

To test for differences between positions (in and out) along the heartwood, the following model was used on *data set 2*:

$$\mathrm{Yijk} = \mu + \mathrm{Ti} + \mathrm{Pj} + \mathrm{TxPij} + \varepsilon\mathrm{ijk}, \quad \text{where}$$
$$\mathrm{Ti} = \text{taxa effect}, \ \mathrm{Pj} = \text{position effect}, \ \varepsilon\mathrm{ijk} = \text{residual}.$$

Individual data for Tax and DHK contents needed to be transformed prior to analysis to normalise data distribution. The transformation used was of the type $(\mathrm{Var}^{\lambda}-1)/\lambda$ with $\lambda = 0.4$ for both Tax and for DHK contents.

When significant differences between taxa were shown, taxa means were compared with a Scheffe's test.

Results

Heartwood extractives contents

Significant differences between the three taxa were detected for overall sample variables. JL had the highest amount of total phenols, EL the lowest and HL was intermediate (Table 1). Tax content for JL and HL did not differ significantly from each other, but differed significantly from EL which had the lowest content. The DHK content was much lower than the Taxifolin content with JL the smallest and HL the highest amount. A broad variability among samples was observed with all three parameters in all species. The range for extractives among genotypes within each taxon was larger for all the traits, especially for Tax and DHK contents (Table 1 and Fig. 1).

Table 1 Descriptive statistics of global extractives content and *F* test for taxa

	Species	Mean	CV (%)	Min.–Max. (individuals)	Min.–Max. (genotypes)	F^a
Total phenols	EL	18.23	31	5.57–33.64	10.87–26.08	a
(mg eq. Tax/g DW)	JL	34.49	19	21.75–50.45	28.9–40.42	c
	HL	26.70	28	5.96–55.94	16.59–37.43	b
Taxifolin	EL	7.36	49	1.29–17.79	3.91–12.72	a
(mg/g DW)	JL	19.67	42	1.68–38.71	13.98–29.79	b
	HL	16.47	41	2.67–41.73	7.78–24.93	b
DHK	EL	3.46	44	1.08–8.49	1.45–5.22	b
(mg eq. Tax/g DW)	JL	1.50	29	0.67–2.56	1.19–1.90	a
	HL	4.40	55	0.51–13.66	1.71–7.99	c

EL European larch, *JL* Japanese larch, *HL* hybrid larch

[a] Different letters mean statistical differences between taxa (for $\alpha = 0.05$)

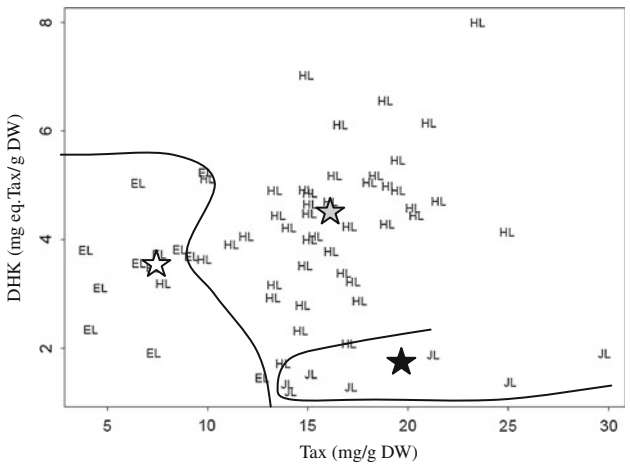

Fig. 1 Relationship between Taxifolin (Tax) and Dihydrokaempferol (DHK) contents at the genotype mean level. (*EL* European larch, *JL* Japanese larch, *HL* hybrid larch; *the white, grey and black stars* means for EL, HL and JL, respectively)

Significant differences between positions in the heartwood were detected for all three extractives content (Table 2). The position effect was particularly strong compared with taxa effect for total phenols and Taxifolin content; differences between positions were still significant for DHK content, but the main source of variability was obviously among taxa. In all taxa, outer heartwood was always richer in phenolics than inner heartwood (Fig. 2): in EL, total phenols, Tax and DHK contents in outer heartwood were, respectively, 1.6, 1.8 and 1.4 higher than contents in inner heartwood; in HL, these values were even higher (1.7 and 2.0 for total phenols and Tax) excepted for DHK content which did not differ significantly between positions; in JL, these ratios reached 1.6 for total phenols, 1.7 for Tax and equal for DHK.

Interactions between taxa and positions were significant for all traits and corresponded to scale effects and not to any changes in ranking among taxa (Table 2).

Extractives contents of both inner and outer wood samples were well correlated with their respective contents at the overall sample level ($r = 0.78$–0.88). Correlations between total phenols and Tax contents were high (>0.83) irrespective of the sample position but weak (<0.436) between total phenols and DHK contents.

Heartwood colour parameters

Colour parameters showed significant differences between taxa for all three variables (L*, a*, b*) in the overall samples (Table 3). EL and JL had similar L* (characterised by the lightest colour), but it differed from HL. The wood of JL was more reddish, followed by HL and then by EL ones (as shown by a*). Along the b* axis, EL and HL were similar and JL wood had a slightly (but significantly) higher yellow hue.

Altogether, samples showed differences between positions along the heartwood for a* and b* but not for L* (Table 2). When tested for differences between positions within taxa, there were only significant differences for HL. The inner samples of hybrids had slightly, but significantly, redder and yellower hues than the outer ones (a* = 9.6 vs. 9.0; b* = 22.5 vs. 20.6) (Fig. 3).

Individual sample variability for CIE parameters was much lower than that for extractives (CV = 4–18 vs. 19–55 %) (Tables 1, 3). In addition, variability among genotypes within taxa was much smaller than for extractives and quite variable among taxa with EL clones showing the highest variability (Table 3).

Correlations between extractives and colour parameters

Links between extractives contents and colour parameters are given in Table 4 for both the individual and mean genotype levels. Overall, the L* parameter was moderately correlated with all three types of extractives as was the a* parameter with the exception of DHK (not significantly different from zero). Apart from DHK at the mean genotype level, no correlation was found between b* and the extractives.

The highest correlations at the mean genotype level were found between a* and, respectively, total phenols content (Fig. 4) and Tax content and between L* and b*, and DHK content (Table 4).

Table 2 ANOVA tables for extractives and colour parameters

	DDL	Total phenols		Taxifolin		Dihydrokaempferol	
		CM	*F*	CM	*F*	CM	*F*
Taxa	2	6,634.588	80.1***	367.566	149.5***	20.338	20.6***
Position	1	56,900.859	687.2***	1,237.416	503.4***	4.678	4.7*
Interaction	2	744.078	9.0***	15.836	6.4**	3.365	3.4*
Residual	1,158	82.805		2.458		0.988	
	DDL	L*		a*		b*	
		CM	*F*	CM	*F*	CM	*F*
Taxa	2	38,517.492	2,220.1***	122.856	66.5***	31.581	8.4***
Position	1	34.872	2.0 ns	50.235	27.2***	755.817	201.3***
Interaction	2	261.740	15.1***	14.773	8.0***	47.128	12.5***
Residual	1,158	17.349		1.849		3.755	

*, **, *** Significant, respectively, at the 5, 1 and 0.1 % levels

Discussion

Apart from mechanical strength, other properties of heartwood, such as decay resistance and wood colour, are very important when wood with a high level of natural durability or some aesthetic aspects are required. Determination of natural durability through a decay resistance test (NF EN 113 1996) remains laborious, costly and destructive: it impedes genetic studies requiring large sample sizes. Therefore, several authors have attempted to develop indirect predictors of natural durability based on wood extractives content. Indeed, one of the major sources for both decay resistance and for colour of heartwood seems to root from extractives content, among which phenolics seemed to be a determinant as demonstrated by Harju et al. (2003) for Scots pine (*Pinus sylvestris* L.) and by Gierlinger et al. (2004b) and Venalainen et al. (2006) for larches. Qualitatively, Windeisen and Wegener (2003) identified two major components in larch heartwood, Taxifolin and Dihydrokaempferol, demonstrating the prominent role of Taxifolin in natural durability. It is therefore logical that we became interested in quantifying genetic variability for extractives content in larch in relation to the genetic improvement in natural durability.

In this study, comparison between European and Japanese larches and their interspecific hybrids for their heartwood extractives content showed highly significant differences between taxa for total polyphenols content. Japanese larch proved to be much richer in phenolics than European larch (nearly twice as much) in concordance with the results of Gierlinger et al. (2004b). Hybrid larch was intermediate. Taxifolin was the major constituent of total phenols. Hybrid larch did not differ significantly from Japanese larch for Taxifolin content, and both JL and HL had significantly more Taxifolin than EL. However, for the second important phenolic compound of larch wood, Dihydrokaempferol, hybrid larch was closer to the European larch, with a concentration nearly three times higher than that of Japanese larch.

Overall, our results fit well in the range of published values for phenolics and specifically for Taxifolin concentrations (up to 1.8 % in Western larch (Gartner and Barton 1960), between 0.5 and 4.1 % for one EL and one JL (Windeisen and Wegener 2003) and up to 4 % in JL (Kondo and Furuzawa 1954). Obviously with such high concentrations, larches together with Douglas fir are much richer in Dihydroflavonols molecules than in any other gymnosperm species, where concentrations are usually lower than 0.1 %, as noted by Dellus et al. (1997a).

All three extractives variables (total phenols, Tax and DHK) and each taxon showed a large within-tree variability along the radius and among individual trees. The total phenols contents of the outer heartwood were on average more than 1.5 times higher than that of the inner heartwood, and the Tax concentration nearly doubled. This radial increase seems to be a general pattern for wood extractives, as already shown by Gartner and Barton (1960) and Gierlinger and Wimmer (2004). However, this trend seems to be linked to age and is more visible in old trees than in younger ones. Indeed, Gierlinger and Wimmer (2004) observed that total phenols content doubled every 10 cm along the radius in old larch trees (150–260 years old). However, McMillin (1968) and Erickson and Arima (1974), for example, observed instead a constant or even decreasing trend with increasing distance from the pith in young trees (<25 years old). In addition, phenotypic (and perhaps genetic) variability among trees for this trend seems to exist too, as suggested by results from Windeisen

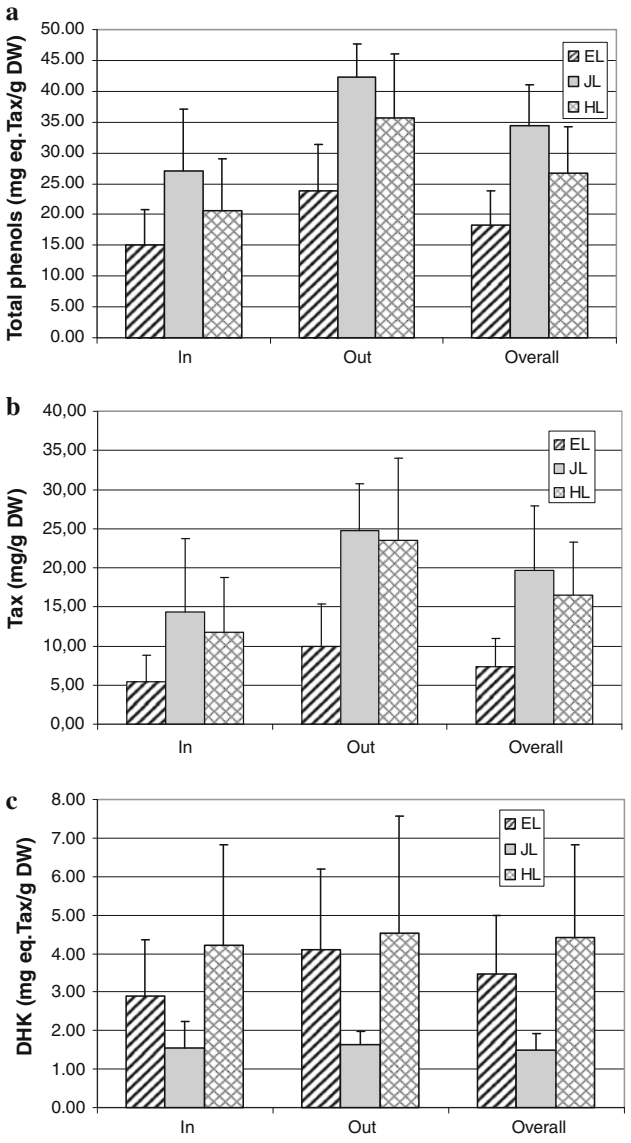

Fig. 2 Extractives contents in inner (in), outer (out) and overall heartwood of European (EL), Japanese (JL) and hybrid (HL) larches. **a** Phenols, **b** Taxifolin (Tax) and **c** Dihydrokaempferol (DHK)

et al. (2002): out of twenty 40-year-old larches, nearly one-third of the trees had the same or even a slightly lower concentration of phenols in the outer compared with the inner heartwood. Dihydrokaempferol concentrations were similar between inner and outer heartwood for all taxa with the exception of EL. This might suggest that DHK is less chemically transformed than Taxifolin during the ageing of wood and is thus accessible in equal quantities from the outside of the heartwood towards the inside.

Differences between full-sib progenies for hybrid larch were larger for extractives with concentrations ranging from 1 to 2.3, 1 to 3.2 and 1 to 4.7. These values were, respectively, for total phenols, Tax and DHK between the poorest and the richest genotypes. Similar ranges were

Table 3 Descriptive statistics of colour parameters and *F* test for taxa

Species	Mean	CV (%)	Min.–Max. (individuals)	Min.–Max. (genotypes)	F^a
L*					
EL	61.03	12	43.29–74.20	53.29–66.77	a
JL	61.40	11	46.71–72.23	56.35–64.65	a
HL	80.85	4	47.11–85.61	77.1–82.38	b
a*					
EL	7.99	18	4.00–12.04	6.04–9.47	a
JL	10.63	11	8.45–13.32	10.15–12.15	c
HL	9.38	12	3.23–13.59	8.14–10.74	b
b*					
EL	22.15	12	13.62–29.17	19.21–24.82	a
JL	23.84	8	19.82–28.29	23.11–25.54	b
HL	21.78	7	10.70–25.30	20.00–23.66	a

EL European larch, *JL* Japanese larch, *HL* hybrid larch

[a] Different letters mean statistical differences between taxa (for $\alpha = 0.05$)

observed among European larch clones although the size of sampling was more limited; the values were smaller for Japanese larch. The variability for extractives content, as observed among European larch populations by Gierlinger et al. (2004b) but also by Fries et al. (2000) in Scots pine among full-sib progeny level, offers real promise for genetic selection.

Differences in colour parameters were more attenuated but still significant among positions and among taxa (except for L*). Inner heartwood was more coloured and in particular more reddish (significantly higher a*-value and higher b*-value) than the outer one. A decreasing a*-value (red hue) from pith to sapwood/heartwood border has already been observed by Dellus et al. (1997b) in Douglas fir, while concomitantly Dihydroquercetin (Taxifolin) content was increasing. The authors suggested then that some reaction involving the colourless Dihydroquercetin (Taxifolin) and leading to its disappearance from extracts should be closely linked to the formation of the red–orange colour of heartwood. In an experimental model, they were able to change the colour of sapwood to that of heartwood by impregnating sapwood with Leucocyanidin, obtained through an enzymatic reduction of Dihydroquercetin and followed by its (non-enzymatic) oxidation. They concluded that Dihydroquercetin was necessary but not sufficient to explain colour formation.

Chemically, the colour of wood is mainly associated with the presence of phenolic compounds but not only because most often the extractable pigments represent only a small fraction of the colouring matter contained in the wood. The remaining fraction consists of polymerised hardly extractable compounds as being strongly associated

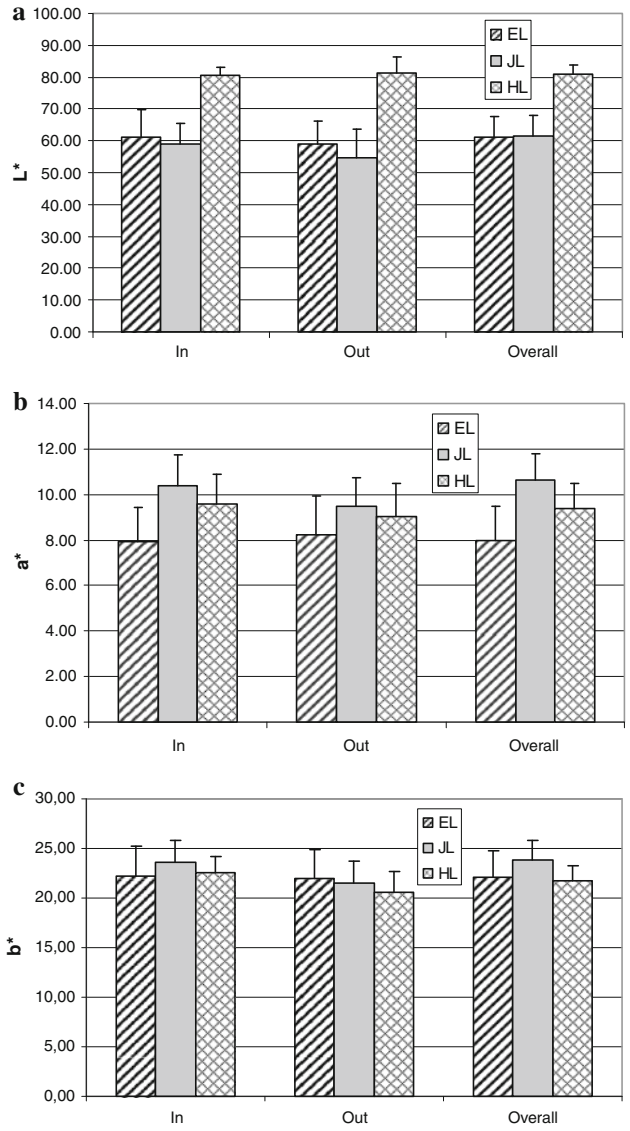

Fig. 3 Colour parameters (CIELAB system) for inner (in), outer (out) and overall heartwood of European (EL), Japanese (JL) and hybrid (HL) larches: **a** L*, **b** a* and **c** b*

Table 4 Correlation coefficients between extractives and colour parameters of larch heartwood at the individual and mean genotype levels

	Total phenols	Tax	DHK
L*			
Individual	0.298***	0.396***	0.313***
Genotype	0.217 ns	0.397**	0.549***
a*			
Individual	0.210***	0.160***	−0.094 ns
Genotype	0.579***	0.516***	−0.184 ns
b*			
Individual	−0.009 ns	−0.097 ns	−0.184 ns
Genotype	0.206 ns	0.025 ns	−0.414***

Tax Taxifolin; *DHK* Dihydrokaempferol

*, **, *** Significant, respectively, at the 5, 1 and 0.1 % levels

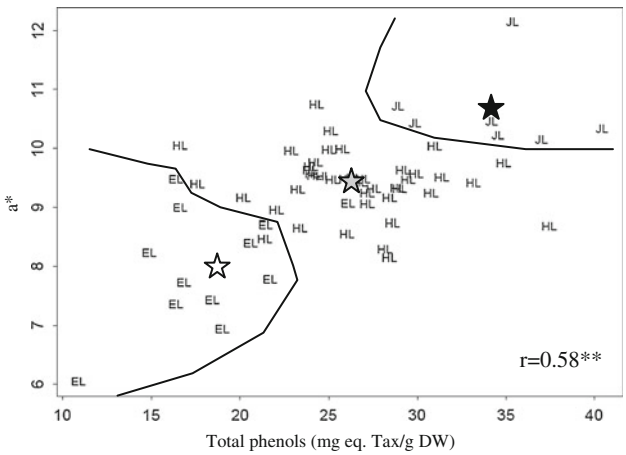

Fig. 4 Relationship between total phenols content and a*-colour parameter at the mean genotype level. (*White, grey and black stars* means for European (EL), hybrid (HL) and Japanese (JL) larches, respectively; *Tax* Taxifolin)

with different cell wall constituents (Macheix et al. 2005). As the wood colour closely depends on the process of heartwood formation, due to the cellular decompartmentalisation, the native phenolic compounds are oxidised and polymerised and very often lead to coloured compounds, while they are naturally colourless (Burtin et al. 1998). They correspond to precursors. That is certainly the case here for Taxifolin.

Among CIELAB parameters, the a*-value was the most discriminant variable with an increasingly redder hue from EL to JL,—which concords with Gierlinger et al. (2004a) results. The taxa ranking for L*- and b*-values was different in the study of Gierlinger et al. (2004a), where EL was on average the lightest in colour, but in our study HL was the lightest; the b*-value was highest in HL, but it was the lowest in our case. Several factors could explain this difference as it is known that the genetic origin of trees, environmental conditions, growth rates and age of trees have an influence on both colour and extractives content (Mosedale et al. 1996a; Moya and Berrocal 2010; Bradbury et al. 2011).

Some links between extractives content and colour parameters have been shown in this study: the best relationships at the individual tree level were found between L* and Taxifolin content. Even if it was significantly different from 0, the correlation was weak. At the mean genotype level, the total phenols and Taxifolin contents were better linked with the red hue (a*) with a correlation coefficient of up to 0.58 with total phenols content. However, according to Fig. 4, a wide range of phenol content

(roughly from 16 up to 40 mg eq. Taxifolin/g DW) corresponded to the highest a*-values (let us say a* above 10).

Colour parameters therefore seem to be rather weak predictors of phenolic content. This is in contrast with results from several authors who found a close link between wood colour and extractives [e.g. for larch: Gierlinger et al. (2004a): $r = 0.84$ between phenolics and a*-value; for Douglas fir: Dellus et al. (1997a); for several broadleaves (Burtin et al. 1998; Mosedale et al. 1996b)]. The much narrower genetic basis of the larch samples used in this study, in contrast to the large set of contrasted genetic origins (covering '*polonica*', '*sudetica*' and 'alpine' ecotypes of European larch in addition to some JL and HL) in the study by Gierlinger et al. (2004b), could explain the weaker correlations observed. However, even in the latter study, the two hybrids tested, which had among the highest a* hues among 13 wood lots, only had an average level of total phenols content.

Based on the previous works on larch, the best link with natural durability proved to be with phenolics and more precisely with Taxifolin concentration. Gierlinger et al. (2004b) associated the high decay resistance of Japanese larch with the high amount of phenolics in its heartwood: up to 3.57 % of dry weight compared with 1.84 % for a European larch population with the lowest durability. Windeisen et al. (2002) and Windeisen and Wegener (2003) found that the tree with the most durable wood contained a Taxifolin concentration of 3.3–4.1 % of dry weight compared with 0.5 % in another tree with a low durability; the concentration of Dihydrokaempferol did not differ. Venalainen et al. (2006) found as well a strong negative correlation (−0.673) between Taxifolin content and mass loss.

This perspective to reliably predict decay resistance through phenolics content, together with the broad variability observed in this study at species and at different genotypic levels for extractives content, opens new and realistic opportunities for breeders to improve natural durability in larch and particularly in hybrid larch. Attempts to predict natural durability by NIR spectroscopy (Gierlinger et al. 2003; Sykacek et al. 2006) based on total amount of phenols proved accurate and particularly efficient for routine assessment of these properties. However, the use of the existing calibration model appears poorly adapted to the larch sample used in this study (results not shown). A new calibration model using either total phenols amount or better Taxifolin is under construction to better fit heartwood characteristics from hybrid larch and from younger trees.

Finally, as revealed by Fig. 1, the combined determination of Taxifolin and Dihydrokaempferol concentrations in heartwood surprisingly seems to be a feasible way to discriminate the European and the Japanese larches and their hybrid from wood. In any case, this approach would remain more laborious than a direct discrimination through a NIRS methodology as already shown by Gierlinger et al. (2004c).

Acknowledgments This study was conducted thanks to a grant from the EFPA INRA department. We acknowledge the field work of F. Millier for core sampling and the laboratory work of N. Boizot and K. Ader. All the chemical results were obtained within the technical platform "GénoBois" (INRA Orléans Laboratory).

References

Bradbury G, Potts BM, Beadle CL, Dutkowski G, Hamilton M (2011) Genetic and environmental variation in heartwood colour of Australian blackwood (*Acacia melanoxylon* R. Br.). Holzforschung 65(3):349–359. doi:10.1515/HF.2011.042

Burtin P, Jay-Allemand C, Charpentier JP, Jannin G (1998) Natural wood colouring process in *Juglans* sp. (*J. nigra*, *J. regia* and hybrid *J. nigra* 23 × *J. regia*) depends on native phenolic compounds accumulated in the transition zone between sapwood and heartwood. Trees 12:258–264

Curnel Y, Jacques D, Gierlinger N, Pâques LE (2008) Decay resistance of larch wood to fungi. Ann For Sci 65(810):1–8. doi:10.1051/forest:2008062

Dellus V, Mila I, Scalbert A, Menard C, Michon V, Hervé du Penhoat C (1997a) Douglas-fir polyphenols and heartwood formation. Phytochemistry 45(8):1573–1578

Dellus V, Scalbert A, Janin G (1997b) Polyphenols and colour of Douglas fir heartwood. Holzforschung 51:291–295

Erickson HD, Arima T (1974) Douglas-fir wood quality studies. Part II: effects of age and stimulated growth on fibril angle and chemical constituents. Wood Sci Technol 8:255–265

Fries A, Ericsson T, Gref R (2000) High heritability of wood extractives in *Pinus sylvestris* progeny tests. Can J For Res 30(11):1707–1713

Gartner JAF, Barton GM (1960) Dihydroquercetin in Douglas-fir and Western larch. For Prod J 10:171–173

Gierlinger N, Jacques D, Schwanninger M, Wimmer R, Hinterstoisser B, Pâques LE (2003) Rapid prediction of natural durability of larch heartwood using Fourier transform near-infrared spectroscopy. Can J For Res 33(9):1727–1736. doi:10.1139/X03-092

Gierlinger N, Jacques D, Grabner M, Wimmer R, Schwanninger M, Rozenberg P, Pâques LE (2004a) Colour of larch heartwood and relationships to extractives and brown-rot decay resistance. Trees 18:102–108

Gierlinger N, Jacques D, Schwanninger M, Wimmer R, Pâques LE (2004b) Heartwood extractives and lignin content of different larch species (*Larix* sp.) and relationships to brown-rot decay-resistance. Trees Struct Funct 18(2):230–236

Gierlinger N, Schwanninger M, Wimmer R (2004c) Characteristics and classification of Fourier-transform near infrared spectra of the heartwood of different larch species (Larix sp.). J Near Infrared Spectrosc 12:113–119

Gierlinger N, Wimmer R (2004) Radial distribution of heartwood extractives and lignin in mature European larch. Wood Fiber Sci 36(3):387–394

Hillis WE (1987) Heartwood and tree exudates. Springer, Berlin

Harju AM, Venalainen M, Anttonen S, Viitanen H, Kainulainen P, Saranpaa P, Vapaavuori E (2003) Chemical factors affecting the brown-rot decay resistance of Scots pine heartwood. Trees Struct Funct 17(3):263–268

Kondo T, Furuzawa N (1954) Chemical studies on *Larix kaempferi* Sarg. J Jpn For Soc 36(1):19–22

Macheix JJ, Fleuriet A, Jay-Allemand C (2005) Les composés phénoliques des végétaux. Presses polytechniques et universitaires romandes, Lausanne, Suisse, ISBN 2-88074-625-6

McMillin CW (1968) Chemical composition of loblolly pine wood as related to specific gravity, growth rate and distance from pith. Wood Sci Technol 2:233–240

Mosedale JR, Charrier B, Janin G (1996a) Genetic control of wood colour, density and heartwood ellagitannin concentration in European oak (*Quercus petraea* and *Q. robur*). Forestry 69(2):111–124. doi:10.1093/forestry/69.2.111

Mosedale JR, Charrier B, Crouch N, Janin G, Savill PS (1996b) Variation in the composition and content of ellagitannins in the heartwood of European oaks (*Quercus robur* and *Q. petraea*). A comparison of two French forests and variation with heartwood age. Ann For Sci 53:1005–1018

Moya R, Berrocal A (2010) Wood colour variation in sapwood and heartwood of young trees of *Tectona grandis* and its relationship with plantation characteristics, site, and decay resistance. Ann For Sci 67:109–117. doi:10.1051/forest/2009088

Pâques LE (2001) Genetic control of heartwood content in larch. Silvae Genet 50(2):69–75

Pâques LE, Millier F, Rozenberg P (2009) Selection perspectives for genetic improvement of wood stiffness in hybrid larch (*Larix* × *eurolepis* Henry). Tree Genet Genome 6(1):83–92

Pometti CL, Palanti S, Pizzo B, Charpentier JP, Boizot N, Resio C, Saidman BO (2010) Durability of five native Argentine wood species of the genera *Prosopis* and *Acacia* decayed by rot fungi and its relationship with extractive content. Biodegradation 21:753–760. doi:10.1007/s10532-010-9340-5

Scheffer TC, Cowling EB (1966) Natural resistance of wood to microbial deterioration. Ann Rev Phytopathol 4:147–160

Sykacek E, Gierlinger N, Wimmer R, Schwanninger M (2006) Prediction of natural durability of commercial available European and Siberian larch by near-infrared spectroscopy. Holzforschung 60(6):643–647. doi:10.1515/HF.2006.108

Venalainen M, Harju AM, Terziev N, Laakso T, Saranpaa P (2006) Decay resistance, extractive content, and water sorption capacity of Siberian larch (*Larix sibirica* Lebed.) heartwood timber. Holzforschung 60(1):99–103

Windeisen E, Lesnino G, Schumacker P, Wegener G (2002) Investigation of the correlation between extractives content and natural durability in 20 cultivated larch trees. Holz als Roh- und Werkstoff 60:373–374. doi:10.1007/s00107-002-0314-0

Windeisen E, Wegener G (2003) Chemische Untersuchingen von Hochzucht-Lärchen. Holz als Roh- und Werkstoff 61:394–397

Standards

AFNOR (1996) Norme NF EN 113 Wood preservatives—test method for determining the protective effectiveness against wood destroying basidiomycetes—determination of the toxic values