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Wood properties of juvenile and mature heartwood in *Robinia pseudoacacia* L.

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Abstract The aim of this study is to characterise the properties of juvenile and mature heartwood of black locust *(Robinia pseudoacacia* L.). Content, composition and the subcellular distribution of heartwood extractives were studied in 14 old-growth trees from forest sites in Germany and Hungary as well as in 16 younger trees of four clone types. Heartwood extractives (methanol and acetone extraction) were analysed by HPLC-chromatography. UV microspectrophotometry was used to topochemically localise the extractives in the cell walls. The natural durability of the juvenile and mature heartwood was analysed according to the European standard EN 350-1. Growth as well as chemical analyses showed that, based on extractives content, the formation of juvenile wood in black locust is restricted to the first 10–20 years of cambial growth. In mature heartwood, high contents of phenolic compounds and flavonoids were present, localised in high concentrations in the cell walls and cell lumen of axial parenchyma and vessels. In juvenile wood, the content of these extractives is significantly lower. Juvenile wood had a correspondingly lower resistance to decay by *Coniophora puteana* (brown rot fungus) and *Coriolus versicolor* (white rot fungus) than mature heartwood.

Introduction

Wood is the most important renewable raw material worldwide. A considerable amount of CO_2 is fixed in the xylem of trees. Therefore, the use of wood in long lasting applications like indoor and outdoor constructions contributes significantly

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to the reduction of CO_2 concentration in the atmosphere and related impacts on global climate. However, in many applications the life cycle of wood is limited by its natural durability. Consequently, during the past years the demand for timber with a high natural durability has increased significantly. Recently, this trend has gained momentum by the increasing demand for wood for exterior use.

For decades, the market of highly durable timber has concentrated on tree species from the tropics, e.g., azobé, doussié from Africa, bangkirai from Asia, massaranduba from Latin America. As a consequence, these tropical timbers have been heavily exploited in natural forests because of their highly desirable physical, mechanical and biological properties, and now the demand exceeds the supply in the international markets. In Europe, very few tree species produce timber with a very high natural durability (Grosser 2003). One of the few promising species is *Robinia pseudoacacia* L., native in North America but cultivated in Europe for more than 400 years (Stringer 1992; Molnar 1995). Many investigations highlight the fast formation of heartwood in black locust and its high natural durability (Hart 1968; Magel et al. 1991, 1994; Richter 2000).

However, several studies also proved a high variability of wood properties in the stem wood of black locust (Chow et al. 1996; Adamopoulus et al. 2005). Some authors suggest that differences between juvenile and mature wood are responsible for the variety in wood quality of black locust (Stringer and Olson 1987). Distinct differences between wood formed during the juvenile and adult growth phase are described for many species, but most of the studies focus on the anatomical structure and the elasto-mechanical properties (Kopitovic et al. 1989; Zobel and van Buijtenen 1989; Zobel and Sprague 1998). However, recent studies also indicate significant differences in chemical properties between juvenile and mature heartwood. Haupt et al. (2003) found a lower concentration of wood extractives in the juvenile wood of teak (*Tectona grandis*) compared to the mature heartwood, corresponding to a lower natural durability of juvenile wood compared to mature wood. In contrast, recent studies on the chemical composition and the natural durability of juvenile and mature heartwood in black locust revealed results that deviate somewhat from that pattern (Chow et al. 1996; Adamopoulus et al. 2005).

With this in mind, the aim of the present study is to investigate the content and the topochemical distribution of phenolic compounds and other wood extractives in juvenile and mature heartwood of *Robinia pseudoacacia* L. The investigations were carried out with special focus on the resistance of black locust heartwood against fungal decay.

Experimental

Selected wood material

Stem discs (DBH, approx. 1.3 m) from 14 old-growth trees (age 41–85 years) from forest sites in Eastern-Germany (Eberswalde) and Eastern Hungary (Nyiracsàd, South-Nyirség 61) were sampled in late September 2005 and in late September 2006, respectively (Table 1). In addition, stem discs (height level approx. 0.5 m) of

Table 1 Number and age of the experimental trees from the sites "Eberswalde", "Waldsieversdorf" (both Germany), and "South-Nyirség", Hungary

Reproduction	Site	Number of trees	Tree age (years)
Seeds	Eberswalde, Germany	10	59–85
Seeds	South-Nyirség, Hungary	4	41
Clones (2402, 2405, 2461, and 2498)	Waldsieversdorf, Germany	16	11

four 11-year-old black locust clones (clones 2402, 2405, 2461, and 2498, four trees per clone) grown at the experimental site of the Institute for Forest Genetics, Federal Research Institute of Rural Areas, Forestry and Fisheries in Waldsieversdorf (Eastern Germany), were sampled in October 2005 (Table 1). The discs were shock-frozen and stored in a freezer (−18°C) until subsequent processing. Samples for increment analyses, chemical extraction and UV microspectrophotometry were taken from pith to cambium adjacent to each other on the discs.

Increment measurements

The annual ring width was measured on polished discs along four radii (north, south, east and west) by means of a digital measuring ocular lens. The increment curves were visually cross-dated between trees of the same site according to Fritts (1976). Cross-dating was successful for the old-growth trees from "Eberswalde" and from "South-Nyirség", whereas cross-dating failed for the 11-year-old clones from "Waldsieversdorf". The individual increment curves were averaged to establish mean increment curves for each site and clone.

Determination of lignin and extractives

The lignin content of the sapwood (sapwood rings closest to the cambium and to the heartwood excluded) and of the heartwood (heartwood ring closest to the sapwood excluded) was determined as "Klason"-lignin according to the TAPPI (Technical Association of the Pulp and Paper Industry 1988)-procedure 222om-88 (extraction solvent 72% H_2SO_4). The acid soluble fraction was quantified photometrically at a wavelength of 205 nm.

Extraction of phenolic compounds

For qualitative and quantitative analyses of the accessory compounds, shavings from juvenile and mature wood of the selected trees were prepared. The shavings were immediately freeze dried and ground in a Retsch mill with a rotating knife using a 3 mm screen, followed by accelerated solvent extraction (ASE 200, Dionex). Extractions were carried out with acetone/water (9:1) and methanol/water (3:1) at 60°C under constant pressure of 100 atm and a static equilibration treatment of 5 min according to Koch et al. (2006).

Reversed-phase high performance liquid chromatography (RP-HPLC)

A total of 5 ml of acetone/water extract of juvenile wood and mature wood without derivatization were directly injected into a *Jasco* system using an Aquasil 5 μ C18 column (250 $\times$ 4.6 mm). The temperature of the column was set at 30°C. Solvent A (0.001 m H_3PO_4) and solvent B (acetonitrile 100%) served as mobile phase in a gradient mode (7.5–15% B at 0–30 min, 15–20% B at 30–40 min, 20–40% B at 40–60 min, 40–100% B at 60–65 min) with a flow rate of 1 ml/min. The separated compounds were detected with a *Jasco* photo-diode array detector. The detection wavelength was set at 280 nm and UV spectra from 200 to 650 nm were also recorded for peak identification. Peak identification was performed by comparison of retention times and UV spectra with purchased standards (*Sigma Aldrich*). For quantification, calibration curves with four calibration points for each substance were set. Quantification was performed in triplicate.

For the quantification of the total content of extractives, the extracts were concentrated in vacuo at 40°C, purged with nitrogen and dried over phosphorus pentoxide. The dry extracts were weighed and their amounts expressed as a percentage of dry mass of the original sample.

Cellular UV microspectrophotometry

The subcellular distribution of lignin and phenolic extractives were topochemically investigated using scanning UV microspectrophotometry according to Koch and Kleist (2001) and Koch and Grünwald (2004). Small wood blocks ($1 \times 1 \times 5$ mm^3) were serially dehydrated in a graded series of acetone and then impregnated with Spurr's resin (Spurr 1969). Semi-thin (1 μm) cross sections of the samples were prepared using a diamond knife. The sections were transferred to quartz microscope slides, immersed in a drop of non-UV absorbing glycerine, and covered with a quartz cover slip. The sections were analysed with a Zeiss UMSP 80 microspectrophotometer. The UV absorbance of the samples was analysed by point measurements (ultrafluar objective 32:1, 1.46 oil, spot size 1 μm^2) between 240 and 700 nm wavelength using the software LAMWIN® (Zeiss). In addition, UV absorbance profiles at constant wavelengths were generated in the scanning mode of the microspectrophotometer using the scan software APAMOS® (Zeiss). The scan creates absorbance profiles with a spatial resolution of 0.25 μm^2 and a photometrical resolution of 4096 grey scales, which are converted into 14 basic colours to visualise the absorbance intensity.

In vitro decay test

The natural durability of juvenile and mature wood was determined according to the European standard EN 350-1 (1994). For the decay test the basidiomycete fungi *Coriolus versicolor* (white rot) and *Coniophora puteana* (brown rot) were chosen. The virulence of the fungi was tested with wood of *Pinus sylvestris* (sapwood) and *Fagus sylvatica*. In total 64 samples of juvenile and 50 samples of mature

heartwood were included in the analyses. The durability class based on mass loss was calculated according to the European standard EN 350-2 (1994).

Results and discussion

Tree ring width of the experimental trees

The annual growth increment of all trees decreased significantly from pith to cambium (Fig. 1). In particular, during the first 10–20 years of growth the annual increment exceeded that of older trees. The analysis of the increment indicates a juvenile growth phase of the black locust trees over a period of approximately 10–20 years, while a distinct phase of adult growth was found in trees older than 20 years. Consequently, for the analyses of chemical composition and natural durability, samples of tree rings 1–10 were selected for the characterisation of juvenile wood, while samples of tree rings formed by trees older than 20 years were selected for the characterisation of mature wood.

The dating of the phases of juvenile and mature wood formation based on increment measurements correspond to results obtained for anatomical characteristics and elastomechanical properties of black locust wood (Dünisch et al. 2007).

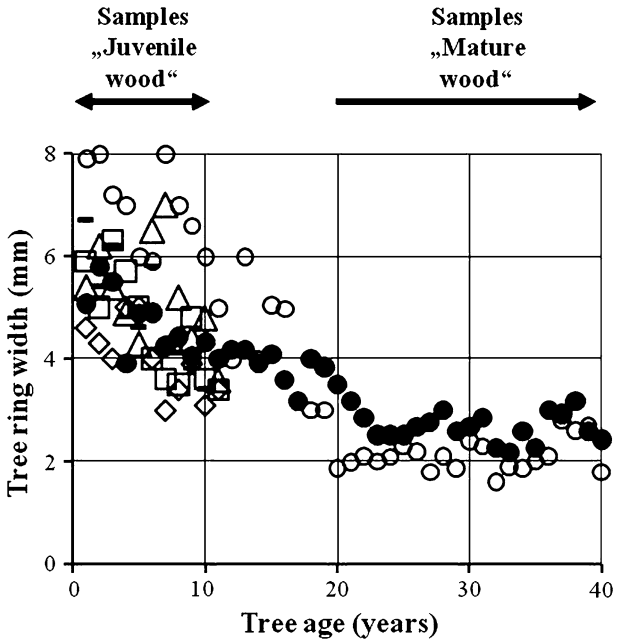

Fig. 1 Tree ring width (mm) of the experimental trees from the sites “Eberswalde” (*filled circle*), “South-Nyirség” (*open circle*), and “Waldsieversdorf” (*triangle* clone 2402, *diamond* clone2405, *square* clone 2461, *hyphen* clone 2498). Samples from the tree rings 1–10 were selected for the analyses of juvenile wood. Samples from the tree rings 20–40 were selected for the analyses of mature wood

This indicates that growth characteristics and wood properties are closely correlated with regard to the differences between juvenile and mature wood.

Lignin and extractive content of juvenile and mature wood

Although the lignin and extractive content in the juvenile and mature sapwood/heartwood of the trees varied with genotype and site conditions, significant differences in the chemical composition between sapwood and heartwood as well as juvenile and mature wood were found (Table 2).

The "Klason"-lignin content in the samples varied between 19.8 and 25.8%. In the heartwood, the lignin content was higher (20.9–25.8%) than in the sapwood (19.8–23.2%). The high content of "Klason"-lignin in the heartwood is supposed to be distorted by non extractable phenolic compounds (Fengel and Wegener 1984). However, in both sapwood and heartwood the lignin content in mature wood was higher than in juvenile wood, a result not likely to be explained by methodological limitations. The ultrastructure of cell walls formed during the juvenile growth phase differs significantly from that of cell walls formed by an older cambium (Abe et al. 1995; Funada et al. 1997; Sahlberg et al. 1997). Various authors (cited in Adamopoulus et al. 2005) consider that particularly the fibrillar orientation in the

Table 2 Lignin and extractive content (%; mean values ± standard deviation) of juvenile and mature sapwood and heartwood of *Robinia pseudoacacia* L.

Sampling position	Trees/sites	Number of samples	Lignin (%)	Extractives MeOH/H_2O (%)	Extractives acetone (%)
Sapwood					
Juvenile wood	Eberswalde	20 (10 × 2)	21.8 ± 2.1	3.9 ± 0.4	n.d.
Mature wood			23.2 ± 2.4	3.8 ± 0.9	n.d.
Juvenile wood	South-Nyirség	8 (4 × 2)	20.7 ± 3.4	3.2 ± 0.8	n.d.
Mature wood			20.9 ± 2.9	3.1 ± 1.1	n.d.
Juvenile wood	Clone 2402, Waldsieversdorf	8 (4 × 2)	19.8 ± 3.1	3.8 ± 0.7	n.d.
	Clone 2405, Waldsieversdorf	8 (4 × 2)	20.2 ± 2.7	4.2 ± 1.2	n.d.
	Clone 2461, Waldsieversdorf	8 (4 × 2)	20.1 ± 3.4	3.6 ± 0.7	n.d.
	Clone 2498, Waldsieversdorf	8 (4 × 2)	22.4 ± 3.0	3.5 ± 0.7	n.d.
Heartwood					
Juvenile wood	Eberswalde	20 (10 × 2)	23.1 ± 1.9	0.9 ± 0.2	5.7 ± 1.3
Mature wood			25.8 ± 3.5	1.3 ± 0.4	8.5 ± 0.8
Juvenile wood	South-Nyirség	8 (4 × 2)	22.4 ± 1.8	1.6 ± 0.4	6.1 ± 0.9
Mature wood			23.7 ± 2.9	1.5 ± 0.5	8.8 ± 1.5
Juvenile wood	Clone 2402, Waldsieversdorf	8 (4 × 2)	22.4 ± 2.8	1.7 ± 0.3	4.9 ± 0.7
	Clone 2405, Waldsieversdorf	8 (4 × 2)	20.9 ± 1.9	2.4 ± 0.3	4.8 ± 0.5
	Clone 2461, Waldsieversdorf	8 (4 × 2)	21.6 ± 2.4	1.9 ± 0.5	5.7 ± 1.3
	Clone 2498, Waldsieversdorf	8 (4 × 2)	23.0 ± 2.7	2.4 ± 0.5	3.9 ± 0.7

Number of samples = number of trees × number of samples per tree

N.d. = not detectable

S_2-layer of the juvenile cells (higher micro fibril angle) is related to the lower lignin content of the juvenile wood.

In agreement with studies by Magel et al. (1991, 1994), the analyses of heartwood extractives in individual tree rings from the cambium to the pith showed that the physiological process of heartwood formation starts in the third or fourth growth increment and is completed after 2 years. In the sapwood, detectable wood extractives were found exclusively in the methanol extracts, whereas heartwood extractives were found in the methanol and in the acetone fraction. In the sapwood of black locust non-structural, methanol soluble carbohydrates and lipids (Magel et al. 1991; Hillinger et al. 1996) are present. The living tree uses these substances particularly in the process of heartwood formation.

The content of heartwood extractives (methanol, acetone) was higher in the mature heartwood (9.8–10.3%) than in the heartwood formed during the juvenile phase of tree growth (6.3–7.7%). The separation of the extractives by HPLC-chromatography showed that in the heartwood of black locust high contents of flavonoids are present, robinetin and dihydrorobinetin being the dominant components (Figs. 2, 3). The comparison of HPLC-chromatograms of heartwood extracts from younger and older trees showed that the chemical composition of extractives in heartwood formed by the younger trees (juvenile heartwood) is very similar to that of heartwood extractives formed by the older trees (mature heartwood). This indicates that in juvenile and mature wood the chemical pathway of heartwood formation is identical (Magel et al. 2001; Yang et al. 2004). However, further analyses are necessary in order to elucidate the chemical structure and the occurrence of higher condensed accessory compounds in the mature heartwood compared to the juvenile heartwood.

Subcellular localisation of lignin and wood extractives

The UV absorbance of lignin depends on the ratio of *p*-hydroxyphenyl-, guaiacyl-, and syringyl units (Sarkanen and Hergert 1971). The lignin in the xylem of the black locust samples showed a maximum UV absorption at a wavelength of 278 nm. Flavonoids also have a strong UV absorbance, but the absorption maxima depend on the chemical bonding to the cell wall. For the detection of flavonoids in the cell walls of the heartwood, the UV absorbance behaviour between 240 and 400 nm wavelength is of special interest (Dietz 2002; Koch et al. 2006).

In all cell walls of sapwood and heartwood, maximum UV absorbance was found at a wavelength of 278 nm indicating that the absorbance was dominated by lignin (Fig. 4a–c). However, over the entire range of wavelengths (240–400 nm), UV absorbance of vessel and axial parenchyma was higher in the heartwood than in the sapwood, while higher UV absorbance of fibre cell walls was restricted to the range of wavelength between 240 and 290 nm. This indicates that a higher concentration of UV absorbing heartwood extractives is present in the cell walls of vessels and axial parenchyma cells than in cell walls of fibres. The exact localisation of the important flavonoids robinetin and dihydrorobinetin was not possible by subcellular UV microspectrophotometry, although the pure substances have distinct maxima of UV-absorbance. Koch et al. (2006) found pure robinetin in heartwood vessels of

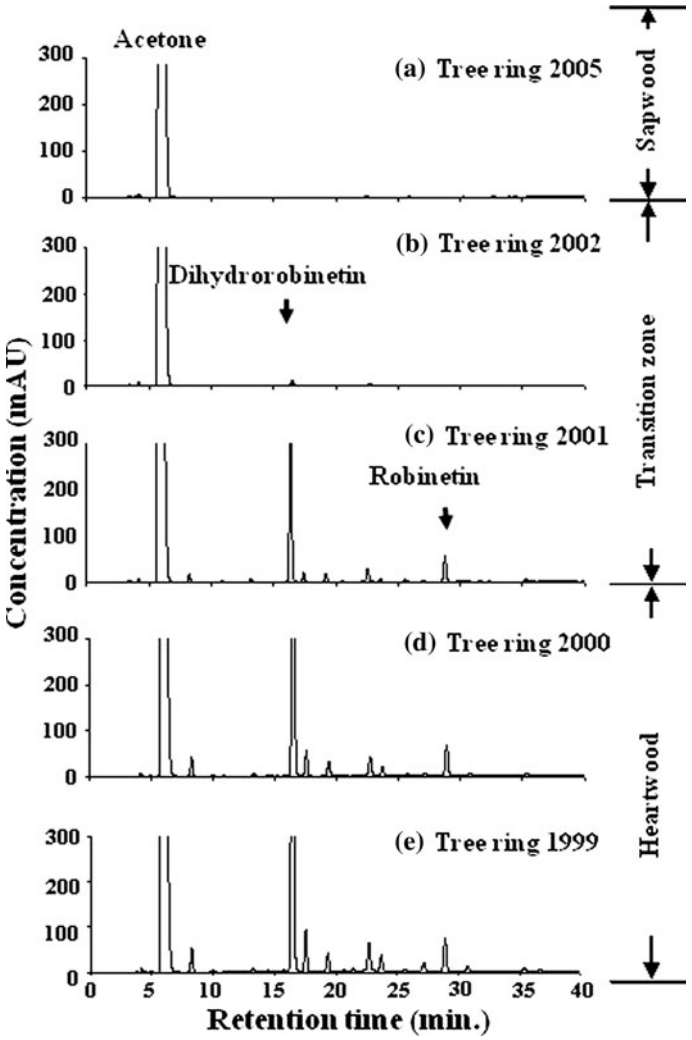

Fig. 2 Extractive content (mAU, acetone extract) in the sapwood, in the sapwood-heartwood transition zone, and in the heartwood of a 11-year-old *Robinia* tree (clone 2498, site "Waldsieversdorf"). HPLC-chromatography, 280 nm wavelength. The retention time of the flavonoids robinetin and dihydrorobinetin are marked by *arrows*

Intsia spp. Differing from that observation, the absorbance spectra indicate that in the heartwood of black locust these flavonoids are not present in their pure form (Fig. 4a–c). The UV-absorbance of robinetin and dihydrorobinetin in situ depends on the chemical structure of the polyphenols (π electron system in the aromatic ring structure) and the chemical bonding to the cell wall (Dietz 2002). The absorbance spectra of black locust heartwood indicate a wide range of aromatic groups such as conjugated double bonds in the molecular structure of robinetin and dihydrorobinetin.

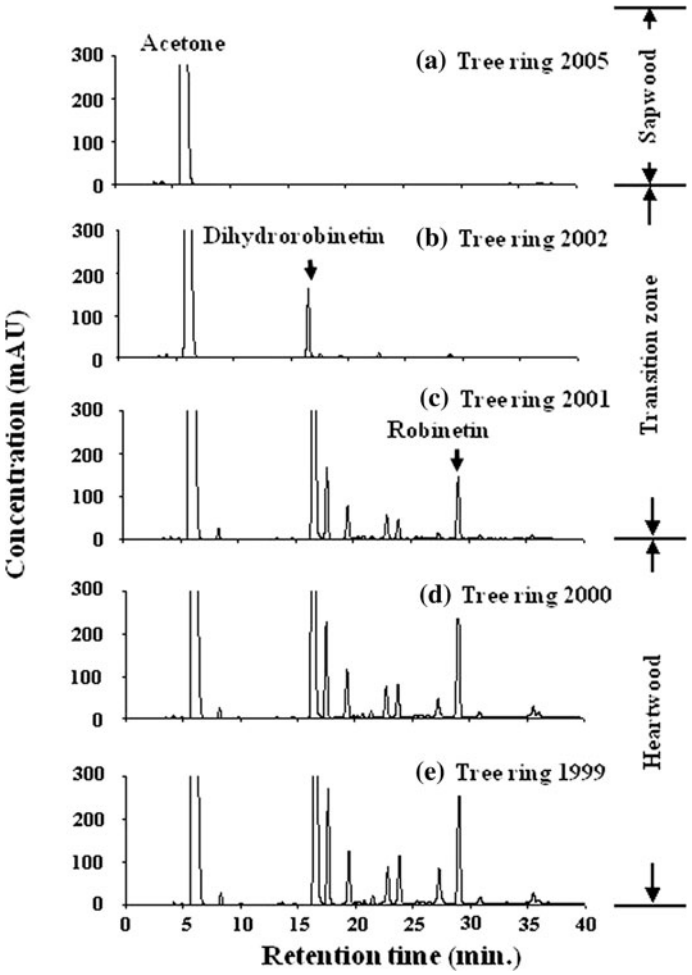

Fig. 3 Extractive content (mAU, acetone extract) in the sapwood, in the sapwood-heartwood transition zone, and in the heartwood of a 59-year-old *Robinia* tree (site "Eberswalde"). HPLC-chromatography, 280 nm wavelength. The retention time of the flavonoids Robinetin and Dihydrorobinetin are marked by *arrows*

The high resolution mapping of the UV absorbance of juvenile and mature heartwood confirmed the results obtained by bulk analyses of heartwood extracts (Table 2). Cell walls of vessels and axial parenchyma of juvenile heartwood had a lower UV absorbance (240–400 nm) compared to cell walls of vessels and axial parenchyma in the mature heartwood (Fig. 5a, b) indicating a higher content of flavonoids deposited in the wall of these cells. Fibre cell walls of juvenile heartwood had a lower maximum of absorbance at a wavelength of 278 nm (UV absorbance $\sim$0.35) compared to fibre cell walls in the mature heartwood (UV absorbance $\sim$0.45). This is evidence for a lower lignin content of fibre cell walls in juvenile wood compared to mature wood (Fergus and Goring 1970a, b), thus explaining the slightly reduced "Klason"-lignin content of the juvenile wood (Table 2).

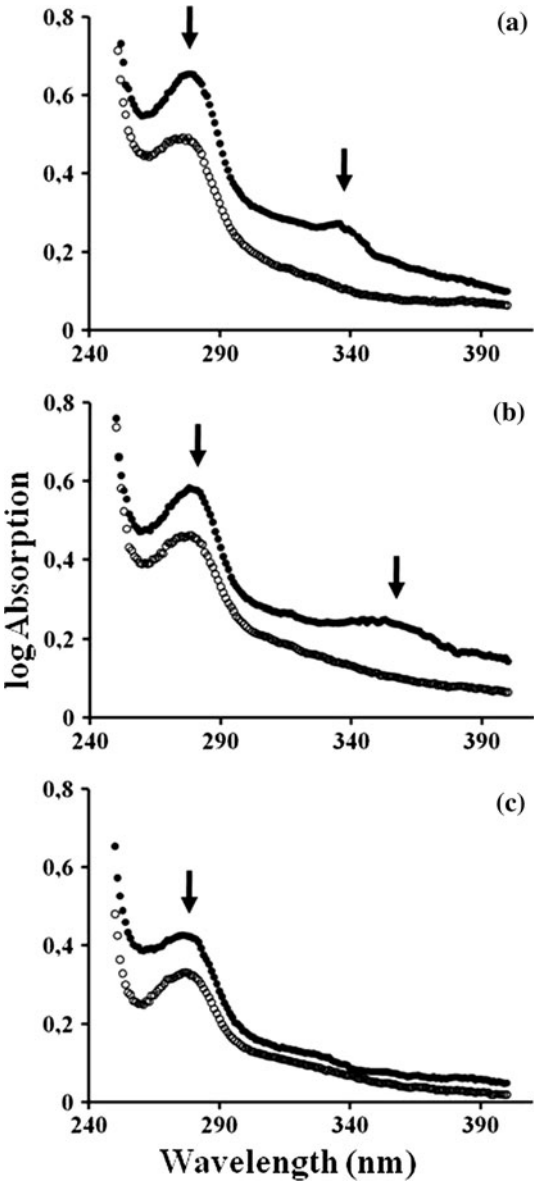

Fig. 4 Representative UV absorption spectra (wavelength 250–400 nm) of the cell walls of (**a**) a vessel, (**b**) an axial parechnyma cell, and (**c**) a fibre in the sapwood (*open circle*) and in the heartwood (*filled circle*) of an old-growth *Robinia* tree (experimental site "Eberswalde"). Absorption maxima in the heartwood are marked by *arrows*

Natural durability of juvenile and mature heartwood

Coriolus versicolor (white rot) caused a higher mass loss of black locust heartwood than *Coniophora puteana* (brown rot). After 16 weeks of exposure the mass loss of juvenile heartwood was 17.0% (*Coriolus versicolor*) and 10.1% (*Coniophora puteana*), while the mass loss of mature heartwood was only 1.7% (*Coriolus versicolor*) and 0.7% (*Coniophora puteana*; Table 3). According to the European

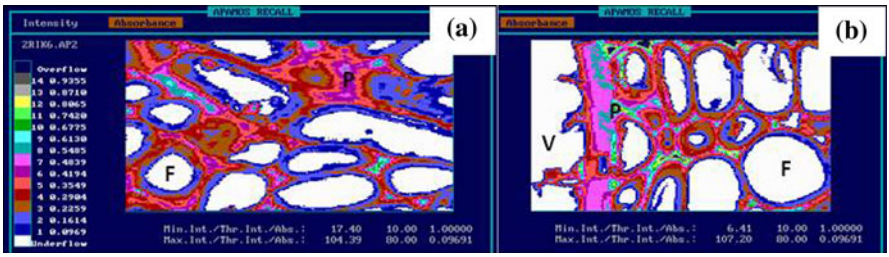

Fig. 5 UV-absorbance (278 nm wavelength) of (**a**) juvenile and (**b**) mature heartwood in the stem of old-growth *Robinia* tree from (experimental site "Eberswalde"). The absorbance scale is given on the left of the figure. *V* vessel, *P* axial parenchyma, *F* fibre

Table 3 Mass loss (%; minimum-mean (underlined value)-maximum) of the juvenile and mature heartwood of *Robinia pseudoacacia* after 16 weeks of exposure to *Coniophora puteana* and *Coriolus versicolor*

Sample (No. of samples)	Coniophora puteana Mass loss (%)	*Coriolus versicolor* Mass loss (%)
Virulence ($n = 18$)	56.6–60.6–64.3 (*Pinus sylvestris* sapwood)	21.7–25.3–35.3 (*Fagus sylvatica*)
Juvenile wood ($n = 64$)	2.9–10.1–32.9 (Durability class EN 350-2: 2)	4.8–17.0–33.0 (Durability class EN 350-2: 4)
Mature wood ($n = 50$)	0.1–0.7–2.5 (Durability class EN 350-2: 1)	0.5–1.7–4.8 (Durability class EN 350-2: 1)

standard EN 350-2, juvenile heartwood conforms to durability class 2–4 (resistant to little resistant), mature heartwood to durability class 1 (highly resistant). The fast increase of durability from the innermost to the outer tree rings during the juvenile phase of growth is supposed to be the reason for the very high variation (up to 30% mass loss, Table 3) of durability found in the juvenile samples.

Conclusion

The results show that the juvenile heartwood of *Robinia pseudoacacia* L. has a lower natural durability than the mature heartwood. The (topo-) chemical analyses indicate that the lower content of phenolic compounds and flavonoids in the juvenile heartwood is the main reason for its lower durability. The results also show that the juvenile growth phase of black locust trees lasts for approximately 10–20 years.

The heartwood of black locust is highly demanded by users especially for exterior applications of long duration. In order to ensure a long lasting performance under exposure of this promising timber, the reduced durability of the juvenile heartwood must be taken into account by silviculture (felling cycle) as well as wood processing (grading).

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