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ORIGINAL

Origin-specific differences in the durability of black locust (*Robinia pseudoacacia*) wood against wood-destroying basidiomycetes

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Abstract

Global climate change is accompanied by a change in tree composition in many regions. In Europe, the distribution areas of many species are expanding towards the north so that, among others, black locust (*Robinia pseudoacacia*), which is native to the USA and has long been established in south-eastern Europe, is also becoming increasingly important in central and northern Europe. Many other tree species are known to have different properties between their original and new locations, including the biological durability of the wood. Hence, the resistance of black locust wood against decay fungi was studied concerning origin-specific differences. Wood was sampled from seven different origins in Europe and original habitats in the United States. Fungal incubation experiments were conducted, wood extractives were analysed, and different anatomical characteristics were quantified such as ring width, vessel size distribution and the presence of tyloses. In addition to differences in durability between juvenile and mature wood, origin-specific differences within the mature heartwood were attributed to extractive contents and the percentages of earlywood vessels containing tyloses. Based on parameters that contributed at least 20% to mass loss, susceptibility to fungal decay was modelled with multiple regressions.

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Introduction

Climate change influences the range of suitability for commercially important tree species (Briscoe et al. 2023; Hama and Khwarahm 2023). Progressive forest management is looking to wood species that are robust to the potential changes in growth conditions. Climate change may influence wood properties, which means that wood properties and performance will change over time in original habitats, and that they may differ between traditional and evolving habitats. Some of the candidate species considered for use in construction show high biological durability and may also serve for outdoor building applications, such as European oak (*Quercus robur* & *Q. petraea*) and sweet chestnut (*Castanea sativa*), which are becoming increasingly available (Conedera et al. 2016; Eaton et al. 2016). There is high demand for European white oaks. While they are well established in Central Europe, their growth and spread potential is increasing in the Nordic countries, such as Norway and Sweden. But other ring-porous hardwood species of higher durability may be seen as alternatives in Central Europe, since they are supposed to better cope with droughts and calamities than the established species. Among them, we find sweet chestnut (Freitas et al. 2022; Conedera et al. 2021) and black locust (*Robinia pseudoacacia*) (Bedbur et al. 2010). The latter is addressed in this study.

Black locust, also called yellow locust, has a disjunct original range in the United States (Huntley 1990). The eastern section is centred in the Appalachian Mountains and ranges from central Pennsylvania and southern Ohio south to north-eastern Alabama, northern Georgia, and south-western South Carolina. The western section ranges from southern Missouri to northern Arkansas and north-eastern Oklahoma. However, black locust has been planted widely and became naturalized throughout the United States, Canada, Europe and Asia (Huntley 1990; Sitzia et al. 2016; Puchałka et al. 2021). It is considered the first North American tree species imported to Europe in the early 17th century and was named after Jean Robin, the gardener of King Henri IV of France, who planted the first trees in Paris (Demené and Merzeau 2007).

Black locust was extensively planted in Central Europe. It is nowadays widespread and the most used non-native tree species on the continent (Brus et al. 2019). Furthermore, black locust is considered a 'climate change winner' tree species since it tolerates drought and nutrient poor soils (DeGomez and Wagner 2001) and is therefore of increasing interest in reforestation. However, black locust mostly regenerates vegetatively by root suckers and may therefore be considered invasive (Vítková et al. 2017). Thus, its suitability for replacement of less drought-tolerant European tree species is debated (Li et al. 2014; Dyderski et al. 2018; Nicolescu et al. 2018; Nadal-Sala et al. 2019; Puchałka et al. 2021).

The wood of *Robinia pseudoacacia* is highly valued and has been appreciated for many different purposes (Cuno 1930; Huntley 1990; DeGomez and Wagner 2001; Page et al. 2013; Vítková et al. 2017).

The durability of the ring-porous heartwoods is a function of water exclusion efficacy, e.g. through the formation of tyloses, and the presence of biologically active secondary metabolites (Ruppitsch et al. 2021). Intra-species variability of moisture dynamics in black locust were reported by Brischke et al. (2024). The high natural

durability of black locust heartwood is often explained by the presence of bioactive phenolic extractives (Pollet et al. 2008; Latorraca et al. 2011). Extractives from black locust heartwood include radical scavengers (Vek et al. 2020a, b; Hosseinihashemi et al. 2016). It is assumed that non-enzymatic antioxidants have an important inhibitory effect against brown rot fungi. Free radicals produced in the first, oxidative radical-based phase of the two-step wood degradation mechanism of brown rots are scavenged by the antioxidant extractives (Belt 2022, Schultz et al. 2000). Triterpenes and simple sugars (e.g., sucrose, glucose and fructose) and non-structural carbohydrates (starch); soluble proteins and phenolic compounds such as simple phenols, phenolic acids, stilbenes, flavonoids and condensed tannins have been identified in the tissues of black locust. Flavonoids dihydrorobinetin (flavanonol), robinetin (flavonol) and derivates of hydroxycinnamic acid have been reported to be the characteristic phenolic compounds present in the hydrophilic extracts of black locust heartwood (Vek et al. 2019a, b). The high natural durability of the heartwood of black locust is also explained by the presence of dihydrorobinetin and robinetin in these tissues (Magel et al. 1994; Sergent et al. 2014). Similar compounds are reported for oak and sweet chestnut (Eichhorn et al. 2017).

The density of ring-porous hardwoods increases with increasing ring width. It has also been reported that the durability of English oak (*Q. robur*) with extremely narrow rings is relatively low, similar to the durability of European beech (*Fagus sylvatica*) (Humar et al. 2008).

The content of heartwood extractives depends also on the position in the stem. It was demonstrated that the heartwood of black locust is characterized by well-defined radial variability, whereas the amount of extractives significantly decreases from the outermost heartwood towards the pith (Magel et al. 1994; Dünisch et al. 2010; Latorraca et al. 2011; Vek et al. 2020b). The axial variability of the extractive content in black locust stemwood is less pronounced (Adamopoulos et al. 2005). The extractives in black locust stemwood have been relatively well studied, but there is little data on the fungal decay resistance of black locust heartwood of different ages.

Wood species can be assigned to five durability classes (DC) of biological durability against wood-destroying fungi (DC 1 ‘very durable’ to DC 5 ‘non-durable’) according to EN 350 (2016). Black locust is assigned to DC 1–2 (1–2), where the classes refer to tests with soil contact and with basidiomycetes (in parentheses) respectively. For some wood species, DCs refer also to different origins, e.g., Douglas-fir (*Pseudotsuga menziesii*) from North America or those cultivated in Europe (EN 350 2016). Previous studies indicate that similar differences can be expected also for black locust and other hardwoods with coloured heartwood (Adamopoulus et al. 2005, Brischke et al. 2009; Dünisch et al. 2010; Meyer et al. 2014; Eichhorn et al. 2017). In particular, the juvenile wood percentage and the amount and composition of extractives with inhibitory effect on fungal growth differs between origins. Differences in durability between sources from original stands and evolving habitats or plantations were reported, for instance for teak (*Tectona grandis*) and Western redcedar (*Thuja plicata*) (Haupt et al. 2003; Stirling et al. 2012). However, the effect of climate and other site-specific effects on the biological durability of European-grown ring-porous hardwood species such as black locust is neither fully understood nor quantified in terms of durability classes or service life expectancies.

The aim of this study was to examine whether the biological durability of black locust varies by origin and to identify potential wood-specific parameters that impact the material-inherent resistance against decay fungi. For this purpose, black locust wood was sampled from different origins in Europe, and from its native range in the United States. Fungal incubation experiments were conducted, wood extractives were analysed, and different anatomical characteristics were quantified such as ring width, vessel size distribution and the formation of tyloses.

Materials and methods

Wood material

Black locust (*Robinia pseudoacacia*) stem sections originating from two to five trees per study region were investigated (Table 1). The origin could only be traced back to the regional level. Stem sections were cut into planks using a band saw and air-dried at the University of Goettingen. Specimens were sampled from central boards at different zones of the stem, i.e., in the outer heartwood (adjacent to the sapwood, hw_{outer}), the central heartwood ($hw_{central}$), and the inner heartwood (juvenile wood, hw_{inner}) as shown in Fig. 1. Juvenile wood specimens were taken within the inner 15 annual rings; the pith itself was excluded. Material from three regions in the United States was sawn board sections, partly without pith, and therefore pooled as shown in Table 1.

For an additional consideration of the entire heartwood (hw_{total}), the data for the three previously separately considered heartwood sections were pooled. Specimens of 15×25×50 (ax.) mm³ were made from central boards of each stem section. Virulence control specimens were made from Scots pine (*Pinus sylvestris*) sapwood and European beech (*Fagus sylvatica*).

Table 1 Origin of black locust timber for testing

Region	Country	Code	Sampled trees			# replicates $hw_{outer}/hw_{central}/hw_{inner}$	
			#	Age [years]	DBH [cm]	*C. puteana*	*T. versicolor*
Brandenburg	Eastern Germany	BB	3	26–33	19.9	25/27/24	25/27/24
Celle	Northern Germany	CE	5	25–33	11.9	28/20/31	28/20/31
Reinhausen	Central Germany	RH	3	33–63	29.9	31/30/27	30/30/27
Calw	Southwestern Germany	CA	3	17–22	15.9	22/22/17	23/22/17
Alsace	Western France	AL	3	42–43	19.9	26/30/21	26/30/20
Dolsko	Slovenia	DO	2	n.a.	n.a.	20/5/20	20/5/20
Sibiu	Central Romania	SI	3	23–27	15.9	28/18/22	26/17/19
Northern Alabama[1]	Eastern United States	NA	3	n.a.	n.a.	1/30/18	1/32/20
Eastern Tennessee[1]	Eastern United States	ET	4	n.a.	n.a.		
Western Tennessee[1]	Eastern United States	WT	3	n.a.	n.a.		

[1]Material was sawn boards, partly containing no pith. Sample sets were pooled for durability tests and named 'US'

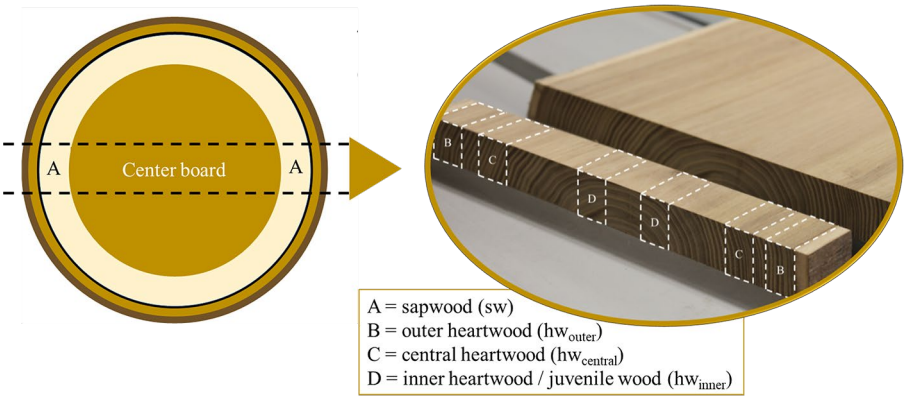

Fig. 1 Stem sections sampled for durability tests

Durability tests with basidiomycetes

The durability of the different black locust timbers was evaluated according to EN 113-2 (2021). Therefore, two to 38 replicate specimens were used for each combination of test fungus, origin, and stem zone. The number of replicates varied due to varying availability of the test material.

Virulence control specimens were made from Scots pine sapwood and European beech. The following fungal strains were used for the tests: the brown rot fungus *Coniophora puteana* = (Schum.:Fr.) P. Karsten BAM Ebw. 15 and the white rot fungus *Trametes versicolor* = (L.:Fr.) Pilat CTB 863 A.

Before incubation for 16 weeks, the test specimens were conditioned at 20 °C / 65%*RH* to constant mass, weighed to the nearest 0.001 g, steam sterilized in a steam pot for 20 min and another 10 min on the following day. Afterwards, sets of two specimens of the same combination of wood species, origin, and stem section were placed on fungal mycelium in Kolle flasks filled with 100 ml malt extract agar (4%). Additional moisture content control specimens were oven-dried at 103 ± 2 °C. Their average equilibrium moisture content (*EMC*) was used to calculate the theoretical initial oven-dry mass of all test specimens according to EN 113-2 (2021).

After incubation, the specimens were cleaned of adhering mycelium, weighed to the nearest 0.001 g, oven-dried at 103 ± 2 °C, and weighed again. The *ML* of the specimens were determined and median *ML* was used to assign durability classes (DC) between DC 1 (very durable) and DC 5 (not durable) according to EN 350 (2016).

Anatomical analysis

The anatomical analysis included measuring the dimensions of vessels and the analysis of tylosis-bearing vessels within the samples of *Robinia pseudoacacia* from different wood origins (Table 1). Before image collection, specimens with the size of 15 × 25 × 50 (ax.) mm³ were prepared according to EN 113-2 (2021), and then cut into smaller specimens with cross-sectional surfaces of 12.5 × 15 mm² (R×T). Subsequently, the surface of these specimens was smoothed using a microtome. The image

collection was performed using the digital 3D reflected light microscope Keyence VHX-7000 (Keyence, Neu-Isenburg, Germany). The Keyence VHX-7000 (VHX) allows to obtain true-colour images of the observation surface non-destructively. All images were acquired at a lens magnification of 100× and saved as TIFF files. Each saved image was the result of several 2D images stitched together into a composite image. Using VHX composite images, the entire sample surface was captured.

The image analysis was performed by the open-source GIMP software (GIMP Development Team). Prior to the analysis, the pixel size of the images was set in GIMP based on the pixels of their scale bars. For each cross-section, three rectangular boxes, whose size radially covered one annual ring and tangentially 2000 µm, were extracted for the analyses as indicated in Fig. 2. Within these boxes four anatomical traits were considered for earlywood and latewood: (i) the number of vessels, (ii) the radial diameter of vessel lumina, (iii) the percentage of vessels containing tyloses, and (iv) the width of earlywood and latewood. Vessels smaller than 10 µm in diameter were not considered. The examinations were performed for the different growing locations, stem sections (i.e., outer heartwood (hw_{outer}) and inner heartwood (hw_{inner}) zones, and annual ring zones (i.e., earlywood and latewood).

Extraction and chemical analysis

Extraction

Prior to extraction and chemical analyses, blocks of black locust heartwood were ground with a Retsch SM 2000 cutting mill using a 1 mm bottom sieve. The ground samples were freeze-dried for 24 h at -85 °C and 4.5 kPa in a Telstar LyoQuest CC1930 lyophilizator, weighed into the 10 mL SST extraction cells, and extracted in a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor. Accelerated extraction was performed with aqueous acetone (90%, v: v) at 110 °C and 10.342 MPa. One gram (dw) of the heartwood sample was extracted with 4×5 min

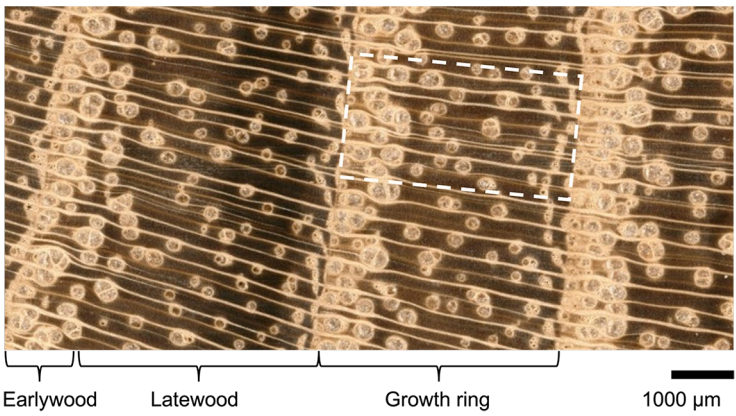

Fig. 2 Different zones of interest on a cross-section of black locust heartwood. The dashed rectangle indicates the position and size of the observation area that was considered for the anatomical analysis

static cycles under nitrogen. Final volumes of the acetone heartwood extracts were defined to 100 mL.

Gravimetrical analysis

Total hydrophilic extractives (THE) were determined gravimetrically. Aliquots of heartwood extracts were dried in an oven to constant weight using a temperature gradient from 60 °C to 105 °C. THE were measured in milligrams per gram of dry heartwood sample (mg/g, dw) (Vek et al. 2019a). The results of the gravimetric analysis are expressed as a percentage of THE per dry weight of the sample (% w/w, dw).

Spectrophotometric analysis

Total phenol contents (TPC) in heartwood samples were measured according to a protocol previously described by Singleton and Rossi (1965) and Vek et al. (2019a). Briefly, aqueous Folin–Ciocalteu phenol reagent (diluted 10 times) and aqueous solution of sodium carbonate (75 g/L) were added to 0.25 mL of a heartwood extract. After incubation of the reaction mixtures, absorbance was measured at 765 nm using a Perkin-Elmer UV/Vis spectrophotometer. The results were expressed in milligrams of gallic acid equivalents per gram of dried wood sample (mg GAE/g dw). The method for semi-quantitative determination of TPC was linear in the selected concentration range ($R^2 > 0.99$).

HPLC analysis

Reference compounds in black locust heartwood, i.e., dihydrorobinetin (DHR) and robinetin (Rob) were measured with a high-performance liquid chromatography system equipped with a photodiode array detector (Thermo Scientific, Accela 600 HPLC-PDA) (Vek et al. 2022). Samples were separated on a Thermo Accucore C18 column with dimensions of 4.6 mm (I.D.) × 150 mm, particle size of 2.6 µm). Water + 0.1% formic acid (v/v) (A) and methanol + 0.1% formic acid (v/v) (B) served as a mobile phase. The flow rate of the mobile phase was 1 mL/min. The gradient used was from 5 to 95% of solvent (B). The temperature of an auto-sampler with sample trays and the temperature of a column oven were 5 and 30 °C, respectively. Analytical HPLC standards for DHR and Rob were purchased from Extrasynthese (HPLC assay, ≥ 95%). Absorbance of separated compounds of heartwood extracts and those of HPLC standards were measured at 280 nm and UV spectra were recorded from 200 nm to 400 nm. The calibration curves for DHR and Rob used for quantitative analysis were linear ($R^2 > 0.99$).

Antioxidant assay

After the chemical analyses, the heartwood extracts of the same growth site were pooled. Eleven samples of black locust heartwood, one sample per site (Table 1), were prepared. The extracts were dried using a vacuum chamber (at 10 kPa and room temperature), lyophilizator (same conditions as described above) and then re-

dissolved in water. Gallic acid (GA) and ascorbic acid (AA) were used as reference antioxidants. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to the protocol described by Vek et al. (2020a). Briefly, the references and the heartwood extracts were prepared in ten testing concentrations (1000 mg/L – 1,6 mg/L). Water and methanol were used as a control. A 2.25 mL of DPPH methanol solution was mixed with a 90 µL of sample. The reaction mixtures were incubated for 30 min in the dark at room temperature. After incubation, the reduction of DPPH radical was determined colorimetrically by measuring absorbance at 517 nm with UV-Vis (see above). Results are expressed as DPPH radical scavenging activity (%RSA), together with the IC50 (50% inhibition concentration, mg/L).

Statistical analysis

Each combination of origin and stem zone was one group of statistical interest. The two preconditions normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene test) were checked first. Depending on the results, the compatible tests for further analysis were chosen. If there were more than two groups to compare (e.g., decay tests and the chemical analysis) an omnibus-test (analysis of variance, Welch or Kruskal-Wallis test) and the compatible post-hoc analysis (Tukey, Games Howell or Steel-Dwass test) were applied to do pairwise comparisons. Only two stem zones were considered per origin for analysis of anatomical features (see sect. Anatomical analysis). Hence, different tests were used for their analysis (i.e., t-test, Mann-Whitney test). Multiple or multifactorial regressions were conducted to examine which extractives had an influence on mass loss by fungal decay (see sect. Multifactorial analysis of impact variables).

The chemical structures of flavonoids and stilbenes presented with figures were prepared by using a Perkin Elmer's ChemDraw 20.1 software.

Results and discussion

Mass loss by fungal decay

Mass loss (*ML*) of black locust heartwood by fungal decay differed between stem sections, test fungi and wood origins (Fig. 3). Generally, the white rot fungus *T. versicolor* caused higher *ML* on black locust, compared to the brown rot fungus *C. puteana*, which coincides with the general association of white rot fungi and hardwoods. *T. versicolor* was generally more virulent than *C. puteana* (Table 2), but both test fungi caused *ML* well above the threshold of 20%, and *C. puteana* even well above 30%. Hence, the test was valid according to EN 113-2 (2021).

The *ML* data and the corresponding DCs are shown in Fig. 3 and Table 3 and do basically coincide with the durability classification provided by EN 350 (2016), i.e., DC 1–2 (1–2). The median *ML* of both the inner and central black locust heartwood

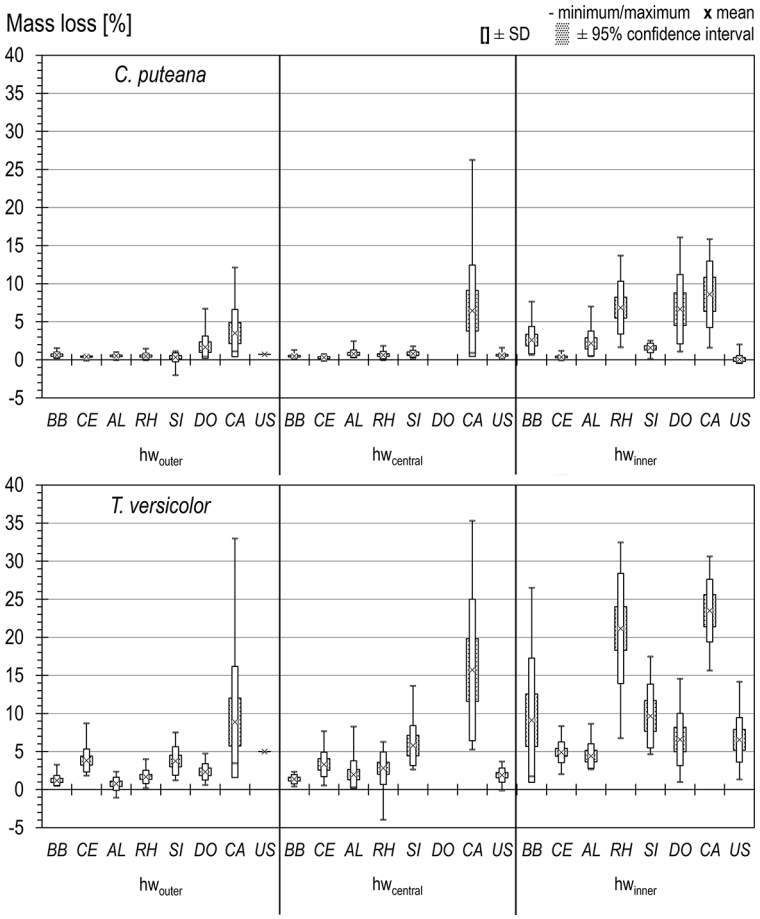

Fig. 3 Mass loss of different black locust origins and stem sections due to fungal decay after 16 weeks of incubation with *Coniophora puteana* and *Trametes versicolor.* No specimens of $hw_{central}$ from DO were available

Table 2 Mass loss [%] of virulence control specimens after 16 weeks of incubation

Wood species	Test fungus			
	C. puteana		*T. versicolor*	
European beech	36.9	±8.8	29.2	±2.7
Scots pine sapwood	35.6	±9.8	29.5	±10.5

of all origins was below 5% except for the material from Calw in south-western Germany (Fig. 3). The latter showed a median *ML* of 6.4% (hw_{outer}) and 14.3% ($hw_{central}$) after incubation with *T. versicolor* and should therefore be assigned to durability class (DC) 2 and 3, respectively (EN 350 2016). Furthermore, the $hw_{central}$ from Sibiu (Romania) had a median *ML* of 5.01% and was thus assigned to DC 2. Black locust from all other origins was assigned to DC 1 (≤5% *ML*) including the wood from the

Table 3 Median mass loss (*ML*) and corresponding durability classes (DC)

Wood origin	Stem zone	*T. versicolor*		*C. puteana*	
		Median *ML*	DC	Median *ML*	DC
BB	hw_{outer}	1.1	1	0.4	1
	$hw_{central}$	1.5	1	0.4	1
	hw_{inner}	5.6	2	2.1	1
CE	hw_{outer}	3.5	1	0.4	1
	$hw_{central}$	3.1	1	0.4	1
	hw_{inner}	5.0	2	0.3	1
AL	hw_{outer}	0.5	1	0.5	1
	$hw_{central}$	1.4	1	0.7	1
	hw_{inner}	4.0	1	1.5	1
RH	hw_{outer}	1.5	1	0.4	1
	$hw_{central}$	2.7	1	0.5	1
	hw_{inner}	22.6	4	5.8	2
SI	hw_{outer}	3.2	1	0.4	1
	$hw_{central}$	5.0	2	0.8	1
	hw_{inner}	7.8	2	1.5	1
DO	hw_{outer}	2.3	1	1.2	1
	$hw_{central}$	n.a.			
	hw_{inner}	6.2	2	6.4	2
CA	hw_{outer}	6.4	2	2.6	1
	$hw_{central}$	14.3	3	4.2	1
	hw_{inner}	23.2	4	9.6	2
US	hw_{outer}	5.0	1	0.7	1
	$hw_{central}$	1.9	1	0.6	1
	hw_{inner}	6.0	2	-0.0	1

US. *T. versicolor* caused small differences in *ML* between the different heartwood groups.

Larger differences in *ML* between the origins were found for the inner heartwood specimens of black locust (Fig. 3). The highest median *ML* caused by *T. versicolor* was obtained for inner heartwood from Calw (23.2%) followed by that from Reinhausen (22.6%), Sibiu (7.7%) and Brandenburg (5.7%). The lowest median *ML* was determined for inner heartwood from the Alsace (4.0%). The generally lower durability of the inner heartwood, which is likely juvenile wood, was expected and confirms previous findings, e.g., by Dünisch et al. (2010) who found 17% *ML* of juvenile wood, while mature heartwood had only 1.7% *ML* both caused by *T. versicolor.*

Anatomical traits

Inner and outer heartwood differed visibly in their anatomical traits, such as ring width, and number and diameter of vessels (Table 4). On average for all wood origins, the annual tree-ring width was 2.8 mm for the outer heartwood (hw_{outer}) and 4.5 mm for the inner heartwood (hw_{inner}, Table 4). In other words, the ring width of hw_{inner} was 1.6 times of that of hw_{outer}. Unlike the similar width of the earlywood zone in both heartwood sections (approx. 0.6 mm), the widths of the latewood zones differed considerably. This consistency of earlywood, and the variation in latewood is typical for ring-porous hardwoods (Carlquist 2013). Thereby, the latewood width

Table 4 Anatomical results of black locust wood for all wood origins and the two heartwood zones. EW: Earlywood; LW: latewood; *n*: number of replicates

Wood origin	Stem zone	*n*	Growth ring width [µm]		Mean vessel number		Vessels without tyloses [%]	
			EW	LW	EW	LW	EW	LW
BB	hw_{outer}	6	593.1	992.9	12.7	20.4	4.4	25.5
BB	hw_{inner}	6	485.1	2767.4	17.0	50.9	5.2	19.0
CE	hw_{outer}	8	583.9	1091.8	17.1	36.0	10.9	45.4
CE	hw_{inner}	10	574.5	1899.3	18.3	47.9	3.6	40.6
AL	hw_{outer}	6	545.9	1158.6	20.1	45.6	2.8	28.8
AL	hw_{inner}	6	659.4	3953.7	28.9	88.9	4.2	16.5
RH	hw_{outer}	6	764.4	1902.2	23.6	62.9	14.6	26.7
RH	hw_{inner}	8	735.3	4677.0	20.6	80.3	4.3	18.9
SI	hw_{outer}	6	728.2	1098.5	18.7	81.1	7.1	27.1
SI	hw_{inner}	6	741.3	4778.0	19.3	66.0	0.6	21.7
DO	hw_{outer}	4	785.4	6142.8	23.8	120.0	2.3	22.9
DO	hw_{inner}	4	633.0	6057.5	22.1	139.2	2.5	23.0
ET	hw_{outer}	3	767.2	1234.6	25.3	73.7	5.3	58.8
ET	hw_{inner}	3	718.8	3651.6	22.0	118.0	3.0	45.5
ET	hw_{inner}	3	641.0	3877.0	19.7	107.6	14.1	36.2
CA	hw_{outer}	6	567.3	3665.8	19.2	85.7	10.4	23.3
CA	hw_{inner}	6	542.4	4465.0	25.6	82.3	11.3	23.1
WT	hw_{inner}	3	728.7	2335.5	23.1	91.4	5.8	28.8

Table 5 Anatomical results of vessel diameter of black locust wood averaged for all growing locations

Heartwood zone	Annual ring zone	Vessel diameter [µm]	
hw_{outer}	Growth ring	89.5	±87.6
	Earlywood	180.7	±103.5
	Latewood	61.4	±58.1
hw_{inner}	Growth ring	104.9	±81.4
	Earlywood	172.5	±87.6
	Latewood	87.1	±69.6

of 3.8 mm for hw_{inner} was 1.8 times of that for hw_{outer} (width of 2.1 mm). Even though the hw_{inner} tended to larger ring widths for most of the locations, stems from Dolsko and Calw showed marginally greater ring widths for hw_{outer}. Previous studies (Adamopoulos et al. 2010; Adamopoulos and Voulgaridis 2002; Dünisch et al. 2010) showed that the tree-ring widths of black locust decrease with cambial age in the first 20 years distinctly.

The wood of black locust showed a typical ring-porous vessel distribution (IAWA Committee 1989; Wagenführ 1999; Table 5). In this study, earlywood vessels in hw_{inner} and hw_{outer} were two and three times larger than latewood vessels (Table 4). Average earlywood vessel size of hw_{inner}, (105 µm) was greater than that of hw_{outer} (90 µm). However, comparing the earlywood and latewood separately, an opposite trend became obvious. The earlywood vessels were marginally smaller in the inner heartwood zone than those in the outer heartwood zone (Table 3). An opposite trend was observed for latewood vessels (Table 5).

Because of the small size of latewood vessels and the large width of latewood sections, it was not surprising that the number of vessels in the latewood was considerably higher compared to the earlywood (Table 4). Unlike the number of earlywood vessels, which was similar for all origins, the number of latewood vessels varied between origins from 20 in Brandenburg to 139 in Dolsko.

Black locust is known as a hardwood species with a high rate of vessel occlusions (i.e., tyloses) of heartwood. That fact was confirmed in this study. Regarding the vessels without tyloses, there was no significant difference between the heartwood zones (Table 4) with average values of 26% and 23%, respectively. Within the growth rings, the highest rate of tyloses in vessels was found in earlywood. Here, only 6% of the vessels were free of tyloses. In latewood, 27% of vessels were free of tyloses. It should be noted that the examination of vessel occlusion was accomplished on 2D microscopy images. Vessels that revealed no tyloses during the 2D examination, however, might be occluded by tyloses elsewhere within the same vessel.

Content of extractives

The chemical analysis revealed significant differences in the amounts of total hydrophilic extractives (THE) among the heartwood samples of different geographical origins (ANOVA, $p=0.01$) (Table 6). The highest amounts of THE were extracted from the heartwood samples from northern Alabama followed by Alsace, Celle, Dolski, Sibiu, western Tennessee, and Calw. The results of the gravimetric analysis are supported by the results of the colorimetric analysis, which also showed significant differences in total phenol contents (TPC) among the heartwood samples examined (ANOVA, $p<0.01$, Table 6). The largest TPC were measured in heartwood samples from northern Alabama (51.84 mg/g) and Alsace (36.31 mg/g) (LSD test). In contrast, the lowest TPC was characteristic for samples from Calw (20.07 mg/g) (LSD test). It has already been demonstrated that the heartwood of black locust is characterized by a significant radial gradient in extractive content with higher amounts of THE, TPC and DHR in the outermost heartwood (Vek et al. 2020b). In the present study, a significant decrease of THE, TPC and DHR in the centripetal direction ($hw_{outer} \rightarrow hw_{central} \rightarrow hw_{inner}$) was also found for black locust heartwood of other geographical origins (ANOVA, $p_{THE,\ TPC,\ DHR,\ Rob} > 0.051$). On average, the hw_{outer} contained significantly higher amounts of THE, TPC, DHR, and Rob than the juvenile hw_{inner} (LSD test, Fig. 4).

The applied HPLC method has already been demonstrated to be efficient for the chemical monitoring of dihydrorobinetin (DHR) and robinetin (Rob) in wood and bark extracts of black locust (Vek et al. 2019b, 2020b; Keržič et al. 2023). The most abundant and characteristic peak on the HPLC chromatograms was assigned to DHR (Fig. 4). Besides DHR and also other identified compounds that are extracted from wood of black locust (Sanz et al. 2012), Rob is referred to be one of the characteristic flavonoids (Sergent et al. 2014; Bostyn et al. 2018; Atwi-Ghaddar et al. 2023). The stilbenes piceatannol and resveratrol were also found in the heartwood samples but only in traces (Fig. 4). However, DHR and Rob are related to the higher decay resistance of black locust heartwood (Smith et al. 1989; Rademacher et al. 2016). The HPLC showed significant differences in the content of DHR in Rob in the inves-

Table 6 Contents of extractives in the heartwood samples of black locust of different origin. Results are expressed on a dry matter basis (% or mg/g, dw)

Origin and stem zone	THE				TPC			DHR				Rob		
	% (w/w, dw)				mg GAE/g (dw)			mg/g (dw)				mg/g (dw)		
	n	mean	SD		mean	SD		**n**	mean	SD		mean	SD	
Alsace	**9**	**11.55**	**±3.07**	**A**	**36.31**	**±10.72**	**A**	**9**	**27.97**	**±11.17**	**ABC**	**4.79**	**±3.64**	**AB**
hw_{outer}	3	13.60	±2.44	a	44.24	±9.78	a	3	38.03	±9.54	a	8.52	±4.22	a
$hw_{central}$	3	12.80	±2.59	ab	38.63	±10.1	a	3	24.48	±8.81	a	2.79	±0.91	a
hw_{inner}	3	8.26	±0.40	b	26.07	±0.79	a	3	21.40	±9.77	a	3.07	±1.76	a
Brandenburg	**9**	**9.88**	**±2.61**	**AB**	**33.42**	**±10.37**	**A**	**9**	**29.23**	**±10.54**	**AB**	**5.56**	**±2.04**	**AB**
hw_{outer}	3	11.77	±1.75	a	40.09	±9.37	a	3	36.60	±7.91	a	5.96	±1.28	a
$hw_{central}$	3	11.12	±1.39	a	38.01	±7.18	ab	3	32.44	±10.73	a	6.20	±2.92	a
hw_{inner}	3	6.75	±0.17	b	22.14	±1.20	b	3	18.63	±1.24	a	4.53	±2.02	a
Calw	**9**	**6.77**	**±1.50**	**B**	**20.07**	**±4.33**	**B**	**9**	**14.78**	**±5.04**	**BC**	**3.51**	**±1.71**	**B**
hw_{outer}	3	7.97	±0.77	a	23.76	±2.52	a	3	20.15	±3.47	a	5.41	±1.16	a
$hw_{central}$	3	6.94	±1.79	a	20.96	±3.86	ab	3	13.67	±2.65	ab	3.33	±0.51	b
hw_{inner}	3	5.41	±0.49	a	15.47	±0.57	b	3	10.51	±3.18	b	1.78	±0.33	b
Celle	**15**	**8.98**	**±2.61**	**AB**	**28.23**	**±7.43**	**AB**	**15**	**15.19**	**±6.33**	**C**	**4.62**	**±1.74**	**B**
hw_{outer}	5	10.34	±1.77	a	32.59	±6.09	a	5	16.42	±2.22	a	5.50	±1.12	a
$hw_{central}$	5	8.84	±3.82	a	27.60	±10.3	a	5	12.90	±7.67	a	4.20	±2.68	a
hw_{inner}	5	7.77	±1.36	a	24.51	±2.64	a	5	16.25	±8.17	a	4.15	±0.82	a
East. Tennessee	**4**	**8.68**	**±2.28**	**AB**	**25.65**	**±5.83**	**AB**	**4**	**17.10**	**±10.90**	**ABC**	**7.03**	**±2.73**	**AB**
$hw_{central}$	1	10.93			32.78			1	2.60			3.03		
D	3	7.93	±2.10		23.28	±4.14		3	21.93	±6.17		8.36	±0.68	
North. Alabama	**1**	**14.36**			**51.84**			**1**	**27.96**			**8.36**		
hw_{inner}	1	14.36			51.84			1	27.96			8.36		
Reinhausen	**9**	**9.17**	**±3.73**	**AB**	**28.11**	**±12.62**	**AB**	**9**	**26.15**	**±16.39**	**ABC**	**9.75**	**±8.15**	**A**
hw_{outer}	3	12.01	±0.90	a	36.46	±1.14	a	3	38.49	±3.64	a	14.82	±10.24	a
$hw_{central}$	3	11.05	±1.10	a	35.91	±6.13	a	3	27.01	±19.74	a	9.38	±7.73	a
hw_{inner}	3	4.44	±1.59	b	11.98	±3.50	b	3	12.96	±13.48	a	5.05	±5.42	a

Table 6 (continued)

Origin and stem zone	THE				TPC			DHR				Rob		
	% (w/w, dw)				mg GAE/g (dw)			mg/g (dw)				mg/g (dw)		
	n	mean	SD		mean	SD		**n**	mean	SD		mean	SD	
Sibiu	**8**	**8.04**	**±1.54**	**AB**	**23.47**	**±3.14**	**AB**	**8**	**18.32**	**±4.29**	**BC**	**6.21**	**±0.84**	**AB**
hw_{outer}	3	9.62	±1.31	a	26.67	±2.99	a	3	22.68	±2.67	a	6.32	±1.13	a
$hw_{central}$	2	7.60	±0.13		21.82	±0.70		2	15.74	±3.10		6.02	±0.21	
hw_{inner}	3	6.75	±0.32	b	21.37	±0.86	b	3	15.68	±2.63	a	6.24	±1.05	a
Dolsko	**4**	**8.18**	**±1.50**	**AB**	**32.16**	**±7.34**	**AB**	**4**	**39.50**	**±15.39**	**A**	**2.58**	**±0.80**	**B**
hw_{outer}	2	8.67	±0.79		36.44	±8.11		2	47.99	±13.65		2.71	±0.87	
hw_{inner}	2	7.70	±2.28		27.87	$\pm 4.72^{-}$		2	31.00	±15.36		2.44	±1.04	
West. Tennessee	**3**	**7.45**	**±0.96**	**AB**	**23.64**	**±1.41**	**AB**	**2**	**7.84**	**±3.23**		**1.27**	**±0.38**	
hw_{inner}	3	7.45	±0.96		23.64	±1.41		2	7.84	±3.23		1.27	±0.38	

The content of total hydrophilic extractives (THE) is expressed as percent THE (w/w, %) per dry weight of sample, dw). The total phenols (TPC) are given as mass equivalents of gallic acid (mg GAE) per dry weight of wood sample (g, dw) (mg GAE/g, dw). The contents of dihydrorobinetin (DHR) and robinetin (Rob) is given in milligrams per gram of dry wood sample (mg/g, dw). The contents of total hydrophilic extractives (THE), total phenols (TPC), dihydrorobinetin (DHR) and robinetin (Rob) are expressed as the number of specimens (n) and the mean value of measurements (mean) with standard deviations (SD) of the geographical origins (bold) and stem sections. Statistical comparisons were done as post-hoc analysis using the Tukey test. Groups with $n<3$ were excluded. Different small letters mark significant differences between the stem zones per origin at a 95% confidence level. Different capital letters mark significant differences between the origins at a 95.0% confidence level

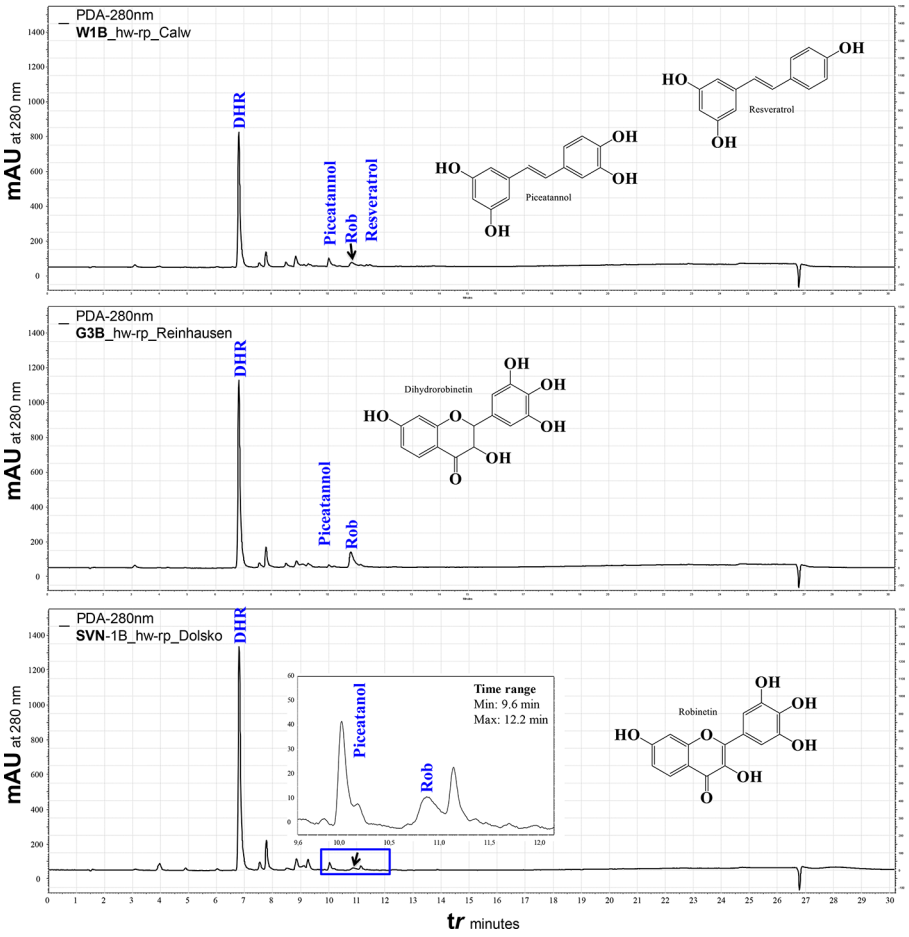

Fig. 4 HPLC-PDA chromatograms of acetone extracts of black locust monitored at 280 nm. The phenolic compounds targeted were dihydrorobinetin (DHR) and robinetin (Rob), as the high natural durability of the heartwood of black locust is associated with the presence of robinetins. Calw (W1B), Reinhausen (G3B), and Dolsko (SVN). HPLC of the heartwood extracts gave the following elution order: Dihydrorobinetin (DHR), t_r = 6.9 min; Piceatannol, t_r = 10.0 min; Robinetin (Rob), t_r = 10.9 min, and Resveratrol, t_r = 11.5 min. HPLC run time was 30.2 min

tigated heartwood samples (ANOVA, $p_{DHR,\ Rob} > 0{,}051$) (LSD test). On average, among the trees of investigated geographical regions, the heartwood of black locust from Dolsko and Brandenburg contained the highest amounts of DHR, while heartwood from Reinhausen was the richest in Rob (Table 1; Fig. 4).

The protective role of phenolic extractives against wood decay is explained also with nonbiocidal properties, e.g., with their free radical scavenging activity (Schultz et al. 2002), and extractives of black locust have already been demonstrated to be good natural antioxidants rather than fungal growth inhibitors (Vek et al. 2020a, b). Hence, a preliminary analysis was performed and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) of heartwood extracts of different geo-

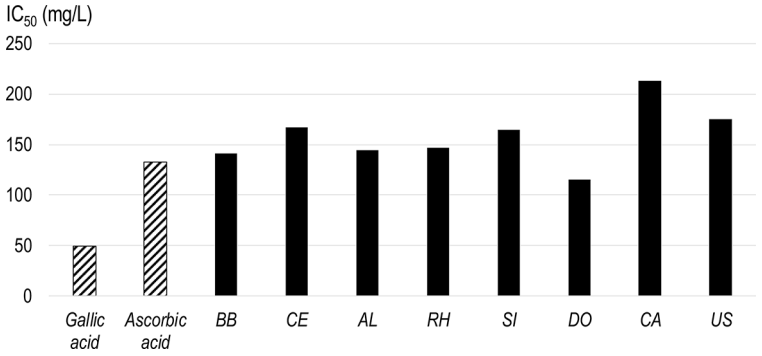

Fig. 5 DPPH radical scavenging IC_{50} values (mg/L) of the heartwood extracts of black locust of different geographical origins. The IC_{50} values (mg/L) of heartwood samples were compared with the IC_{50} values (mg/L) of gallic and ascorbic acid

graphical origin was measured. The results of the antioxidant assay are presented as IC_{50} values (Fig. 5), i.e., with the concentration of a heartwood extract that scavenges 50% of the initial DPPH radicals, and with the plots showing DPPH radical scavenging activity regarding the test concentration of the heartwood extract.

Comparison of DPPH RSA to those of reference natural antioxidants, i.e., gallic and ascorbic acid, showed that the tested heartwood extracts are good free radical scavengers (Fig. 5). Heartwood extracts at 250 mg/L showed DPPH RSA around 80% or even higher. The tested heartwood extracts performed well as natural antioxidants also at the lower test concentrations. For example, heartwood extracts at 100 mg/L from northern Alabama, Brandenburg, Alsace and Reinhausen showed RSA comparable with the RSA of ascorbic acid of the same concentration. Heartwood extract of black locust from Dolsko at 100 mg/L was an even better scavenger of free radicals than ascorbic acid. Almost the same DPPH RSA number has been reported for the heartwood extracts of black locust that were sampled at the suburban forest Panovec near Nova Gorica, Slovenia (Vek et al. 2020a; Fig. 5). An average concentration of the heartwood extracts that scavenge 50% of the initial DPPH radicals, was measured to be 162.9 mg/L. IC_{50} values for gallic acid and ascorbic acid were 49.5 and 133.0 mg/L. Phenolic compounds are believed to protect the wooden cell wall after a fungal attack by scavenging free radicals produced by the Fenton reaction (Schmidt 2006; Schultz et al. 2000, Valette et al. 2017). In summary, the results of the chemical analysis showed that the geographical origin has an important influence on both the content of extractives and the antioxidant properties of the heartwood extracts of black locust.

Multifactorial analysis of impact variables

Possible influences of the chemical and anatomical parameters on the fungus-induced *ML* were investigated. Therefore, stem sections per fungus were differentiated first, followed by further differentiations in a second run. The interrelationships between *ML* by fungal decay and the content of total hydrophilic extractives (THE), total phenols (TPC), dihydrorobinetin (DHR), and robinetin (Rob) separately are shown in Figs. 6 and 7. It became evident that these parameters were better correlated with *ML*

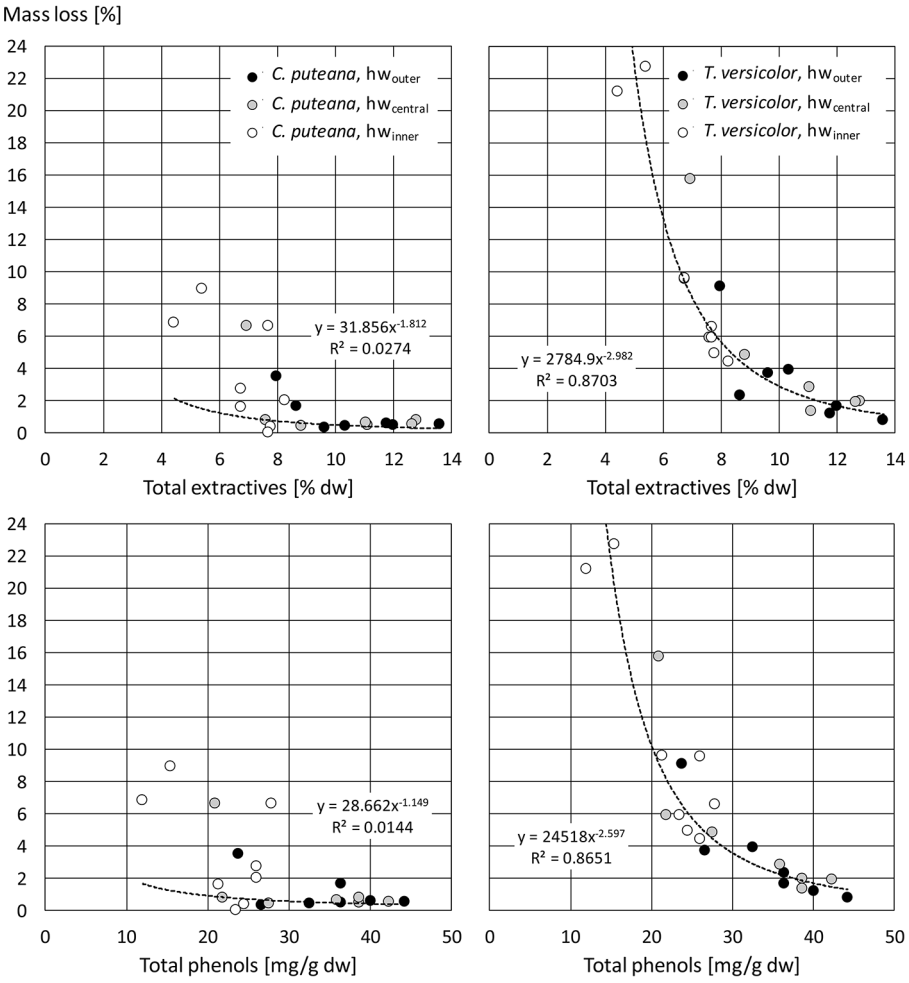

Fig. 6 Interrelationship between the content of total extractives (%), the amount of total phenols (mg/g) and mass loss (%) by *C. puteana* and *T. versicolor*. Each dot represents the mean of one wood origin. Fitting power functions refer to data pooled from all three heartwood sections

by *T. versicolor* compared to those caused by *C. puteana*, which was also generally lower in *ML* values. The best correlation was obtained between *ML* by *T. versicolor* and THE, followed by TPC, DHR, and Rob which coincided with previous findings by Vek et al. (2020), who showed the variability of extractives in black locust stems with gradients from hw_{outer} to hw_{inner}. The lower decay resistance of the juvenile wood of black locust might be attributed to extractive gradients, but the latter became evident also within one stem zone (Figs. 6 and 7). In contrast to the extractives, the *ML* was barely influenced by the percentage of vessels closed with tyloses.

The results of a predictor screening, where THE, TPC, DHR, Rob and the percentage of different vessels without tyloses were checked all together, are summarized in

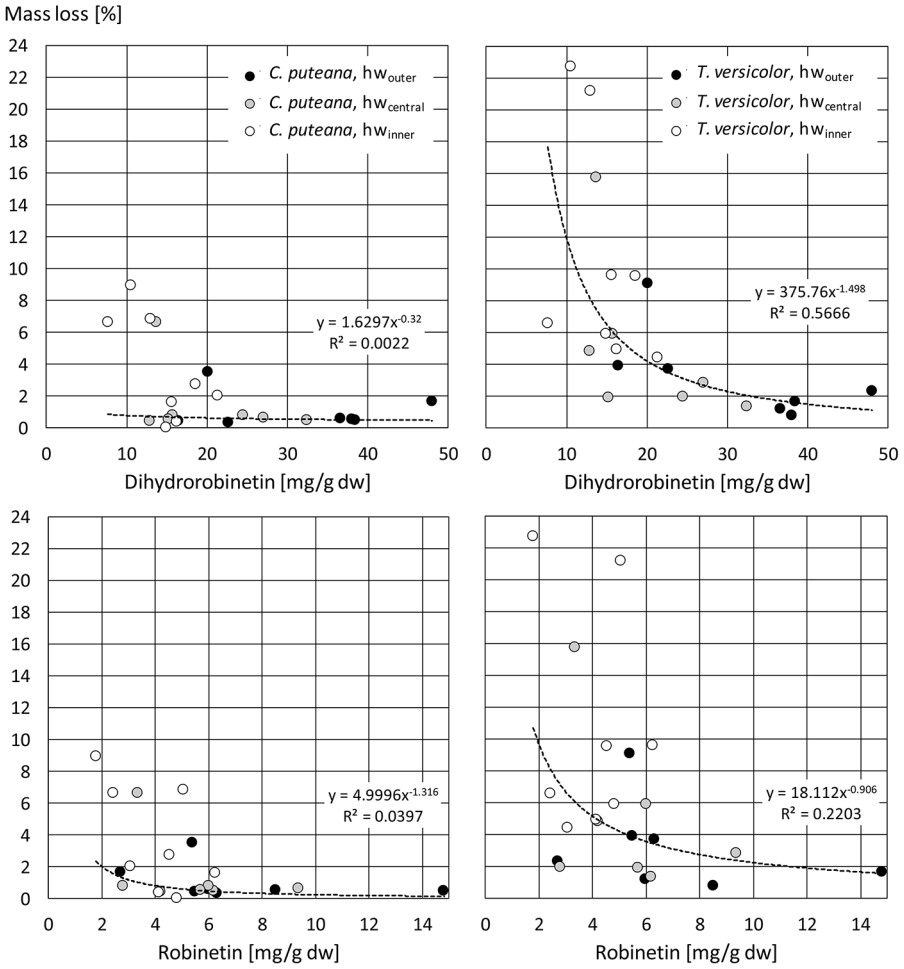

Fig. 7 Interrelationship between the amount of dihydrorobinetin (mg/g) and robinetin (mg/g) respectively and mass loss (%) by *C. puteana* and *T. versicolor*. Each dot represents the mean of one wood origin. Fitting power functions refer to data pooled from all three heartwood sections

Table 7. Differences in contributions between the single stem sections and the total stem are partly explained by the lack of information for $hw_{central}$. The parameters, that contributed at least 20% to the *ML*, were in case of both *ML* by *T. versicolor* and *ML* by *C. puteana* TPC and THE. Due to the small number of replicates, potential interactions between the parameters remained unconsidered. We got the following two linear models:

$$C.puteana \quad ML = 8.40 - 0.85 \times THE + 0.05 \times TPC, R^2 = 0.44$$

$$T.versicolor \quad ML = 23.97 - 0.45 \times THE - 0.46 \times TPC, R^2 = 0.70$$

Table 7 Contribution [%] of different chemical and anatomical variables to the mass loss (*ML*)by fungal decay

Test fungus	Stem section	THE	TPC	DHR	Rob	Vessels without tyloses		
						EW	LW	all
C. puteana	hw_{outer}	37	9	7	13	8	22	4
	$hw_{central}$	74	16	4	6			
	hw_{inner}	30	9	14	19	6	2	20
	all	20	33	6	16	2	18	5
T. versicolor	hw_{outer}	47	18	9	1	5	13	7
	$hw_{central}$	73	19	6	2			
	hw_{inner}	53	15	17	3	11	0	1
	all	37	49	12	0	0	1	1

THE total hydrophilic extractives, TPC Total phenolic compounds, DHR dihydrorobinetin, Rob Robinetin, EW earlywood, LW latewood

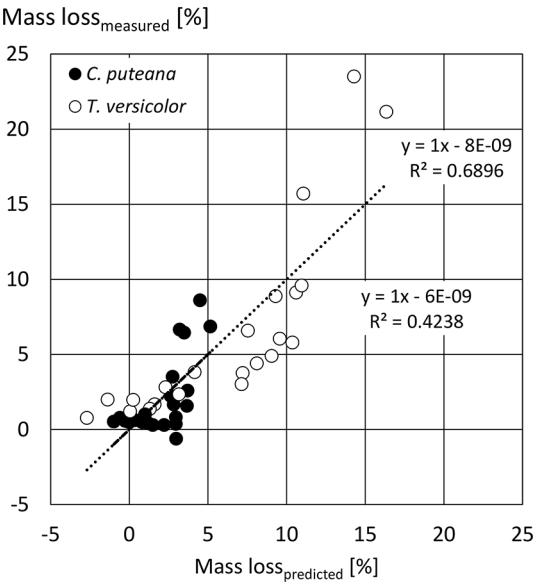

Fig. 8 Mass loss prediction. Each dot represents the mean of one stem zone from one origin

and calculated the theoretical values for *ML* with these formulas, Table 7. The measured and predicted *ML* were compared in Fig. 8.

Again, it became clear that the relationships between the influencing variables and the resulting *ML* were more pronounced for *T. versicolor* compared to *C. puteana*. Generally, the fit between predicted and measured *ML* was surprisingly striking.

Conclusion

The results of this study made clear that the biological durability of the black locust heartwood is subject to considerable variability. Black locust from most origins was assigned to DC 1 including the wood from the US, but wood from Calw (Germany)

was assigned to DC 2 and 3, material from Sibiu (Romania) to DC 2. These differences could partly be attributed to the origin of the test material and associated differences in the extractive content of the wood. The influence of vessels blocked by tyloses was negligible. In general, the results obtained are indicative, since the underlying sample size was limited. Future studies with a larger sample size and considering further wood origins are needed to validate the recent findings. Nevertheless, the influence of the most important influencing variables could be quantified and used for a simple prediction model of *ML* with a surprisingly high level of accuracy. Increased understanding of source-associated variability in wood durability will be helpful as forest management and climate change alter the distribution of useful tree species.

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Author contributions C.B., H.S., T.K., V.V. and M.H. planned and conducted the experiments, analysed the data, wrote the main manuscript text and illustrated the results. C.M.C.C. conducted the anatomical and durability studies. B.S. did the statistical analysis. A.M.T. planned the experiments and interpreted the results. All authors reviewed the manuscript.

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Data availability Data are available from the authors on request.

Declarations

Competing interests The authors declare no competing interests.

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