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Phenolic extractives of wound-associated wood of beech and their fungicidal effect

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ABSTRACT

Extracts of wound-associated beech wood (*Fagus sylvatica* L.) were spectrophotometrically analyzed and a paper disc screening test was applied to estimate their fungicidal potential against selected brown (*Gloeophyllum trabeum*) and white (*Trametes versicolor*) rot fungi. Colorimetric analysis revealed that higher amounts of total phenols, flavonoids and proanthocyanidins were characteristic of the reaction zone, and especially of wound-wood, while the lowest contents were measured in red heart samples. Estimation of the fungicidal properties of wound-associated wood extracts revealed that the evident inhibitory effect on wood decaying fungi can be ascribed to methanolic extracts of wound-wood, as well as to healthy sapwood. Extracts of reaction zones did not exhibit a corresponding inhibitory effect toward the chosen fungi. These results indicate a potential defensive function of wound-wood and sapwood in living trees, whereas already formed reaction zones behave as physical barriers rather than chemically inhibiting fungal growth.

1. Introduction

European beech (*Fagus sylvatica*) is one of the economically most important tree species in Europe. It is widely used in the furniture industry, for impregnated railway sleepers, plywood, particle boards and bentwood (Hakkou et al., 2006).

Beech is generally susceptible to the formation of false "red heart" at the location of ripe wood, due to enzymatic oxidative discoloration (Torelli, 1984). This is a significant problem, since the value of discolored beechwood is usually lower than unaffected wood. Discolored beechwood is hard to impregnate, problems can occur during drying, the cutting and slicing of logs results in cracked veneer and it is sometimes treated as esthetically defective material (Zell et al., 2004).

Wood from trees in sustainably managed forests is usually additionally depreciated due to wounding, which may occur as a result of forestry operations, as well as numerous biotic and abiotic factors. Trees respond to wounding in a predictable way. Compromised wood becomes discolored and walled-off from the sound sapwood by reaction zones (Shigo and Marx, 1977; Shain, 1979), whereas new wood formed after wounding is intended to close up the scar. This wood is referred to as wound-wood (Shigo, 1986).

Reaction zones in beech are dark colored wood layers, which are characterized by an intensive formation of tyloses in vascular tissues, an accumulation of phenolic substances and suberization of parenchyma cells and tyloses (Schwarze and Baum, 2000). A protective and defensive function of reaction zones has been variously attributed to both structural and chemical features, which can act as physical, antifungal, antimicrobial barriers and hydraulic sealants (Pearce, 1996).

Information about the content of phenolics in wound-associated wood, as well as their inhibitory effect on the growth of wood decaying fungi is sparse (Baum and Schwarze, 2002). It is assumed that the content of phenolic extracts might be higher in these tissues due to the compartmentalization function they have in the wood of living trees. It is also assumed that bioactive compounds may occur in wound-associated wood of beech. It is important to determine the influence of these bioactive compounds on fungal growth, since these chemicals might influence the performance of susceptible beech wood against wood decay fungi.

The objectives of this investigation were (*a*) to determine the content of total phenols, flavonoids and proanthocyanidins in different categories of wood tissue altered by wounding of European beech and (*b*) to estimate the fungicidal effect of hydrophilic

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extracts of wound-associated wood of European beech against representative white and brown rot fungi.

2. Materials and methods

2.1. Wood inhabiting fungi

White rot fungus *Trametes versicolor* (ZIM L057) and brown rot fungus *Gloeophyllum trabeum* (ZIM L018) were used for this study. Both fungi were stored in the culture collection of industrial microorganisms (Raspor et al., 1995) of the Biotechnical Faculty, University of Ljubljana, Slovenia. Each fungus was maintained on a previously prepared Petri dish containing potato dextrose agar (DIFCO). The white and brown rot fungi were incubated in a growth chamber at 25 °C and 85% RH for one week (Humar and Pohleven, 2007).

2.2. Plant material

The wood samples that were included in this investigation were obtained from two mechanically wounded beech trees (*Fagus sylvatica* L.) with lesions several meters long. In both cases this was superficial wound of the stem, where bark was peeled off. Wounds were similar in both trees but tangential dimensions of the original and already overgrown wound as well as extent of decayed and discolored wood varied along the stem (Fig. 1). The origin of the wound is not known. Three sample discs of approx. 10 cm in thickness were taken at 1 m, 2 m and 3 m above ground. Samples of intact sapwood (S), reaction zone (RZ), discoloration or "red heart" (RH) and wound-wood (W) were sawn from the discs (Fig. 1), dried at room temperature and milled in a Retsch ZM 200 rotary mill, by which 0.5 mm sample fractions were obtained. The dust was then stored at − 20 °C until further processing.

2.3. Extraction

Hydrophilic extractives were extracted from powder of intact sapwood, reaction zones, "red heart" and wound-wood wood powder with 70% methanol (*aq*) after removal of lipophilic extractives by cyclohexane. Two extraction techniques were applied. Cold extraction was performed on a multi-position magnetic stirrer at 22 °C and lasted for 6 h (Albert et al., 2003). Secondly, samples were extracted in a Soxhlet apparatus for 6 h with 250 ml of solvent.

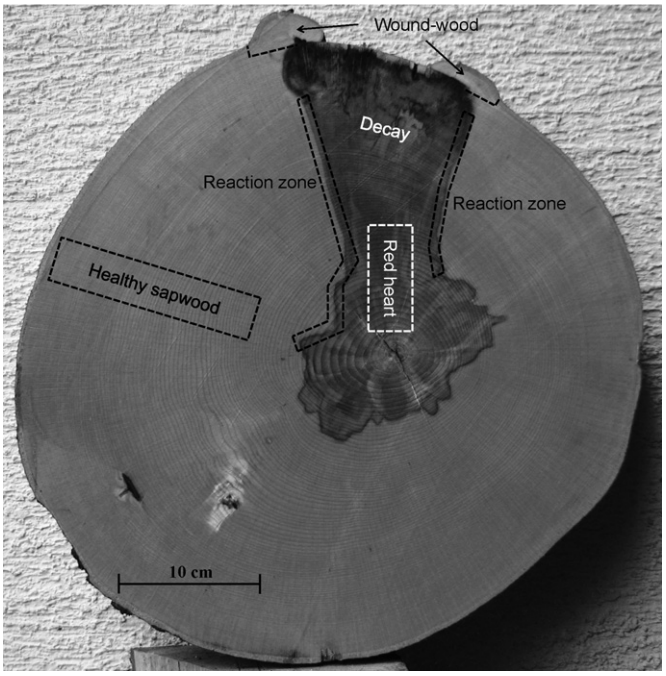

Fig. 1. Cross-section of the wound with marked positions of samples for extraction. The disk was dissected from beech tree no. 2.

2.4. Spectrophotometric analysis

The content of total phenols, flavonoids and proanthocyanidins in samples of intact sapwood, reaction zone, discoloration or "red heart" and wound-wood were determined colorimetrically with a Perkin–Elmer Lambda 2 UV–Vis spectrophotometer in extracts obtained by cold extraction. Three aliquots of each extract were prepared and measured. The results were subsequently expressed as a mean value. Total extractives were additionally determined gravimetrically (%).

2.4.1. Content of total phenols

The content of total phenols in wound-associated beechwood was estimated by the Folin–Ciocalteu method, according to Singleton and Rossi's protocol (Singleton and Rossi, 1965; Scalbert et al., 1989). Calibration was achieved with gallic acid aqueous solutions. Diluted Folin–Ciocalteu phenol reagent and aqueous sodium carbonate were added to methanol extracts and gallic acid solutions (*aq*). According to Scalbert et al. (1989), sodium carbonate was added within 8 min after the addition of the Folin–Ciocalteu phenol reagent. Incubation lasted for 2 h at room temperature. Absorbances were measured at a wavelength of 765 nm and the content of total phenols was expressed as gallic acid equivalents per mass of dry wood.

2.4.2. Content of total flavonoids

Total flavonoids were determined by applying the $AlCl_3$ method (Brighente et al., 2007; Diouf et al., 2009). A methanol solution of aluminum chloride was mixed with the same volume of wood extracts and to standard solutions of quercetin. After 1 h of incubation at room temperature, absorbances were measured at a wavelength of 415 nm and the results were expressed in quercetin equivalents per mass of dry wood.

2.4.3. Content of total proanthocyanidins

The content of total proanthocyanidins was measured by vanillin assay as described by Scalbert et al. (1989). Methanol was removed under reduced pressure, the obtained water fractions were acidified by 6 N hydrochloric acid to pH = 2 ± 0.5 and extracted again with a non-polar solvent (diethyl ether). Two ml of vanillin reagent, which had been prepared as vanillin solution in 70% sulfuric acid, was added to 1 ml of acidified water solution of each extract. After the samples had been incubated for 15 min at 20 °C, the reaction was stopped in an ice bath and absorbances were measured at a wavelength of 500 nm. Results were defined as (+)-catechin equivalents.

A comparison of the content of total phenols, flavonoids and proanthocyanidins in beechwood samples from the two trees was done by statistical methods, whereby significant differences were investigated by means of ANOVA at 0.95 interval of confidence. Yields of phenolic extractive groups for different categories of wood tissue in an individual stem were further compared by means of the multiple range test (LSD procedure).

2.5. Fungicidal properties of extractives

The effect of lipophilic and hydrophilic extractives on the growth of white rot (*T. versicolor*) and brown rot (*G. trabeum*) fungi was investigated by means of a paper disc screening test according

to the non-standard method proposed by Humar and Pohleven (2007, Hashim et al., 2009). By using extractives only, we excluded influence of anatomy of regular and traumatic structures of wood as well as influence of variability in distribution of structural compounds of wood on decay rate. Samples of intact sapwood, reaction zones, "red heart" and wound-wood extracted by magnetic stirrer and Soxhlet apparatus were included in this research. Whatman cellulosic antibiotic assay discs with a diameter of 13 mm were used as carriers of the beechwood extracts. The assay with each fungus was performed separately on 5 parallels for non-polar and polar beechwood extracts. Four cellulosic discs, which were placed in a suitably marked Petri dish containing potato dextrose agar, were impregnated ten times with a volume of 100 μl of sapwood, reaction zone and wound-wood extracts. The methanol aqueous solution and cyclohexane, the solvents that had been used for extraction, were used for impregnation of the controls in the same quantities as for the impregnation of other paper discs. As the number of the testing position in the petri dish is limited, and as we tried to have all test setups as comparable as possible fungicidal properties of extractives of three tissues were determined only. RH extractives were excluded, as they did not exhibited any influence on the growth of the fungi in the preliminary experiments.

After the extracts had been pipetted on cellulosic discs, the solvent was evaporated by keeping the Petri dish open at room temperature for approximately half an hour under sterile conditions. White and brown rot fungal inoculums were placed in the center of the Petri dish and stored in a growth chamber, in which incubation followed at 25 °C and 75% relative humidity. Growth of mycelium on each of the impregnated discs was monitored after 3, 4, 7 and 10 days as indicated in Table 1 and described by Humar and Pohleven (2007). Fungal growth toward the paper discs with different extractives was visually estimated and compared to the growth in control direction for every respective Petri dish. The estimate of the influence of beech wood extracts on the growth of *T. versicolor* and *G. trabeum* was determined as an average of all observations. It should be taken to consideration, that some extractive promotes fungal growth, while some other retarded it, therefore the scale chosen covers all possible responses.

3. Results

3.1. Content of phenolic extractives

The content of total extractives, total phenols, flavonoids and proanthocyanidins for different types of wound-associated wood from two beech trees sampled at different heights are presented in Table 2 and Figs. 2 and 3.

The amount of compounds soluble in methanol was not significantly different between the trees (ANOVA, $F = 0.83$, $p = 0.37$) (Fig. 2).

Statistically significant differences in the content of total phenols (TP), flavonoids (Fla) and proanthocyanidins (PAC) between the two trees (ANOVA, $F_{TP} = 11.96$, $p_{TP} = 0.0009$, $F_{Fla} = 8.47$, $p_{Fla} = 0.0048$, $F_{PAC} = 28.43$, $p_{PAC} = 0.0000$) were found. This coincides with the average values of each group of phenolic extractives, where amounts were appreciably higher in samples from the first stem (1st tree = 9.86, 0.320 and 1.29 mg g^{-1}; 2nd tree = 5.88, 0.215 and 0.444 mg g^{-1}) (Fig. 3). The differences in content of total phenols (ANOVA, $F = 0.65$, $p = 0.53$) and flavonoids (ANOVA, $F = 0.23$, $p = 0.79$) were not statistically significant among different sampling heights (Table 2). A comparison of total proanthocyanidins by ANOVA and the multiple range test revealed that samples taken at 3 m contained statistically significantly less proanthocyanidins (ANOVA, $F = 4.23$, $p = 0.0185$) than samples from lower parts of the investigated stems (Table 2).

Significant differences in the content of extractable compounds (ANOVA, $F = 5.6$, $p = 0.0052$) (Fig. 2) and in the content of different groups of phenolic extractives among different categories of wound-associated beechwood (ANOVA, $F_{TP} = 44.13$, $p_{TP} = 0.0000$, $F_{Fla} = 24.59$, $p_{Fla} = 0.0000$, $F_{PAC} = 14.53$, $p_{PAC} = 0.0000$) (Fig. 3) were found.

The average amount of extractable compounds was significantly higher for wound-wood (Fig. 2). No significant differences were found in the average content of extractable compounds from sapwood, reaction zones and red heart.

The average content of total phenols in reaction zones and wound-wood were significantly higher than in red heart and sapwood at a 95% confidence level (LSD test). Neither reaction zones and wound-wood nor sapwood and red heart differed in respect of total phenols.

The average content of flavonoids was significantly higher in reaction zones than in the other three categories of wood at a 95% confidence level (LSD test). Analysis of the average proanthocyanidin contents revealed that their amount was lower in red heart and was significantly different from the content of proanthocyanidins found in reaction zones and wound-wood (LSD test, 95% confidence level).

Examination of the different categories of wood tissues of an individual tree revealed that RZ contained significantly higher amounts of TP, Fla and PAC than adjacent sapwood and red heart in the 1st tree (Fig. 3 and Table 3). RZ contained significantly higher amounts of TP and Fla than wound-wood, although the amount of PAC did not differ among these categories of wood in the first beech tree. The average content of TP and Fla was also higher in RZ than in sapwood and reaction zones of the 2nd tree (Fig. 3 and Table 4). The amount of proanthocyanidins was below the limit of detection in RH. In contrast to the 1st tree, the average amount of proanthocyanidins of RZ in the 2nd tree was lower than in wound-wood.

3.2. Influence of beech extracts on wood decaying fungi

The inhibitory effects of cyclohexane and methanol beechwood extracts on the wood decaying fungi *T. versicolor* and *G. trabeum* are shown in Table 5.

A screening test indicated that wood extracts did not exhibit observably different influences on the growth of *T. versicolor* and *G. trabeum*. Polar extractives, which were obtained with aqueous methanol, showed a noticeably stronger inhibitory impact on the growth of wood decaying fungi than compounds soluble in cyclohexane (Table 5). The growth of the chosen rotting fungi was not inhibited by control carriers that were impregnated with the solvents used for extraction.

Among the four examined wood extracts, three showed a relevant inhibitory effect on the growth of the selected rotting fungi. The strongest influence on fungi was demonstrated by the methanol extract of wound-wood. The progress of both fungi was also impeded by cyclohexane wound-wood extracts. A particularly strong inhibitory effect of methanolic extracts of wound-wood and sapwood was shown against brown rot fungus *G. trabeum*. The strong impact of wound-wood extract on the growth of the fungi

Table 1
Visually determined mark for estimation of fungal growth within screening test.

Mark	Visually estimated feature
+	Promotes the growth of mycelia
/	Normal growth, no retardation
−	Inhibits the growth of mycelia
—	Markedly inhibits the growth of mycelia

Table 2
Amount of extractives soluble in methanol and content of total phenols, flavonoids and proanthocyanidins in different types of wound-associated wood for two investigated beech trees.

Tree	Height	Sample	Total extractives (%)	Total phenols (mg g^{-1})	Flavonoids (mg g^{-1})	Proanthocyanidins (mg g^{-1})
1st beech tree	1 m	S	2.8	7.3 ± 0.09	0.15 ± 0.001	1.58 ± 0.106
		RZ	3.4	19.1 ± 0.30	0.78 ± 0.007	2.19 ± 0.063
		RH	2.4	3.1 0.09	0.24 ± 0.003	0.49 ± 0.102
		W	4,7	11.4 ± 0.10	0.22 ± 0.002	1.94 ± 0.088
	2 m	S	1.5	6.6 ± 0.10	0.14 ± 0.000	0.98 ± 0.052
		RZ	4.0	21.7 ± 0.32	0.60 ± 0.003	2.75 ± 0.452
		RH	2.0	2.6 ± 0.11	0.21 ± 0.016	0.22 ± 0.034
		W	3.1	10.0 ± 0.11	0.19 ± 0.001	1.63 ± 0.047
	3 m	S	0.5	6.3 ± 0.12	0.14 ± 0.002	0.55 ± 0.097
		RZ	1.4	13.6 ± 0.12	0.51 ± 0.003	1.30 ± 0.034
		RH	3.2	3.3 ± 0.13	0.39 ± 0.009	0.03 ± 0.020
		W	3.1	13.3 ± 0.23	0.25 ± 0.002	1.83 ± 0.066
2nd beech tree	1 m	S	2.0	5.1 ± 0.05	0.21 ± 0.001	0.65 ± 0.077
		RZ	2.0	9.3 ± 0.17	0.32 ± 0.003	0.87 ± 0.057
		RH	1.4	1.2 ± 0.02	0.11 ± 0.001	0.00 ± 0.000
		W	4.7	9.7 ± 0.09	0.24 ± 0.001	0.99 ± 0.048
	2 m	S	2.8	5.6 ± 0.08	0.22 ± 0.003	0.46 ± 0.082
		RZ	2.3	8.5 ± 0.07	0.30 ± 0.005	0.58 ± 0.099
		RH	0.8	1.4 ± 0.08	0.13 ± 0.001	0.00 ± 0.000
		W	4.4	11.3 ± 0.12	0.34 ± 0.003	1.47 ± 0.110
	3 m	S	1.3	3.9 ± 0.08	0.17 ± 0.001	0.10 ± 0.069
		RZ	1.7	7.9 ± 0.10	0.26 ± 0.005	0.03 ± 0.004
		RH	0.3	1.5 ± 0.01	0.13 ± 0.001	0.00 ± 0.000
		W	2.8	5.3 ± 0.04	0.17 ± 0.006	0.17 ± 0.027

Results are expressed by mean value of measurements accompanied with standard deviation. Units represent mg of each group of phenolic compounds per gram of absolute dry wood.

might be explained by the increased content of phenolic extractives, which also corresponded to spectrophotometric analysis (Fig. 3; Table 2).

4. Discussion

Analysis of different categories of wood in the wounded stems of beech showed that reaction zones with a compartmentalization function in living trees contained a higher content of total phenols and two classes of phenolic compounds than adjacent discolored wood or sound sapwood. It was also shown that wound-wood can possess a higher amount of phenolic extractives than functionally similar sound sapwood. Our research demonstrated that extracts obtained from tissues that are altered by or formed after wounding had a conspicuous effect on the growth of wood decaying fungi.

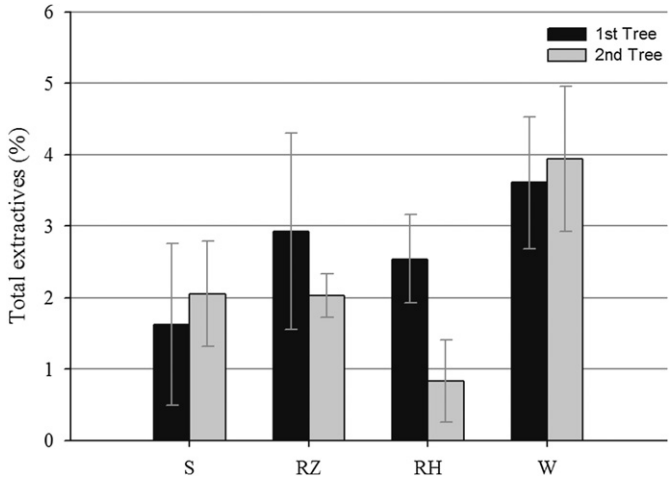

Fig. 2. Extractable compounds of different types of wound-associated tissue for two beech trees. Bars indicate standard deviation.

Pearce (1996) reported that a crude determination of total phenols might be 5–10 times higher in reaction zones than in intact sapwood. In our research, the maximum value (21.69 mg g^{-1}) was measured in the reaction zone sample taken from the disc of the first investigated stem. Essentially lower amounts of total phenols were determined for intact sapwood and discolored wood extracts.

Statistically significant differences were identified in the content of flavonoids between reaction zones and other categories of investigated tissue. Markedly high amounts were determined in the reaction zone samples from the first tree, among which the maximum value (0.78 mg g^{-1}) was measured in the sample taken at 1 m above the ground. Red heart samples from this tree contained higher amounts of flavonoids than intact sapwood and wound-wood, while discoloration extracts from the second stem included the lowest concentrations. The minimum value of total flavonoids was thus measured in the second tree, in a red heart sample (0.11 mg g^{-1}) taken at 1 m height. Higher amounts were determined for wound-wood and intact sapwood extracts.

Distribution trends of total proanthocyanidins for both beech stems were comparable to distributions of total phenols. The proportion of proanthocyanidins was thus highest in reaction zone and wound-wood extracts, whereby the maximum value was determined for the reaction zone sample of the first stem and amounted to 2.75 mg g^{-1}. In the first stem, the lowest amount measured was from the discoloration sample from the upper part of the tree. Red heart extracts from the second tree contained no proanthocyanidins.

With exception of proanthocyanidins, no differences were detected in content of investigated phenolic compounds in samples taken at different heights along the wounds. Increased amount of proanthocyanidins in lower parts of the stem could be related to age of wounded wood tissues. It appears that tissues in the lower parts of the tree, which are physiologically older, respond to wounding more intensively than those in the upper part of the tree.

The high content of total phenols, flavonoids and proanthocyanidins in the reaction zone and wound-wood extracts can be explained by the active response of living parenchyma cells to the

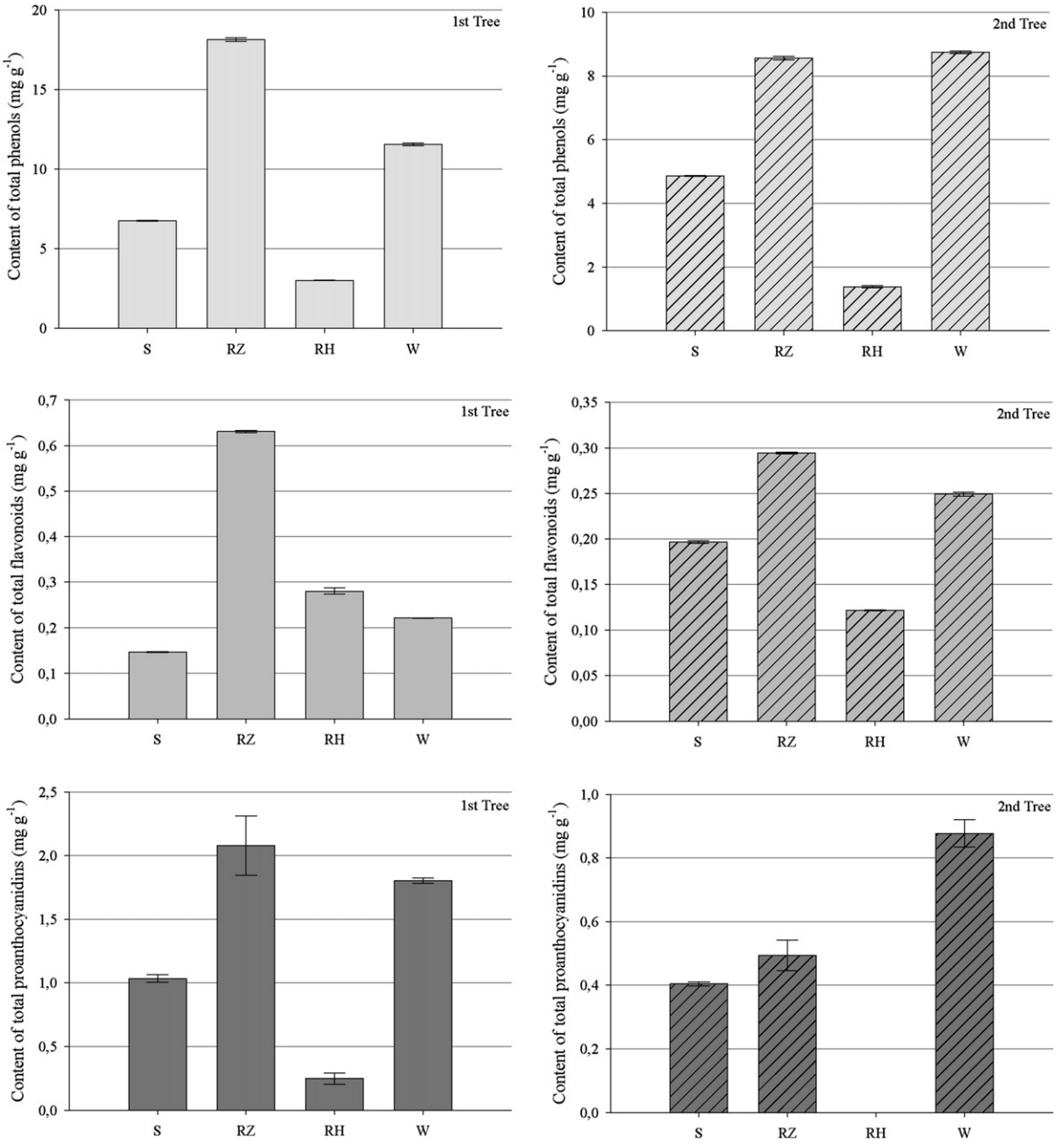

Fig. 3. Average content of total phenols, flavonoids and proanthocyanidins in wound-wood (W), red heart (RH), reaction zone (RZ) and sapwood (S) for two investigated beech trees. Bars indicate standard deviation.

negative consequences of tree wounding (Oven et al., 2008). The protective and defensive role of these compartmentalization barriers might be due in particular to the high amounts of flavonoids because some of them, e.g., flavanonols, flavonols and flavanols, have a proven fungicidal effect (Malterud et al., 1985; Pohjamo et al., 2003; Välimaa et al., 2007). Our results are in accordance with the findings of previous studies (Baum and Schwarze, 2002; Hofmann et al., 2004), demonstrating that

Table 3
Statistically significant differences in the average content of total phenols (TP), flavonoids (Fla) and proanthocyanidins (PAC) among different categories of tissues in the first beech tree. The presence of an asterisk indicates statistical significance at a 95% confidence level (LDS test).

	Reaction zone			Red heart			Wound-wood		
	TP	Fla	PAC	TP	Fla	PAC	TP	Fla	PAC
Sapwood	*	*	*	*	*	*	*	*	*
Reaction zone				*	*	*	*	*	
Red heart							*		*

Table 4
Statistically significant differences in the average content of total phenols (TP), flavonoids (Fla) and proanthocyanidins (PAC) among different categories of tissues in the second beech tree. Asterisk indicates statistical significance at a 95% confidence level (LDS test).

	Reaction zone			Red heart			Wound-wood		
	TP	Fla	PAC	TP	Fla	PAC	TP	Fla	PAC
Sapwood	*	*		*	*		*	*	*
Reaction zone				*	*			*	*
Red heart							*	*	

Table 5
Inhibition of white and brown rot producing fungi by cyclohexane and methanol extracts of different categories of wound-associated beechwood (*Fagus sylvatica* L.).

Extraction	Solvent	*Trametes versicolor*				*Gloeophyllum trabeum*			
		C	S	RZ	W	C	S	RZ	W
Magnetic stirrer	Methanol	/	—	/	—	/	/	+	−
	Cyclohexane	/	/	+	−	/	−	−	—
Soxhlet	Methanol	/	—	+	—	/	—	/	—
	Cyclohexane	/	−	/	−	/	−	−	−

Sample abbreviations: C – control, S – healthy sapwood, RZ – reaction zone, W – wound-wood.

reaction zones contain much higher contents of total proanthocyanidins than intact sapwood. A high content of total phenols in colored boundary layers has also been reported for other deciduous tree species (Shortle and Smith, 1990; Shortle et al., 1995; Deflorio et al., 2008). The high amount of phenolic extractives in wound-wood might reflect the protective role of this type of wood, which is essential for cambium survival after wounding. The relatively high content of phenolic extractives in methanolic extracts of intact sapwood can be explained by the presence of flavan-3-ols, which are regularly present in sapwood (Koch et al., 2003). The lowest content of phenolic extractives determined for discolored beechwood indicates that these compounds might participate in the formation of colored substances due to interactions with enzymes and atmospheric oxygen (Albert et al., 2003; Mayer et al., 2006). Furthermore, color changes in beech are frequently ascribed to chemical reactions of the phenolic extractives and cell wall components (lignin and hemicelluloses) (Koch et al., 2003; Jamalirad et al., 2012). It has been suggested that catechin and epicatechin, and presumably other flavan-3-ols, have an important role in the formation of colored chromophores of red heart in beech (Hofmann et al., 2004). Furthermore, catechin has been reported to be a valuable biomarker indicating the initial stages of wood decay (Mounguengui et al., 2007).

The strong impact of wound-wood extract on the growth of fungi might be explained by the increased contents of phenolic extractives (Fig. 3 and Table 5). Interestingly, a relatively strong influence on the growth of wood decay fungi was also exhibited by cyclohexane and, particularly, methanol extracts of intact sapwood (Table 5). This can be ascribed to the increased formation of phenolic compounds in tissues adjacent to the reaction zone (Albert et al., 2003), since some of them have proven bioactive properties. In spite of the ascribed protective and defensive function of reaction zones in wood of living trees (Shain, 1979; Pearce, 1996), the results of the screening test did not show any notable effect on the growth of the chosen wood fungi. The progress of white rot fungi *T. versicolor* toward the carrier with the reaction zone extract was noticeable faster than with the control and other extracts. *T. versicolor* is one of the most efficient wood degraders, able to decompose lignin, cellulose and hemicelluloses. With its enzymes, this fungus can also easily degrade phenolic extractives (Mounguengui et al., 2007; Lekounougou et al., 2008). It has been reported that laccases from *T. versicolor* are able to degrade beechwood extractives and that they presumably have a detoxification function. For example, catechin, which is one of the important compounds of beech extract, represents laccases substrate in beechwood (Lekounougou et al., 2008). Nevertheless, it can be seen from Table 5 that cyclohexane extract of the reaction zone had an inhibitory effect on the progress of brown rot fungus *G. trabeum*. The weak inhibitory effect of reaction zone extract on wood decaying fungi might be ascribed to the occurrence of insoluble phenolic compounds in these tissues, which behave as physical barriers rather than chemical inhibitors. In a living tree, these compounds may serve either as mechanical barriers to further fungal penetration or as permeability barriers, preventing the spread of drying. The low inhibitory influence of reaction zone extract in our case may be also explained by the assumption that the activity of reaction zone extractives might be greater in a living tree than in the relatively old samples included in assay systems (Pearce, 1996).

5. Conclusions

An overview of the results obtained by spectrophotometric analysis indicates that tissues formed as a consequence of tree wounding were characterized by a higher content of extractives with a phenolic character. High amounts of phenolics in our case were particularly distinctive of wound-wood and reaction zone extracts; the lowest concentrations of the mentioned compounds were measured in red heart samples.

The paper disc screening test proved that the strongest inhibitory effect on rot producing fungi was exhibited by methanol extract of wound-wood and intact sapwood. In spite of the ascribed protective and defensive function of reaction zones in wood of living trees, extracts of these tissues did not reveal a relevant fungitoxic effect on the chosen fungi. Further characterization of compounds in hydrophilic extracts of wound-associated beechwood is in progress.

Acknowledgments

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