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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.

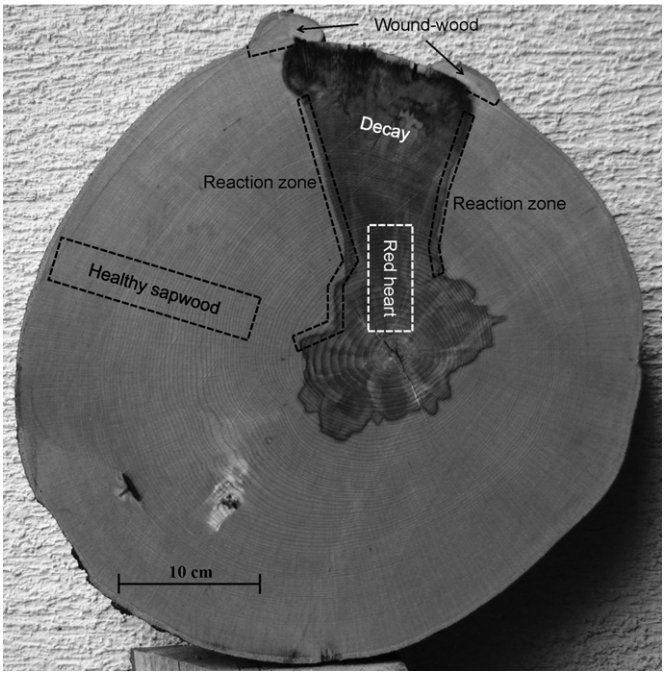

Fig. 1. Cross-section of the wound with marked positions of samples for extraction. The disk was dissected from beech tree no. 2.

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