



HAL
open science

The effect of brown-rot decay on water adsorption and chemical composition of Scots pine heartwood

Outi Karppanen, Martti Venäläinen, Anni M. Harju, Tapio Laakso

► **To cite this version:**

Outi Karppanen, Martti Venäläinen, Anni M. Harju, Tapio Laakso. The effect of brown-rot decay on water adsorption and chemical composition of Scots pine heartwood. *Annals of Forest Science*, 2008, 65 (6), pp.1. hal-00883390

HAL Id: hal-00883390

<https://hal.science/hal-00883390>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The effect of brown-rot decay on water adsorption and chemical composition of Scots pine heartwood

Outi KARPPANEN¹, Martti VENÄLÄINEN^{1*}, Anni M. HARJU¹, Tapio LAAKSO²

¹ Finnish Forest Research Institute, Punkaharju Research Unit, FIN-58450 Punkaharju, Finland

² Vantaa Research Unit, Finnish Forest Research Institute, Box 18, FIN-01301 Vantaa, Finland

(Received 4 September 2007; accepted 25 March 2008)

Abstract –

- The effect of brown-rot (*Coniophora puteana*) decay on the water adsorption capacity and concentration of extractives of Scots pine (*Pinus sylvestris* L.) heartwood were studied by comparing corresponding properties of decayed and undecayed wood samples.
- The samples derived from 39 felled trees having a large between-tree variation in the extractive concentrations, and subsequently in the mass loss in the decay test. The water adsorption capacity, expressed as equilibrium moisture content (EMC), was measured at a high relative humidity (RH ~100%, 21 °C).
- In contrast to the widely held belief, the water adsorption capacity of brown-rotted heartwood appeared to be significantly higher than that of undecayed heartwood.
- The chemical composition of heartwood was changed radically by the fungus: the concentration of stilbenes, resin acids and free fatty acids decreased, while the concentration of soluble sugars increased as a result of decay. In addition, fungal sugars were found in the decayed samples. The concentration of total phenolics increased, which obviously reflected chemical changes in cell wall constituents other than extractives.
- As a conclusion, the information concerning the hygroscopicity of brown-rotted wood might be valuable e.g. when carrying out repairs on buildings damaged by advanced decay.

brown-rot decay / extractives / Scots pine heartwood / mass loss / moisture content

Résumé – Effet des pourritures brunes sur l'adsorption de l'eau et la composition chimique du bois de Cœur du pin sylvestre.

- Nous avons étudié l'effet de la présence de pourriture brune (*Coniophora puteana*) sur la capacité d'adsorption de l'eau et sur la concentration d'extractibles du bois de cœur du pin sylvestre (*Pinus sylvestris* L.) en comparant des échantillons contaminés et sains obtenus pour 39 arbres échantillonnés.
- Dans les essais de décomposition, on obtient une grande variation entre arbres de la concentration en extractibles et de la perte de masse. La capacité d'adsorption de l'eau, exprimée comme l'humidité d'équilibre, a été mesurée à une humidité relative de 100 % à 21 °C.
- Contrairement à ce qui était attendu, la décomposition augmente la capacité d'adsorption de l'eau du bois de cœur en atmosphère très humide. La différence entre arbres des variations de l'humidité d'équilibre (décomposé-contrôle) augmente significativement avec l'augmentation de la perte de masse.
- La composition chimique du bois de cœur est radicalement modifiée par le champignon : la concentration de stilbènes, de résines acides et d'acides gras libres décroît tandis que la concentration de sucres solubles augmente, cela résultant de la décomposition. La concentration de composés phénoliques totaux, mesurée par le test de décomposition de Folin-Ciocalteu, augmente. De plus des sucres fongiques dérivant des hyphes de *C. puteana* ont été retrouvés dans les échantillons décomposés.
- En conclusion, les informations concernant l'hygroscopicité du bois brun pourraient être utiles par exemple au moment de procéder à la réparation de bâtiments endommagés par une dégradation avancée.

pourriture brune / extractibles / perte de masse / teneur en eau / humidité relative

1. INTRODUCTION

The close relationship between wood and water has a fundamental effect on the properties of wood. Among the most important consequences of the wood-water relationships are the hygro-expansion of wood (Skaar, 1988) and the role of moisture in the biodegradation of wood (Zabel and Morrell, 1992). The hydroxyl groups of hemicellulose and cellulose have an affinity to bind water molecules by hydrogen bonds,

which explains the hygroscopicity of woody cell walls. The dependence of the equilibrium moisture content (EMC) of wood on the relative humidity (RH) of the surrounding air at a constant temperature is described with a sorption isotherm (e.g. Haygreen and Bowyer, 1996; Skaar, 1988). The sigmoidal form of the sorption isotherm can be satisfactorily explained by the Brunauer-Emmett-Teller sorption theory (BET) (Brunauer et al., 1938), except at a very high relative humidity (RH 90–100%) (Simpson, 1980).

* Corresponding author: martti.venalainen@metla.fi

In order to better explain the sharp increase in the moisture content (MC) of wood at very high humidity, the BET theory is complemented with the theory of capillary condensation (Simpson, 1980; Walker, 1993). According to this theory, close to the fiber saturation point (FSP) the small voids in wood (diameter less than 1.5 μm) rapidly begin to fill up with capillary water (Griffin, 1977). It is challenging to experimentally study and model the adsorption behaviour of wood at very high humidity (Griffin, 1977). However, this is important because the wood material frequently reaches the FSP under normal service conditions, resulting e.g. in a risk of decay.

The hygroscopicity of dried wood enables the MC to increase again over 28–30%, which is a prerequisite for the initiation of fungal degradation. Soon after colonization, the fungi begin to change the physical properties of the wood, including the hygroscopicity. According to the general conclusion presented by Zabel and Morrell (1992), “the EMC of brown-rotted wood is lower than that of sound wood”. The sharp drop of EMC in the early stages of brown-rot decay is supposed to reflect the degradation of the most hygroscopic hemicellulose and amorphous cellulose molecules (Zabel and Morrell, 1992).

Extractives integrated into the ligno-carbohydrate matrix complicate the wood-water-decay relationship in two ways. Firstly, the EMC of wood is affected by the presence of extractives. For example, Celimene et al. (1999) suggested that stilbenes lower the moisture content of pinewood by acting as hydrophobic compounds. Koponen (1985) determined the sorption isotherms of wood and found that the hygroscopicity of extractive-rich Scots pine heartwood was lower than that of the sapwood. Thus the decay resistance of extractive-rich wood may be partly due to the lower EMC. Secondly, the extractives inhibit the degradation processes of the decay fungi. The association between the increasing extractive concentration of Scots pine heartwood and the decreasing degradation rate by the brown-rot fungus *Coniophora puteana* has been reported in several studies (Harju et al., 2002; 2003; Heijari et al., 2005; Karppanen et al., 2007; Venäläinen et al., 2003; 2004).

As far as we know, no reports have been published on the hygroscopicity of brown-rotted Scots pine wood in high humidity. Neither have we seen any reports on the effect of brown-rot fungi on the extractive concentrations of Scots pine wood.

The aim of this study was to investigate the difference in the water adsorption capacity (expressed as EMC) and the difference in the chemical composition between undecayed and brown-rot (*C. puteana*) decayed Scots pine heartwood. Moreover, we investigated whether the change in the chemical composition of decayed wood distorts the negative correlation between the result of the Folin-Ciocalteu assay and the decay resistance (Harju and Venäläinen, 2006). The wood material covered a wide range of heartwood extractive concentrations and, accordingly, a wide range in the stage of decay attained in a 7-week decay test. High air humidity (RH ~100%) was used in the adsorption experiment because wooden structures are often unintentionally exposed to high humidity.

2. MATERIAL AND METHODS

2.1. Wood samples

The wood material was obtained from a 5.4 ha Scots pine (*Pinus sylvestris*) stand growing in Leppävirta (62° 25' N, 27° 45' E), Finland. The stand was established in May 1968 by planting 2-year-old, bare-root seedlings, managed in the same way as in normal commercial forestry, and thinned in 1997. A sample of 40 trees had earlier been selected from the stand (Harju and Venäläinen, 2006). The sampling followed a two-stage procedure. First, core samples were taken from a large number of standing trees with an increment borer in March 2003 and screened for the concentration of total heartwood phenolics, after which a sub-sample of 40 trees was felled and measured at the end of September 2003. The felled trees represented the whole range of total heartwood phenolics in the stand.

A 25 cm long stem section was cut from each of the 40 stems at a height of ca. 1 m. A 5 × 15 × 200 mm strip (tangential, radial, and longitudinal dimensions) was cut from the section and oven-dried at 60 °C for 48 h. The dried strip was cut into five 30 mm long blocks (average density 0.384 g/cm³, standard deviation 0.043 g/cm³). One of the five blocks was subjected to the water adsorption treatment and one used for the chemical analyses. The remaining three blocks were subjected to an in vitro decay test, after which two of the decayed blocks were subjected to the water adsorption treatment and one to the chemical analyses.

2.2. Decay test

VTT Technical Research Centre of Finland carried out the decay test. Three wood blocks per sample tree were exposed to a pure culture of a brown-rot fungus, *Coniophora puteana* (Schum. ex Fr.) Karst. (strain BAM Ebv. 15), for seven weeks (Harju and Venäläinen, 2006). The average mass loss of the samples was 22.5% (standard deviation 15.6%), which was determined on oven-dry (60 °C) samples. The texture of decayed wood was examined under a scanning electron microscope (XL30 ESEM TMP).

2.3. Determination of water adsorption capacity

The surface of the heartwood blocks was sterilized with ethanol (96%) to prevent the growth of mould, after which the blocks were dried at 60 °C for 48 h and the dry mass was measured. A higher temperature was not used in order to avoid hysteresis. A tightly closed steel tank was half-filled with tap water and the relative humidity of the tank atmosphere (21 °C) was stabilised close to 100% (humidity sensor, Davis Instruments). The blocks were placed on steel racks immediately above the water surface. The decayed and undecayed heartwood blocks were exposed in separate experiments. The similarity of the experimental conditions was controlled by means of an equal set of Siberian larch (*Larix sibirica* Ledeb.) blocks in both experiments. The mass of the blocks was measured 11 times at increasing intervals. The duration of the test was 388 h (16 d 4 h) for the undecayed samples, and 432 h (18 d) for the decayed samples. After the last measurement the blocks were dried at 60 °C for 48 h, the mass measured, and then dried at 103 °C for 48 h in order to obtain the absolute dry mass. The result of the adsorption test was expressed as the ratio between the mass of adsorbed water and the mass of the

Table I. Means, ranges, and coefficients of variation (CV, %) of the initial moisture content (MC after drying at 60 °C), equilibrium moisture content (EMC) at RH ~100%, concentration of total phenolics, and chemical composition of undecayed and decayed heartwood blocks, and the result of sign test (*p*-values). *n* = 39.

Variable	Undecayed			Decayed			Difference Mean	Sign test <i>p</i> -value
	Mean	Range	CV%	Mean	Range	CV %		
Initial MC	0.025	0.02–0.03	7	0.025	0.01–0.05	30	0.000	0.302
EMC	0.292	0.23–0.33	9	0.430	0.25–0.65	25	0.137	0.000
TP (mgTAE/g)	10.90	1.9–21.7	49	22.47	12.6–51.3	32	11.57	0.000
PS (mg/g)	3.55	0.4–7.4	49	1.54	0.0–5.5	99	–2.01	0.000
PSM (mg/g)	6.11	0.4–14.7	50	2.83	0.0–11.0	97	–3.27	0.000
STB (mg/g)	9.66	0.8–21.1	47	4.37	0.0–16.1	97	–5.28	0.000
RAC (mg/g)	52.32	4.1–160.2	87	43.88	3.9–156.5	91	–8.44	0.200
SS (mg/g)	0.30	0.1–0.6	42	0.72	0.0–1.7	76	0.41	0.025
FFA (mg/g)	4.51	0.6–10.2	56	2.45	0.4–8.7	66	–2.06	0.000
Arabitol (mg/g)				2.94	0.0–8.9	86		
Mannitol (mg/g)				0.03	0.0–1.1	113		
Trehalose (mg/g)				1.72	0.0–5.0	78		

TP = Total phenolics; TAE = tannic acid equivalent; PS = pinosylvin; PSM = pinosylvin monomethyl ether; STB = PS + PSM; RAC = resin acids; SS = soluble sugars; FFA = free fatty acids.

absolutely dry wood, i.e. as the moisture content (MC). The number of studied trees decreased to 39 since, due to the advanced decay, the blocks of one tree broke into several small pieces, which had to be omitted from the experiment.

2.4. Analysis of extractives

Powdered heartwood (50 mg undecayed, 20 mg decayed) was sonicated with 2 mL methanol (p.a.) for 30 min; 0.4 mg diethylstilbestrol (Sigma D 4628) and 0.2 mg m-erythritol (Fluka 45670) were used as internal standards for undecayed heartwood, and 0.1 mg m-erythritol (Fluka 45670) for sugars and 0.3 mg heptadecanoic acid (Sigma H3500) for fatty acids, resin acids and for stilbenes pinosylvin (PS) and pinosylvin monomethyl ether (PSM) for decayed heartwood. After centrifugation 1 mL extract solution was evaporated to dryness under nitrogen. Before GC/MS analysis, the dried samples were silylated with 0.5 mL 20% TMSI-pyridine mixture (TMSI = 1-(trimethylsilyl)imidazole). The GC/MS analyses were performed using a HP 6890 GC system equipped with a mass selective detector 5973 and HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as carrier gas, at a flow rate of 1.5 mL/min. For undecayed heartwood the chromatographic conditions were as follows: initial temperature 180 °C; temperature rate 5 °C/min; final temperature 300 °C for 5 min; injector temperature 280 °C and split ratio 1:20, and respectively for decayed heartwood: initial temperature 110 °C; temperature rate 10 °C/min to 180 °C; 3 °C/min to 240 °C; 10 °C/min to final temperature 300 °C for 5 min; injector temperature 280 °C and split ratio 1:20. In both cases the MS-interface temperature was 300 °C and ion source temperature 230 °C. Mass spectra were obtained by electron impact (EI mode) ionization energy 70 eV. Quantification was performed using internal standards and pure reagents (Arbonova) for stilbenes PS and PSM, heptadecanoic acid (Sigma H 3500) for fatty acids, 75% abietic acid (Fluka 00010) for resin acids, and m-erythritol for soluble sugars. For the fungal-specific carbohydrates (trehalose, arabitol and mannitol) in decayed heartwood, quantification was performed with m-erythritol corrected by the response with pure reagents.

2.5. Analysis of total phenolics by the Folin–Ciocalteu assay (FC assay)

The FC assay was carried out as described in Harju and Venäläinen (2006). Tannic acid (Merck; 1.59446.0010) was used as a reference standard, and the results are therefore expressed as milligrams of tannic acid equivalents (TAE) per gram dry wood. Heartwood contains compounds other than phenolic compounds, e.g. resin acids and sugars, which react in the FC assay (Harju and Venäläinen, 2006; Prior et al., 2005). However, we have used the expression “concentration of total phenolics” for the results of the FC assay.

2.6. Data analysis

The difference in initial MC (initial MC after drying at 60 °C), in EMC, and in the concentration of extractives and total phenolics between the undecayed and decayed sample sets was tested by a sign test. Spearman’s coefficient of rank order correlation was estimated between the tree-wise mass loss and the difference in EMC ($EMC_{\text{decayed}} - EMC_{\text{undecayed}}$).

In order to demonstrate more clearly the effect of the stage of decay we divided the material into three groups (13 trees/group) according to the mass loss of the decayed samples. The first group represented heartwood with an incipient decay (Decayed 1 = mass loss 1–12%), the second one with intermediate decay (Decayed 2 = mass loss 13–30%), and the third one with advanced decay (Decayed 3 = mass loss 31–49%) during the 7-week decay test. Another three groups including the undecayed samples (Undecayed 1–Undecayed 3) of the corresponding trees were created for the comparison.

3. RESULTS

The average MC was low in Scots pine heartwood blocks dried at 60 °C and there was no significant difference between the MC of decayed and undecayed samples (Tab. I). In the

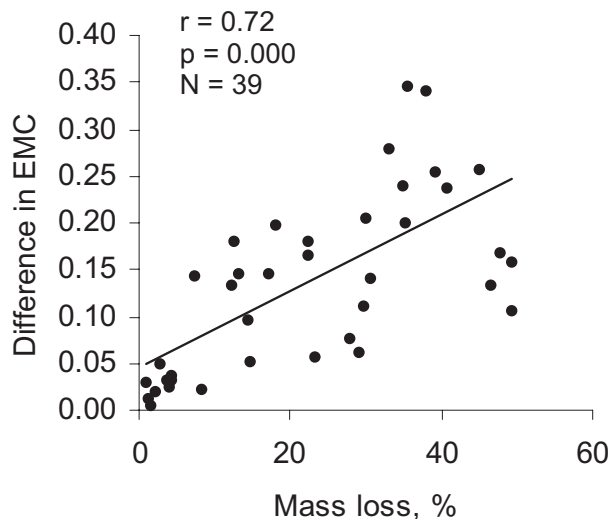


Figure 1. The difference in EMC ($EMC_{\text{decayed}} - EMC_{\text{undecayed}}$) as a function of mass loss. r = Spearman's rank order correlation.

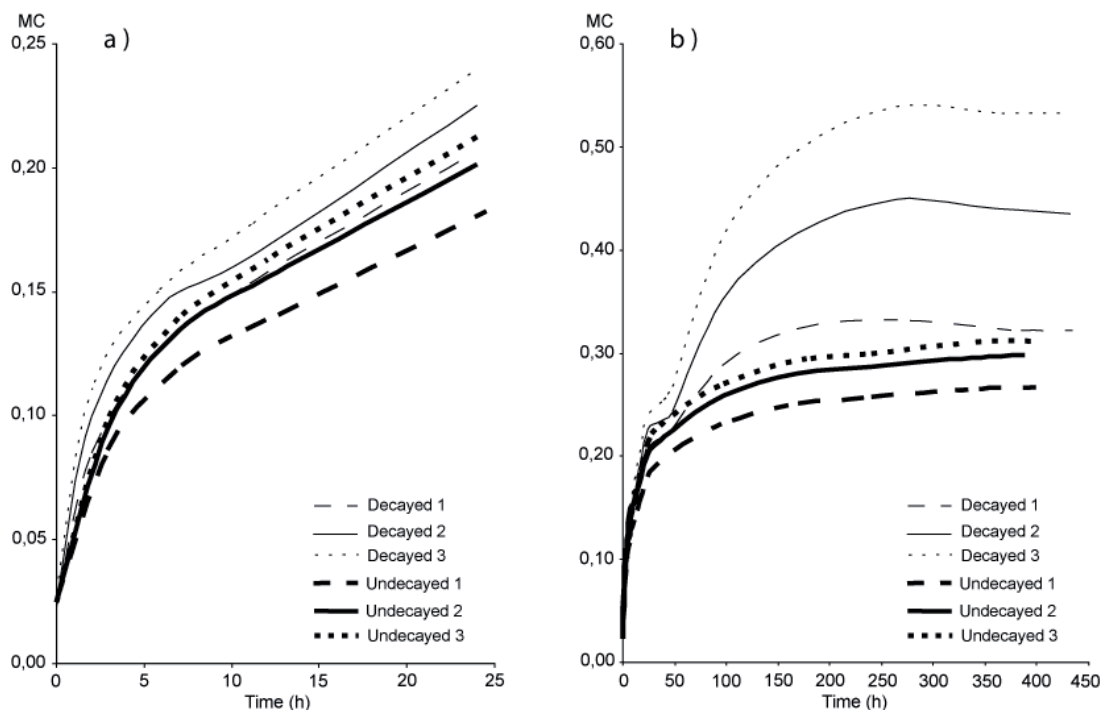


Figure 2. The water adsorption curves of undecayed and decayed Scots pine heartwood at high relative humidity ($RH \sim 100\%$, 21°C) as a function of time: (a) in the beginning of the experiment, (b) during the whole experiment. The groups were formed according to the mass loss level: Decayed 1 = incipient decay (mass loss 1–12%), Decayed 2 = intermediate decay (mass loss 13–30%), Decayed 3 = advanced decay (mass loss 31–49%). Undecayed samples were tree-wisely grouped into respective sets of samples Undecayed 1, Undecayed 2 and Undecayed 3.

moistening experiment, where water was abundantly available in the atmosphere, the water adsorption capacity of the decayed heartwood proved to be significantly higher than that of undecayed heartwood. Due to the wide variation in the stage of decay in the material, the variation in EMC at high humidity, expressed as CV%, was three-fold larger in the decayed samples than in the undecayed ones (Tab. I). Furthermore, there

was a statistically highly significant correlation between the tree-wise difference in the EMC ($EMC_{\text{decayed}} - EMC_{\text{undecayed}}$) and the mass loss of heartwood: the difference increased along with increasing mass loss (Fig. 1).

The average group-wise development of water adsorption as a function of the exposure time is demonstrated by adsorption curves in Figure 2. Within a few hours at high humidity

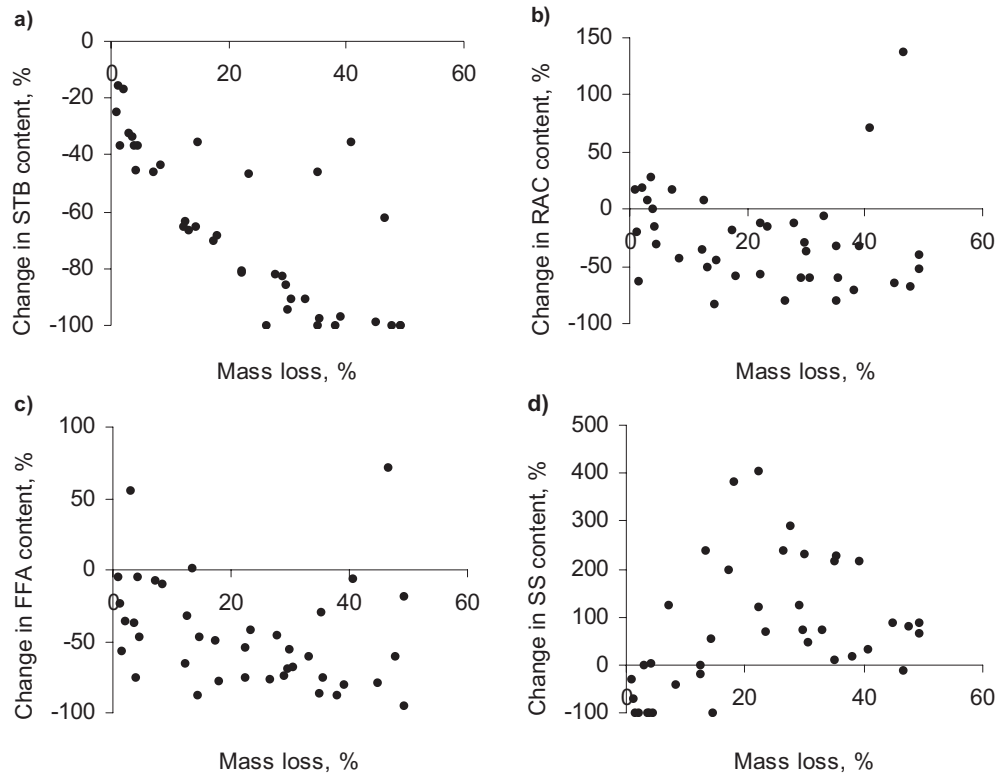


Figure 3. The relative change in the concentration of (a) stilbenes (STB = PS + PSM), (b) resin acids (RAC), (c) free fatty acids (FFA) and (d) soluble sugars (SS) as a function of mass loss. The relative change = $100\% \times (\text{concentration}_{\text{decayed}} - \text{concentration}_{\text{undecayed}}) / \text{concentration}_{\text{undecayed}}$.

(RH ~100 %), the decayed heartwood showed stronger hygroscopicity than the undecayed one at each stage of decay (i.e. Decayed 1 versus Undecayed 1 etc.) (Fig. 2). Furthermore, the water adsorption capacity of heartwood with incipient decay appeared to differ considerably from that with advanced decay soon before the equilibrium phase was reached (Fig. 2b).

The concentration of stilbenes (PS and PSM), resin acids and free fatty acids decreased along with increasing decay, while the concentration of total phenolics and soluble sugars increased (Tab. I). The difference between the concentration of stilbenes, free fatty acids and total phenolics in the undecayed and in the decayed heartwood was significant (Tab. I). In addition, we found the fungal carbohydrates arabinitol, mannitol and trehalose (Söderström et al., 1988; Tibbett et al., 2002) in the decayed heartwood (Tab. I). The tree-wise observations showing the association between the mass loss and the relative change in the concentrations of stilbenes, resin acids, free fatty acids and soluble sugars are presented in Figure 3, and between the mass loss and the relative change in the concentration of total phenolics in Figure 4.

4. DISCUSSION

The degradation by a brown-rot fungus, *C. puteana*, progressively increased the water adsorption capacity of Scots

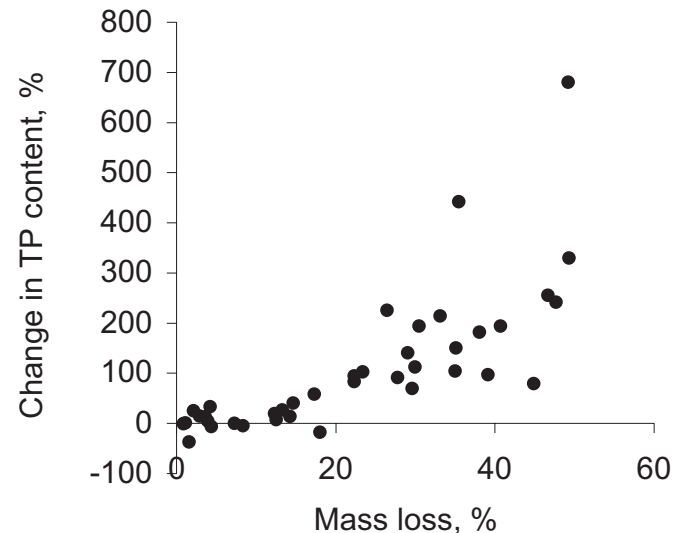


Figure 4. The relative change in the concentration of total phenolics (TP) as a function of mass loss. The relative change = $100\% \times (\text{concentration}_{\text{decayed}} - \text{concentration}_{\text{undecayed}}) / \text{concentration}_{\text{undecayed}}$.

pine heartwood in an extremely humid atmosphere. This result, which is contrary to the widely held assumption (Zabel and Morrell, 1992), applies to humidity conditions that are

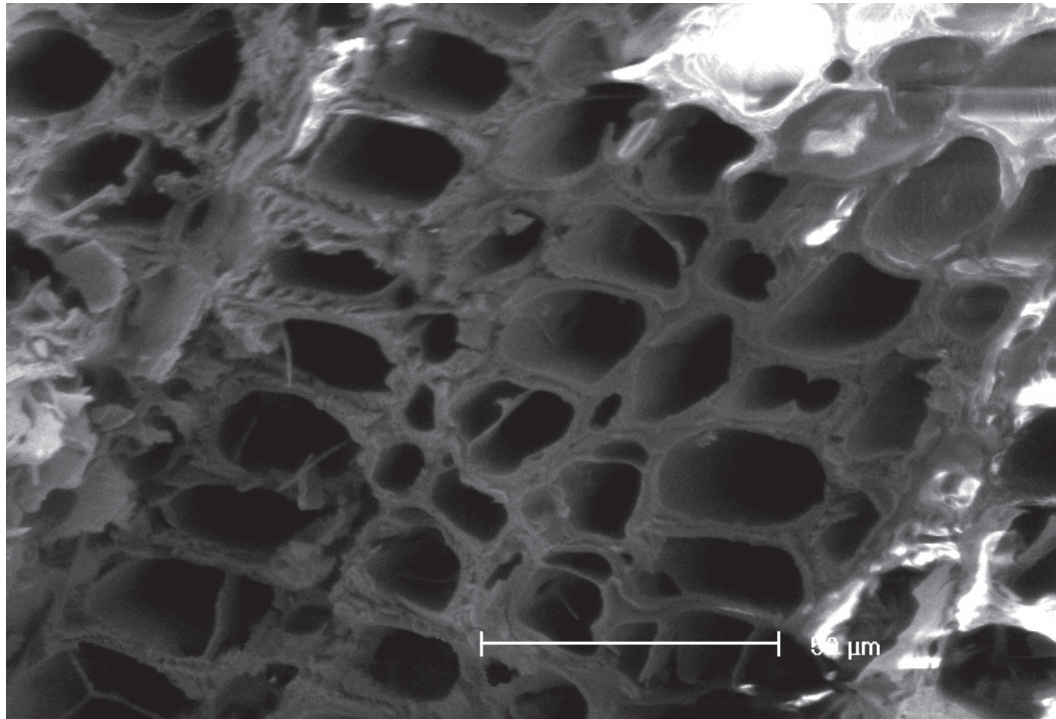


Figure 5. The degradation of cell walls visible in a transverse section SEM image of a block of Scots pine heartwood with advanced decay (mass loss 31–49%) caused by *C. puteana*. Bar 50 μm .

difficult to model and to control in an experiment, but which represent the ordinary service conditions for timber. The adsorption curves presented in this report differ from the conventional adsorption curves: only one relative humidity was applied, and the change in MC of the samples was followed as a function of time. The MC of the decayed wood blocks increased until about 20% within one day. According to the BET theory (Brunauer et al., 1938; Simpson, 1980), that was the phase of mono- and multilayer adsorption. The increase in MC above 20% up until FSP at high humidity can be explained by the theory of capillary condensation (Brunauer et al., 1938; Griffin, 1977; Simpson, 1980; Walker, 1993). Thus our results primarily reveal the increased capacity of brown-rot decayed Scots pine heartwood to adsorb water vapour by capillary condensation.

The increased capacity for capillary condensation can be explained by the structural changes in decayed wood. The degradation of the cell walls and the increased cavity formation (Flournoy et al., 1991; Highley et al., 1983; Rawat et al., 1998), especially in the S_2 layer (Kleist and Schmitt, 2001; Larsen et al. 1995; Lee et al., 2004), might have increased the number of capillaries as well as the surface area. In our material these kind of changes were visible in the scanning electron microscope (SEM) image (Fig. 5). Not only the number, but also the size of capillaries is important at high humidity. The radius of a capillary that is able to fill up with water increases from 0.01 to 1.06 μm when the relative humidity increases from 90% to ~100% (Walker, 1993).

In addition to the changes in the structure of the cell walls, the hygroscopicity of decayed wood may differ from that of sound wood owing to the presence of fungal mycelium. For example, Jones and Worrall (1995) found that about 7% of the residual mass of pinewood blocks after a 7-week decay test was fungal biomass. However, we do not know how the hygroscopic properties of fungal biomass differ from those of wood.

Several studies have been carried out on the effect of decay on the hygroscopicity of wood. In these studies, a large number of tree and fungus species have been used, and there is considerable variation in the decay stage and in the experimental humidity conditions. Rawat et al. (1998) studied Scots pine sapwood decayed by *C. puteana* (mass loss values 23.5%, 32%, 48%), and found that the hygroscopicity of decayed sapwood was higher than that of undecayed sapwood below RH 37%, but lower above RH 37% up until RH 93%. The MC of their decayed material varied from about 0.05 to 0.17. Winandy and Morrell (1993) studied brown-rot decayed Douglas fir heartwood (mass loss range 2.5–19.8%). They found that the hygroscopicity of decayed wood was slightly higher, and suggested that the increased EMC from 0.117 to 0.127 at 25 °C and RH 65% reflected the increased initial availability of water-binding sites and the lack of utilization of carbohydrate decomposition products. Anagnost and Smith (1997) studied brown-rot decayed red maple sapwood (mass losses 12.5% and 24.5%) and heartwood (mass loss 5.2%). They found the hygroscopicity of decayed samples to be lower than that of undecayed samples at RH 40, 68 and 80%. It is not possible

to say whether the conclusions of earlier studies and our conclusions are in agreement or not because the experiments are not similar. Thus we would rather consider that the findings are complementary to each other and describe a complex phenomenon.

In addition to the changes in the hygroscopicity of wood we also discovered a change in the concentration of extractives due to fungal degradation. The average concentration of stilbenes, resin acids and free fatty acids decreased in the decayed heartwood (Tab. I). This indicates that *C. puteana* was able to degrade or modify the structure of these compounds at a faster rate than it degraded the main constituents of the cell wall. Thus it seems that the brown-rot fungus eliminates stilbenes (PS, PSM) and resin acids, which are considered to act as decay-inhibiting extractives (Celimene et al., 1999; Henriks et al., 1979; Karppanen et al., 2007; Rennerfelt, 1945; Scheffer and Cowling, 1966), before utilizing hemicellulose and cellulose, its principal source of nutrients. At the tree-wise level the relative change in the stilbene concentration depended on the mass loss, but the relationship was not linear (Fig. 3a). The relationship is difficult to interpret because the mass loss was dependent on the initial concentration, e.g. the trees that had lost nearly 100% of their stilbenes had a low initial stilbene concentration and had high mass loss values after the 7-week decay test. The relative change in the concentrations of resin acids (Fig. 3b) and free fatty acids (Fig. 3c) does seem to depend on the mass loss as strongly as the relative change in stilbenes. The variation among the individual trees is clearly evident in the scatter plot figures (Fig. 3). In Figure 3a there are five trees in which the decrease in the relative stilbene concentration was slow compared to the mass loss. These trees were the thinnest ones in the material (diameter in heights of 1.3 and 3.5 m), but we could not find any explanation for their deviating behaviour.

Soluble sugars showed a different kind of pattern than the other extractives (Fig. 3d). The concentration of soluble sugars decreased at a low mass loss level. This supports the hypothesis that fungi use sugars as a source of energy during the incipient stage of decay (Goodell, 2003). In contrast, we have no plausible explanations for the strong average increase and wide variation in the concentration of soluble sugars at the intermediate mass loss levels (Fig. 3d). The existence of fungal sugars in the decayed heartwood was explained by the presence of mycelia within the tracheids, which was verified with the SEM.

The FC assay has been found to be a suitable method for predicting the decay resistance of Scots pine heartwood (Harju and Venäläinen, 2006; Heijari et al., 2005), despite the fact that it is an unspecific method that also detects compounds other than phenolics, e.g. resin acids and sugars (Harju and Venäläinen, 2006; Prior et al., 2005). In the decayed heartwood, the relative concentration of stilbenes and resin acids decreased along with increasing mass loss, while the concentration of compounds detected by the FC assay increased strongly (Fig. 4). Obviously constituents other than stilbenes and resin acids were responsible for the strong response in the decayed heartwood. Possible constituents could be fungal sugars or degradation products of hemicelluloses, cellulose and

lignin (Goodell, 2003). Anyhow we suggest that already decayed heartwood material should be avoided when applying the FC assay for predicting the decay resistance.

5. CONCLUSIONS

In contrast to the widely held belief, at extremely high humidity the hygroscopicity of brown-rotted Scots pine heartwood appeared to be significantly higher than that of undecayed heartwood. This information might be valuable e.g. when carrying out repairs on buildings damaged by advanced brown-rot decay. Furthermore, according to our results, the brown-rot fungus radically changed the extractive concentrations of Scots pine heartwood: the concentrations of stilbenes, resin acids and free fatty acids decreased, while the concentration of soluble sugars increased as a result of decay. The increase in the concentration of total phenolics, determined by the FC assay, obviously reflects chemical changes in cell wall constituents other than extractives.

Acknowledgements: We thank Heikki Kinnunen, Jussi Tiainen, Matti Muukkonen, and Pekka Paakkinen for the careful sampling of numerous specimens in the stand, as well as on the felled trees, and Arja Ronkanen and Taina Naukkarinen at Metla, Leena Paajanen and Liisa Seppänen from VTT Technical Research Centre of Finland for their contribution to the decay tests, and Arto Koistinen for his contribution to the SEM images. We also thank John Derome for revising the language and Professor Matti Kärkkäinen for his invaluable comments on the manuscript.

REFERENCES

- Anagnost S.E. and Smith W.B., 1997. Hygroscopicity of decayed wood: implications for weight determinations. *Wood Fiber Sci.* 29: 299–305.
- Brunauer S., Emmett P.H., and Teller E., 1938. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* 60: 309–319.
- Celimene C.C., Micales J.A., Ferge L., and Young R., 1999. Efficacy of pinosylvins against white-rot and brown-rot fungi. *Holzforschung* 53: 491–497.
- Flournoy D.S., Kirk T.K., and Highley T.L., 1991. Wood decay by brown-rot fungi: changes in pore structure and cell wall volume. *Holzforschung* 45: 383–388.
- Goodell B., 2003. Brown-rot fungal degradation of wood: our evolving view. In: Goodell B., Nicholas D.D., and Schultz T.P. (Eds.), *Wood deterioration and preservation. Advanced in our changing world*, American Chemical Society, Washington DC, pp. 97–118.
- Griffin D.M., 1977. Water potential and wood decaying fungi. *Ann. Rev. Phytopathol.* 15: 319–329.
- Harju A.M. and Venäläinen M., 2006. Measuring the decay resistance of Scots pine heartwood indirectly by the Folin-Ciocalteu assay. *Can. J. For. Res.* 36: 1797–1804.
- Harju A.M., Kainulainen P., Venäläinen M., Tiitta M., and Viitanen H., 2002. Differences in resin acid concentration between brown-rot resistant and susceptible Scots pine heartwood. *Holzforschung* 56: 479–486.
- Harju A.M., Venäläinen M., Anttonen S., Viitanen H., Kainulainen P., Saranpää P., and Vapaavuori E., 2003. Chemical factors affecting the

- brown-rot decay resistance of Scots pine heartwood. *Trees* 17: 263–268.
- Haygreen J.G. and Bowyer J.L., 1996. *Forest products and wood science, an introduction*, Iowa State University Press, Iowa, 484 p.
- Hejjari J., Nerg A.-M., Kaakinen S., Vapaavuori E., Raitio H., Levula T., Viitanen H., Holopainen J.K., and Kainulainen P., 2005. Resistance of Scots pine wood to Brown-rot fungi after long-term forest fertilization. *Trees* 19: 728–734.
- Henriks M.-L., Ekman R., and von Weissenberg K., 1979. Bioassay of some resin acid and fatty acid with *Fomes annosus*. *Acta Academiae Aboensis, Ser. B.* 39: 1–7.
- Highley T.L., Murmanis L., and Palmer J.G., 1983. Electron microscopy of cellulose decomposition by brown-rot fungi. *Holzforchung* 37: 271–277.
- Jones H.L. and Worrall J.J., 1995. Fungal biomass in decayed wood. *Mycologia* 87: 459–466.
- Karppanen O., Venäläinen M., Harju A., Willför S., Pietarinen S., Laakso T., and Kainulainen P., 2007. Knotwood as a window to the indirect measurement of the decay resistance of Scots pine heartwood. *Holzforchung* 61: 600–604.
- Kleist G. and Schmitt U., 2001. Characterisation of a soft rot-like decay pattern caused by *Coniophora puteana* (Schum.) Karst. in Sapelli wood (*Entandrophragma cylindricum* Sprague). *Holzforchung* 55: 573–578.
- Koponen H., 1985. Sorption isotherms of Finnish birch, pine and spruce. *Paperi ja Puu* 67: 70–77.
- Larsen M.J., Winandy J.E., and Green F., 1995. A proposed model of the tracheid cell wall of southern yellow pine having an inherent radial structure in the S2 layer. *Mater. Org.* 29: 197–210.
- Lee K.H., Wi S.G., Singh A.P., and Kim Y.S., 2004. Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus *Coniophora puteana*. *J. Wood Sci.* 50: 281–284.
- Prior R.L., Wu X., and Schaich K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in food and dietary supplements. *J. Agric. Food Chem.* 53: 4290–4302.
- Rawat S.P.S., Khali D.P., Hale M.D., and Breese M.C., 1998. Studies on the moisture adsorption behaviour of brown-rot decayed and undecayed wood blocks of *Pinus sylvestris* using the Brunauer-Emmett-Teller Theory. *Holzforchung* 52: 463–466.
- Rennerfelt E., 1945. The influence of the phenolic compounds in the heartwood of Scots pine (*Pinus sylvestris* L.) on the growth of some decay fungi in nutrient solution. *Svesk Botanisk Tidskrift* 39: 311–318.
- Scheffer T.C. and Cowling E.B., 1966. Natural resistance of wood to microbiological deterioration. *Ann. Rev. Phytopathol.* 4: 147–170.
- Simpson W., 1980. Sorption theories applied to wood. *Wood Fiber* 12: 183–195.
- Skaar C., 1988. *Wood-water relations*, Springer-Verlag, Berlin, Heidelberg, 283 p.
- Söderström B., Finlay R.D., and Read D.J., 1988. The structure and function of the vegetative mycelium of ectomycorrhizal plants, IV. Qualitative analysis of carbohydrate contents of mycelium interconnecting host plants. *New Phytol.* 109: 163–166.
- Tibbett M.F., Sanders E., and Cairney J.W.G., 2002. Low-temperature changes in trehalose, mannitol and arabitol associated with enhanced tolerance to freezing in ectomycorrhizal basidiomycetes (*Hebeloma* spp.). *Mycorrhiza* 12: 249–255.
- Venäläinen M., Harju A.M., Kainulainen P., Viitanen H., and Nikulainen H., 2003. Variation in the decay resistance and its relationship with other wood characteristics in old Scots pines. *Ann. For. Sci.* 60: 409–417.
- Venäläinen M., Harju A.M., Saranpää P., Kainulainen P., Tiitta M., and Velling P., 2004. The concentration of phenolics in brown-rot decay resistant and susceptible Scots pine heartwood. *Wood Sci. Technol.* 38: 109–118.
- Walker J.C.F., 1993. Water and wood. In: Walker J.C.F. (Ed.), *Primary wood processing. Principles and practice*, Chapman & Hall, London, pp. 68–94.
- Winandy J.E. and Morrell J.J., 1993. Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. *Wood Fiber Sci.* 25: 278–288.
- Zabel R. and Morrell J., 1992. *Wood microbiology: Decay and its prevention*. Academic Press, San Diego, 476 p.