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Resistance of thermo-hygro-mechanically densified wood to colonisation and degradation by brown-rot fungi

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Abstract

Colonisation and wood degradation by three brown-rot fungi, *Coniophora puteana*, *Gloeophyllum trabeum* and *Poria placenta*, were studied in wood of Norway spruce (*Picea abies*) subjected to three different treatments: hygro-thermal (TH) (160 and 180°C), mechanical densification and thermo-hygro-mechanical (THM) treatment including densification and post-treatment under saturated steam conditions at different temperatures (140, 160 and 180°C). The weight loss induced by all three fungi was lowest in THM-densified wood post-treated at 180°C. Highest weight losses were recorded for controls and TH-treated wood. Fungal colonisation varied in its intensity, depending on the treatment applied to the wood. Hyphal growth in controls and TH-treated wood was abundant, whereas in densified and THM-densified wood it was sparse and confined predominantly to the cell lumina of earlywood tracheids. Also, penetration of large-diameter hyphae and associated degradation in THM-densified wood was impeded by occlusion of the lumina, associated with irreversible compression (loss in shape memory). In contrast to *C. puteana* and *P. placenta*, which showed typical brown-rot behaviour, *G. trabeum* frequently showed hyphal tunnelling within the secondary walls of tracheids and xylem ray parenchyma of controls and thermally treated wood. Such growth was never observed in THM-densified wood post-treated at 180°C.

Keywords: brown rot; hyphal growth; light microscopy; soft-rot tunnelling basidiomycetes; thermo-hygro-mechanical (THM) wood; wood densification.

Introduction

There is a need for cost-effective wood protection methods that do not employ the toxic preservatives used in the past. This need has so far been addressed mainly by the development of various thermal and hydrothermal modification techniques, which reduce the hygroscopicity of wood and increase its resistance to fungal degradation (Boonstra et al. 1998; Tjeersdma et al. 1999, 2000; Militz 2002). Heat treatments can also improve the durability and mechanical properties of wood (e.g., for moulding), and there have been considerable efforts in several countries to develop such techniques (Militz 2002). Some of these treatments involve permanent fixation with steam or a combination of heat and chemicals (Militz 2002).

A process was recently developed for densifying wood by thermo-hygro-mechanical (THM) treatment (Navi and Giradet 2000). The resulting product is several-fold denser than the raw material and shows reduced hygroscopicity, significantly improved mechanical performance and little shape memory (Navi and Giradet 2000). Its resistance to fungal degradation has, however, not been tested.

The purpose of the present study was to test the brown-rot resistance of wood modified by different thermal and densification treatments and to determine whether such resistance is related to patterns of fungal colonisation and cell wall degradation. Wood samples from Norway spruce (*Picea abies*) were used for this purpose and the brown fungi used in the study were *Coniophora puteana*, *Gloeophyllum trabeum* and *Poria placenta*.

Materials and methods

Thermo-hygro-mechanical densification

A total of 60 wood blocks of *Picea abies* Karst., Norway spruce, (dimensions 150 mm×25 mm×15 mm) were subjected to the two-stage THM procedure of Navi and Giradet (2000), involving densification and then post-treatment in saturated steam at 140, 160 and 180°C. Densification was conducted at 140°C in saturated steam for approximately 20 min. The wood samples were compressed under a controlled force (maximum 22 kN) until they reached a radial compression ratio of 60–70%. The dimensions of the samples after densification were approximately 50 mm×25 mm×15 mm. During the subsequent post-treatment, samples were kept under mechanical pressure in saturated steam to eliminate the shape memory. The rate at which this change is effected decreases in proportion to the temperature. The post-treatment time was 180 min for the samples that had been densified at 140°C, with periods of 60 and 30 min used for samples densified at 160 and 180°C, respectively (Navi and Giradet 2000).

For comparison, 80 wood blocks (20 for each treatment) were either untreated, TH-treated or densified without heat treatment. Hygro-thermal treatment took place in saturated steam at 160 and 180°C, either for 60 or 30 min. A detailed account of the

Table 1 Condition and mean density of Norway spruce wood samples before incubation with brown rot fungi (n = 20).

Condition of Norway spruce wood		Temperature (°C)		Duration (min)		Set recovery (%)	r_0 (g cm^{-3})
		Densification	Post-treatment	Densification	Post-treatment		
Control	Untreated	–	–	–	–	–	0.387 ± (0.02)
TH 160°C	Heat treatment at 160°C under SSC	–	160	–	60	–	0.406 ± (0.03)
TH 180°C	Heat treatment at 180°C under SSC	–	180	–	30	–	0.389 ± (0.02)
Densified	Mechanical densification under SSC, without post-treatment	140	–	20	–	71	1.171 ± (0.01)
THM 140°C	Densification and post-treatment at 140°C under SSC	140	140	20	210	4.3	1.197 ± (0.01)
THM 160°C	Densification and post-treatment at 160°C under SSC	140	160	20	60	1.3	1.190 ± (0.01)
THM 180°C	Densification and post-treatment at 180°C under SSC	140	180	20	30	0.9	1.174 ± (0.01)

r_0, mean density before incubation; SSC, saturated steam conditions.

methods and of the recovery-set for different post-treatments is given by Navi and Giradet (2000).

Inoculation of wood blocks

The fungi used were as follows:

- *Coniophora puteana* (Schum.: Fr.) Karst (isolate no. EMPA 62);
- *Gloeophyllum trabeum* (Pers.: Fr.) Murrill (isolate no. EMPA 100); and
- *Poria placenta* (Fr.) Cooke (isolate no. EMPA 229)

Identification of pure cultures was confirmed using mycelial character as observed on plates of malt extract agar (MEA) (Stalpers 1978). Test wood blocks, 50 mm × 50 mm × 150 mm, were obtained from the heartwood of living 40–50-year-old trees of *Picea abies* Karst. and were prepared according to EN 113 (European Committee for Standardisation 1997). The blocks were sterilised with ethylene oxide for approximately 5 h, dried at 100°C for 48 h, cooled in a desiccator and then weighed. They were then inoculated and incubated in units with each of the above fungi according to EN 113.

For each treatment/fungus combination and incubation period, 20 wood blocks were set up, together with 20 controls. The units were incubated at a moisture content between 6% and 10% in a random array at 22 ± 1°C and 70 ± 5% relative humidity for 16 weeks. Before the incubated wood blocks were dried (as above) for measurement of weight loss, they were cleaned and sampled at random points by removing chips of negligible weight. These were plated onto MEA to check whether the decay fungi were the only micro-organisms present, and this was confirmed in all cases.

One-way analysis of variance (ANOVA) of the recorded dry weight losses was performed in Excel with the significance level set at $P < 0.05$. A Tukey HSD post hoc test was performed in SPSS to demonstrate differences in means.

Light microscopy

The incubated test blocks were cut into sub-samples of approximately 20 mm × 5 mm × 5 mm, with transverse, radial, and tangential faces exposed for examination. These were fixed in 2% glutaraldehyde buffered at pH 7.2–7.4, dehydrated with acetone and embedded in a methacrylate medium (Schwarze and Fink 1998). They were then sectioned at approximately 2 and 4 μm using a rotary microtome (Leica® 2040 Supercut) fitted with a diamond knife. For general observation of cell wall degradation and hyphal growth, sections were stained for 12 h in safranin and then counter-stained for 3 min in methylene blue and for 30 min in auramin. Early stages of brown rot (observed as loss of birefringence due to cellulolysis) were detected by viewing sections between crossed Nicol prisms (Lohwag 1937; Schulze and Theden 1937; Wilcox 1993). Micrographs were taken on colour film (Kodak® EPY 64T) with a Leitz® Orthoplan microscope fitted with a Leitz-Vario-Orthomat® camera system.

Results and discussion

Densification resulting from wood treatment

The treatments resulted in increases in density ranging from three- to four-fold (Table 1). The density of wood blocks exposed to TH-treatment was similar to that of the untreated controls.

THM treatment with post-treatment at 140°C compressed the tracheid lumina to a radial width ranging from 1 to 5 μm in the latewood and from 1 to 10 μm in the earlywood (Figure 1). With post-treatment at 160°C, the corresponding values were 0. 5–2 μm in latewood and 1–5 μm in earlywood (Figure 1). With post-treatment at 180°C, most of the latewood tracheid lumina were completely occluded, whereas those of earlywood tracheids were reduced to a width of 0.5–3 μm (Figure 1). Such differences were noted by Navi and Giradet (2000), who also showed that "set-recovery" (the tendency for cells to partially re-expand after densification) was reduced by raising the temperature of post-treatment).

Fungal colonisation and wood degradation

Most test blocks in all the wood treatments were completely colonised by external mycelia after the 16-week incubation period, as assessed by EN 113 criteria (European Committee for Standardisation 1997).

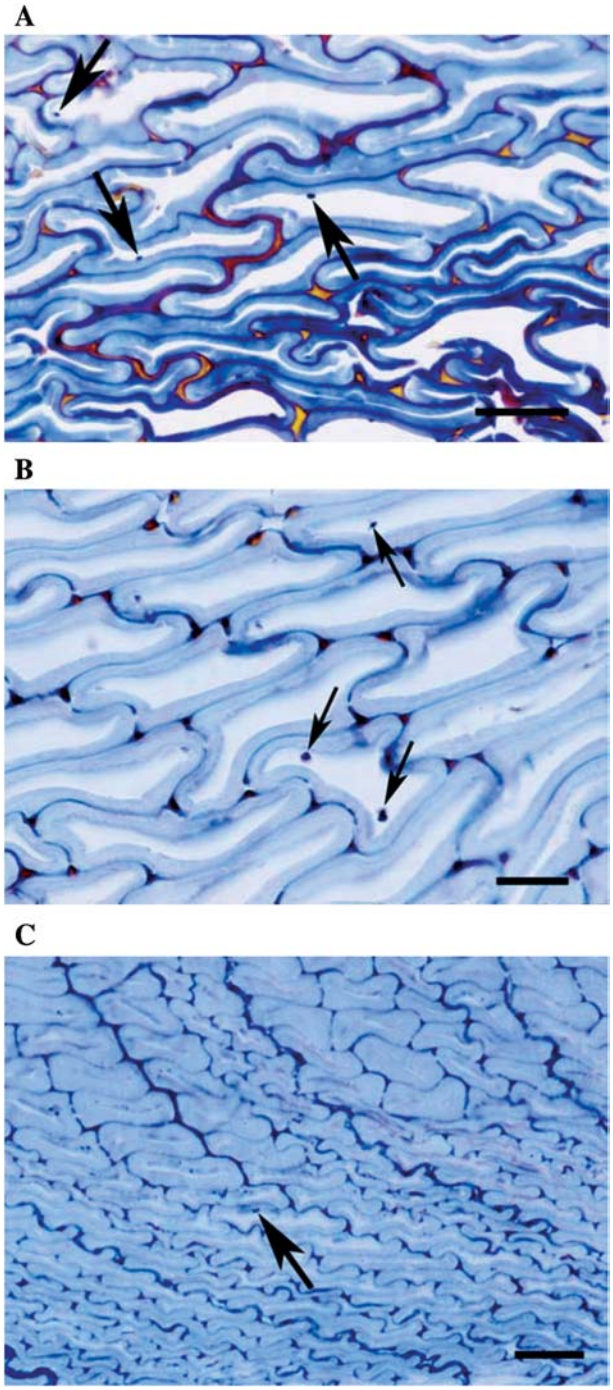

Figure 1 Transverse sections of densified Norway spruce wood; bar represents 10 μm. (A) Post-treated at 140°C and incubated with *Poria placenta*. Note that the cell lumina of all tracheids are only partially closed. (B) Post-treated at 160°C and incubated with *Gloeophyllum trabeum*. Note that in both (A) and (B) fine hyphae (arrows) are visible within the lumina of earlywood growing on S_3. Note that in (B) the cell lumina of some tracheids are completely sealed. (C) Post-treated at 180°C and incubated with *Gloeophyllum trabeum*. Note that hyphal growth (arrow) is very sparse and restricted to the earlywood tracheids. The cell lumina of most tracheids are completely sealed.

For each fungus, the weight losses were significantly different ($P < 0.05$) amongst the pre-incubation wood treatments (Figure 2). For all the fungi, the highest weight losses were observed in untreated and TH-treated (160 and 180°C) wood. The lowest weight losses occurred in THM-densified wood post-treated at 180°C. Also, the fungi differed in the amount of weight loss that they induced.

Microscopy examination of the wood blocks showed that the differences between treatments regarding weight loss could be partly attributed to the restriction of fungal growth by the occlusion of tracheid lumina. In wood subjected to THM treatment and post-treated at 180°C, fungal hyphae failed to grow in cell types with completely occluded lumina and were confined to the partially occluded lumina of earlywood tracheids. Comparisons of unoccluded and partially or fully occluded lumina and of the fungal growth within them are illustrated in Figures 1 and 3 for *Poria placeta* and Figure 4 for *G. trabeum*. Complete occlusion of all cell lumina would be needed to prevent brown-rot activity, since degradative substances can diffuse from a single hypha in a cell lumen.

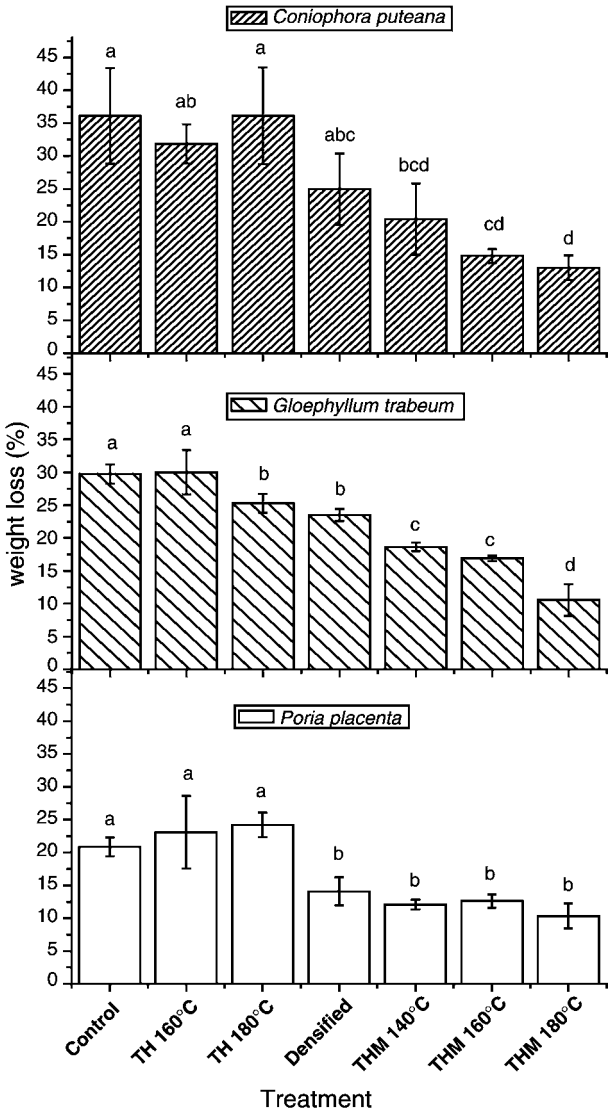

Figure 2 Dry weight losses of wood blocks in untreated controls, TH-treated wood (160 and 180°C), densified wood without thermal treatment and THM-densified wood (140, 160 and 180°C) incubated with *Coniophora puteana*, *Gloeophyllum trabeum* and *Poria placenta* ($n = 20$). Bars show standard deviation. Columns sharing the same letters show no significant differences ($P > 0.05$); those not sharing letters show significant differences ($P < 0.05$).

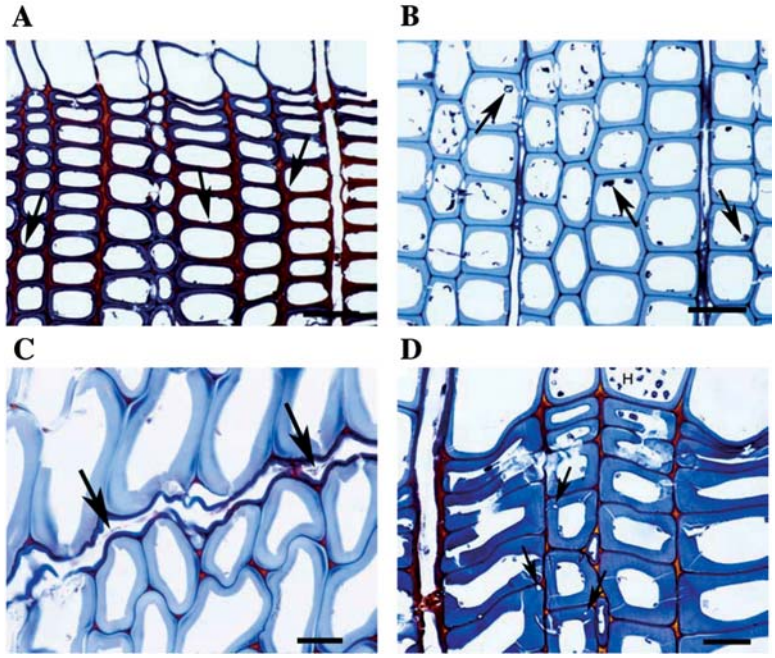

Figure 3 Transverse sections of Norway spruce wood. (A) Untreated wood incubated with *Coniophora puteana*. Note the fine hyphae (arrows) within the cell lumina of tracheids; bar represents 50 μm. (B) Untreated spruce wood incubated with *Poria placenta*. Note the abundant fine large hyphae (arrows); bar represents 50 μm. (C) Densified wood incubated with *Poria placenta*. Note that earlywood tracheids are only slightly compressed. Hyphae (arrows) are growing within the cell lumen of xylem ray parenchyma; bar represents 10 μm. (D) Untreated wood incubated with *Gloeophyllum trabeum*. Note the formation of boreholes and cavities (arrows) within the secondary walls of tracheids; bar represents 10 μm.

However, if a high proportion of lumina are completely occluded, this will clearly restrict fungal ingress overall sufficiently to explain why the rate of decay was reduced.

Although fungal hyphae were unable to enter fully occluded lumina and showed restricted development when occlusion was almost complete, the species tested in this study were able to produce narrow hyphae, which may enable them to enter cells with partially occluded lumina. *Coniophora puteana* produced much narrower hyphae in wood than in agar media and all three fungi showed some effect of substrate (agar versus wood) on their hyphal widths and morphology (Table 2). *Poria placenta* produced wider hyphae than *C. puteana*, even in wood, and may therefore be less able to enter partially occluded cell lumina. In wood, the hyphae of *C. puteana* all bore simple clamp connections, whereas those of *P. placenta* were either very fine (1–2 μm) and bearing simple clamps, or somewhat wider (3–9 μm), with either medallion clamps or simple clamps (Figure 3). The hyphae of *G. trabeum*, which were abundant within the lumina of tracheids and xylem ray parenchyma in undensified wood, possessed numerous clamp connections, sometimes in the form of medallions (Figures 3 and 4).

Hyphal tunnelling through cell walls is another means by which certain fungi may be able to grow through wood in which the lumina are inaccessible (Daniel et al. 1992; Schwarze et al. 1995, 2004; Schwarze and Fink 1997, 1998; Worrall et al. 1997). Only a few brown-rot fungi have been observed to grow in this way (Duncan 1960; Schwarze et al. 2000; Kleist and Schmitt 2001; Kleist et al. 2002) but *G. trabeum* was found to do so in the present study (Figures 3 and 4). It penetrated the secondary walls transversely with very fine hyphae of <0.5 μm in diameter. Tunnelling within the secondary walls began when these hyphae reached the compound middle lamella and changed direction without penetrating it (Figure 4). Enzyme activity along the length of the tunnelling hyphae produced individual cavities of 0.5–2 μm in diameter within the S_2 layer, following the alignment of the microfibrils (Figures 3 and 4). Longitudinal sections revealed the early stages of cavity formation, which began within the chambers of simple pits, together with localised multiple branching of the hyphae (Figures 3 and 4). Subsequently, individual cavities coalesced to form larger cavities (Figure 4).

Despite the hyphal tunnelling activity of *G. trabeum*, the weight loss caused by this fungus was reduced significantly by THM densification, as in the case of the other two fungi. However, the tunnelling activity was predominantly observed in thermally treated wood, rarely in untreated and densified wood and in THM-densified wood post-treated at 140 and 160°C. It was never observed in THM densified wood post-treated at 180°C. The effect of heat treatment is discussed below in relation to the availability of low-molecular-weight carbohydrates and of moisture.

When cell lumen occlusion was absent; (i.e., in untreated wood and in TH-treated non-densified wood), *C. puteana* and *P. placenta* preferentially degraded hemicellulose and cellulose in the tracheid walls, as demonstrated by histochemical staining (red instead of blue; Figure 3). A partial loss of birefringence under polarised light revealed cellulolysis in earlywood tracheids, but not to any significant extent at this stage in the epithelial cells of resin canals and in latewood tracheids. Hyphae were visible within the cell lumina (Figure 3) and there was typ-

Table 2 Hyphal width measured in agar media culture and in wood.

Fungal species	Hyphal width (μm)		Medallion clamps
	In agar	In wood	
Coniophora puteana	2.5–8	0.5–2	Absent
Gloeophyllum trabeum	1–4.5	1–5	Present
Poria placenta	2–8	1–9	Present

ical cracking of the cell walls extending from the S_3 layer into the S_2 and S_1 layers.

It seems likely that, in addition to causing occlusion of cell lumina, densification reduces the size of voids within cell walls. If so, the diffusion of fungal enzymes through the walls of densified wood may be retarded. A slow rate of diffusion may explain why tree species with naturally dense and highly lignified wood are relatively resistant to decay (Rayner and Boddy 1988; Schwarze et al. 2004). This may be particularly important in resistance to brown rot, which mainly involves the diffusion of degradative substances rather than the direct erosion of cell walls by fungal hyphae. Even without densification, pores within undecayed woody cell walls are no more than 2 nm in diameter (Hill and Papadopoulos 2001) and are therefore too small to allow the diffusion of cellulolytic enzymes (Cowling and Kirk 1976). For this reason, non-enzymatic systems involving substances of low molecular weight have been implicated in the early stages of brown rot, prior to pathways being opened up for the diffusion of enzymes (Koenigs 1974a,b; Murmanis et al. 1987). Evidence for the operation of such systems has, however, been challenged (Kerem et al. 1999).

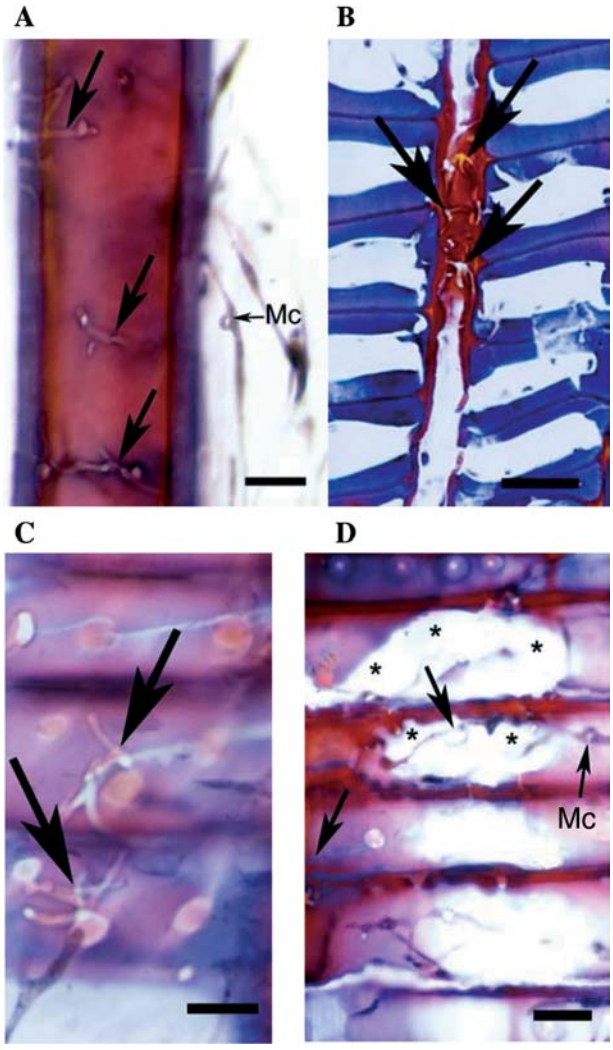

Figure 4 TH treated (160°C) Norway spruce wood incubated with *Gloeophyllum trabeum*; bar represents 10 μm. (A) Radial longitudinal section showing boreholes, multiple hyphal branching (arrows) and cavity formation within the secondary wall of tracheids. Mc, medallion clamp. (B) Transverse section showing hyphal growth (arrows) along the spiral alignment of the cellulose microfibrils. Cavity formation is apparent around the hyphae. (C,D) Radial longitudinal sections showing hyphal growth (arrows) within the secondary walls of xylem ray parenchyma. In regions of the secondary wall where hyphal tunnelling (arrows) has formerly occurred, secondary walls are completely degraded (asterisk).

Apart from the effects of densification on the size of cell lumina and voids within the cell wall, TH treatment may affect the suitability of wood as a substrate for fungal growth. In the present study, the moisture content of TH-treated wood was found to be far below the fibre saturation point by the end of the 16-week incubation period. Thus, no free water was available within the cell lumina, so that conditions for hyphal growth and wood degradation were unfavourable. These conditions were, however, circumvented by *G. trabeum*, which switched its mode of action to hyphal tunnelling within the secondary wall to utilise the water that was bound there. The relatively rapid degradation of such wood may be also explained by the fact that its density is reduced due to the depolymerisation of polysaccharides; this is estimated to amount to approximately 5–25% in coniferous wood.

Further studies are currently in progress with the objective of resolving the mechanisms underlying the resistance of THM densified wood post-treated under saturated conditions against brown-, white- and soft-rot fungi.

Conclusions

Hygro-thermal treatment of wood densified at relatively high temperatures not only increases the dimensional stability, but also seems to make conditions less conducive to hyphal growth within the secondary walls of tracheids and xylem ray parenchyma. The extent to which fungal decay is retarded in this way may be influenced by the hyphal width and colonisation strategies of the fungi concerned.

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