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Critical moisture conditions for fungal decay of modified wood by basidiomycetes as detected by pile tests

Abstract: The aim of cell wall modification is to keep wood moisture content (MC) below favorable conditions for decay organisms. However, thermally modified, furfurylated, and acetylated woods partly show higher MCs than untreated wood in outdoor exposure. The open question is to which extent decay is influenced by the presence of liquid water in cell lumens. The present paper contributes to this topic and reports on physiological threshold values for wood decay fungi with respect to modified wood. In total, 4200 specimens made from acetylated, furfurylated, and thermally modified beech wood (*Fagus sylvatica* L.) and Scots pine sapwood (sW) (*Pinus sylvestris* L.) were exposed to *Coniophora puteana* and *Trametes versicolor*. Piles consisting of 50 small specimens were incubated above malt agar in Erlenmeyer flasks for 16 weeks. In general, pile upward mass loss (ML) and MC decreased. Threshold values for fungal growth and decay ($ML \geq 2\%$) were determined. In summary, the minimum MC for fungal decay was slightly below fiber saturation point of the majority of the untreated and differently modified materials. Surprisingly, *T. versicolor* was able to degrade untreated beech wood at a minimum of 15% MC, and growth was possible at 13% MC. By contrast, untreated pine sW was not decayed by *C. puteana* at less than 29% MC.

Keywords: acetylated wood, critical moisture content, decay resistance, furfurylated wood, mass loss, moisture performance, moisture content, pile test, thermally modified wood, wood degradation by basidiomycetes

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Introduction

The protective mechanism of modified timber is based on its hydrophobic character and improved moisture performance (Hill 2007; Ringman et al. 2014). Different physiological studies on wood destroying fungi (Viitanen and Ritschkoff 1991; Huckfeldt and Schmidt 2006) showed considerable impact of moisture content (MC) on fungal decay. By keeping the MC below conditions favorable for decay organisms, the wood is sufficiently durable for many outdoor applications (Ibach and Rowell 2000; Hill 2007; Welzbacher 2007). The hydrophobicity and the durability of chemically or thermally modified timber (TMT) are strongly dependent on the treatment intensity (TI) (Epmeier et al. 2004; Esteves and Pereira 2008; Thybring 2013).

The TI of TMT is usually expressed by the percentage of mass loss (ML) during heat treatment (HT) (Welzbacher et al. 2007). ML is frequently correlated with TMT intensity and other properties such as durability, moisture behavior, and strength properties (Metsä-Kortelainen et al. 2006; Brischke et al. 2007; Paul et al. 2007; Welzbacher et al. 2007; Meyer et al. 2011). By contrast, for chemical modification (CM) processes, the uptake of solid agents remaining after drying (weight percent gain [WPG]) is considered as a measure of modification intensity (Li et al. 2000; Lande et al. 2004; Mai and Militz 2004; Hill 2007). However, the parts of the modification agent left in the cell lumens do not contribute to cell wall modification. In addition, it is not known to which extent the modification agents react/polymerize within the cell wall after impregnation, and this parameter can have a significant effect on the resulting wood properties. Although the acetylation process decreases the hygroscopicity of wood (Rowell 2006), it might also increase its capillary water uptake (Larsson and Simonson 1994). Similar changes in water uptake behavior were reported for TMT (Metsä-Kortelainen et al. 2006). Information is scarce in terms of the changed water uptake behavior and the critical MC for onset of fungal growth (Meyer et al. 2012). Furthermore, it is still controversially discussed to which extent decay is influenced by the presence of liquid water in cell lumens (Thygesen et al. 2010; Ringman et al. 2014).

Therefore, it seems necessary to determine physiological threshold values for wood decay fungi with respect to modified timber. Critical moisture levels are material-specific characteristics and need to be considered when estimating the resulting moisture and temperature-induced risk for decay of wood-based materials. Hence, this study focused on determining moisture threshold values for wood decay fungi with respect to the three currently most widely commercialized modified wood products, i.e., acetylated, furfurylated, and TMT. In focus was the relationship between treatment-induced mass changes (ML or WPG) and equilibrium moisture content (EMC) as these parameters indicate the TI. The pile test approach, previously described by Schmidt et al. (1996), was applied to determine the minimum MC needed for fungal growth and decay as a function of the type and intensity level of modification.

Materials and methods

Specimens, 5 (long.) \times 40 \times 40 mm³, were made from Scots pine sW (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.). In total, 10 000 specimens were prepared, whereby always 50 replicate specimens were axially matched and used together for (1) acetylation, (2) furfurylation, and (3) thermal modification. Each specimen was modified with high and low TI.

Acetylation was performed at SP Technical Research Institute of Sweden in Borås, Sweden, in a pilot plant reactor and consisted of an initial vacuum-pressure impregnation step with acetic anhydride. The excess anhydride was drained off, and the impregnated wood was reacted at elevated temperature for different length of times to achieve two different TIs. The by-product acetic acid was removed by the evacuation of the reactor during heating.

Furfurylation was performed at the Norwegian Forest and Landscape Institute in Ås, Norway, with two different aqueous furfuryl alcohol (FA) solutions: (1) 62% water, 35% FA, and 3% additives (FA55) (low TI); and (2) 57% water, 40% FA, and 3% additives (FA70, Kebony AS, Skien, Norway) (high TI). The impregnation schedule for both solutions was 30 min at 0.004 MPa and 60 min at 0.9 MPa.

HT was performed at Leibniz University Hannover, Germany, at 220°C in a drying oven for beech: 1.5 h (low TI) and 4.0 h (high TI). Pine sW specimens were modified for 4.0 h (low TI) and 8.0 h (high TI). All specimens were tightly wrapped in aluminum foil to reduce access of oxygen.

Change in mass due to modification: The mass change was determined as one of the important parameters. All modified specimens were oven-dried at 103°C until constant mass and weighed to the nearest 0.001 g before (m_0) and after ($m_{0,1}$) the treatments. The WPG of acetylated and furfurylated wood was calculated according to Eq. (1), and the ML by heat treatment (ML_{TMT}) was calculated according to Eq. (2).

$$WPG\% = 100 \times (m_{0,1} - m_0) / m_0 \quad (1)$$

$$ML_{TMT}\% = 100 \times (m_0 - m_{0,1}) / m_0 \quad (2)$$

Afterward, piles for the fungal tests were selected according to the WPG and ML_{TMT} values. For every pile, the highest possible similarity between specimens was decisive. Hereby, every pile consisted of axially matched specimens exclusively.

Equilibrium moisture content: EMC was also determined as a crucial parameter before the fungal tests. The specimens were placed in a closed but ventilated small-scale climate chamber over a saturated solution of KCl (85% RH, 20°C). After a constant mass was achieved, the specimens were weighed again (m_{EMC}) to determine EMC according to Eq. (3):

$$EMC\% = 100 \times (m_{EMC} - m_{0,1}) / m_{0,1} \quad (3)$$

Fiber saturation point: Wood MC at fiber saturation point (FSP) was determined for every material based on $n=6$ specimens. The specimens were exposed at 20°C/100% RH in water-saturated atmosphere in a closed but ventilated small-scale climate chamber over deionized water. After a constant mass was achieved, the specimens were weighed again (m_{FSP}) to determine FSP according to Eq. (4):

$$FSP\% = 100 \times (m_{FSP} - m_{0,1}) / m_{0,1} \quad (4)$$

Pile tests: The decay test with piled wood specimens was described by Ammer (1964), Schmidt et al. (1996, 1997), and Stienen et al. (2014). This method (Figure 1a) allowed for determining the cardinal points of wood MC for different fungal species and wood-based substrates in terms of mycelial growth and decay activity (Huckfeldt et al. 2005; Huckfeldt and Schmidt 2006). Here, the pile direction was identical with the longitudinal direction of the wood specimens, allowing easy capillary water transport and mycelial growth through the wood pile upward. The brown rot fungus *Coniophora puteana* (Schumacher) P. Karsten (DSM 3085) and the white rot fungus *Trametes versicolor* (L) Lloyd (DSM 2086) were inoculated on malt agar (approximate formula in grams per liter: malt extract, 12.75; glycerin, 2.35; dextrin, 2.75; gelatin peptone, 0.78; and agar, 15.00; pH 4.7 \pm 0.2). The decay tests were performed with $n=3$ piles (i.e., 150 specimens) per material, TI, and test fungus (in total: 6 materials, 2 TIs, 2 test fungi; 3600 specimens, 72 piles). In addition, $n=3$ piles of untreated controls were included (2 materials, 2 test fungi; 600 specimens, 12 piles). For each material/fungus combination, 3 \times 50 specimens were labeled and piled. A metal ring (\varnothing 30 mm, h=25 mm) was placed between the seventh and the eighth specimens to avoid direct moisture flow between the agar and the test specimens. The piles were tied up with thin, synthetically covered binding wires, dipped in tap water for 45 s, and put into wide-necked 2-l Erlenmeyer flasks, filled with 500 ml, freshly cooked malt agar. The flasks were then covered with a cotton plug and aluminum foil and sterilized in a steam oven at 120°C for 30 min. The flasks were stored in 20°C/65% RH to generate a moisture gradient within the piles. After 2 weeks of storage, 10 inoculated wood samples (10 \times 10 \times 5 mm³) were placed on the agar next to the pile (Figure 1a). The inoculation samples were preincubated in small Petri dishes for 2 weeks. During incubation, the mycelium growth height was measured visually by marking the maximum height to which the mycelium has grown on the outside of the flasks once a week. After 16 weeks of incubation at a room condition (20°C/65% RH), all specimens were cleaned from adhering mycelium, weighed, oven dried, and weighed again to determine MC and ML. The maximum growth height of mycelium was determined. Therefore, it was

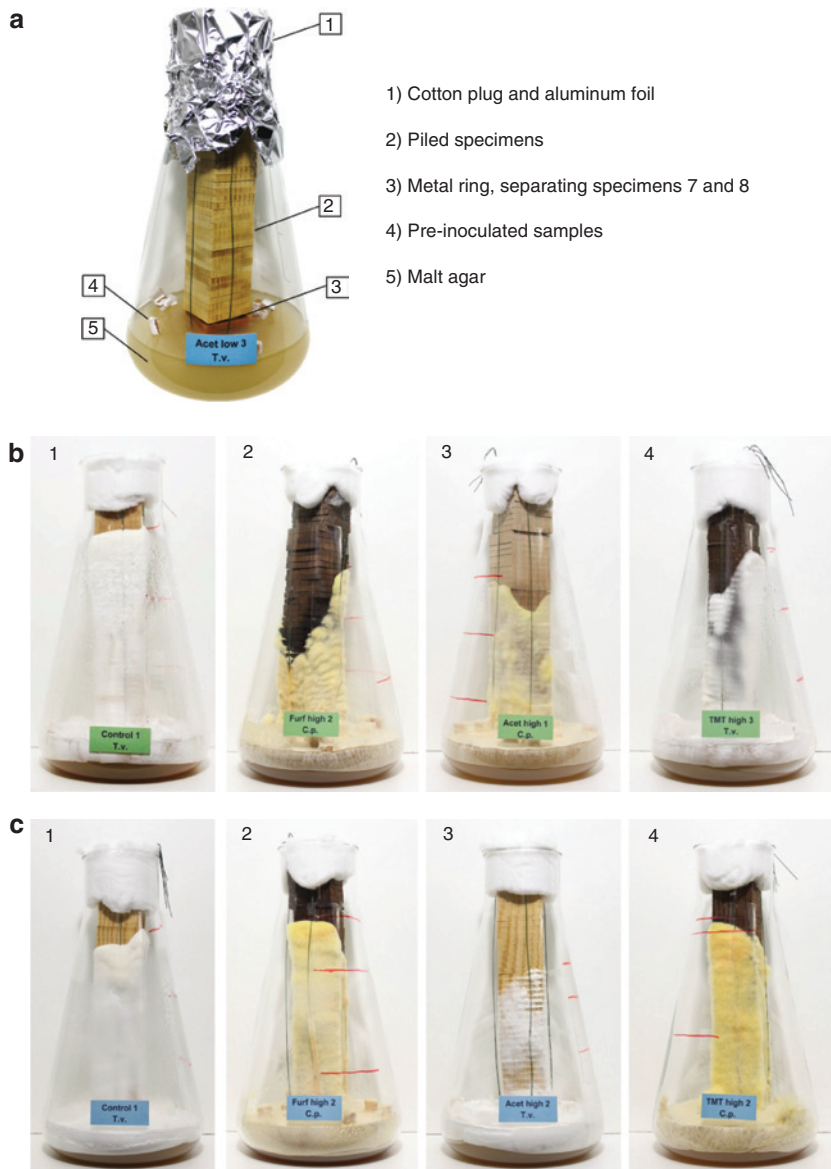


Figure 1: (a) Erlenmeyer flask with piled specimens. (b) Beech and (c) Scots pine sW piles. Mycelium growth after 3 weeks of incubation with *Coniophora puteana* and *Trametes versicolor* on (1) control, (2) furfurylated, (3) acetylated, and (4) thermally modified specimens. Modification level was high.

distinguished between internal mycelium growth through the wood specimens and growth on the pile's outer surface.

Results and discussion

Mass changes as a function of modification

The mass changes (ML_{TMT} as well as WPG) summarized in Table 1 reflect expectedly the TIs. Although the treatment time for TMT was less for the beech specimens, the

ML_{TMT} data are higher than those of pine specimens. This can be explained by the higher content of pentosans of hardwoods, which are less thermally stable than the hexosans of softwoods (Tjeerdsma et al. 2002; Weiland and Guyonnet 2003; Hill 2007). The ML_{TMT} of beech (high TI) was more than three times higher than that of pine after the same treatment time (4 h).

Equilibrium moisture content

The EMC of all modified materials was significantly reduced in a range between 31% and 64%. TMT pine sW

Table 1: Mean±standard deviation of ML due to thermal modification (ML_{TM}) and weight percent gain for acetylated (WPG_{ac}) and furfurylated (WPG_{furf}) samples for $n=150$ replicate specimens.

Material	ML_{TM} (%)	WPG_{ac} (%)	WPG_{furf} (%)
Beech, TI_{low}	8.2±0.7	13.8±1.3	29.5±8.2
Beech, TI_{high}	15.3±0.5	18.2±0.5	38.0±5.7
Pine sW, TI_{low}	4.9±0.3	15.1±2.6	35.5±8.2
Pine sW, TI_{high}	8.6±0.4	22.3±0.6	55.5±5.1

(low TI) showed the lowest and acetylated pine (high TI) the highest sorption reduction (Table 2). For all materials, EMC reduction corresponds to higher WPG or ML_{TM} . The reduced water sorption is explained by the reduced number of hydroxyl groups in the cell wall that is accessible or available for water (Hill 2007; Esteves et al. 2011; Thybring 2013; Ringman et al. 2014).

Mycelium growth

The setup allowed fast mycelium growth upward the pile, especially in the first 3 weeks. An example for this is presented in Figure 1b and c for the different materials with high TI. After only 3 weeks of incubation with *C. puteana* and *T. versicolor*, almost all piles were covered with mycelium to half of their height or more. For most materials, mycelium growth remained static after approximately 5 weeks. However, none of the fungi suffered from dry conditions due to the high amount of malt agar serving also as nutrient source. Consequently, results from all 84 piles can be considered valid.

ML and MC of untreated wood

The relationship between MC and ML was determined for every single pile. Figure 2a shows the results for untreated beech exposed to *T. versicolor*. In general, ML decreased with decreasing MC, which coincides with findings of Huckfeldt and Schmidt (2006). As expected, the highest ML

and the highest MC were determined for the lowest sample directly above the malt agar. By contrast, the moisture threshold for decay ($ML \geq 2\%$) was surprisingly low; an ML of 2.2% was obtained at an MC of only 15.4%. Consequently, the fungus was able to degrade wood clearly below FSP.

Figure 2b shows the results for a pine sW pile exposed to *C. puteana*. In analogy to beech wood (Figure 2a), a continuous moisture gradient developed in the piles within the 16 weeks of incubation and ML decreased with decreasing MC. Hereby, water might be transported from the malt agar by fungal mycelium and strands (Schmidt 2006). In addition, water might be derived from fungal metabolism, as suggested, e.g., by Ammer (1964). The highest ML and the highest MC were again determined on sample no. 8. In contrast to *T. versicolor*, the brown rotter *C. puteana* caused mass increase instead of ML on the upper samples (nos. 41–48). Because the internal mycelium growth border was determined on the 48th specimen, this observation might be explained to some extent by ingrown mycelium with little metabolizing effect of wood substance, as earlier described by Huckfeldt and Schmidt (2006). Brischke et al. (2008) conducted a high-energy multiple impact test with specimens previously exposed to different fungi in pile tests. The tests revealed that a remarkable decrease in structural integrity is detectable for specimens with mass increment due to ingrown mycelium. However, as *C. puteana* caused mass increment up to 5–10%, the argument of ingrown mycelium is not enough. It is also possible that nutrients were transported from the malt agar to the growth front at the upper part of the piles. When modeling decay as a function of MC and TI, setting a threshold of only $\geq 2\%$ ML, might be considered for determining the lower moisture limit as nonconservative because the actual threshold might be even lower.

ML and MC of modified wood

In many cases, a continuous moisture gradient developed in the piles of modified wood similar to the untreated

Table 2: Mean±standard deviation of equilibrium moisture content (EMC) for modified materials and controls as well as reduction in EMC (EMC_{red}) for modified materials; $n=150$ replicate specimens.

Material	Control	Thermal modification		Acetylation		Furfurylation	
	EMC (%)	EMC (%)	ΔEMC (%)	EMC (%)	ΔEMC (%)	EMC (%)	ΔEMC (%)
Beech, TI_{low}	15.83±0.59	8.51±0.15	46.24	9.00±0.50	43.15	9.36±0.77	40.87
Beech, TI_{high}		7.71±0.24	51.30	6.60±0.14	58.31	9.00±0.32	43.15
Pine sW, TI_{low}	15.15±0.61	10.44±0.43	31.09	8.22±0.88	45.74	8.79±0.55	41.98
Pine sW, TI_{high}		8.45±0.15	44.22	5.36±0.14	64.62	8.41±0.32	44.49

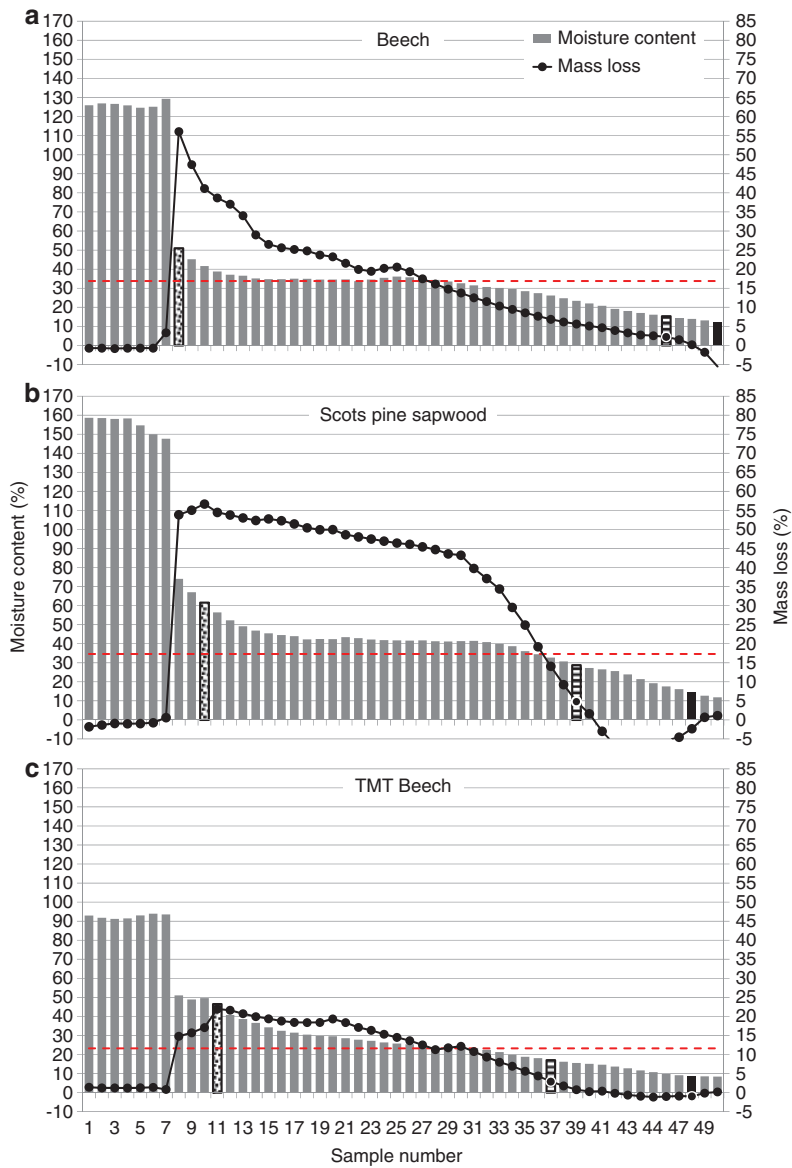


Figure 2: MC and ML for every single sample in (a) untreated beech, (b) untreated Scots pine sW, and (c) thermally modified “low” beech piles exposed to *Trametes versicolor* (a, c) and *Coniophora puteana* (b) for 16 weeks (No. 1=bottom, No. 50=top, metal ring spacer between No. 7 and No. 8). MC at maximum ML (dotted column), MC minimum for ML ($\geq 2\%$) (striped column), and mycelium growth border (black column). Dotted red line shows FSP.

controls and ML decreased with decreasing MC, although FSP was significantly reduced compared with the untreated wood. This is exemplarily shown in Figure 2c for TMT beech exposed to *T. versicolor*.

The highest ML was found for sample no. 11. The threshold for decay ($ML \geq 2\%$) was determined below fiber saturation with an ML of 2.9% obtained at an MC of 17.0%. This also demonstrates that TMT was degradable below FSP.

The relationship between MC and ML for acetylated beech is presented in Figure 3. In general, MC and ML decreased upward the pile up to sample no. 16. A new

increase of MC and ML was observed at the samples no. 24 and 34. The question arises whether the MC was increased through moisture transport by high fungal activity or the increased ML was caused by a high MC. Additional exposure times could give more insight into the development of MC and ML. Looking at the corresponding WPG and EMC, it can be stated that for every specimen with a remarkably higher ML and MC, the WPG was comparably low. The slight drops in TI within one pile became also evident based on the EMC. Hereby, it becomes clear that a small change in TI can have an enormous effect on fungal degradation. The same effect was found for the furfurylated piles.

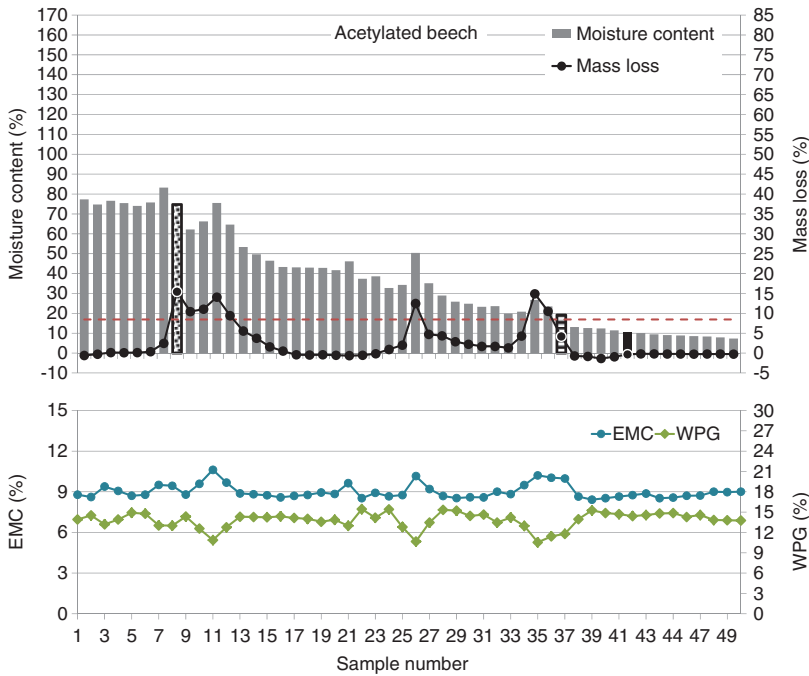


Figure 3: MC and ML as well as equilibrium moisture content (EMC) and weight percent gain (WPG) for every single sample in an acetylated “low” beech pile exposed to *Coniophora puteana* for 16 weeks (No. 1=bottom, No. 50=top, metal ring spacer between No. 7 and No. 8). MC at maximum ML (dotted column), MC minimum for ML (≥2%) (striped column), and mycelium growth border (black column). Dotted red line shows FSP.

Wood moisture thresholds

In Tables 3 and 4, the results concerning moisture thresholds for mycelium growth and ML obtained from untreated

and modified beech and pine sW exposed to *T. versicolor* and *C. puteana* are presented, respectively. Internal and external growth borders were distinguished and refer to mycelium growing on the outer surface of the pile and

Table 3: Wood moisture content, fiber saturation, and growth heights (growth b.) of *Trametes versicolor* after 16 weeks of incubation for acetylated (Acet.), furfurylated (Furf.), and thermally modified (TMT) materials.

Material	Treatment	MC _{opt} : MC at ML _{max} ^a	MC _{growth b.} ^a	MC _{min} for ML≥2% ^a	FSP ^b	Mean max growth b. ^{a,c}
		(%)	(%)	(%)	(%)	(n)
Beech	Control	46.6	12.3	15.4	33.8±2.6	50
Pine sW	Control	53.2	12.9	21.4	34.6±3.3	48
Beech	Acet. TI _{low}	43.6	8.7	25.0	16.9±1.1	46
	Acet. TI _{high}	n.a.	8.7	n.a.	14.8±1.1	39
Pine sW	Acet. TI _{low}	n.a.	7.9	n.a.	17.1±1.9	43
	Acet. TI _{high}	n.a.	6.1	n.a.	14.4±0.8	45
Beech	Furf. TI _{low}	82.1	12.1	17.9	24.6±2.4	41
	Furf. TI _{high}	60.7	14.4	20.7	25.5±1.4	37
Pine sW	Furf. TI _{low}	62.5	17.0	28.3	30.0±3.7	33
	Furf. TI _{high}	69.1	21.3	37.4	29.4±1.7	23
Beech	TMT TI _{low}	45.5	8.2	16.0	23.2±3.7	49
	TMT TI _{high}	35.2	7.7	15.9	22.3±3.4	48
Pine sW	TMT TI _{low}	34.8	13.1	23.6	21.4±1.7	36
	TMT TI _{high}	n.a.	11.2	n.a.	20.0±2.3	35

Data are presented as mean±standard deviation. n.a., not available; i.e., ML was <2% for all specimens in the pile.

^aMean and minimum/maximum values have been calculated based on n=3 replicate piles.

^bMean value of n=6 replicate samples per material.

^cMaximum height=30 cm.

Table 4: Wood moisture content, fiber saturation, and growth heights (growth b.) of *Coniophora puteana* after 16 weeks of incubation for acetylated (Acet.), furfurylated (Furf.), and thermally modified (TMT) materials.

Material	Treatment	MC _{opt} : MC at ML _{max} ^a	MC _{growth b.} ^a	MC _{min} for ML \geq 2% ^a	FSP ^b	Mean max. growth b. ^{a,c}
		(%)	(%)	(%)	(%)	(n)
Beech	Control	73.4	13.5	29.7	33.8 \pm 2.6	49
Pine sW	Control	76.1	13.8	28.5	34.6 \pm 3.3	47
Beech	Acet. TI _{low}	73.9	10.0	19.2	16.9 \pm 1.1	44
	Acet. TI _{high}	n.a.	7.1	n.a.	14.8 \pm 1.1	45
Pine sW	Acet. TI _{low}	49.4	8.5	14.2	17.1 \pm 1.9	42
	Acet. TI _{high}	n.a.	7.7	n.a.	14.4 \pm 0.8	40
Beech	Furf. TI _{low}	41.6	12.1	17.5	24.6 \pm 2.4	42
	Furf. TI _{high}	82.0	12.7	30.6	25.5 \pm 1.4	40
Pine sW	Furf. TI _{low}	31.1	10.8	17.5	30.0 \pm 3.7	40
	Furf. TI _{high}	46.6	7.9	44.1	29.4 \pm 1.7	40
Beech	TMT TI _{low}	49.1	8.5	21.3	23.2 \pm 3.7	46
	TMT TI _{high}	n.a.	9.3	n.a.	22.3 \pm 3.4	42
Pine sW	TMT TI _{low}	39.2	12.1	24.4	21.4 \pm 1.7	41
	TMT TI _{high}	n.a.	10.6	n.a.	20.0 \pm 2.3	41

Data are presented as mean \pm standard deviation. n.a., not available; i.e., ML was $<$ 2% for all specimens in the pile.

^aMean and minimum/maximum values have been calculated based on $n=3$ replicate piles.

^bMean value of $n=6$ replicate samples per wood species.

^cMaximum height=30 cm.

through the wood samples inside the pile. Because the fungi generally grew higher inside the pile, the internal border was considered for the threshold definition.

The lowest growth height (mean maximum growth border) was found for *T. versicolor* on furfurylated pine sW. This is in agreement with findings reported by Alfredsen et al. (2008), who investigated fungal colonization on furfurylated, acetylated, and TMT and found that furfurylated pine sW showed the lowest amount of *T. versicolor* DNA. They assumed that this might be explained by wood cell wall blocking due to polymerization and reduced accessibility of wood polymers, which hinder the enzymatic degradation of the cell wall and therewith growth of *T. versicolor*. For all other materials, the piles were overgrown by mycelium to at least one half of their height.

In many cases, the fungi were both able to colonize and decay the untreated and the modified materials at MCs below the respective FSP. This coincides with findings from Stienen et al. (2014), who reported that also *Antrodia xantha*, *C. puteana*, and *Gloeophyllum abietinum* were able to grow and subsequently decay pine sW at MCs below FSP, if a moisture source (malt agar) was neighboring. However, Stienen et al. (2014) did not observe ML \geq 2% at less than 24.6% MC. For *C. puteana*, an MC_{min} of 21.5% for a degradation $>$ 2% was determined by Huckfeldt and Schmidt (2006). Ammer (1963) conducted test with *C. puteana* on spruce and observed an ML of 2.4% at an MC of only 18.5%. Schmidt (2006), however,

concluded that data on cardinal points for MC can vary depending on the wood species, test parameters, and fungal isolate. The lower threshold MC for fungal decay determined in this study for *T. versicolor* on beech wood can indicate that there is a relationship between fungus and material combinations and the respective moisture thresholds.

Similarly, in this study, the thresholds for all three modification types were partly below their respective FSP, e.g., *T. versicolor* on furfurylated beech and TMT pine sW (6.7% and 6.4% below FSP) and *C. puteana* on acetylated and furfurylated pine sW (2.9% and 12.5% below FSP, respectively). However, as shown in Figure 2, ML was depending on weak points, e.g., reduced TI of the more durable modified materials. Low modification levels in combination with increased MC – even below FSP – might therefore lead to fungal colonization and subsequent decay.

For some materials in this study, in particular the high modification levels, no ML \geq 2% occurred. Therefore, thresholds could not be determined for the most durable materials.

Conclusions

The pile test method could be confirmed as a useful tool for determining moisture thresholds of decay fungi also

for thermally and chemically modified timbers. For interpretation of the thresholds obtained, it has to be considered that an external moisture source was available for the fungus. For future studies, it might be worth investigating growth and decay conditions also without external moisture source as well as the conditions for spore germination. The latter is to reflect the most relevant exposure situations, such as in the building envelope of timber frame houses. The interpretation of the results of moisture monitoring and modeling of the moisture-induced risk for decay requires exact knowledge about MC thresholds for fungal decay and corresponding limit states. The pile test setup lacks information on (1) whether moisture is evenly distributed within one sample or gradually decreasing from decayed to nondecayed areas and (2) whether water is located in the cell lumen or bound in the cell wall. More sophisticated techniques, such as NMR, in combination with the pile test may further contribute to answering these questions.

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