

Christian Brischke\*, Arved Soetbeer and Linda Meyer-Veltrup

# The minimum moisture threshold for wood decay by basidiomycetes revisited. A review and modified pile experiments with Norway spruce and European beech decayed by *Coniophora puteana* and *Trametes versicolor*

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**Abstract:** To know the minimum moisture threshold (MMThr) in wood allowing for the initiation of the fungal decay by basidiomycetes is relevant both from a theoretical and a practical point of view. The present work summarizes the knowledge about MMThr and presents experimental data obtained by improved laboratory decay tests on *Picea abies* (Norway spruce) and *Fagus sylvatica* (European beech) with the fungi *Coniophora puteana* and *Trametes versicolor* under different exposure scenarios well suited for simulation in real applications. The three experimental set-ups, in which the pile tests play a pivotal role, differed in terms of external moisture supply and the inoculation strategies. It was confirmed that wood-destroying basidiomycetes are able to degrade wood at high relative humidity without any external source of available liquid water. The method of moistening the wood samples has an effect on the MMThr before initiation of the fungal decay, but different basidiomycetes were able to cause significant decay at moisture contents considerably below the fiber saturation point.

**Keywords:** brown rot, durability, fungi, minimum moisture threshold, physiological limit, pile test, white rot, wood decay, wood decay fungi

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\*Corresponding author: Christian Brischke, University of Goettingen, Faculty of Forest Sciences and Forest Ecology, Department of Wood Biology and Wood Products, Buesgenweg 4, D-37077 Goettingen, Germany, Tel.: +49-(0)-551-39-19514, Fax: +49-(0)-551-39-9646, e-mail: christian.brischke@uni-goettingen.de, <http://www.holz.uni-goettingen.de>

Arved Soetbeer and Linda Meyer-Veltrup: Leibniz University Hannover, Faculty of Architecture and Landscape Sciences, Institute of Vocational Sciences in the Building Trade, Herrenhaeuser Str. 8, D-30419 Hannover, Germany

## Introduction

Wood moisture content (MC) and temperature are recognized as key environmental variables to quantify the risk of fungal decay and to predict the service lives of wooden structures (Bavendamm and Reichelt 1938; Griffin 1977; Brischke et al. 2006a,b; Schmidt 2006; Viitanen et al. 2010; Naumann et al. 2012; Thybring 2013; Zelinka et al. 2015). Many different models were developed to describe the effects of temperature, relative humidity (RH), and in some cases also rain events on decay (Brischke and Thelandersson 2014), but among all parameter the minimum moisture threshold (MMThr) value is the most important one, below which the decay cannot be initiated.

MMThr and other physiological requirements of decay fungi are usually tested in laboratory experiments (Viitanen 1997; Viitanen et al. 2010), and also in the field (Scheffer 1971; Augusta 2007; Van den Bulcke et al. 2009; Meyer-Veltrup et al. 2017). It is agreed that available free water in the cell walls is critical, but not that in the cell lumens (Schmidt 2006; Stienen et al. 2014). Nevertheless, there are reports on fungal decay in cases when the MC was significantly below the fiber saturation point (FSP) (Bavendamm and Reichelt 1938; Theden 1941; Griffin 1977; Viitanen and Paajanen 1988; Carll and Highley 1999; Stienen et al. 2014; Höpken 2015; Meyer and Brischke 2015; Meyer et al. 2015; Zelinka et al. 2015). The data about the MMThr in the literature deviate because of the different experimental designs for their determination.

Bavendamm and Reichelt (1938) conducted growth tests on malt agar with sawdust and small wood blocks with seven different basidiomycetes. Various RHs between 81.5 and 99% were generated by various concentrations of sodium chloride (NaCl) solution in small glass vessels and 4 months of exposure, and more than 2% of mass loss (ML) were detected for wood blocks kept under 85.6% RH. The MC after incubation was not determined and thus the MMThr could not be determined. Theden (1941) examined

the MC requirements of various wood rot fungi by a similar approach and generated RHs from 83 to 100% at 20°C. The MMThr was achieved at 98.2% RH for different basidiomycetes, but decay below the FSP was not seen, because the MC of the specimens exceeded by far the theoretical equilibrium moisture content (EMC) after 4 months of incubation. The higher ML by fungal decay, the higher was the MC after incubation, and the author explained this by the biochemical production of water during degradation of carbohydrates:



Apart from this, the nominal wood MC increases due to ML of the dry wood mass, and thus the relative amount of water is also elevated in the case of the constant water amount in wood. In the quoted work the decay started at RH below 100%, but MMThr below FSP was not observed.

Ammer (1963) tested pre-inoculated specimens and stored them in screw-top jars above different saturated salt solutions at RHs from 22 to 100%. Here also, drastic differences occurred between the target wood EMC and the actual MC after incubation of the specimens with significant MLs. This author found MMThr at 24% MC in a 96% RH environment, a value, which is ca. 2% below the expected EMC and 7% below the FSP of Norway spruce (*Picea abies*).

Saito et al. (2012) conditioned Japanese red pine (*Pinus densiflora*) in small vessels at 93, 97, and 100% of RH and incubated the specimens with *Fomitopsis palustris* up to 12 months. No decay was observed below FSP at 100% RH.

Schmidt et al. (1996), Huckfeldt et al. (2005), Huckfeldt and Schmidt (2006) and Stienen et al. (2014) performed experiments with small piled wood samples in Erlenmeyer flasks. The bottom of the piles was exposed to malt agar inoculated with fungal mycelium serving as the nutrition and water source at the same time. This set-up provided a moisture gradient upwards in the pile and limited fungal growth and decay in the upper layers. Meyer and Brischke (2015) and Meyer et al. (2015) modified the set-up to obtain more exact data and found MMThr below FSP. However, as was critically discussed by the authors themselves, malt agar at the pile bottom provides an external moisture and nutrition source and the fungus is able to transport water and nutrition from the agar upwards through mycelium and strands. This is the reason why the results of this approach are frequently questioned.

Höpken (2015) modified the pile test method, where the specimens with a drilled hole in the center are on a stainless steel bar, to examine the water transport ability of decay fungi. Stainless steel washers between the specimens supposed to disrupt capillary water transport. The

tests were run with and without malt agar at the pile bottom for a total of 61 days of incubation. Fungal growth was slower in the absence of malt agar (substituted with water instead) and the ML was less compared to tests with malt agar. Obviously, different fungi are able to transport water actively within the piles.

The objective of the present study was to further evaluate the effect of an external moisture source on the physiological requirements of wood-destroying basidiomycetes with a focus on MMThr. Different experimental set-ups were applied according to Ammer (1963) without an external moisture source to determine more exactly the MMThr. The RH was adjusted by means of different saturated salt solutions, and the pile tests according to Meyer and Brischke (2015) were applied without malt agar as nutrition and moisture sources.

## Materials and methods

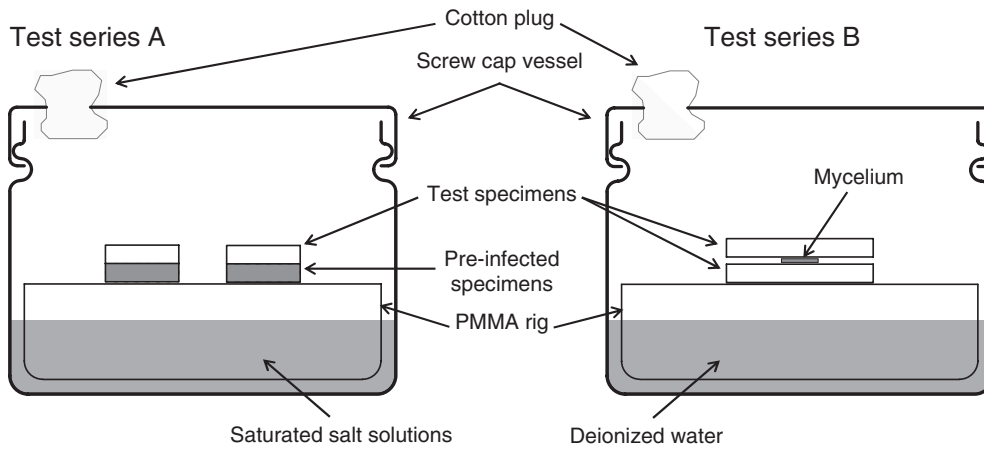
**Wood specimens:** Wood specimens with dimensions of 5 (axial) × 40 × 40 mm<sup>3</sup> were prepared from Norway spruce (*Picea abies* Karst.) and European beech (*Fagus sylvatica* L.). All specimens were made from the same wood samples described already by Meyer and Brischke (2015). Sapwood and heartwood of Norway spruce were not distinguished. For decay tests with inoculated samples, the specimens were split into halves with dimensions of 5 (axial) × 20 × 40 mm<sup>3</sup>. Specimens for the pile tests were axially matched within each pile. All specimens were free of cracks, decay, discoloration or any other visible defects. Before decay, all specimens were oven-dried at 103°C ± 2°C for 24 h and afterwards weighed to the nearest 0.001 g to determine their oven-dry weight ( $m_0$ ).

**Determination of the FSP:** Wood MC at the FSP was determined for every material in nine replicates. Then the specimens were exposed at 20°C and 100% RH in a closed but ventilated small-scale climate chamber over deionized water. After equilibrium, the specimens were weighed again ( $m_{FSP}$ ) and FSP was calculated:

$$\% \text{ FSP} = 100 \times (m_{FSP} - m_0) / m_0 \quad (2)$$

where:  $m_0$  is the oven dry mass before incubation (g), and  $m_{FSP}$  the mass after storage in a water saturated atmosphere (g).

**Decay tests:** Wood specimens were exposed to different RHs and inoculated either with previously infested wood specimens (series A) or mycelium (series B). The following fungal strains were selected: the brown rot (BR) fungus *Coniophora puteana* (Schum.: Fr.) P. Karsten; 62, Ebw. 15 and the white rot (WR) fungus *Trametes versicolor* (L.: Fr.) Pilát; 159, CTB 863a. The specimens were placed on polymethylmethacrylate (PMMA) rigs in screw cap vessels ( $\varnothing = 105$  mm,  $h = 80$  mm) filled with different saturated salt solutions (Figure 1) to generate seven different RHs at 20°C according to ISO 12571 (2013): NaBr (56%), NaCl (75%), KCl (85%),  $NH_4H_2PO_4$  (93%),  $KH_2PO_4$  (96%),  $K_2SO_4$  (98%), and deionized water ( $H_2O$ , 100%). RH was selected according to Ammer (1963). The screw cap vessels together with the



**Figure 1:** Experimental set-up for decay tests in different climates with wood specimens in screw cap vessels.

Test series A: climate via different saturated salt solutions. Inoculation of test specimens through pre-inoculated wood samples. Test series B: climate via deionized water. Inoculation of specimens through the mycelium.

test specimens were sterilized in an autoclave at 120°C for 30 min. Afterwards, approx. 75 ml of the salt solutions were transferred into the vessels.

In total, 560 specimens were tested in series A (280 × beech; 280 × Norway spruce). A total of 140 specimens of each wood species were pre-inoculated with *T. versicolor* and *C. puteana*, respectively, incubated in Petri dishes inoculated with mycelium on the malt agar surface. The incubation time was 2 and 3 weeks for *C. puteana* and *T. versicolor*, respectively. To estimate the ML before the fungal degradation, 20 additional specimens per wood species and fungus combination were incubated in Petri dishes for 2 or 3 weeks, respectively,  $MC_i$  and ML were determined. The specimens were cleaned from the adhering mycelium and then weighed to the nearest 0.001 g ( $m_i$ ), dried at 103°C ± 2°C until constant mass was obtained and weighed again ( $m_{i,0}$ ). MC after incubation ( $MC_i$ ) and ML were calculated:

$$\% MC_i = 100 \cdot (m_i - m_{i,0}) / m_{i,0} \quad (3)$$

where:  $MC_i$  moisture content after incubation,  $m_i$  mass after incubation (g), and  $m_{i,0}$  oven-dry mass after incubation (g).

$$\% ML = 100 \cdot (m_0 - m_{i,0}) / m_0 \quad (4)$$

where:  $m_0$  and  $m_{i,0}$  are the oven-dry mass before and after incubation (g), respectively.

The specimens were placed on PMMA rigs to avoid direct contact between wood and any liquid. Two specimens were placed into each vessel in series A to assure that the same wood volume was exposed per vessel in both test series. To allow exchange of oxygen and for possible refill of salt solutions, a hole of 10 mm diameter was drilled in the vessel lid and plugged with cotton.

The MC was determined before inoculation and after 2 or 3 weeks of conditioning in the screw cap vessels with two out of 10 replicate specimens. The specimens were weighed to the nearest 0.001 g ( $m_c$ ) and the EMC was calculated:

$$\% EMC = 100 \cdot (m_c - m_0) / m_0 \quad (5)$$

where:  $m_c$  is the mass after conditioning (g), and  $m_0$  oven-dry mass before incubation (g).

The pre-inoculated specimens were removed under sterile conditions from the Petri dishes and then placed beneath the conditioned test specimens in the screw cap vessel. In a similar way, the specimens from test series B were prepared and conditioned above deionized water corresponding to 100% RH. Those specimens were inoculated with fungal mycelium grown on malt agar. A block of well-developed mycelium of 10 × 10 mm<sup>2</sup> was taken and placed between two specimens as illustrated in Figure 1.

All specimens were then incubated for a period of 16 weeks before cleaning off the adhering mycelium, weighed to the nearest 0.001 g, oven-dried at 103°C ± 2°C till constant mass, and weighed again.  $MC_i$  and ML were calculated according to Eqs. 3 and 4.

In addition, two out of 10 replicate specimens in test series A served for determination of swelling as a measure of bound water in the cell walls, which is considered as a prerequisite for fungal decay. The longitudinal dimensions of the specimens were measured to the nearest 0.01 mm after oven-drying before incubation; the same was done after incubation and after final oven-drying. The mean swelling orthogonal to the grain was calculated:

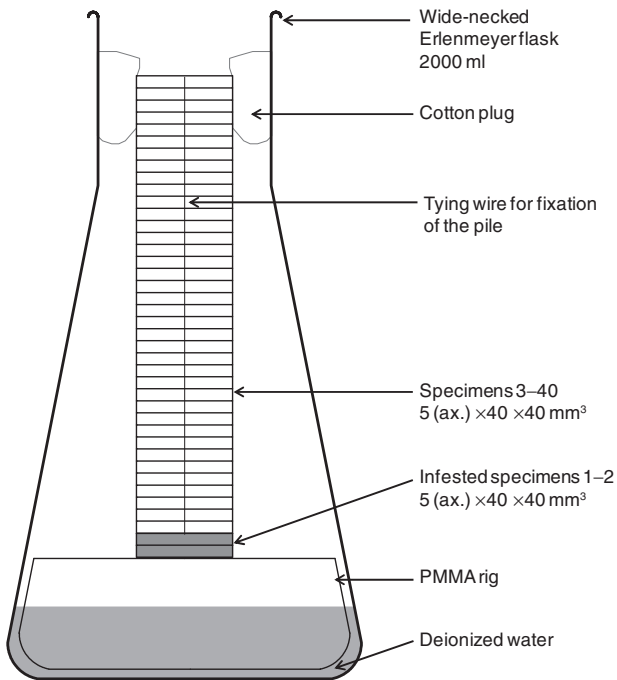
$$\% \alpha_{\text{mean}} = \left( \frac{s_i}{s_{i,0}} \right) \cdot 100 - 100 \quad (6)$$

$$\% \alpha_{\text{mean, max}} = \left( \frac{s_{\text{max}}}{s_{i,0}} \right) \cdot 100 - 100 \quad (7)$$

where:  $\alpha_{\text{mean}}$  is the mean swelling;  $\alpha_{\text{mean, max}}$  is the maximum mean swelling;  $s_i$  is the mean dimension of specimens after incubation (mm);  $s_{i,0}$  is the mean oven-dry dimension (mm);  $s_{\text{max}}$  is the mean maximum dimension at water saturation (mm).

The specimens were vacuum-pressure impregnated with deionized water (30 min at 4 kPa; 60 min at 800 kPa) and afterwards kept submerged for a further 24 h to determine maximum swelling. The dimensions of maximum swelling ( $s_{\text{max}}$ ) were determined to the nearest 0.01 g and used to calculate the  $\alpha_{\text{mean, max}}$  according to Eq. 7.

**Pile test set-up** The tests were performed according to Ammer (1964), Schmidt et al. (1996) and Meyer and Brischke (2015), but in a modified form with respect to the number of piled specimens and the



**Figure 2:** Pile test set-up: 40 specimens [5 (ax.) $\times$ 40 $\times$ 40 mm<sup>3</sup>] piled up and placed on a PMMA rig above deionized water. The axial direction of the specimens are parallel to the pile's vertical direction.

avoidance of malt agar, i.e. substituted with distilled water, to create a moisture gradient in the wood pile. The specimens were piled in their axial direction allowing an easy water transport and mycelia growth upwards through the wood pile (Figure 2). For each wood and fungal species combination, 5 $\times$ 40 specimens were piled and placed on a PMMA rig (50 mm high) at the bottom of a wide-necked Erlenmeyer flask of 2000 ml volume. The piled specimens were fixed with tying wires. To achieve 100% RH, 250 ml deionized water were filled into the flask and its neck was closed with a cotton plug. The set-up allowed a moisture gradient in the wood pile to be established. In the first step, only 38 specimens were placed in the flask for steam sterilization. After 2 weeks, the two pre-inoculated bottom specimens were added after 2 and 3 weeks of incubation in Petri dishes in a similar way to the procedure described above for the decay tests with screw-cap vessels (test series A). Before inoculation and incubation of the pile specimens, the specimens from one out of five replicate piles were removed aseptically and weighed to the nearest 0.001 g for calculation of the initial EMC (Eq. 5).

In total, 800 specimens in 20 piles were thus exposed to the two test fungi for 16 weeks. After incubation, the mycelium growth height was measured and marked on the flask. The specimens were carefully removed from the flasks and cleaned from the adhering mycelium, weighed, oven-dried and weighed again to determine  $MC_i$  (Eq. 3) and ML (Eq. 4). In addition, the maximum growth height of the mycelium was determined. Therefore, it could be distinguished by visual inspection between internal mycelium growth through the wood specimens and growth on the pile's outer surface. Swelling was determined on specimens from one out of five replicate piles according to the procedure described above. Mean swelling and mean maximum swelling orthogonal to the grain was determined according to Eqs. 6 and 7.

## Results and discussion

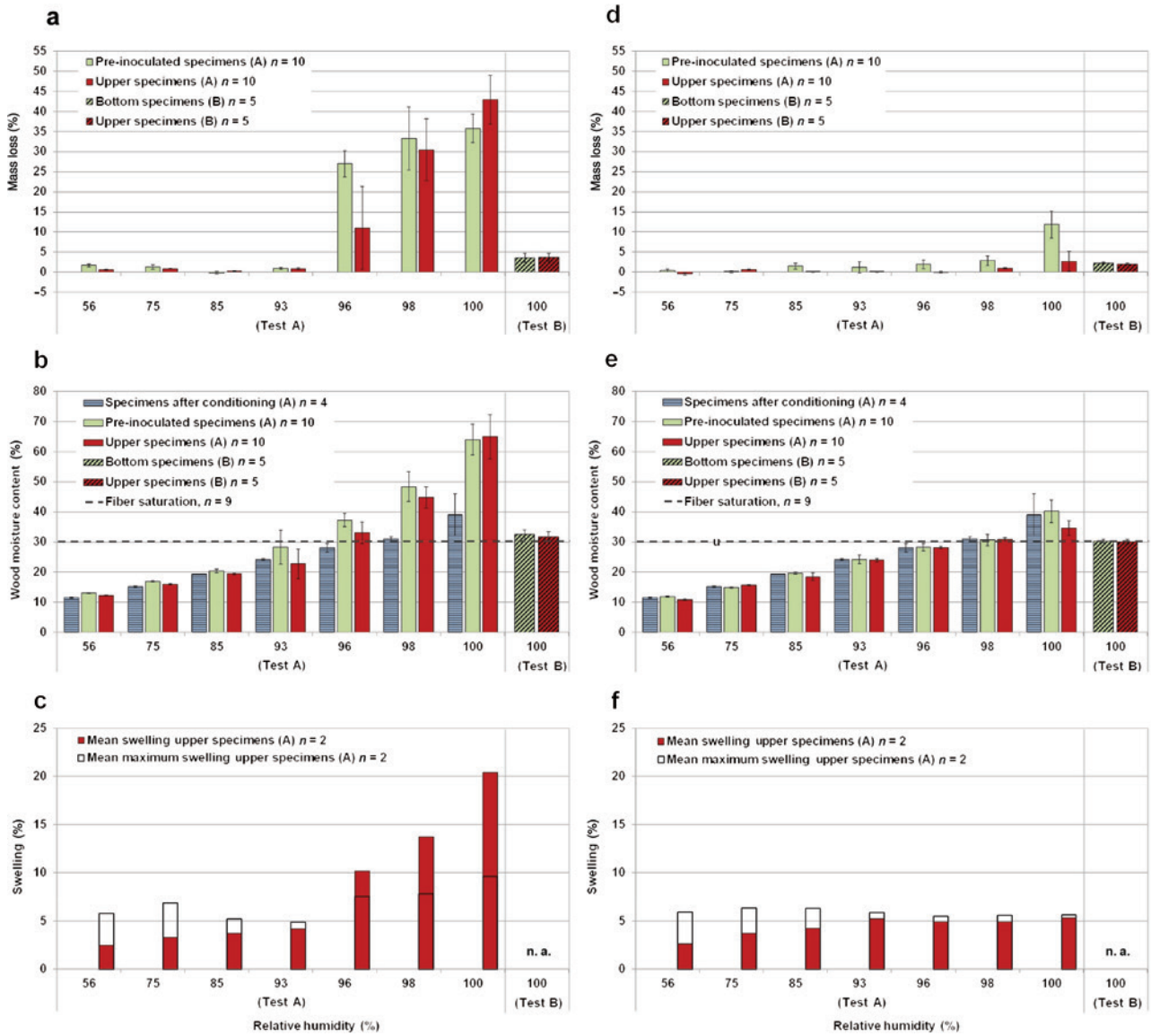
### MC and decay in different climates

Norway spruce specimens, which had been inoculated by pre-inoculated specimens (test series A), showed significant ML at 96% RH and even higher for *C. puteana* (Figure 3a), whereas *T. versicolor* did not cause significant ML below 100% RH. The ML by *T. versicolor* was less compared to *C. puteana* on Norway spruce, in general. The significant threshold for  $ML \geq 2\%$  is in agreement with previous studies (Huckfeldt et al. 2005; Stienen et al. 2014; Meyer and Brischke 2015). As shown in Figure 3a and b, both ML and  $MC_i$  increased at higher RH, and, consequently, ML was also higher with increasing  $MC_i$ . The lower MMThr of Norway spruce was on average 33.1% and 34.6% caused by *C. puteana* and *T. versicolor*, respectively.

Ammer (1963) determined in a similar test set-up ML 7.2% by *C. puteana* on Norway spruce at only 93% RH, but the incubation time was longer (26 weeks) and the temperature was also higher (25°C).  $MC_i$  of the more severely decayed specimens at 98 and 100% RH was clearly above the FSP of Norway spruce (30.3%). Furthermore, after incubation  $MC_i$  of pre-inoculated and upper test specimens, was higher than EMC of specimens before incubation (Figure 3b and e). At 100% RH, the  $MC_i$  exceeded FSP, which is likely due to condensation leading to extra moistening of the specimens. Furthermore, the  $MC_i$  of specimens increased with increasing ML probably because of the water liberated according to Eq. 1 (Theden 1941; Saito et al. 2012). Even if the  $MC_i$  after incubation was corrected by the reduced oven-dry weight of the samples, there still would be a difference between the actual  $MC_i$  after incubation and the theoretically expected  $MC_i$  in equilibrium with ambient air. For instance, the pre-inoculated Norway spruce specimens exposed to 98% RH (Figure 3b) had 48%  $MC_i$  after incubation. Considering the ML caused by *C. puteana* of around 33% (Figure 3a), the EMC at 98% RH should have been 45% instead of 30% (see the conditioned specimens in Figure 3b) as the amount of evaporated water is negligibly small at 98% RH. Similarly, *T. versicolor* induced decay of Norway spruce at 100% RH, where the  $MC_i > FSP$ .

In contrast to test series A at 100% RH, the BR fungus *C. puteana* caused ML between 2.3 and 4.9% on Norway spruce in test series B, where the specimens were inoculated by the mycelium. *Trametes versicolor* led to ML between 1.7 and 2.5%. Even though ML was significantly less compared to series A, where pre-inoculated and thus pre-moistened specimens were applied, the MMThr for fungal decay were very similar in test series B without extra ingress of moisture.



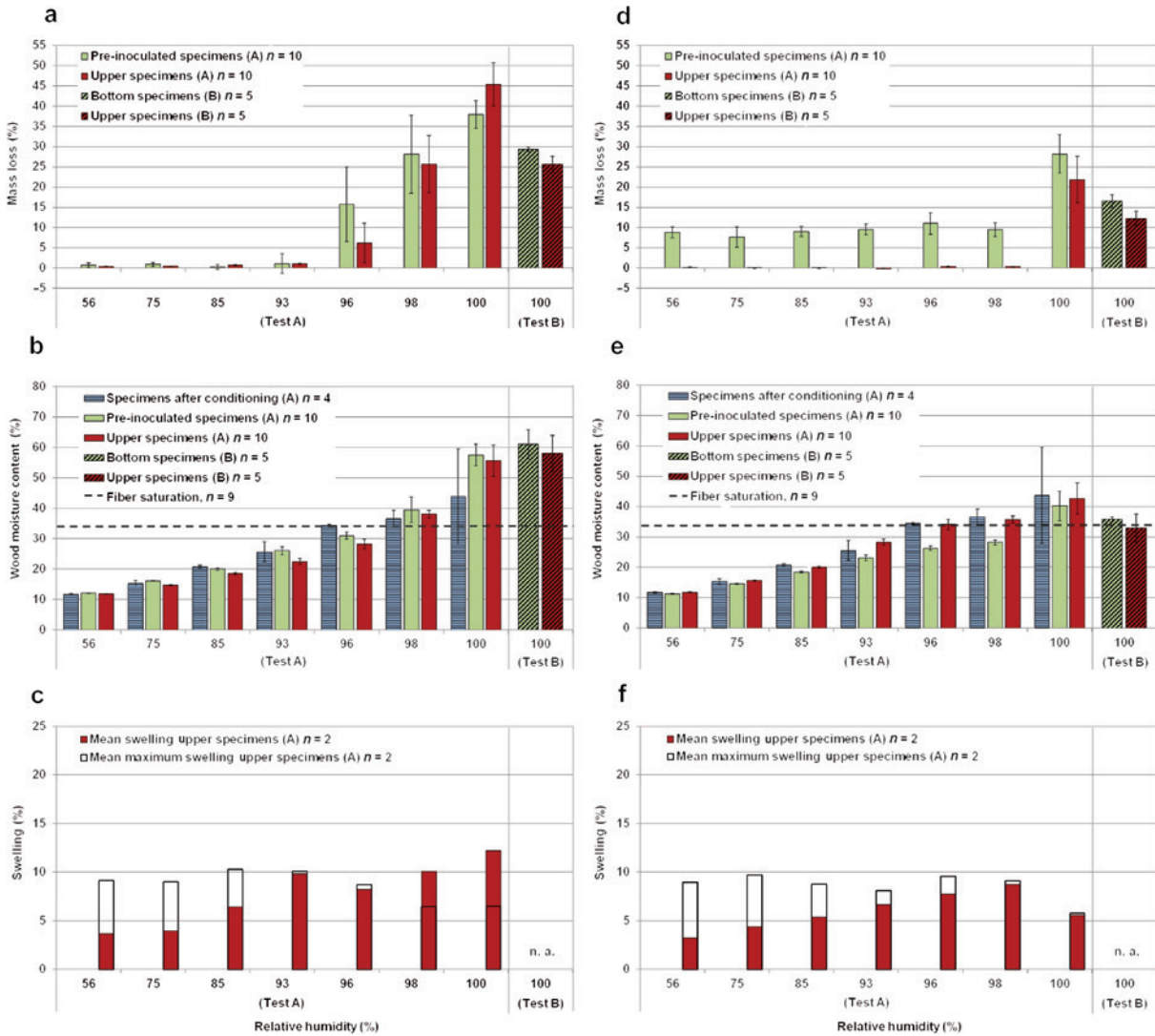


**Figure 3:** Mass loss ML, moisture content  $MC_i$ , and mean swelling  $\alpha_{mean}$  of Norway spruce specimens after exposure to *Coniophora puteana* (a–c) and *Trametes versicolor* (d–f) for 16 weeks in different climates (n.a., not available; error bars indicating standard deviation).

Between 56 and 93% RH, the wood  $MC_i < FSP$ , and mean swelling ( $\alpha_{mean}$ ) did not reach a maximum mean swelling ( $\alpha_{mean,max}$ ), and no  $ML \geq 2\%$  occurred (Figure 3f). Specimens exposed to *C. puteana* at 96% RH and higher showed  $MC_i > FSP$ . In extremis, the  $\alpha_{mean}$  was 12.1% higher than  $\alpha_{mean,max}$ . Buro (1954) reported that BR decayed wood shrinks much more than WR decayed wood during drying, which is related to the higher cellulose degradation in the former case. For the same reason, BR decay usually leads to a higher strength loss at the same ML level caused by WR, where lignin, cellulose and hemicelluloses are degraded successively or simultaneously. The drastically increased swelling of BR decayed wood is related to its permanent shrinkage. Consequently, the

$\alpha_{mean}$  of specimens decayed by WR are in the same range as  $\alpha_{mean,max}$  even at  $RH > 96\%$ .

*Coniophora puteana* caused similarly high ML on beech as on Norway spruce. The lower MMThr was at  $MC_i = 25.3\%$  achieved at 96% RH and was thus 8.5% below FSP (Figure 4a and b). Again, at 100% RH the EMC of specimens before incubation exceeded the FSP indicating that water condensed on the specimens' surface, but further increase in  $MC_i$  might be explained again by Eq. 1. *Trametes versicolor* caused no ML on beech below 100% RH, but ML was between 7.7 and 11.0% of the pre-inoculated specimens under all conditions between 56 and 98% RH, and thus was remarkably higher than all other wood/fungus combinations. However, the ML of those pre-inoculated

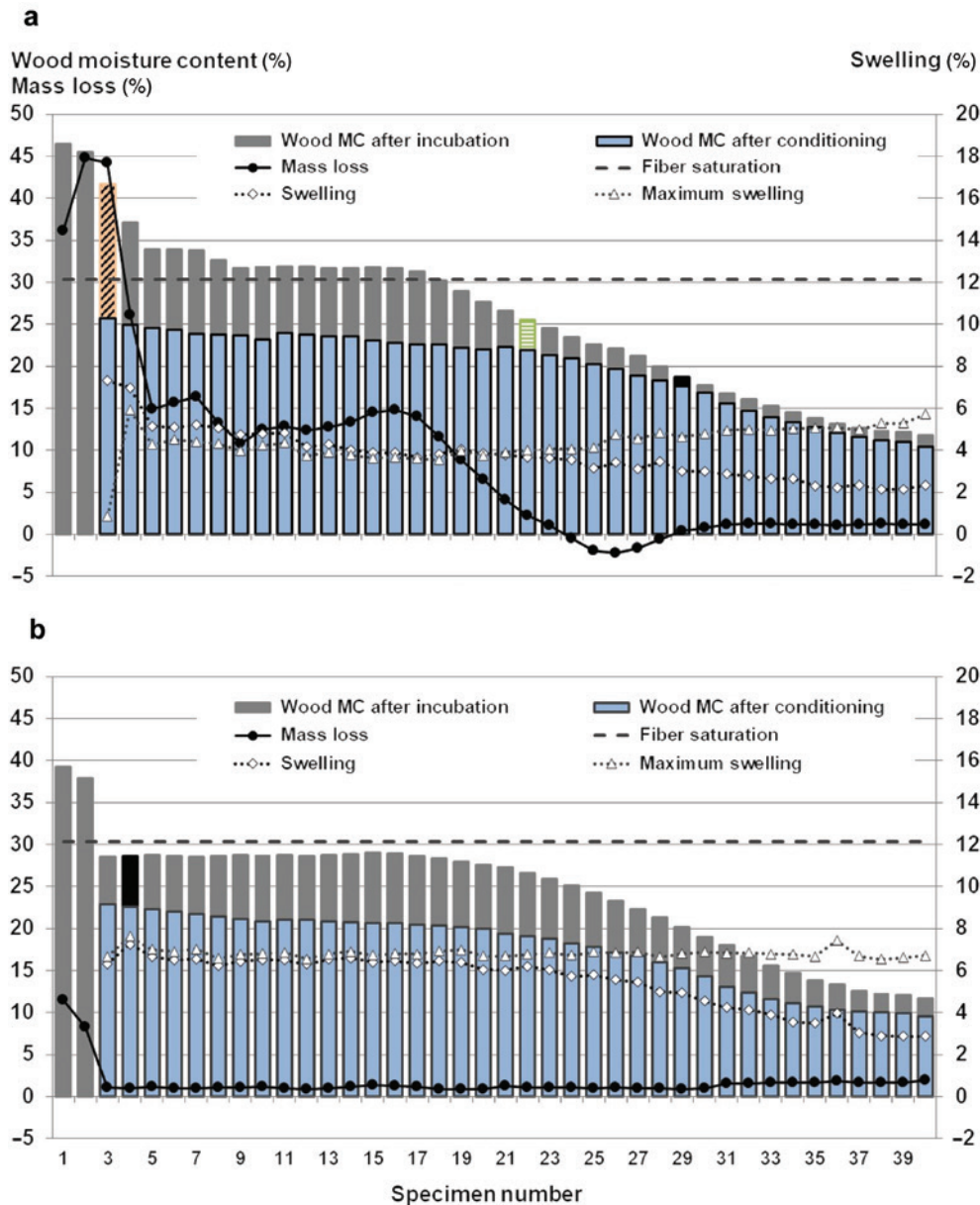


**Figure 4:** Mass loss ML, moisture content  $MC_i$ , and mean swelling  $\alpha_{mean}$  of beech specimens after exposure to *Coniophora puteana* (a–c) and *Trametes versicolor* (d–f) for 16 weeks in different climates (n.a., not available; error bars indicating standard deviation).

**Table 1:** Minimum moisture thresholds (MMThr) for fungal decay as determined in different studies using different test climates and set-ups with different ways of moisture supply.

Inoculation of specimens	Moisture source	MMThr at $ML \geq 2\%$ (ML, RH, wood, fungus, $MC_i$ )	References
Sawdust, pre-inoculated with mycelium	Humidity, sawdust	2.2% ML, 85.6% RH, <i>P. sylvestris</i> sW, <i>Coniophora cerebella</i> , no $MC_i$ data	Bavendamm and Reichelt (1938)
Specimens, pre-inoculated with mycelium	Humidity, mycelium was allowed to grow into liquid	3.0% ML, 98.2% RH, <i>P. sylvestris</i> sW, <i>Lenzites abietina</i> , $MC_i = 28.0\%$	Theden (1941)
Specimens, pre-inoculated with mycelium	Humidity, increased MC of specimens due to pre-infection on malt agar	2.4% ML, 85.0% RH, <i>Picea abies</i> sW, <i>Coniophora puteana</i> , $MC_i = 19.0\%$	Ammer (1963)
Specimens, pre-inoculated with mycelium	Humidity, increased MC of specimens due to pre-infection on malt agar	n.a.	Saito et al. (2012)
Specimens, pre-inoculated with mycelium	Humidity, mycelium was allowed to grow into liquid	2.1% ML, 96% RH, <i>F. sylvatica</i> , <i>Trametes versicolor</i> ; $MC_i = 25.3\%$	Present study

*P.*, *Pinus*; *F.*, *Fagus*. sW, sapwood.



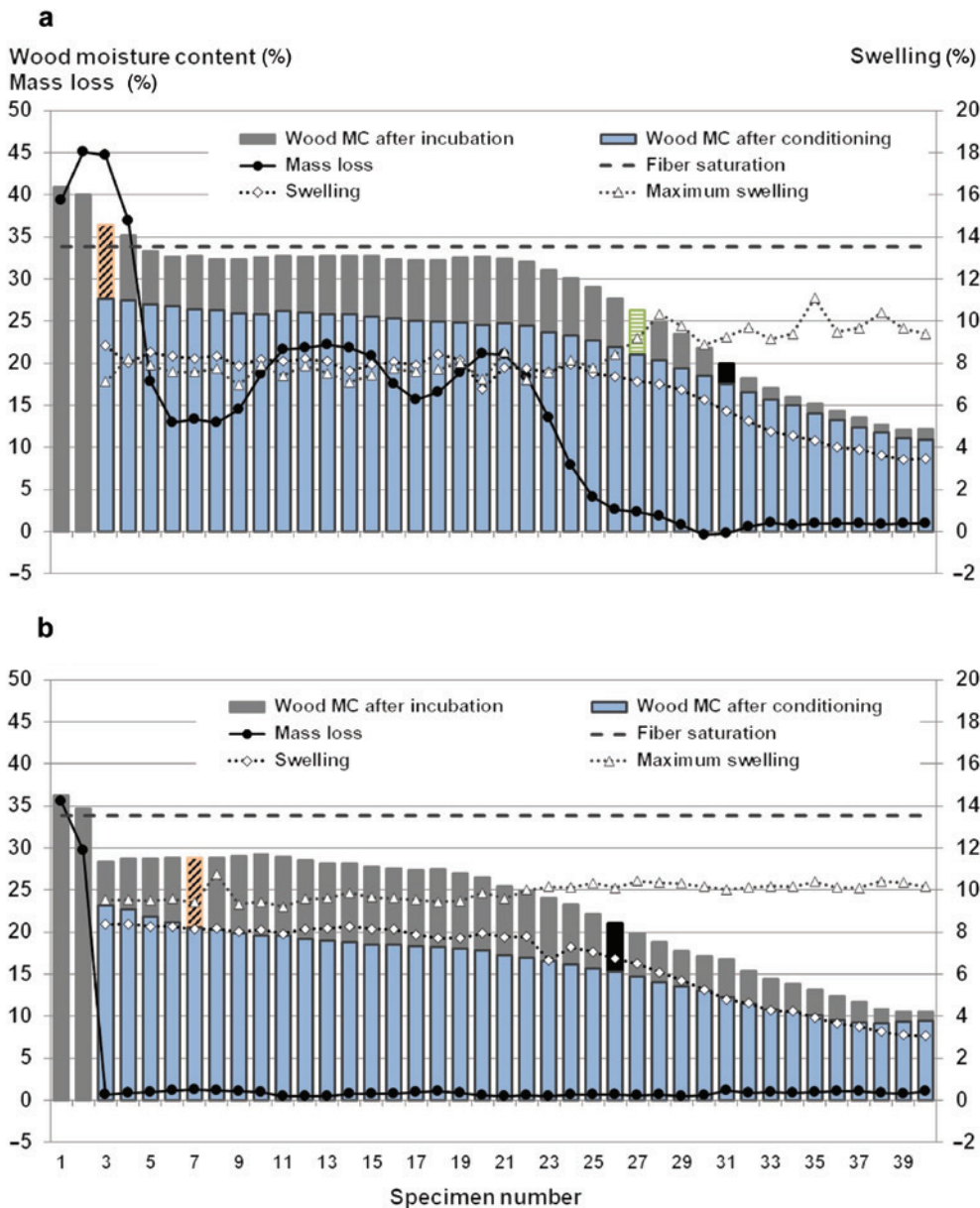
**Figure 5:** Mass loss, ML, moisture content after incubation  $MC_i$ , swelling  $\alpha_{mean}$ , and maximum swelling of Norway spruce specimens after exposure to (a) *Coniophora puteana* and (b) *Trametes versicolor* for 16 weeks (Specimen # 1: bottom of pile; specimen # 40: top of pile).  $MC_i$  at maximum mass loss ML (dotted column),  $MC_i$  at  $ML \geq 2\%$  (striped column), mycelium growth border (dark grey column). Results from one out of five replicate piles.

specimens was on average 7.2% and increased by 1.4% only after the 16 weeks of incubation. The fungus was able to continue degradation only at 100% RH; at lower RH the initial decay was obviously disrupted. This coincides fairly well with observations of Saito et al. (2012), where ML of *P. densiflora* by *F. palustris* was detected below 100% RH.

Both, *C. puteana* and *T. versicolor* also caused high ML on beech wood (23.2%–29.9% and 9.7%–18.1%) in test series B after inoculation with pure mycelium.  $MC_i$  after exposure to *C. puteana* was far above FSP. In contrast,  $MC_i$  was at FSP after exposure to *T. versicolor*.

Between 56 and 96% RH, the wood  $MC_i$  was below or at FSP, but  $\alpha_{mean}$  did not reach  $\alpha_{mean,max}$ . In a similar way to decayed Norway spruce, *C. puteana* caused permanent shrinkage of beech specimens, wherefore  $\alpha_{mean}$  clearly exceeded  $\alpha_{mean,max}$ , when significant ML was observed at 96% RH and higher (Figure 4c). Such an effect was not seen with WR caused by *T. versicolor* on beech wood.

The MMThr was 25.3%  $MC_i$  at 96% RH obtained in test series Ammer (1963) showed that the low MMThr for fungal decay can be even lower following the same minimum ML (with  $ML \geq 2\%$ ), but differences between



**Figure 6:** Mass loss ML, moisture content after incubation MC, swelling  $\alpha_{\text{mean}}$ , and maximum swelling of beech specimens after exposure to (a) *Coniophora puteana* and (b) *Trametes versicolor* for 16 weeks (Specimen # 1: bottom of pile; specimen # 40: top of pile).  $MC_i$  at maximum mass loss ML (dotted column),  $MC_i$  at  $ML \geq 2\%$  (striped column), mycelium growth border (dark gray column). Results from one out of five replicate piles.

the results of this study and those of literature data can partly be explained by differences in the test set-ups, in particular in terms of different moisture supply, as detailed in Table 1.

### MC and decay in piled specimens

Growth of fungal mycelium was observed on piled wood upwards, but its intensity differed between combinations

of wood and fungal species and was generally less intensive compared to data obtained by piled experiments with malt agar (Meyer et al. 2015). Höpken (2015) also found that growth of fungal mycelium is less on piles without agar. The relationship between  $MC_i$  and ML was determined for every single pile. A moisture gradient from the bottom to the top was found in each pile (Figure 5), which is in agreement with results of pile studies with malt agar as the nutrition medium (Schmidt et al. 1996; Huckfeldt et al. 2005).



The lowest MMThr ( $ML \geq 2\%$ ) in case of *C. puteana* on Norway spruce was 25.6%, which is 4.8% below FSP (Figure 5). The MMThr varied only slightly and was 27.6%  $MC_i$  at maximum. *Trametes versicolor* caused ML above 2% at a minimum  $MC_i$  of 16.3% (i.e. 14% below FSP) and 29.6%; in three out of the five piles no  $ML > 2\%$  was observed. In contrast to the assumption that decay fungi might require higher wood MC in the absence of an external moisture source, the lower MMThrs determined in this study were in the same range or even lower compared to those reported by Meyer and Brischke (2015) for piled specimens on malt agar, i.e. 25.9% and 28.4%  $MC_i$  for Norway spruce decayed by *C. puteana* and *T. versicolor*, respectively.

Moisture gradients in the wood piles were already observed before incubation (Figure 5). After 16 weeks of exposure to *C. puteana*, the  $MC_i$  exceeded FSP, which agrees with findings of Theden (1941) and Saito et al. (2012), where  $MC_i$  increment with increasing ML was seen because of water generation according to Eq. 1. *Trametes versicolor* did not result in  $MC_i > FSP$  in case of Norway spruce in pile #5 (Figure 5), because ML was here as insignificant as in case of two other piles. However in two out of five piles, significant ML was achieved and  $MC_i$  exceeded FSP. Short below the growth limit of *C. puteana*, an ML increase was observed on the Norway spruce (# 29, Figure 5a) as well as on beech (# 31, Figure 6a). This phenomenon was observed previously by Huckfeldt and Schmidt (2006) and Meyer et al. (2015) and might be explained to some extent by ingrown mycelium with little metabolizing effect on wood constituents.

The lower MMThr for fungal decay on beech were between 21.9 and 29.7%  $MC_i$  for *C. puteana* and 27.1%  $MC_i$  for

*T. versicolor* (Figure 6), which were 12.8 and 6.7% below FSP. The ML limit of 2% was not reached by *T. versicolor* in four out of five piles. Furthermore the lower MMThr was remarkably higher compared to the MMThr determined on piled specimens on malt agar found by Meyer and Brischke (2015).

As expected, the mean swelling of test specimens was equal or almost equal to their mean maximum swelling underneath the point, where specimens showed  $MC_i < FSP$  (Figures 5 and 6) and with decreasing  $MC_i$  above this point mean swelling decreased. In contrast, the “decay border”, i.e. the first specimen in the pile that showed  $ML < 2\%$ , was found in specimens located several centimeters upwards in the pile. This corroborates the hypothesis that fungal degradation can take place at MCs significantly below FSP independently of the presence of an external moisture source.

The findings from the various studies concerning the physiological threshold values for wood decay fungi based on the pile test method are summarized in Table 2. Irrespective of the variation of test results associated with any biological test, it is evident that fungal infestation and significant decay of wood (here defined at  $ML \geq 2\%$ ) can occur at MC clearly below the respective FSP in both scenarios, in the presence or absence of an external source of available liquid water.

## Conclusions

Wood-destroying basidiomycetes were able to degrade wood at high RH without an external source of liquid water. The tested basidiomycetes caused significant decay

**Table 2:** Minimum moisture thresholds (MMThr) for fungal decay as determined in different studies by piled tests with different ways of moisture supply.

Inoculation of specimens	Moisture source	MMThr at $ML \geq 2\%$ (ML, Wood, Fungus, $MC_i$ )	References
Mycelium	Contact to water or liquid malt agar, humidity	2.0% ML, <i>P. sylvestris</i> sW <i>Physisporinus vitreus</i> ; $MC_i = 31.0\%$	Schmidt et al. (1996)
Mycelium on malt agar	Contact to malt agar, humidity	2.0% ML, <i>P. sylvestris</i> sW, <i>Serpula lacrymans</i> ; $MC_i = 26.2\%$	Huckfeldt et al. (2005)
Mycelium on malt agar	Contact to malt agar, humidity	5.4% ML, <i>P. sylvestris</i> sW <i>Coniophora puteana</i> $MC_i = 27.4\%$	Stienen et al. (2013)
Mycelium on malt agar	Contact to malt agar, humidity	2.2% ML, on <i>F. sylvatica</i> , <i>Trametes versicolor</i> ; $MC_i = 15.4\%$	Stienen et al. (2013)
Pre-inoculated specimens at bottom of pile	Presence of malt agar, humidity	2.5% ML, <i>Q. robur</i> sW <i>Serpula lacrymans</i> ; $MC_i = 25.5\%$	Höpken (2015)
Pre-inoculated specimens at bottom of pile	Presence of water, humidity	2.9% ML, <i>Q. robur</i> sW <i>Serpula lacrymans</i> ; $MC_i = 24.9\%$	Höpken (2015)
Pre-inoculated specimens at bottom of pile	Humidity	2.0% ML, <i>Picea abies</i> , <i>Trametes versicolor</i> ; $MC_i = 16.3\%$	Present study

*P.*, *Pinus*; *F.*, *Fagus*; *Q.*, *Quercus*. sW, sapwood.

considerably below the FSP. Conditioning single or pairs of wood specimens above saturated salt solutions allows the fungus to take up adsorbed moisture in equilibrium with the humidity of the air and to some extent from pre-inoculated specimens. In real life, this refers to situations, where decay is already established, but further ingress of moisture is limited or completely restricted. In piling experiments, fungal decay is established at the bottom of the pile. Additional water by biodegradation of carbohydrates can be produced and transported upwards to the dryer area of the pile. ML decreased at lower MCs in the pile so that mycelium growth stops. This scenario refers to situations, where after damage (e.g. leakage) fungi infested the material and decay expands from an area with RH around 100% to drier areas. A third scenario to be expected from exposed wooden building components is the infestation by spores. The MMThr for spore germination should be investigated in future experiments.

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