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CHANGES IN THE SURFACE PROPERTIES OF WOOD DURING BROWN ROT

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ABSTRACT

We investigated the applicability of the instrumented microscratching and microindentation methods in the examination of fungal decay of wood. Samples of Norway spruce wood (*Picea abies*) were exposed to the brown rot fungus *Gloeophyllum trabeum* using the mini-block method. The decay progression was monitored at one-week intervals during the 8-week fungal exposure. The results were complemented by hyperspectral imaging. In contrast to the progression of fungal decay, measured by mass loss of wood samples and where decay could only be detected five weeks after exposure, signs of decay could be detected by microscratching and microindentation measurements after only two weeks of exposure.

Key words: Norway spruce wood, fungal decay, hyperspectral imaging, microscratching, microindentation.

INTRODUCTION

Fungal decay is a constant threat to wood and wood products that are frequently exposed to a humid environment, usually outdoors, but under certain conditions also indoors, e.g. wooden floors, furniture in bathrooms and kitchens or joinery. Visible signs of wood rot caused by fungal attack usually only become apparent when it is too late for repair or less demanding remediation action.

Wood decomposition by fungi begins with infection by spores or fungal mycelia and ends when the wood is completely decomposed. In the initial phase of decomposition, the fungi penetrate the lumen of the cell walls. At this stage, a wood mass loss is not yet detectable (Humar *et al.* 2020). As the wood rot progresses, discolouration and changes in the wood texture occur, which is referred to as the early stage. A considerable mass loss occurs. The stage at which we first recognise decayed wood is called the intermediate stage of decay. In the final stage, the wood completely loses its original texture (Humar *et al.* 2020). The brown rot fungi mainly decompose hemicelluloses and cellulose, and oxidise lignin, what results in a dark brown colour, cracks and a loss of mechanical properties. During decomposition, the fungi weaken the mechanical properties of the wood, especially the impact bending and tensile strength. (Humar *et al.* 2020).

In the past, brown rot of different wood species has been extensively studied by different brown rot species. Various methods have been used to clarify the mechanism of brown rot at all stages of decay, examining a range of wood properties. For example, Arantes and Goodell (2014) published a review of the mechanisms of biodegradation by brown rot fungi with emphasis on chemical mechanisms by brown rot fungi via •OH Fenton reactions. According to Råberg and co-authors (2005), the most commonly used methods for detecting and assessing the extent of decay are visual assessment, image analysis, microscopic assessment, the pick or splinter test, density and mass loss and various strength tests. Some other methods include magnetic resonance imaging (Müller *et al.* 2002), antibody tests and immunoassays (Clausen 2003), nuclear magnetic resonance and UV spectroscopy (Fackler and Schwaninger 2012), Fourier transform infrared spectroscopy (Jelle and Hovde 2012), changes in the transparency of wood to microwave radiation (Brodie *et al.* 2011), anatomical-microstructural investigations (e.g. by Bader *et al.* 2012) and even some more "exotic" methods, such as machine olfaction (Suzuki *et al.* 2021).

Hyperspectral imaging (HSI) is an instrumental technique that is spreading rapidly in wood research. This technique combines spectroscopy and imaging. A variety of applications have been reported (Schimleck *et al.* 2023), covering the following topics: property variations within trees or samples, reaction wood detection, wood product applications, pulp and paper, wood degradation and preservation, wood–water interactions, wood waste or wood in waste, and species identification. As far as fungi are concerned, only the study of the colonisation of wood with blue stain fungi is described in this overview. To the best of our knowledge, we could find only three reports on hyperspectral imaging in fungal wood decay studies. Confalonieri *et al.* (2024) showed that it is possible to detect and distinguish four categories using this technique: clear wood, soft rot, brown rot and blue stain. In the other study (Belt *et al.* 2022), hyperspectral near-infrared imaging was applied to sapwood samples of Scots pine exposed to the brown rot fungi *Coniophora puteana* and *Rhodonia placenta* to obtain spatially resolved chemical information about the fungal decay process. The hyperspectral camera was also used to investigate the possibilities of using HSI for rapid and non-destructive detection of fungal decay (Jochemsen *et al.* 2022) and it was found that it is suitable for the quantification of basidiomycetes.

Due to the significant influence of brown rot on structural applications, numerous studies have been carried out on the mechanical properties of decayed wood. Bader *et al.* (Bader *et al.* 2012a) investigated the changes in moduli of elasticity and stiffness data in all anatomical directions of pine (*Pinus sylvestris*) degraded by *Gloeophyllum trabeum* (brown rot) and *Trametes versicolor* (white rot). It was shown that the transverse stiffness properties are much more affected by degradation than the longitudinal properties. In the case of *G. trabeum* decay, the radial stiffness decreased by up to 70.0 %, while the corresponding longitudinal stiffness decreased by only 29.8 % Ge *et al.* (Ge *et al.* 2017) investigated the influence of *G. trabeum* on selected mechanical properties of poplar wood (*Populus cathayana* Rehd.). During the decay process, losses in impact bending strength and modulus of rupture increased logarithmically with the degree of decay, while losses in modulus of elasticity and compressive strength parallel to the grain increased slowly with linear trends.

With regard to the mechanical properties of wood, our overview showed that the techniques of instrumented microindentation and microscratching are extremely useful and versatile techniques that have the potential to investigate the mechanical properties of wood surfaces and surface-treated wood (Petrič *et al.* 2023, Petrič 2023). Our preliminary instrumented microindentation studies of the mechanical surface properties of Scots pine wood (Petrič *et al.* 2023) showed that the hardness and stiffness of the late wood is greater than that of the early wood. However, a large dispersion of the measured values was observed, due to the large variability of wood as a material and the experimental difficulties in performing measurements either only on late wood or only on early wood. Instrumented studies of wood surfaces, exposed to wood pests were rarely reported. For example, Shangguan *et al.* (Shangguan *et al.* 2014) investigated the changes in cell wall properties of larch wood during the white rot process. Nanoindentation measurements showed that the average MOE of the infested wood decreased from 24.0 GPa to 17.1 GPa and the hardness decreased from about 528 MPa to 428 MPa after 12 weeks of infestation.

The aim of this study was to investigate the influence of exposure of spruce (*Picea abies* Karst.) wood to the brown rot fungus *Gloeophyllum trabeum* (Pers.) Murrill on some selected mechanical properties of the wood surface. We performed instrumented microindentation and microscratching measurements to evaluate the stiffness, hardness and elastic recovery of the infested surfaces after 1, 2, 5 and 8 weeks of exposure. In addition, preliminary hyperspectral imaging experiments were performed to visually track the progression of surface changes caused by the fungus.

EXPERIMENTAL METHODS

EXPOSURE TO GLOEOPHYLLUM TRABEUM

Samples made of Norway spruce (*Picea abies* Karst.) wood (30 mm × 15 mm × 5 mm), (longitudinal, radial, tangential), were steam sterilised (121˚C; 0.15 MPa; 20 min). In the meantime, we prepared a nutrient medium (DifcoTM Potato Dextrose Agar) and transferred it into the Petri dishes. The solidified nutrient medium was inoculated with the brown rot fungus *Gloeophyllum trabeum* (Pers.) Murrill (ZIM L018). After one-week incubation (25°C; 85% RH), the samples were exposed to fungal mycelium above HDPE mesh, which prevented direct contact between the nutrient medium and the wood (Fig.1). The experiment was performed according to the modified Bravery procedure (Bravery 1979). After the time periods specified in Table 1, the samples were isolated and cleaned of mycelia, and the mass loss was determined gravimetrically. The experiment was carried out with six replicate samples per treatment.

Figure 1: Exposure of samples in Petri dishes: (left) control samples in a Petri dish without mycelium and (right) 8th week of exposure

HYPERSPECTRAL IMAGING

The presence of decay was mapped with hyperspectral imagining. Hyperspectral analysis was performed with a high-resolution ClydeHSI Hyperion A3 Scanner, using visible and near-infrared (VNIR; 400–1000 nm, $\Delta\lambda = 3$ nm) or short-wavelength infrared (SWIR; 900–2500 nm, $\Delta\lambda = 10$ nm) hyperspectral cameras. Subsequently, data analysis was made using Principal Components Analysis (PCA) to determine the spread of decay.

INDENTATION AND SCRATCHING MEASUREMENTS

After completion of the decay test, the samples were stored in a dry and dark place under laboratory conditions (room temperature, normal indoor humidity). The storage period prior to the mechanical property measurements was quite long at five months. All instrumented microindentation and microscratching tests were performed using the MCT3 (Anton Paar) with

100 CR6 steel balls (d = 6 mm). The measurements were carried out on surfaces that were in Petri dishes in contact with the plastic mesh, i.e. directly exposed to the fungal mycelium on the growth medium.

Microscratching and determination of elastic recovery

The scratch tests were carried out over a length of 15 mm with linearly increasing loads from 0.03 N to 15 N. The actual penetration depth and the residual depth were determined using the pre- and post-scan function. One sample was randomly selected from each series (0 (control), 1, 2, 5, 8 weeks of exposure). The scratch was performed on a radial surface, parallel to the grain direction for both early and late wood. Three scratching experiments were performed per sample. The penetration depth at this test represents the total amount of deformation, with the residual depth and elastic recovery depth representing the amounts of plastic deformation and elastic deformation, respectively (Liu and Wang 2023). The elastic recovery rate can be defined as d_e/d_p, indicating the proportion of elastic deformation in total deformation, where penetration depth d_p represents the total deformation, and elastic recovery depth $d_e = d_p – d_r$ represents elastic deformation (Liu and Wang 2023).

Microindentation

Initially, several single-load indentation tests were planned, some of which had already been carried out. However, considering the number of all samples (30) and at least 12 measurements per sample (6 on earlywood and 6 on latewood), the measurement time would be quite long. And above all, the exact positioning of the ball on the early or late wood was quite tricky and uncertain. Therefore, we performed the matrix measurements on a randomly selected sample from a series that performs a matrix of identical indentations with an (equally) defined X and Y distance. The maximum loading force was set to 5 N, and a 10-second pause was inserted between the loading and unloading phases. The measurement matrix was 5.60 mm x 4.00 mm and the distances between the individual indentations were 0.4 mm and 1.00 mm. This resulted in 75 instrumentally generated indentations per sample. In some cases, the indentations were not possible due to the uneven surface caused by fungal decay, so that the minimum number of indentations was 66. The indenter indiscriminately hit early or late wood or the edge area between the two.

Multicycle microindentation measurements

While single indentations provide a value for the hardness and modulus of elasticity at a given depth, cyclic indentations provide a better insight into the hardness and elasticity gradient and in our case could reflect the depth of the fungus-infested area. A progressive multicycle measurement was performed, i.e. a single indentation measurement performed in several loading and unloading cycles. The maximum load was increased with each measurement cycle. The maximum load was set at 5 N and 9 indentations were performed in the range between 0 and 5 N. 3 multi-cycle measurements were performed on a randomly selected sample from a series on early and 3 on late wood.

RESULTS AND DISCUSSION

MASS LOSS OF EXPOSED SAMPLES

The mass losses of the samples after 1 to 8 weeks of exposure are shown in Table 1. As can be seen, mass loss was only observed after 5 weeks of exposure and, as expected, was increased after 8 weeks of exposure. The differences between the minimum and maximum values of mass loss (5th and 8th week of exposure) show an uneven decay rate of the small miniblock samples.

Table 1: Mass loss of the samples (%)

Weeks of exposure	Number of samples	Mass loss (%)	Minimal mass loss (%)	Maximal mass loss (%)
0	6			
1	6	-0.9	-1.9	2
2	6	0.3	-0.2	0.9
5	6	22.8	15.5	26.4
8	9	35.3	11.8	72.0

MULTISPECTRAL IMAGES

As can be seen from the hyperspectral analysis, the decay was not uniform. The first signs of decay were evident after the first few weeks of exposure, although mass loss had not yet been detected. This is consistent with the reports of Jochemsen *et al*. (2022) and Belt *et al*. (2022). It should be noted that the hyperspectral analysis only reflects the changes on the surface. Few differences were observed in the samples exposed to the fungi for 5 and 8 weeks. The chemical composition of the wood changed, as did the density, as indicated by the light shades of green (Fig. 2, right). It should be noted, however, that this was a preliminary experiment to support the microindentation and microscratching studies and that we did not perform an analysis of the infrared spectra as was done by Jochemsen *et al*. (2022) and Belt *et al*. (2022).

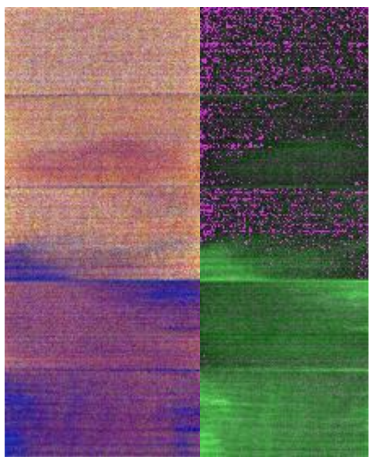

Figure 2: Various examples of wood degradation (0, 1, 2, 5 and 8 weeks of exposure, from top to bottom) determined with the hyperspectral camera. The left images are representations resulting from the reflectance determined at the most prominent wavelength. The right images indicate the differences resulting from the principal component analysis

SCRATCHING RESULTS AND ELASTIC RECOVERY

Fig. 3 is an example of the results of the microscratching test (unexposed wood). In the case of early wood (Fig. 3A), the 15-N load caused scratches up to 200 µm deep, and when the load was removed, the depth of the residual scratch was 75 µm. The comparison between the microscratching test on early wood and late wood is shown in Fig. 3B, and both the scratch depth and the residual depth have lower values for late wood. This confirms that the latewood is harder than the early wood, as reported previously (Petrič *et al.* 2023, Golovin *et al.* 2022).

We also investigated the influence of the fungal infestation on the scratch and residual depths (Fig. 4). While the depth curves after one week of exposure were very similar to those in Fig. 3 for the unexposed control wood (not shown here), a gradual increase in scratch depth was observed after 2 and 5 weeks of exposure (not shown) to reach values of about 500 µm. This reflects the fact that the wood became much softer due to decay. However, it is interesting to note that the residual depths of the rotted wood were quite similar to those of the unaffected wood.

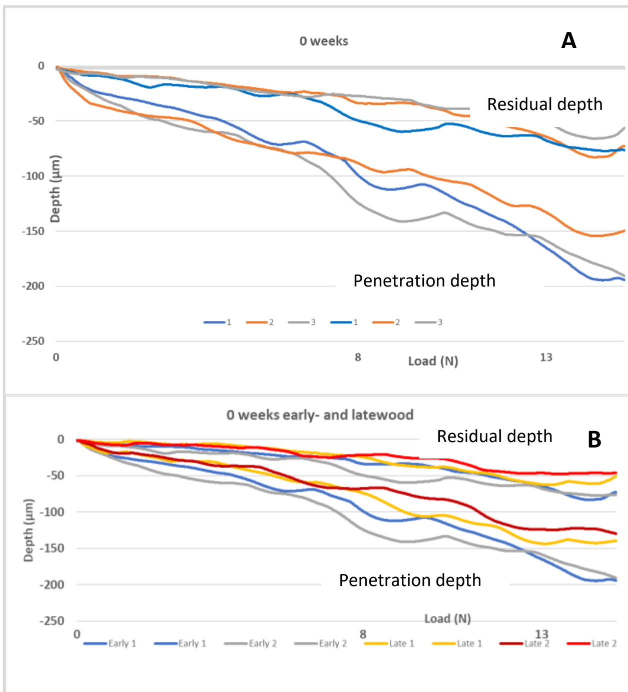

Figure 3: Comparison of the penetration depth and the residual depth on the early wood of control samples (A) and on the early and late wood of control samples (B)

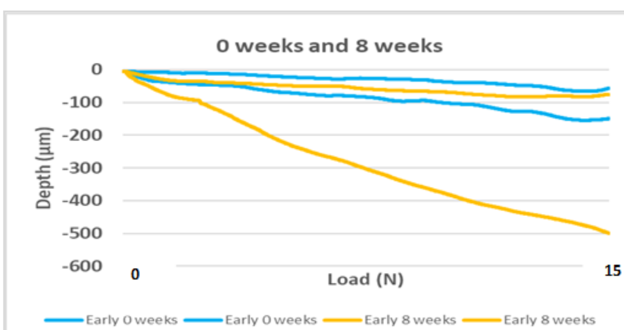

Figure 4: Comparison of the penetration depth and the residual depth on the early wood of control samples (blue) and on the early and late wood that was exposed to the fungus for 8 weeks (yellow)

The elastic recovery can be easily determined by comparing the penetration depth (Pd, sometimes also called scratch depth) with the residual depth (Rd). The smaller the residual depth, the better the recovery, in other words, the wood is more elastic. The elastic recovery (%) curves of 3 samples of early and 3 samples of late wood control, unexposed wood are shown in Fig. 5. In the second half of the scratch, the elastic recovery values are very similar, irrespective of the wood tissue type (early or late wood). The values ranged roughly between 70 and 60 %. The elastic recovery curves of the degraded samples (Fig.6) are less regular than those presented in Fig. 5 for undecayed wood, especially in the first half of the scratch. The reasons are very probably in an uneven decay process – while some parts of the samples have been already decayed – in various depths of the samples, the others were still unaffected. It should be noted that the elastic recovery reached the values of around 90% after 8 weeks of exposure.

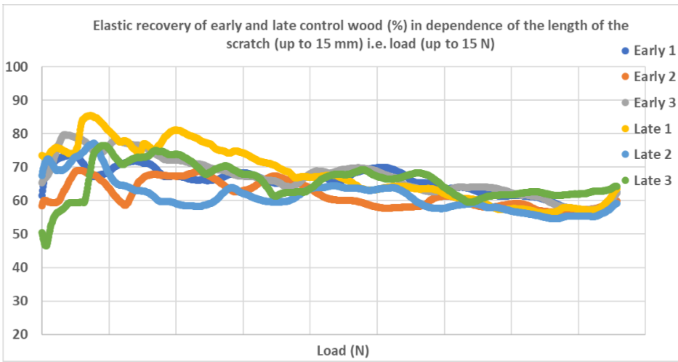

Figure 5: Elastic recovery (%) of early unexposed (3 measurements) and late unexposed wood (3 measurements)

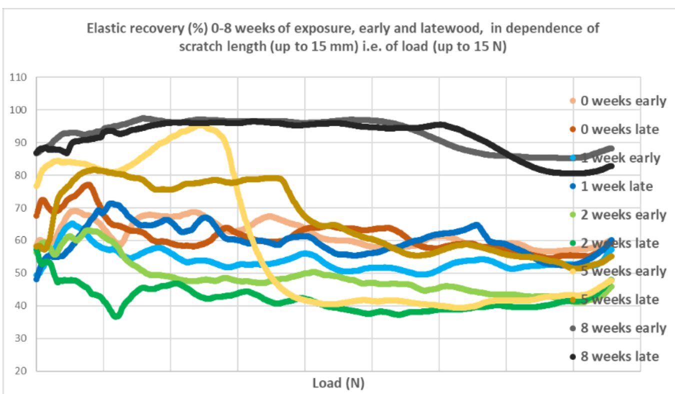

Figure 6: Elastic recovery (%) of early and late wood that was exposed to fungal degradation for 0, 1, 2, 5 and 8 weeks

MICROINDENTATION RESULTS

Hardness and stiffness

As shown in Fig. 7 (left), the indentation hardness (HIT) was influenced by the effect of the fungus. We did not perform a detailed statistical analysis of the results, but the trend is evident: after 5 and 8 weeks of exposure, softer wood was recorded. However, the scatter of the results was significant (as expected, due to the high variability of the wood samples and the uneven decay). The indentation hardness values obtained confirm the results of micro-scratching (e.g. Fig. 4), where the load (15 N) caused the deepest scratch in the wood that had been rotting for 8 weeks. Less clear was the influence of the exposure time on the indentation modulus (Fig. 7, right).

In accordance with our previous research (Petrič *et al.* 2023) and some references therein, harder late wood should be distinguished from early wood. As previously described, we made up to 76 indentations in the given matrix. The ball hit either the latewood, the earlywood or – probably most frequently – the area at the edge between latewood and earlywood, so that our experiments did not allow a clear distinction between latewood and earlywood. We performed a distribution analysis of the obtained values, and only indications of different indentation hardness values between late wood and early wood were found. For example, in the distribution of hardness results on the surface of wood exposed to the fungus for two weeks, a distribution peak was observed between 80-120 MPa and another between 150-190 MPa (Fig. 7).

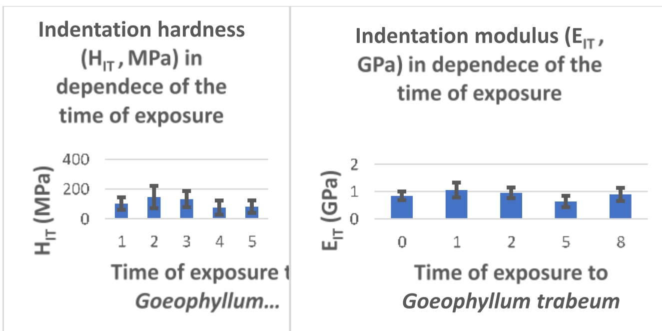

Figure 7: Indentation hardness (MPa) (left) and indentation modulus (GPa) (right) as a function of the decay time

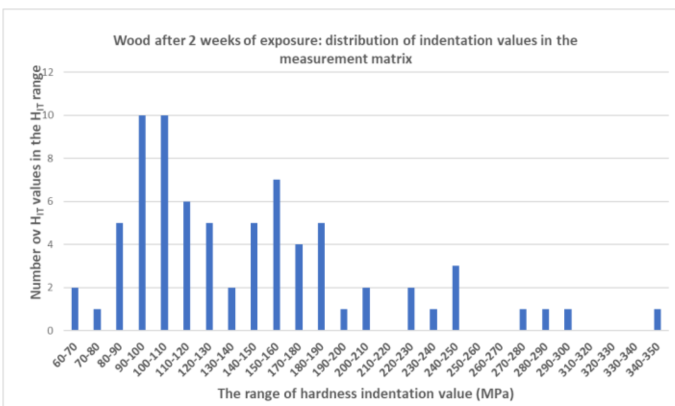

Figure 7: Distribution of the recorded indentation hardness values (MPa)

Depth profile of the modulus of elasticity

Finally, we performed cyclic indentations to gain a better insight into the hardness gradient and indentation modulus of elasticity of the surface layers of rotten wood. The graph in Fig. 8 clearly shows that the control wood and the wood exposed to the fungus for one week have different depth profiles of indentation hardness and indentation modulus than the wood degraded for 2, 5 or 8 weeks. Therefore, this measurement method detects the influence of decay as early as the second week of exposure, when no mass loss was observed (Table 1).

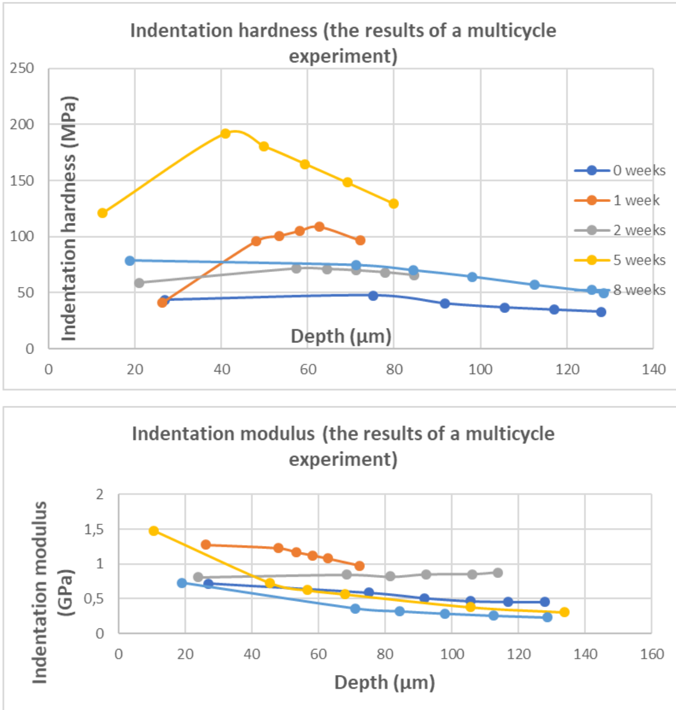

Figure 8: Depth profiles of indentation hardness (MPa) and of indentation modulus (right, GPa)

CONCLUSIONS

We performed microscratching and microindentation experiments to follow the decay of mini-block test specimens of Norway spruce wood during 8-week exposure. Hyperspectral images of decayed wood complemented these experiments. While the scratch depth curves after one week of exposure were very similar to those of unexposed wood (100 µm – 200 µm depth), a gradual increase in scratch depth was observed after 2 and 5 weeks of exposure, reaching values of about 500 µm after 8 weeks. This reflects the decrease in hardness with the progression of decay. The cyclic indentation measurements showed that the control wood and the wood exposed to the fungus for one week had different depth profiles of indentation hardness and indentation modulus than the wood that had been rotting for 2, 5 or 8 weeks. With this method, it is therefore possible to determine the influence of decay as early as the second week of exposure, when mass loss has not yet been observed. In summary, in contrast to the progression of fungal decay measured by mass loss of the wood samples, where decay could only be detected 5 weeks after exposure, the signs of decay could be detected by microscratching and microindentation measurements after only two weeks of exposure to *Gloephyllum trabeum*. These results were complemented by hyperspectral imaging, which demonstrated the progression of decay even in the early stages of decay.

Our first experiments on the application of the microscratching and microindentation method in the study of wood decay have provided us with some experience that will be useful in the careful planning of such experiments in the future (measurement parameters, number, size and orientation of the different wood species, different fungi, statistical analyses).

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