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Cover image based on the beech specimens of Radim Rousek and Mátyás Báder by Miklós Bak, 2021

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Can acetylation make hornbeam wood last? Results of 6-year-long field stake test

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ABSTRACT

This test aimed to discover if industrially acetylated hornbeam can tolerate real-field conditions in Hungary, where various microorganisms can attack the wood separately or cooperatively. Untreated samples accompanied the modified wood to assess the degradation capacity of the soil. The test also focused on weather parameters, the Scheffer index, and soil properties. All of the untreated stakes broke after 6 years, and showed insect damage, soft rot decay, white rot decay, wasp stripping, moss, and cracks. The acetylated hornbeam stakes showed no decay after 6 years of exposure, and they became dry shortly after being taken from the soil. Acetylated hornbeam stake number 7 had superficial brown rot decay after 18 months, which gradually worsened over the years. The Fourier transform infrared spectroscopy analysis revealed that this stake had lower acetyl content. It was associated with hornbeam wood; it had a wet pocket or a part that was not as permeable and achieved a lower grade of acetylation.

INTRODUCTION

The degradation rate caused by microorganisms depends on many factors, such as wood structure, natural or artificial toxicity of the wood, tolerance to temperature, moisture content, pH, and oxygen range. Although fungi and bacteria can degrade the wood, *Basidiomycetes* are usually more aggressive but less tolerant of extreme conditions than the bacteria or soft rot fungi. When colonizing wood, bacteria, moulds, blue stain, and soft rot fungi decay initially, and then *Basidiomycetous* fungi take over (Raberg et al. 2005). Several authors have reported that acetylation of lignocellulosic materials increases their resistance to biological degradation. Laboratory tests and field tests have shown considerably improved protection against attack by white rot, brown rot, soft rot fungi, tunnelling bacteria, and marine borers in various species such as southern yellow pine, beech, Scots pine, and poplar (Takahashi et al. 1989, Militz 1991, Larsson-Brelid et al. 2000, Mohebby and Militz 2010).

Fungal colonization requires moisture for oxalate production. Moisture is also necessary for movement in the cell wall, in Fenton chemistry to degrade cell wall polysaccharides, for gene expression and enzyme activity, for glycoside hydrolysis, and for the movement of soluble nutrients. Wood-rotting fungi and insects have their specific enzyme systems, which degrade wood polymers into digestible units. Hydroxyl groups are biological enzymatic reaction sites; if these are chemically changed, the fungal enzymatic action cannot take place (Takahashi 1996, Suttie et al. 1999).

Since 2008, Accsys Technologies (Arnhem, the Netherlands) has been commercially acetylating radiata pine and selling it on the market as Accoya® wood. The biological durability of Accoya® wood is DC 1, the highest class according to EN 350: 2016 with WPG of 20% (Larsson-Brelid et al. 2000, Mohebby 2003, Mohebby and Militz 2010, Rowell 2016). In a 10-year-long ground stake test in Greece (southern Mediterranean zone), Accoya® wood exhibited very good performance with no visual signs of decay (Mantanis et al. 2020).

Hornbeam (*Carpinus betulus* L.) can be found all over Europe, except in the Mediterranean. It is a diffuse-porous wood species; it does not form coloured heartwood and has low natural durability (DC 5 according to EN 350: 2016). Hence, it is not recommended for outdoor use.

By improving its properties with acetylation, hornbeam could be used to expand the range of durable species for outdoor applications in Hungary such as oak, black locust, Scots pine, and larch (Molnár and Bariska 2002). Only a few research papers have focused on the acetylation of hornbeam to increase its durability.
In a previous laboratory test (Fodor et al. 2017), hornbeam and acetylated hornbeam were exposed to three different fungal cultures according to EN 113: 1996. Hornbeam had DC 4-5 against white and brown rot fungi, while acetylated hornbeam's weight loss was below 1%, which makes it DC 1 against all three fungi according to EN 350: 2016. Another research study tested the durability of hornbeam acetylated with acetic acid and liquid formalin (Bari et al. 2019). The study found that the most effective treatment to achieve DC 2–3 was 10% liquid formalin and 5% acetic acid against white and brown rot fungi. In spite of this, microscopic studies revealed a scarce appearance of hyphae, so fungi were able to colonize in lumina of acetylated hornbeam (Rousek et al. 2022).
This research aimed to see if acetylated hornbeam could be utilized as an outdoor product in real-field conditions, with many different microorganisms that can attack the wood separately or cooperatively. The test was conducted for 6 years according to EN 252, and it also considered soil and weather characteristics of the field, as well. The difference in the rate of degradation was determined visually, by microscopy, and by calculating density and mass loss.

EXPERIMENTAL METHODS

Sample preparation

The long-term field test was performed according to EN 252:2015 with some slight modifications: the sample dimensions were changed from 25 × 50 × 500 mm to 20 × 50 × 300 mm (thickness × width × length) because the dimensions of raw material were limited. There were 12 stakes of each type: untreated hornbeam and industrially acetylated hornbeam, supplemented with beech and Scots pine sapwood according to standard. Acetylation was carried out at Accsys Technologies (Arnhem, the Netherlands) on 28 × 160 × 2500 mm boards (Fodor et al. 2017). The stakes were cut from these boards, having WPG levels ranging from 13.55 to 16.15%. The average WPG was 15.10 ± 1.03%. The beech and pine stakes indicated the intensity of the decaying mechanism of the soil. Table 1 shows the important characteristics and parameters of the stakes. The stakes were conditioned at 20 ± 2 °C and 65 ± 5% relative humidity before measuring their parameters and weight.

Table 1: Information about the stakes

Average	Acetylated hornbeam	Hornbeam	Beech	Scots pine sapwood
No. of annual rings on 10 mm surface	10	8	8	9
Air-dry density [kg/m3]	794 ± 49	745 ± 45	719 ± 7	525 ± 19
Mean density [kg/m3]	804 ± 50	822 ± 55	799 ± 10	614 ± 23
Moisture content [%]	3.35 ± 0.06	14.16 ± 0.96	13.50 ± 0.16	13.34 ± 0.50

The stakes were buried in the outdoor exposure testing field at the University of Sopron (47°40′41.4″ N 16°34′32.6″ E) in April 2016. The stakes were buried half of their length, one by one from each type. The distance between stakes was 30 cm. The vegetation on the field was cut regularly, and no chemicals or herbicides were used during the test. The presence of wood-decaying fungi and insects was also observed during the evaluation.

Soil of Testing Field

Five samples were taken from the soil of the testing field in order to examine the soil properties that influence the intensity of degradation. The samples were taken from four corners and the middle of the field. Skeletal grain content was determined by their dry weight relative to the dry weight of the soil. The measurement of soil pH was performed electrometrically at a soil/liquid ratio of 1/2.5 according to MSZ-08-0206-2: 1978. The calcium carbonate ($CaCO_3$) content of the soil was determined with a Scheibler calcimeter according to MSZ-08-0206-2: 1978. Humus or organic matter content was measured by the wet incineration process using potassium dichromate for the oxidation of organic matter. Particle content or fine-earth fraction was determined by the pipetting method according to MSZ-08-0205: 1978.

Weather and Climate

The weather parameters of the testing field were received from the Department of Ecology and Bioclimatology of the University of Sopron. These included average and maximum monthly temperature, monthly precipitation, number of days with precipitation above 0.25 mm, sunshine duration per month, solar irradiance per month, and monthly relative humidity. The Scheffer climate index (SCI) was also calculated, which Scheffer (1971) proposed to estimate decay hazard by geographic location within the conterminous United States for wood exposed above ground to exterior conditions. The index is calculated from local weather data using the mean monthly temperature and mean number of days with at least 0.25 mm of precipitation over the exposure period. An index of less than 35 represents the least favourable conditions for decay; 30 to 65, intermediately favourable conditions; and greater than 65, conditions most conducive to decay.

Rate of Degradation

Every six months, the level of degradation of each stake was determined from 0 to 4 according to EN 252:2015. Photos were taken before, during, and after exposure to observe the changes perceivable to the eye. After the failure of a stake, the broken pieces were dried at 103 °C in a drying kiln until a constant mass was reached, and the dry weight and parameters were determined to calculate the dry mass loss and dry density loss caused by degradation.

Microscopic Evaluation

Cubes with 10 × 10 × 10 mm dimensions were cut from the lower part of the samples and dried at 103 °C in a drying kiln to constant weight. Then, they were placed into a desiccator. As the cubes were cut with a circular saw, which resulted in rough surfaces, the surfaces were smoothed with a razor blade/scalpel before examination with a Hitachi S-3400N PC-Based Variable Pressure Scanning Electron Microscope (Hitachi, Tokyo, Japan) and its software. Cross and longitudinal sections were examined as well. Microscopic analysis was performed at a 60 Pa vacuum and a 10 kV accelerating voltage using a BSE detector. The working distance was 10 mm. The surfaces were not coated with a sputter coater before imaging.

Fourier Transform Infrared Spectroscopy (FTIR)

Acetylated hornbeam stake number 7 showed local signs of decay (see Results and discussion), which was examined with Fourier transform infrared spectroscopy. The measurement was performed using a Specac Golden Gate ATR (attenuated total reflection) with zinc selenide (ZnSe) lenses. Sound (control) and decayed parts of the stake were ground separately into smaller pieces using mortar and pestle to improve the contact area of the sample with the diamond on the ATR FT-IR. The FT-IR spectra were measured in transmittance mode (%T). A three-point baseline correction was applied to 850, 1180 and 1800 cm^{-1} to maximum transmittance (100%). The peak intensity was determined at peaks 1740 cm^{-1} and 1230 cm^{-1}. Then, it was translated to absorbance mode (%A), using the following formula: %A = 2–log (%T). Normalization was performed at peak 1030 cm^{-1} having 1% absorbance. Then, the peak height ratios (PHR) were calculated by dividing the absorbance at 1740 cm^{-1} and 1030 cm^{-1} ($\%A_{1740}/\%A_{1030}$), and the absorbance at 1230 cm^{-1} and 1030 cm^{-1} ($\%A_{1230}/\%A_{1030}$). Both measurements for the sound part and decayed part were performed in triplicates.

RESULTS AND DISCUSSION

Soil of Testing Field

The skeletal grain content ranged from 4 to 11%, having an average of 7 ± 3%. This does not significantly influence the water and nutrient holding capacity of the soil.

In the case of pH_{H2O} or current acidity, there was no significant difference between the samples, as the values ranged from 7.1 to 7.3. This is neutral (< 7.2) and slightly alkalic (> 7.2). As expected, pH_{KCl} was lower than pH_{H2O}, and it ranged from 6.7 to 6.9. There was no significant difference between pH_{KCl} and pH_{H2O}, which means there was no latent (hidden) acidity rate. The pH of this soil is sufficient for most plant species, as most nutrients are best absorbed by plants and are most mobile in this pH range.

The calcium carbonate ($CaCO_3$) content ranged from 2 to 3%, which corresponded to the expected values based on soil pH. This amount of $CaCO_3$ is favorable because it improves the soil structure. It is also advantageous in that there was no expected calcium deficiency.

The particle content (≤ 2 mm) is made up of 19% clay, 18% silt, 43% fine sand, and 10% coarse sand. Based on the tests, the amount of sludge (clay and silt fraction) ranged from 31 to 43%, with an average of 37%, which indicates a sandy loam type. This type has favourable water management properties because it allows water to enter well, retains it well, and makes it available for plants.
Humus or organic matter content was between 2.7 and 4.9%, having an average of 4-0 ± 0.8%, which is classified as a good/medium supply. This result also corresponded to the other soil properties.
According to these results, the soil in which the wooden specimens were tested was rich in nutrients and had good aeration and drainage properties. These characteristics were favourable for not just plant growth but also fungal growth, such as soft, white and brown rot fungi.
Basidiomycetes live in conditions with high oxygen content (soil with good aeration), moist wood with moisture content between 40 and 80%, and their optimal temperature range is from 24 °C to 32 °C. White rot fungi require higher moisture content and higher pH than brown rot fungi. They can decay wood during a short period when the temperature and the moisture are at optimal levels, e.g., summer, end of spring, and early autumn seasons. Soft rot fungi activate in a wide range of moisture contents, from relatively dry wood to saturated conditions, and a wide range of temperatures from 0 °C to 60 °C. They are active at a pH close to neutral and have better adaptability properties than other fungi during the whole year when soil moisture and temperature change periodically (Raberg et al. 2005, Mohebby and Militz 2010).

Weather and Climate

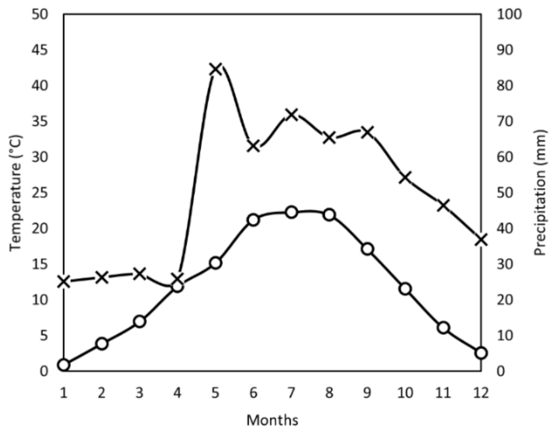

Figure 1: Ombrothermic diagram, which summarizes trends in temperature (O) and precipitation (X) of 6** years* ***of exposure (2016–2022). The wet period is typical for the whole year; there is no dry period

According to its solar-climatic classification, Hungary is situated about halfway between the Equator and the North Pole, in the temperate climatic zone. Hungary has a continental climate, with hot summers with low overall humidity levels but frequent showers and frigid to cold snowy winters. According to Péczely (1988), Sopron is in the moderately cool–moderately dry climatic region.
The area of the study site has a warm and wet summer season (May–September) with mean temperatures between 13–23 °C, with a maximum of 37 °C, and monthly average precipitation between 63–85 mm. It has a drier winter season (October–April) with 25–54 mm of average precipitation per month, mean temperatures between −4–16 °C, with a maximum of 28 °C. The average annual rainfall during the test period was approximately 594 mm; the average annual temperature was 12 °C; the maximum temperature was 37.2 °C. The relative humidity ranged from 45 to 91%. According to the ombrothermic diagram (Fig. 1), there was no dry season during the year, which would be the area below the temperature line and above the precipitation line. On the other hand, the wet season—the area below the precipitation line and above the temperature line—was typical for the whole year. The wet and warm periods at the exposure site were quite long, which enabled fungal growth. For *Basidiomycetes*, the summer period was favourable, while for soft rot, the conditions for growth were good throughout the whole year when temperatures did not drop below zero. The moisture content of the soil and that of the wood specimens are related to each other, and the change in their moisture content is related to the oxygen level of the soil. The oxygen level and aeration properties of the soil enable the growth of microorganisms in the wood. The Scheffer climate index of this site is 46.1, which indicates intermediately favourable conditions for decay.

Rate of Degradation

Mold started growing and cracks appeared on untreated stakes already after 1 month. There was no sign of decay on the acetylated hornbeam after 1 year. Wasp stripping was observed on untreated hornbeam and beech after 8 months. Moss appeared on Scots pine stakes after 10 months. Signs of fungal decay were visible on untreated stakes already after 5 months. After 1 year, three untreated hornbeam and one untreated beech stake broke during evaluation. Signs of soft rot, white rot, and insect damage were observed. Whitish discoloration, long-fibered splinter and fibered structure indicated white rot attack. Mushrooms of *Coprinellus micaceus* were identified on untreated hornbeam stakes. Soft rot decay attacks hardwoods more than softwoods because the lignin is more methoxylated in hardwoods (Raberg et al. 2005, Mohebby and Militz 2010).

After 15 months, mushrooms of *Bjerkandera adusta* and *Coprinellus micaceus* formed on beech stakes. Moss was found later on acetylated hornbeam stakes after 17 months. One of the acetylated hornbeam stakes showed signs of brown rot decay after 18 months (Fig. 2). The rate of white rot and soft rot decay, as well as insect damage in untreated stakes, worsened after another year.

After 2 years of exposure, three hornbeam and seven beech stakes failed the test. Scots pine stakes were heavily degraded by soft rot and brown rot. All untreated hornbeam stakes failed after 3.5 years, while beech stakes failed already after 2.5 years. Scots pine stakes started to fail after 3.5 years, and all of them broke after 6 years. Small cubic breaks, softened surface, dark discoloration, insect damage, and moss were observed on them. The soil was probably less optimal for the growth of brown rot fungi, which can explain the slower decaying mechanism compared to soft rot and white rot.

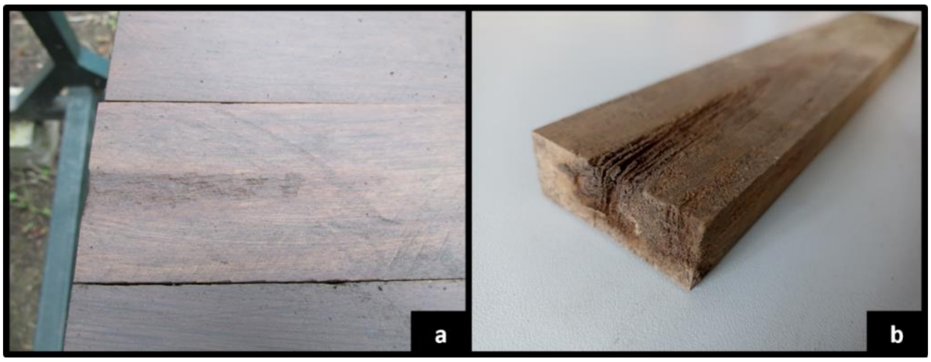

Figure 2: Brown rot in acetylated hornbeam stake Number 7 after 1.5 years (a) and 5.5 years (b)

After 5.5 years, acetylated hornbeam stake Number 7 was taken out from the test to examine its properties as the only locally decayed stake. Brown rot was observed on acetylated wood, as it can attack it even at this WPG (15%) or higher due to its non-enzymatic system (Mohebby 2003, Rowell 2020).

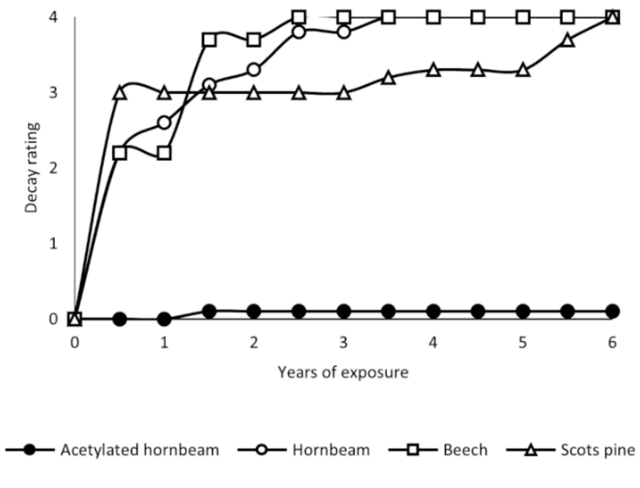

Figure 3: Average rating of in-ground stakes during exposure period (2016–2022). Decay rating or durability class is given according to EN 252 (2015): 0—sound, 1—slight attack, 2—moderate attack, 3—severe attack, 4—failure

Fig. 3 summarizes the rating and lifetime of each stake. The average lifetime of untreated hornbeam, beech and Scots pine was 2.0 ± 1.0, 1.8 ± 0.6, and 5.1 ± 1.0 years, respectively., and all of the stakes broke. Acetylated hornbeam stake number 7 had mild local brown rot decay, while the others showed no signs.
On average, one-third of mass (28-39%) and one-third of density (23-29%) were lost during the exposure in the case of untreated stakes. The local brown rot-decayed acetylated hornbeam sample lost 6% of its mass and density after 5.5 years of field test. Depth of degradation was calculated by halving the thickness loss of samples, which was 0.02, 0.18 ± 0.34, 0.34 ± 0.11, and 1.98 ± 0.82 mm for acetylated hornbeam, beech, hornbeam, and Scots pine sapwood, respectively. In Scots pine, there was a great difference between the degradation of earlywood and latewood because earlywood was more susceptible to fungal attack. There were no correlations found between density, annual ring density, and lifetime.

Microscopic Evaluation

The results of acetylated hornbeam show that some parts were not damaged or only the presence of hyphae was observed without cell wall damage. This corresponds to other reports in the literature (Ringman et al. 2019, Rowell 2020, Rousek et al. 2022). Hyphae can colonize cell lumina and ray cells of acetylated wood because they penetrate the wood across open ways like vessel lumina and rays, then into fiber cell lumina through inter-fiber pits and cross-fields between rays and fibers (Mohebby 2003, Mohebby and Militz 2010).
In the case of decayed parts in acetylated hornbeam, typical signs of soft and brown rot decay have been found, such as hyphae and cavity formation in cell walls, amorphous cell walls, erosion, and the thinning of cell walls with intact middle lamella (Fig. 4) (Raberg et al. 2005, Schwarze 2007).

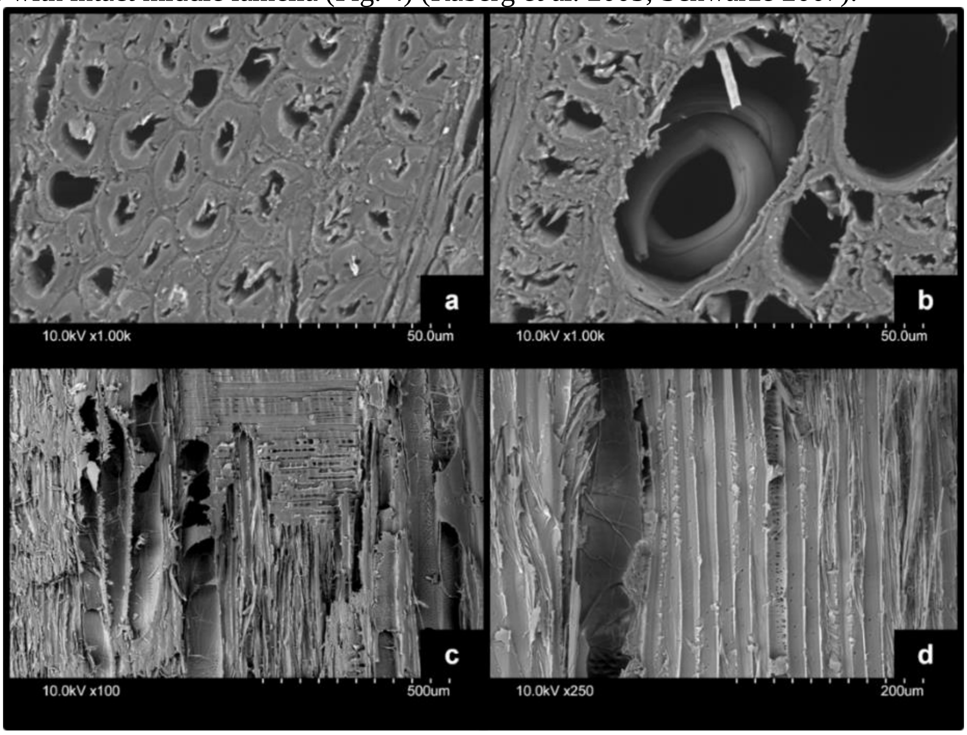

Figure 4: SEM pictures of cross section (above) and longitudinal section (below) of partly decayed acetylated hornbeam stake: (a) non-decayed parts with thick cell walls, (b) worm in cell lumen, (c) disappeared bordered pits, fibrous tissue, (d) hyphae in lumen, gradual thinning of cell walls, typical signs of soft rot decay

Fourier Transform Infrared Spectroscopy

The wood that originated from the "initial decay" area was relatively brittle, which made working with the mortar and pestle relatively easy. The piece of wood that originated from the intact part of the board was much less brittle and required more force than could be applied through the mortar and pestle.
Fig. 5 displays typical spectrums for wood from the two different parts. The dotted line corresponds to the spectrum of acetylated hornbeam wood part with initial decay, while the solid line represents the spectrum

of acetylated hornbeam wood that is considered normal in appearance. The spectra have been shifted with respect to each other for better visualization.

The largest peak with maximum intensity and absorbance was caused by C-O vibration in hemicellulose (1034 cm^{-1} for decayed, and 1032 cm^{-1} for sound part) (Fodor et al. 2018). This peak was considered constant (absorbance equals 1) in order to compare the 1730 cm^{-1} and 1230 cm^{-1} peaks between both spectra. Thus, the PHR between examined peak and 1030 cm^{-1} equaled the absorbance of the examined peak.

The intensity at peak (1) was relatively smaller for the decayed part (1738 cm^{-1}, %T 64.41, %A 0.191) compared to the non-decayed part (1734 cm^{-1}, %T 49.45, %A 0.306). This peak was caused by unconjugated C=O (carbonyl) bond stretching in acetyl in hemicelluloses (Takahashi et al. 1989, Fodor et al. 2018).

There was also a lower absorption at peak (2), which was caused by C-O stretching in the acetyl groups in hemicellulose xylan and mannosan (Fodor et al. 2018). The amount of acetyl-groups present in the decayed part of the wood (1228 cm^{-1}, %T 55.25, %A 0.258) was lower than in the rest of the wood (1224 cm^{-1}, %T 43.62, %A 0.360).

This means that the "affected part" had a much lower acetyl content. It was likely a wet pocket or a place with lower acetylation. This stake had the lowest annual ring density (7 per 2 cm) and the lowest WPG (13.55%) among other acetylated boards from which the stakes were taken.

In a research study concerning acetylated Radiata pine exposed to brown rot fungi (Beck et al. 2018, Thygesen et al. 2021), fungal deterioration was enabled by a de-acetylation mechanism during an initial lag phase. It was concluded that the bonds between chemical groups and biopolymers can be attacked and broken by fungi in optimal conditions for decay. This could also explain partially the lower acetyl content of the acetylated hornbeam stake, although it was of a different wood species. This also means that the acetyl content may not have been as low initially, as it was measured by FTIR.

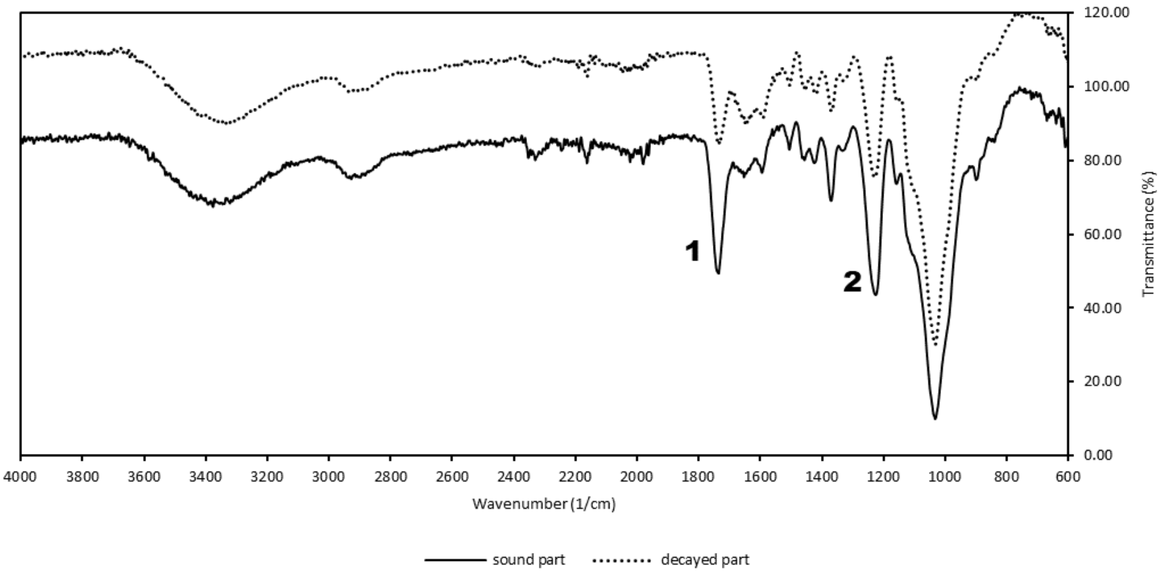

Figure 5: FTIR spectra of acetylated hornbeam wood powder with initial decay (dotted line) and no visible decay (solid line)

CONCLUSIONS

To date, long-term field tests have shown that acetylated hornbeam exhibits greater resistance against fungal decay, mold, insects, and moisture than untreated hornbeam, beech, and Scots pine sapwood do. These tests were evaluated every 6 months. Based on these findings, acetylated hornbeam shows promising results for further research, and for the production of exterior products such as furniture, fencing, decking, cladding, paneling, playground elements, etc. Instead of burning hornbeam wood right away, acetylation can widen its fields of use in order to lengthen its lifespan.

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