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PERMEABILITY OF THREE WOOD SPECIES DEGRADED BY *TRAMETES VERSICOLOR* L. LLOYD

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ABSTRACT

Permeability of wood is an important factor in many technical processes, foremost in treating wood with various chemicals. It depends on the number of pathways in wood and their transmissivity. The more open pores and pathways are, the deeper the chemicals can penetrate wood, and protection will last longer. The focus of this paper is permeability of spruce, beech and sessile oak heartwood intentionally degraded by *Trametes versicolor* L. Lloyd. Porosity of the degraded wood species increased by more than 10 %. A higher permeability was expected because of the increased porosity, but this could not be confirmed, as the values showed a great variability. Longitudinal permeability of the wood degraded with *Trametes versicolor* L. Lloyd was similar to permeability of healthy wood. As expected, the sessile oak heartwood was not permeable at all. To support the findings, all wood species were studied under a light microscope. There were visibly thinned cell walls in the degraded spruce wood, missing toruses in bordered pits, and bore holes in tangential walls of tracheids. Permeability in the radial direction of degraded spruce wood was lower compared to permeability in tangential direction. The degraded beech wood also showed signs of degradation – thinned and disrupted cell walls, sometimes even missing cell walls, pronounced pits and bore holes turning into ruptures. There were numerous tyloses visible in the oak heartwood, as well as the presence of hyphae.

Key words: permeability, light microscopy, spruce, beech, oak heartwood, degraded wood.

INTRODUCTION

Wood is a natural, porous material. Pores in wood ensure the transport of water and nutrients in living trees. This ability to transport masses through pores remains active even after the tree is logged. It is important in many manufacturing processes – drying, vacuum drying, impregnating wood with chemicals, gluing – to mention a few.

Pores in wood are of various sizes, positions (longitudinal, radial or tangential – orientation) and geometry. These properties vary in different wood species. Some wood species pores can be clogged by tyloses or other barriers, which greatly affects the ability to transport mass in wood.

Permeability is the ability of wood to transport mass through its porous structure. Permeability is known to be highly variable (SIAU 1995). Factors affecting permeability can be divided into two groups – inner (anatomical structure of wood) and outer (properties of the mass passing through wood and pressure gradient). The most frequent way to express permeability is Darcy's law.

Inner factors influencing permeability are related to structure of wood; the number and size of cells and elements (POŽGAJ *et al.* 1997). The research by BABIAK *et al.* (1983) showed that 96.3 % of the water transport in beech wood is provided through vessels with a radius bigger than 15 μm. Vessels with a radius smaller than 15 μm provide 3.7 % of the permeability; despite the count of these vessels in a unit of area was 41 % (BABIAK *et al.* 1983). KOVÁČIK (1993) presented similar results for beech wood – 95 % of axial permeability is provided through pores with a radius bigger than 18 μm. Pores with a radius smaller than 18 μm provided only 5 % of the axial permeability; despite pores with this ratio took 28.5% of the pores ratio in the unit of area.

Flow pathways in wood are in axial and lateral directions. In softwoods, axial flow takes place primarily in longitudinal tracheids by passing through the bordered pits that are implemented in their end-walls (CÔTÉ 1963, ERICKSON AND BALATINECZ 1964, COMSTOCK 1965, BAILEY AND PRESTON 1969, ISAACS *et al.* 1971). As far as hardwoods are concerned, the flow of fluids in the longitudinal direction is largely controlled by the size and number of vessels that are un-clogged by tyloses or other obstructions (WARDROP AND DAVIES 1961, CÔTÉ 1963, ISAACS *et al.* 1971). CÔTÉ (1963) mentions that wider lumened fibre tracheids and vasicentric tracheids are often heavily pitted permitting better communication with adjoining cells. Longitudinal parenchyma cells are also described as being more permeable than libriform fibres (WARDROP and DAVIES 1961, HANSMANN *et al.* 2002). As for lateral flow, in general, wood rays offer a significant path (WARDROP and DAVIES 1961, CÔTÉ 1963).

Regarding different pathways of fluids in wood in connection with specific anatomical features and the gross structural factors of wood, it is evident that movement of fluids through wood is easiest along the grain (CÔTÉ 1963). When radial permeability was compared to the tangential one, permeability was greater radially than tangentially (ISAACS *et al.* 1971, PALIN and PETTY 1981).

Heartwood formed in both soft- and hardwoods has a decreased permeability compared to sapwood (WARDROP and DAVIES 1961, CÔTÉ 1963, COMSTOCK 1965 and 1968, ISAACS *et al.* 1971). Low permeability of heartwood is also due to the presence of extractives. Extractives increase the contact angle between aqueous liquids and the cell walls and lead to decreased wettability compared to sapwood (BAILEY and PRESTON 1969, MANTANIS and YOUNG 1997).

Permeability of wood was studied many times before and with various treatments of wood. Experiments conducted on sound beech wood were published in works by HUDEC and DANIHELOVÁ (1992), BABIAK and KÚDELA (1993), KURJATKO *et al.* (1998), POŽGAJ *et al.* (1997), KÚDELA (1999) and BABIAK *et al.* (1995, 2001). Permeability on reaction wood was reported in works by HUDEC (1993) and ČUNDERLÍK and HUDEC (2002). Experiments on permeability on wood treated with microwaves were published in works by LIN and LU (2004) and NASSWETTROVÁ *et al.* (2014). Permeability was also studied on wood treated with wood staining fungi in works by REINPRECHT and PÁNEK (2009), PÁNEK *et al.* (2013), DANIHELOVÁ *et al.* (2018). Treatment of wood with wood decaying fungi and its impact on permeability was presented in works by KURJATKO *et al.* (2002), SOLÁR *et al.* (2003 and 2006), EMAMINASAB *et al.* (2015 and 2016).

Degradation processes in wood caused by wood decaying fungi are driven by different mechanisms. *Trametes versicolor* L. Lloyd is known to thin out cell walls, cause ruptures in cell walls through creating bore holes and eventually make even cells disappear. *Trametes versicolor* primarily attacks lignin, followed by cellulose and hemicelluloses (BARI *et al.* 2015, 2018). Hence, wood becomes more porous, and cell lumina become wider, which could increase the permeability of wood.

A partial removal of lignin is believed to increase porosity and open new pathways for the transport of solutions deep inside the material, which should facilitate a subsequent

impregnation step for further functionalization (VITAS 2019, DONALDSON *et al*. 2015, JAKES *et al*. 2015).

Permeability of three wood species (*Picea abies* L., *Fagus sylvatica* L., *Quercus petraea* Matt. Liebl.) intentionally degraded by white rot fungus *Trametes versicolor* L. Lloyd was the main focus of this work. Porosity of the degraded wood species was higher compared to porosity of undegraded wood species (SLOVÁČKOVÁ 2021b). Because of a higher porosity, permeability of the degraded wood is expected to increase in comparison to permeability of undegraded wood species. The degraded sessile oak heartwood was expected to be permeable. To support the findings, all degraded wood species were studied under a light microscope.

The results and experiments presented in this paper are part of a larger, ongoing experiment on thermal properties of degraded wood and proposing a new bio-based thermal insulation material based on the degraded wood. Thermal properties of the decayed wood species were described in another article (SLOVÁČKOVÁ 2021a). The results on permeability of wood degraded by a wood decaying fungus are expected to contribute to the topic of biological treatment of wood.

MATERIAL AND METHODS

The lumber used to prepare the beech (*Fagus* sylvatica, L.) and the sessile oak heartwood (*Quercus petraea,* Matt. Liebl.) samples was stored at the Department of Wood Science and Technology. Spruce wood (*Picea abies,* L.) lumber was obtained from a wooden windows manufacture located near the University. Permeability was measured in all anatomical directions. There were 16 samples per each anatomical direction. The smallest size of 8 mm was cut according to the anatomical direction, so the sizes of the samples were (dimensions in L × R × T direction) 8 × 50 × 50 mm^3 for permeability measurement in longitudinal direction; 50 × 8 × 50 mm^3 for permeability measurement in radial direction and 50 × 50 × 8 mm^3 for permeability measurement in tangential direction. As it was mentioned in the introduction, thermal properties were measured on these samples as well, so the dimensions were limited by calculations for the thermal properties measurement.

The samples were oven-dried, then weighed and measured. They were then intentionally degraded with *Trametes versicolor* L. Lloyd and the degradation was prepared according to STN EN 113 and it was performed in the laboratory of Department of Wood Technology at the Technical University at Zvolen. The degradation duration was 6 months. Degradation duration according to the STN EN 113 is shorter than in this experiment. The decision for a 6 month long degradation duration was based on results from a previous set of experiments. After the exposure time passed, the samples were taken out of Kolle flasks, cleaned off visible mycelium and they were submerged into containers with distilled water. Submerging the samples caused all cavities to fill up with distilled water so as to remove any air left in the samples. Since there was not any air left, the fungus was not able to survive in these conditions and it stopped its activity. According to RYPÁČEK (1957) wood decaying fungi need at least 5–20 % air in wood in order to be able to survive.

One container per wood species was used. The containers with samples were stored in a dark room with constant temperature to prevent any photochemical reactions. Distilled water was changed periodically. The samples were kept in the water until they reached maximum moisture content. Reaching the maximum moisture content was checked by double weighing the samples in water.

A follow up permeability measurement was performed on non-degraded spruce and beech samples in the longitudinal direction. Non-degraded sessile oak heartwood was not permeable at all.

Measurement and calculation of permeability

Measurement of permeability was performed on a patented apparatus by REGINÁČ *et al*. (1977). Measurement was done on wet samples after they reached maximum moisture content. Values for permeability were obtained for all anatomical directions. Thickness of each sample was measured before every procedure with a Mitutoyo Absolute Digital Digimatic calliper. The samples were fastened into the permeability measurement apparatus, a pressure was set and the valve on the water tank was opened. Water permeating through the samples was collected in a measuring cylinder placed under the apparatus on a KERN KB 1000-2 scale with 0.01g accuracy. The scale was connected to a computer and the increasing mass of the collected water was transferred automatically in desired time intervals into an Excel working sheet. Duration of each run was 2 or 5 minutes, according to the linearity of the flow and anatomical direction.

Permeability was calculated according to Darcy's law:

$$k = C \cdot \frac{\eta_{H2O}}{S} \cdot \frac{l}{\Delta p} \qquad (1)$$

where C is the slope of volume of the permeated water and time, η_{H2O} is the dynamic viscosity of water at a certain temperature, S is the surface of the nozzle of the apparatus (diameter of the nozzle is 1.00 cm), l is the samples' thickness and Δp is the difference between set pressure for water in the apparatus and atmospheric pressure.

Light microscopy

Thin microtome slices were prepared from the degraded samples, one per each species per anatomical direction. Small specimen with dimensions of 3 × 3 × 7 mm were cut from a randomly selected sample. These had to be embedded in epoxy resin because it was impossible to cut thin slices due to samples brittleness. Several slices were cut from each wood species. They were cut with a sledge microtome (Reichert, Wien, Austria). Toluidine Blue stain in liquid state was applied to the samples and rinsed out with distilled water. Microslices were left to dry and then mounted permanently on a microscope slide with Euparal (BioQuip Products Inc., Rancho Dominguez, United States). Microscope slides were covered with cover slides and weighed down for one week. The permanent mounts were examined under a transilluminating microscope at magnifications of 200× and 400×. A Canon EOS 600D camera was attached to the microscope for taking pictures.

RESULTS AND DISCUSSION

Permeability is known to be a very variable property (SIAU 1995). Degraded oak heartwood was expected to be permeable, but the specimen did not let any water pass through, not even under very high pressure of Δp = 55 kPa. Analysis of the sessile oak heartwood microsections showed numerous tyloses in tracheas (Fig. 3B, C) which were the main cause for the sessile oak heartwood being impermeable.

As for the results of spruce and beech wood permeability, the results showed a great variation. The medians, first and third quartiles for all anatomical directions are displayed in Table 1. Average thicknesses of the samples were: 8.2 mm for non-degraded spruce wood in longitudinal direction, 8.1 mm for non-degraded beech wood in longitudinal direction. The thicknesses for degraded spruce wood samples were 7.6 mm in longitudinal direction and 8.0 mm in both radial and tangential directions. The degraded beech wood samples

reached a thickness of 8.0 mm in longitudinal direction, 9.1 mm and 8.4 mm for radial and tangential directions respectively. The pressures varied according to wood species and anatomical direction. In general, the pressure was lower for longitudinal direction than for transversal directions.

Tab. 1 Medians of permeability coefficients for degraded spruce and beech wood for all anatomical directions and reference permeability values in longitudinal direction. N is the number of runs. First and third quartiles are under the median value (Q1 – Q3).

	Reference permeability values in longitudinal direction, healthy wood	Longitudinal direction, decayed wood	Radial direction, decayed wood	Tangential direction, decayed wood
	k [m^2] (n = 15; 15)	k [m^2] (n = 13; 15)	k [m^2] (n = 13; 17)	k [m^2] (n = 11; 15)
Spruce	$1.73 \cdot 10^{-14}$ $1.05 \cdot 10^{-15}$–$3.33 \cdot 10^{-14}$	$1.31 \cdot 10^{-14}$ $8.27 \cdot 10^{-15}$–$2.87 \cdot 10^{-14}$	$2.50 \cdot 10^{-15}$ $1.49 \cdot 10^{-15}$–$2.91 \cdot 10^{-15}$	$3.34 \cdot 10^{-15}$ $1.67 \cdot 10^{-15}$– $6.36 \cdot 10^{-15}$
Beech	$5.28 \cdot 10^{-12}$ $2.55 \cdot 10^{-12}$–$8.25 \cdot 10^{-12}$	$5.90 \cdot 10^{-13}$ $1.48 \cdot 10^{-13}$–$3.96 \cdot 10^{-12}$	$7.61 \cdot 10^{-15}$ $4.87 \cdot 10^{-15}$–$1.05 \cdot 10^{-14}$	$5.93 \cdot 10^{-15}$ $4.20 \cdot 10^{-15}$–$7.45 \cdot 10^{-15}$

The reference longitudinal permeability values of non-decayed wood and longitudinal permeability values of the decayed wood are similar. In fact, the median values of longitudinal permeability of the decayed wood are a little lower than longitudinal permeability values of non-decayed wood.

Permeability values of undegraded beech wood were reported by many researchers: BABIAK and KÚDELA (1993) measured a longitudinal permeability of $4.90 \cdot 10^{-12}$ m^2, POŽGAJ *et al*. (1997) stated a longitudinal permeability of $8.51 \cdot 10^{-12}$ m^2, HUDEC and DANIHELOVÁ (1992) stated a permeability of $7.56 \cdot 10^{-12}$ m^2 and according to KÚDELA (1999), longitudinal permeability of undegraded beech wood was $10.00 \cdot 10^{-12}$ m^2. As stated by BABIAK (1990) the question "whether all variability is caused only by the change of wood characteristics or if at least a part of the variability may be attributed to different physical conditions during the experiment" arises.

Permeability in longitudinal direction is the highest compared to permeability values in transversal directions in both degraded wood species. The degraded spruce wood showed a higher permeability in tangential direction than in radial direction. The degraded beech wood showed the opposite, permeability in radial direction is higher than in tangential direction. These findings are presented in Tab. 2, where ratios of permeabilities were calculated. Degraded spruce wood showed a lower permeability than degraded beech wood in all anatomical directions.

According to various gas permeability data collected by COMSTOCK (1970), the ratios of permeabilities in softwoods are as follows: longitudinal to tangential permeability ratio varies from 520 to 81600, longitudinal to radial permeability ratio varies from 15 to 547000 and the tangential to radial permeability ratio varies from 0.019 to 31.3. Ratios of longitudinal to transverse permeability as high as 10^6 have been observed in some softwood species (COMSTOCK 1970). According to the research by LIHRA *et al.* (2000), the longitudinal permeability of balsam fir was about 2000 and 9000 times higher than the tangential and radial permeability respectively. COMSTOCK (1967) has shown that permeability values measured with gases and liquids are closely related, the relationships can be considered generally applicable. The L/R, L/T and T/R permeabilities ratios of degraded spruce and beech wood are presented in Table 2.

Tab. 2 Permeabilities ratios of degraded spruce and beech wood.

	Longitudinal/Radial	Longitudinal/Tangential	Tangential/Radial
Spruce	5.24	3.92	1.34
Beech	77.53	99.49	0.78

The L/R and L/T permeability ratios in degraded spruce wood are far below the range observed by COMSTOCK (1970) and LIHRA *et al.* (2000), the T/R permeability ratio is within the range observed by COMSTOCK (1970). Degraded spruce wood permeability in transversal directions increased. The L/R and L/T permeability ratios in degraded beech wood are higher than in degraded spruce wood. In comparison to the degraded spruce wood, the degraded beech wood has a higher permeability in longitudinal direction than in transversal directions.

Wood staining fungi like *Sydowia polyspora* were proven to increase permeability of wood as proven by DANIHELOVÁ *et al*. (2018). The coefficient of axial permeability of bio-treated and subsequently dried spruce sapwood increased approximately 5-times (DANIHELOVÁ *et al*. 2018). However, wood staining fungi have different degrading mechanism than wood decaying fungi. Wood staining fungi are known to feed mostly on protoplasmic remnants in cell lumina and pectins in pits' membranes. They are not able to attack cellulose; hemicelluloses or lignin hence wood-staining fungi do not cause major damage to wood structure like wood decaying fungi (REINPRECHT 2016).

The research by EMAMINASAB *et al*. (2015) showed that white-rot fungus and also soft-rot fungus had a negative impact on permeabilities on both poplar normal wood and tension wood. It is suspected that the fungal hyphae in cell lumina are blocking the pathways for the mass flow. This contrasts with findings by GREEN and CLAUSEN (1999), who concluded that both white-rot and brown-rot fungi increase wood permeability in pine wood. Various species of white- and brown-rot fungi were tested in this experiment.

The research of permeability on beech wood degraded by two white-rot fungi *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* by SOLÁR *et al.* (2003) showed various effects of white-rot fungi degradation. Degradation of normal and tension wood specimens by *P. chrysosporium* increased their coefficients of axial permeability in air-dry and saturated state markedly. Degradation of normal and tension beech wood specimens with *C. subvermispora* reduced their axial permeability in both air-dry and saturated states unexpectedly.

The following figures show an analysis of all degraded wood species under the light microscope. All wood species showed clear signs of the degradation process and an advanced stage of cell walls decomposition.

Analysis of decayed wood structure showed visible changes caused by degradation. Bordered pits in degraded spruce wood had visibly damaged or missing toruses. Bore holes were visible on tangential walls of earlywood tracheids and cell walls were visibly thinned, sometimes even disrupted (Figures 1A, B). Hyphae were present in some slices.

Figure 1A shows numerous hyphae present in some cell lumina. The hyphae seem to have been an obstruction for the water flow. The fungus created bore holes in both radial and tangential walls of tracheids (Figures 1B and C). Missing toruses in bordered pits and ruptures in cell walls had a bigger impact on the tangential permeability. In the end result, permeability in tangential direction was higher than permeability in radial direction of the degraded spruce wood.

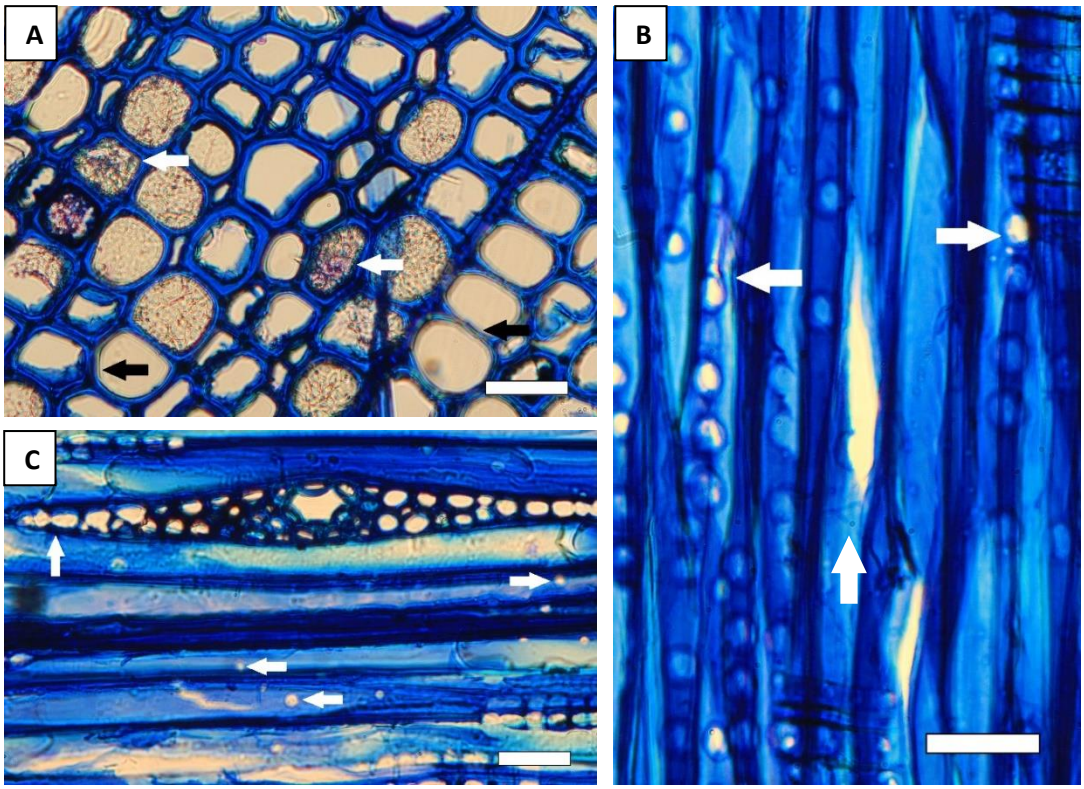

Fig. 1 Degraded spruce wood. White arrows in picture A, transversal cut, shows hyphae. Black arrows show visibly thinned cell walls. Arrows in picture B point at missing toruses in bordered pits which are also visibly degraded. The arrow pointing right shows also some bore holes near the bordered pit. Some hyphae are visible under the big rupture in the middle of the picture. Horizontal white arrow in picture C point at bore holes in tangential side of the cell walls. The vertical white arrow shows disrupted cell wall in ray parenchyma. The scale in all pictures is denoted with a white stripe in the lower right corner and it equals 50 µm.

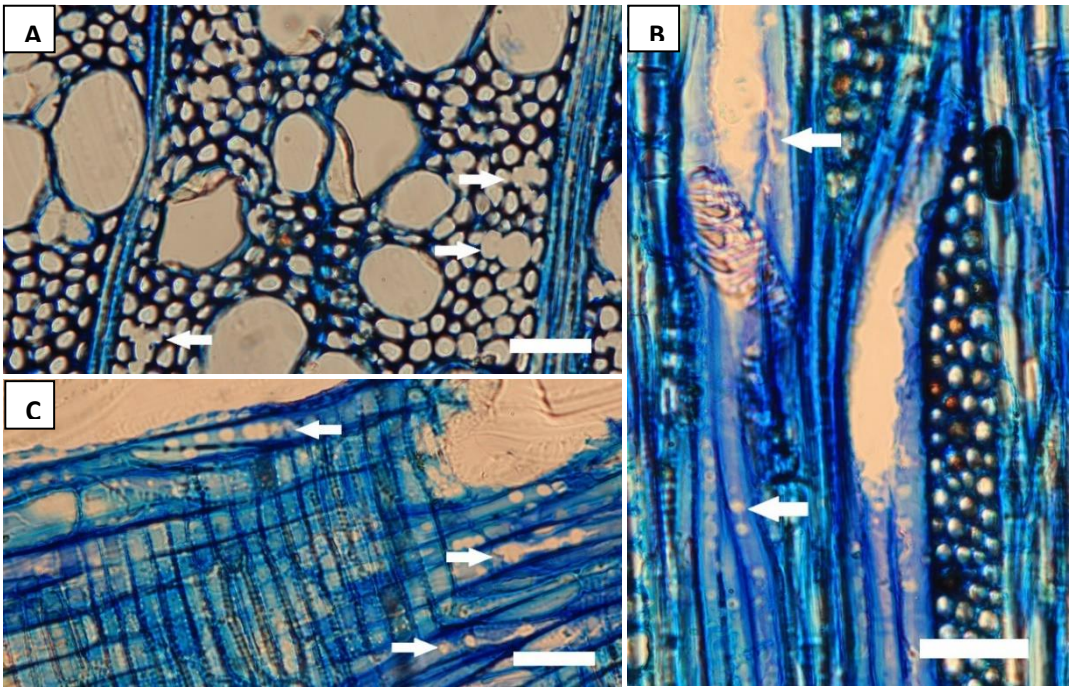

Fig. 2 Decayed beech wood, scale is marked by white stripes in lower right corner of each image, and it equals to 50 µm. Arrows in picture A show missing or severely disrupted cell walls. Pits in ray parenchyma are more pronounced. Arrows in picture B point at rupture and bore holes in cell walls. Ray parenchyma cells are mostly emptied due to activity of fungus. In picture C, arrows show bore holes turning into big ruptures in cell walls.

Similar signs of degradation were found in beech wood. Thinned, disrupted cell walls, pronounced pits and almost completely emptied ray parenchyma cells. Pits in ray parenchyma cells became visible because the fungus degraded most of the compounds stored in ray parenchyma cells.

Unlike in the degraded spruce wood, activity of the fungus did not have an impact on permeability in transverse directions of the degraded beech wood (the permeability in radial direction was higher than in tangential direction). Signs of fungal attack in radial and tangential cuts of the degraded beech wood are visible in Fig. 2C and 2B. They appear to be of a similar extent in both directions. Despite a better cell-to-cell connection through the severely damaged cell walls, vessels not clogged by tyloses, permeability of the degraded beech wood in longitudinal direction was lower compared to permeability of healthy beech wood.

The Fig. 2A – 2C show, that the degraded beech wood was intact even after a 6 months long degradation. As it was already mentioned in the introduction, according to the research by BABIAK *ET AL.* (1983) 96.3 % of the water transport in beech wood is provided in vessels with a radius bigger than 15 µm. Because the fungal activity in the degraded beech wood did not develop more pathways with such radius, the longitudinal permeability did not improve. The longitudinal permeability of the degraded beech wood could increase if the degradation duration was longer. Cell walls digestion extent could develop more pores with a radius bigger than 15 µm through a longer degradation duration.

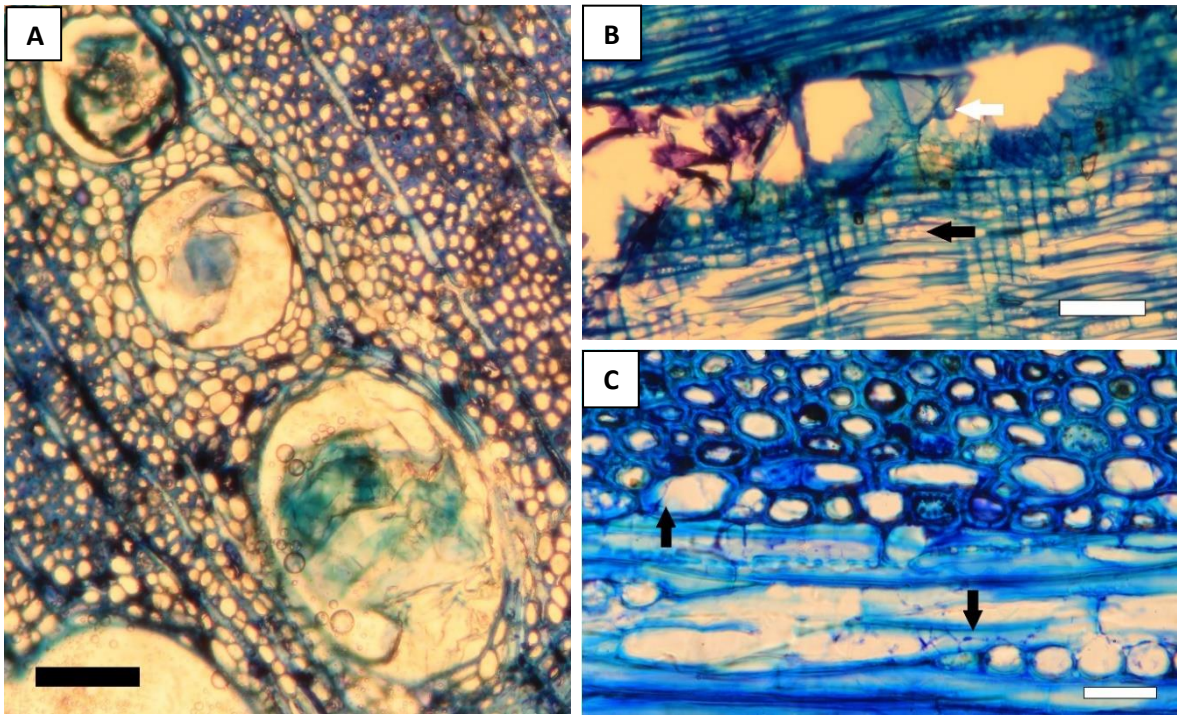

Fig. 3 Degraded oak heartwood. The scale equals 50µm in pictures A and B and 30 µm in picture C, as it is a bigger magnification. Numerous tyloses are visible in picture A. A big tylosis overgrown with hyphae (pointed out with white arrow) is shown in picture A. The black arrow points at bore holes. Picture C shows well-visible hyphae (marked with black arrows). Cell wall disruption is visible as well.

There were numerous tyloses in the degraded sessile oak heartwood which made permeability impossible. Numerous hyphae were observed in the sessile oak heartwood specimen. The hyphae spread also over tyloses as shown in (Fig. 3B). The degradation process in cell walls was not as visible in this sample due to its lower mass loss.

Even though the hyphae visibly attacked tyloses and cell walls, the degradation process did not have an effect on the permeability of sessile oak heartwood. If the hyphae would be able to digest tyloses, the permeability of sessile oak heartwood could increase.

CONCLUSION

The permeability of spruce, beech and sessile oak heartwood degraded with white rot fungus *Trametes versicolor* L. Lloyd was measured. The measurement was done in all anatomical directions.

A higher permeability was expected because of increase in porosity, but this was not confirmed. The permeability of degraded spruce and beech showed great variations. Unexpectedly, permeability in radial direction of the degraded spruce wood was lower compared to permeability in tangential direction. Better permeability in tangential direction was the result of the degradation process. A majority of toruses was digested, hence this pathway became more open.

The degraded beech wood did not have better permeability than healthy beech wood. The degradation process did not develop pathways big enough to significantly increase the longitudinal permeability of the degraded beech wood.

The degraded sessile oak heartwood was not permeable at all. The fungal activity did not digest tyloses which blocked the pathways in the heartwood.

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