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**Section Y** **VVVVVVVVVV**

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## ABSTRACT

The white-rot fungus *Physisporinus vitreus* preferentially degrades the pit membranes of bordered pits in tracheids and subsequently enhances wood permeability. Thus, *P. vitreus* can be used to improve the uptake of wood preservatives and environmentally-benign wood modification substances. This process can be used to enhance the use and sustainability of native conifer wood species by the wood industry.

Mathematical modelling in combination with laboratory experiments is a powerful and efficient investigation method that enables a deeper insight into complex interactions between biological systems and their environment. The objective of mathematical modelling is not to develop an extremely complex system of equations in an attempt to mimic reality. Rather, it is applied to reduce a complex (biological) system into a simpler (mathematical) system that can be analyzed in more detail and from which key properties can be identified, isolated and investigated. In addition, a verified mathematical model enables to optimize hyphal growth and impact of the *P. vitreus*. Enhanced uptake of wood preservatives and environmentally-benign wood modification substances of Norway spruce wood and optimized quality control processes would be of importance for the usability, durability and sustainability of wooden structures in civil engineering.

In this paper we present a mathematical model of hyphal growth and expansion of *P. vitreus* in heartwood of Norway spruce. The model enables to investigate the global penetration front of the fungus in wood as function of the control parameters as well as its shape and penetration depth. This model will serve to assist the choice of pellet concentration and reaction times that are required to induce a defined degree of wood permeability by the fungus.

**Keywords:** modelling, *Physisporinus vitreus*, wood decay, hyphal growth, permeability, bordered pit membranes, *Picea abies*.

## 1. INTRODUCTION

Hyphal growth and impact of fungi are inextricable linked with the underlying substrates and their extracellular digestion of organic matter. The interplay between the chemical composition, the concentration of the components, the fungal enzymes and the geometrical structure of a substratum determine the growth of fungi. Normally, the natural resources of fungi are physically and chemically complex and structured in space. Often, the natural nutrients sources are not continuously uniform but spatial discrete and heterogeneously distributed. The discrete form of the nutrient sources depend on the modelling scale as well as the porous structure of many natural substrates like soils or wood.

In the course of time fungi evolved different strategies to exploit the nutrients of complex substrates such as wood. Wood decay fungi are classified into three types: brown rot, white rot (i.e. selective delignification and simultaneous rot) and soft rot (Type 1 and Type 2). In the present work we study the growth of the white rot fungus *P. vitreus*. Generally, in the primary stage of growth this fungus selectively degrades lignin and hemicelluloses of the wood. In a secondary stage the fungus degrades cellulose in the cell walls (Schwarze & Engels 1999). Additionally, Schwarze & Landmesser (2000) reported that the fungus degrades pit membranes in wood of *Picea abies* (Norway spruce). Preferential degradation of pit membranes is pronounced in the primary stages of growth (Schwarze et al. 2008, Schwarze 2007). Therefore the fungus significantly increases the water uptake of wood whereby the breaking strength of the wood is not significantly reduced (Schwarze et al. 2006, Schwarze et al. 2009). The fungus alters the breaking strength during secondary stages of growth. A detailed review on wood modifications can be found in Lehringer et al. 2009.

Mathematical modelling in combination with laboratory experiments is a powerful and efficient method of investigation that provides a deeper insight into complex interactions between biological systems and their environment. Hyphal growth and expansion of *P. vitreus* was modelled by means of stochastic processes both in time and space (Hermann 1992). It is envisaged that by combining modelling with experimental data from light microscopy (LM), synchrotron radiation tomographic microscopy (TOMCAT) and confocal laser scanning microscopy (CLSM), more detailed quantitative and qualitative results can be obtained (Chris et al.). Irreversible growth has been investigated for a long time in the context of cancer growth, dendritic growth and gelation and penetration in porous media (Araujo et al. 2006, Horvath & Herrmann 1991, Chopard et al. 1991). Mathematical modelling allows identifying, isolating and investigating key properties of fungal growth. By focusing on these fundamental processes, we hope to improve our knowledge on how the complex system (fungus - wood) interacts under defined conditions.

## 2. MODEL

### 2.1 Substrate

During primary stages of growth the filamentous basidiomycete *P. vitreus* preferentially degrades pit membranes of the wood (Schwarze & Landmesser 2000). In earlywood the ratio of the diameter of a hypha and the mean distance between two neighbored pit membranes is approx.1:10; in latewood approx. 1:1000. Thus, the continuous progression of hyphae from pit membrane to pit membrane is the simplest description for its spatial growth. Cell walls and nutrients sources form the structure of the wood.

The *nutrient source* is the substrate of the fungus on which its enzymes act. The model assumes that all essential substances for fungal growth are concentrated in the pit membranes. Additionally, during growth relative humidity, temperature of the system and the wood moisture (about 30%) remain constant. The fungus degrades the pit membranes by extracellular digestion. The pit membranes are modelled as points with the attributes

$$\begin{aligned} & r_p^{(i)} \quad i = (1 \ldots N_p) \\ & f_p^{(i)} \in [0,1], \end{aligned} \tag{1}$$

where *rp* denotes the coordinates of an arbitrary nutrient point. *Np* is the number of nutrient point in the system and the variable *fp* describes the available amount of nutrients at the site *(i)*.

In contrast *cell walls* are the substratum on which the hyphae grow. During the primary growth phase extracellular enzymes secreted by hyphae do not interact with the cell walls. Hyphal degradation of cell walls commences during secondary stages. In the present model only the primary stage of hyphal growth is taken into account. Cell walls are additional boundary conditions within the system. Firstly, cell walls determine the distance between the pit membranes. Secondly, the cell walls limit the available pit membranes for hyphal growth due to the opacity of wood. Resulting from hyphal growth, *P. vitreus* degrades pit membranes and exploits the wood stepwise.

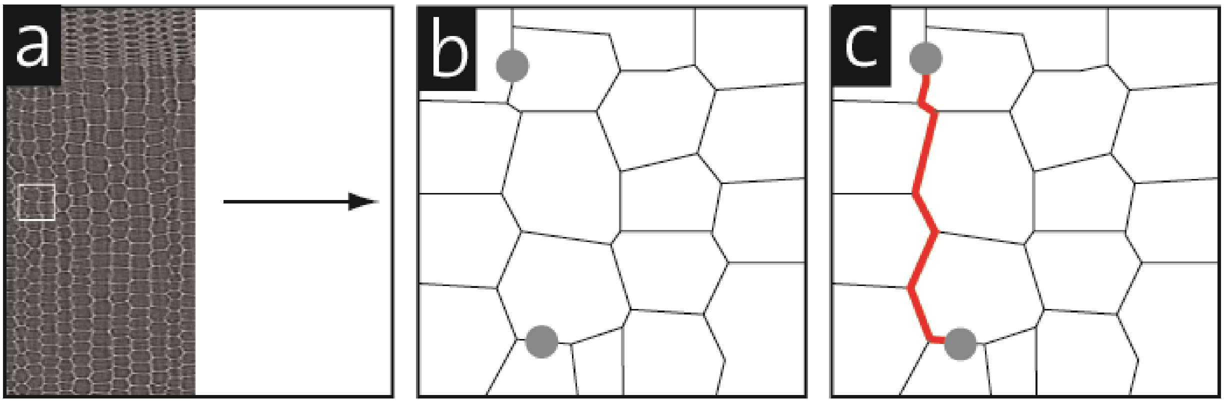

Figure 1: The modelling process using TOMCAT pictures: (a) Transverse section of Norway spruce wood. (b) The substratum is formed by skeletonising pictures. The position of the pit membranes is marked manually. (c) The distance between the pit membranes is the shortest course on the graph.

The model handles the wood structure as a graph consisting of vertices and edges. The position of the pit membranes and the cell walls are generated using TOMCAT, CLSM or by applying data from the literature. Figure 1 shows schematically the modelling process from a raw tomography image (1a) of the wood to the graph used for simulating hyphal growth of the fungus.

## 2.2 Mycelium

An aggregation of edges and nodes form the mycelium. The model defines two kinds of nodes: Links and hyphal tips. The edges connect the nodes and form filaments. These filaments and the hyphal tips are the framework forming the mycelium. The location of the *nodes* is restricted to the location of the pit membranes. Thus,

$$r_k^{(i)} \in r_p \qquad i = (1 \ldots N_k), \tag{2}$$

where $N_k$ denotes the number of nodes in the system. $R_k$ is a vector containing the coordinates of the node *(i)*. Degradation of nutrients is irreversible. The nodes are connected by *edges*, which is the shortest path on a graph formed by the cellular structure of wood (see Fig. 1). The shortest path between two arbitrary points on a graph could be calculated e.g. using the algorithm of Dijkstra (1959). There is no restriction due to crossing of edges. We defined the degree of a node as the number of its associated edges. A node with the degree of one is called *hyphal tip* and nodes with more than one edge are *links*.

The key processes hyphal growth, polarization of hyphal tips, uptake of nutrients and branching (lateral and apical) describe the dynamic growth of the mycelium. The continuous growth of every hyphal tip is modelled by a sequential growth algorithm, called fungal growth model (FGM): At each time step τ the mycelium is extended by maximum one edge. Thus, τ denotes an artificial time, which is related to the real (laboratory) time by

$$t = \frac{\tau}{N_t}. \tag{3}$$

Therefore, the algorithm of the FGM has to run *Nt* time to simulation the simultaneous growth of *Nt* active hyphal tips.

*Uptake* and *concentration* of nutrients are taking place at the nodes. The variable *fk* qualifies the concentration of nutrients at the node *(i)*. The function

$$f(\lambda, i) = a + g(\tau), \tag{4}$$

describes the uptake of nutrients and the degradation of the pit membranes at the node *(i)*. The constant *a* is a mass for the initial degradation of a pit membrane by a hyphal tip. The function *g(τ)* describes the continuous degradation of the pit membranes by enzymes and depends on the length of the connected edges to the node *(i)*.

Fungi have to grow to acquire nutrients. *Hyphal growth* is located at the tip for most filamentous fungi. The *polarization of growth* distinguishes filamentous fungi and is a key aspect of their morphogenesis (Sudbery & Court 2007). The variable *pt* describes the polarization of a hyphal tip. The higher the value of *pt* as active, the faster a hyphal tip grows. A hyphal tip is inactive if *pt* = 0. The probability of an active hyphal tip to growth in time τ is

$$P(\tau, X = i) = \frac{p_t^{(i)}}{\sum_{j=1}^{N_t} p_t^{(j)}} \qquad i = (1 \ldots N_t), \tag{5}$$

where *Nt* is the number of active hyphal tips of the mycelium. The polarization of a hyphal tip is directly related to the concentration of nutrients at a hyphal node, i.e. *pt* ~ *fk*. Thus, the polarization increases due to the degradation of pit membranes.

Fungi require nutrients in order to grow and degrade e.g. the cell walls. In this model the *growing costs* depend linearly of the length of a new edge. Thus,

$$gc(l) = b \cdot l, \tag{6}$$

where *l* denotes the length of the new edge added to the hyphal tip *k* and the concentration of the node *fk* is reduced by *gc(l)*. If *fk* < *gk* the hyphal tip becomes inactive and growth stops.

During each time step a pit membrane is selected due to Eq. 5. If growth costs are lower than the amount of tip nutrients the hyphae grows randomly to a adjacent pit membrane.

*Branching* is one of the most common modes of growth in nature and can be found from river networks to living organisms like fungi (Fleury et al. 2001). In fungi there are two kinds of branching modes: apical and lateral branching. In our model branching occurs if $f_k$ in a link (lateral) or hyphal tip (apical) exceeds the specific threshold $b_l$ or $b_t$ and if a new hyphal tip is added to the specific node. Thus, we can interpret apical branching as global phenomena while lateral branching is more strongly related to the local condition of the hyphal network.

## 3. SIMULATION

The simulation process starts by selecting the substrate of the fungus. In this paper we discuss the penetration of *P. vitreus* in axial direction of the tracheids, i.e. as observed in longitudinal tangential sections (TLS). We modelled the substrate according to Lewis (1935) as polyhedrons. The dimensions of the tracheids and the distribution of the pit membranes were selected according to (Brändström 2001, Sirviö & Kärenlampi 1997). At the beginning of the simulation for every pit membrane there is $f_p^{(i)}=1$. After placing a pellet with a fixed number of hyphal tips on the substrate (starting node) the simulation runs until the the total amount of nutrients is zero, i.e. hyphae have degraded all available pit membranes in the system, or when a specific number of time steps is completed.

## 4. RESULTS AND DISCUSSION

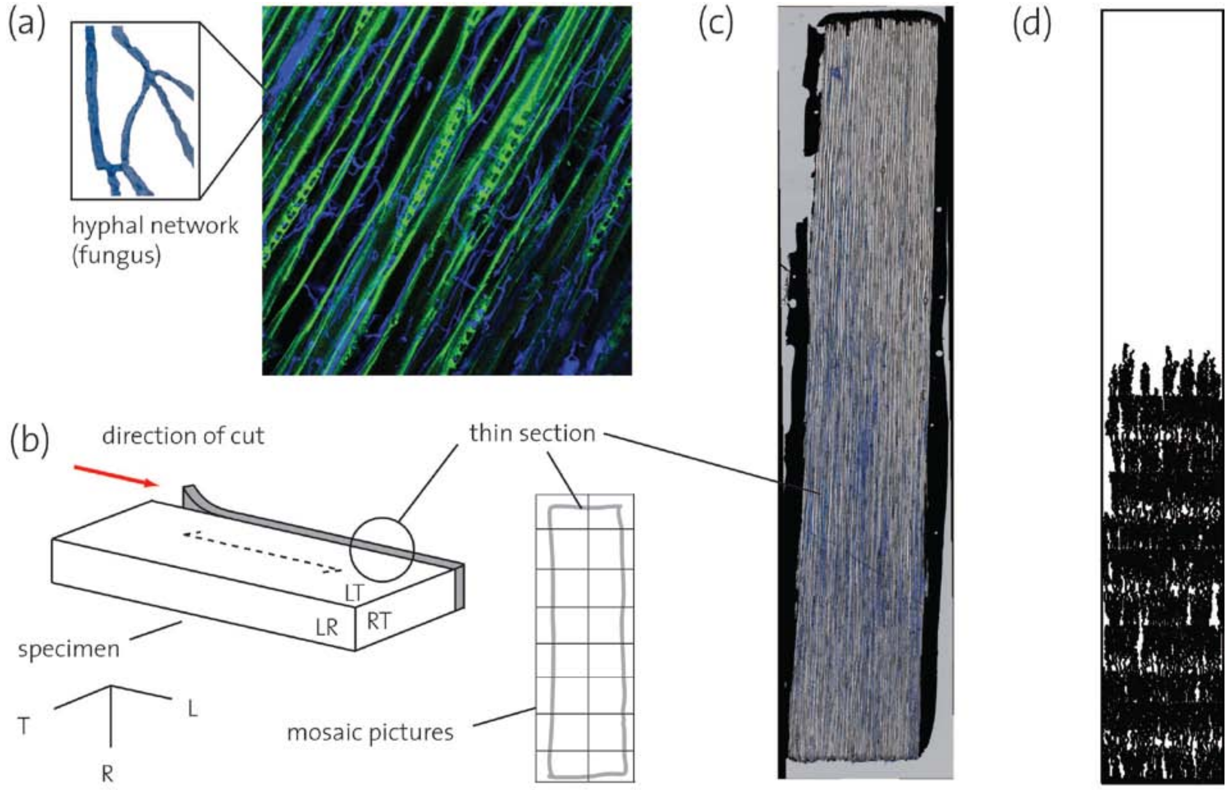

Figure 2: (a) Wood decay fungi exploit a substrate by forming a network (mycelium) consisting of filaments (hyphae), which are (b) visible under the laser scanning microscope. (c) Mosaic pictures of thin sections (LM, TOMCAT or CLSM) allow analysis of the mycelium's morphology and enable the calibration (d) of mathematical models.

We simulated hyphal growth of *P. vitreus* in tangential longitudinal sections of Norway spruce wood using a two dimensional hyphal growth model. Simulations revealed that the mycelium density correlated with the distribution of the pit membranes and the density was higher at the tapering ends of tracheids. The simulations show a fingering shape of the growth front. The penetration of hyphae within tracheids is faster in longitudinal direction than in the tangential directions.

A qualitative comparison of laboratory experiments and simulations showed a good agreement, e.g. the penetration depth of hyphae in longitudinal and tangential direction (see Fig. 2). A quantitative comparison of biomass, hyphal growth unit, fractal dimension and branching mode using CLSM and TOMCAT is currently in progress. First results suggest that a two dimensional growth model cannot describe hyphal growth in the radial direction by neglecting xylem rays. Thus, for simulating hyphal growth in the radial direction a three-dimensional model is required.

## 5. CONCLUSIONS

The presented two dimensional mathematical model describes the growth and impact of the wood decay fungus *P. vitreus*. Qualitative comparison of the model with laboratory experiments shows a good agreement. However, for simulating the growth in radial direction a three-dimensional model is required due to the complex three-dimensional structure of wood. Quantitative comparisons of laboratory experiments and simulations are currently in progress. The FGM is extensible to transport processes as well as hyphal anastomosis.

## 6. REFERENCES

Araujo, A D, Bastos, W B, Andrade, J S and Herrmann, H J (2006): The distribution of local fluxes in porous media. *Physical Review E*, **74**(1), 010401.

Brändström, J (2001): Micro- and ultrastructural aspects of Norway spruce tracheids. *IAWA Journal*, **22**(4), 333-353.

Dijkstra, E W (1959): A note on two problems in connexion with graphs. *Numerische Mathematik*, **1**,269–271.

Chopard, B, Herrmann, H J and Vicsek, T (1991): Structure and growth mechanism of mineral dendrites. *Nature*, **353**(6343), 409–412.

Fleury, F, Gouyet, J F and Léonetti, M (2001); *Branching in nature*. Springer-Verlag Berlin.

Lewis, F T (1935): The shape of the tracheids in the pine. *The American Journal of Botany*, **22**(8), 741–762.

Herrmann, H J (1992): Simulation of random growth processes. *Topics in Applied Physics*, **71**, 93–120. Springer Berlin / Heidelberg.

Horvath, V K and Herrmann, H J (1991): The fractal dimension of stress corrosion cracks. *Chaos, Solitons and Fractals*, **1**(5),395–400.

Lehringer, C, Arnold, M, Richter, K, Schubert, M, Schwarze, F W M R, Militz, H (2009): Bioincised Wood as Substrate for Surface Modifications. *European Conference on Wood Modification*.

Lehringer, C, Richter, K, Schwarze, F W M R (2009): A Review on Promising Approaches For Liquid Permeability Improvement in Softwoods. *Wood and Fiber Science*, **41**(4), 374-385.

Schwarze, F W M R (2007): Wood decay under the microscope. *Fungal Biology Reviews*, **1**, 133–170.

Schwarze, F W M R and Engels, J (1998): Cavity formation and the exposure of peculiar structures in the secondary wall (s2) of tracheids and fibres by wood degrading basidiomycetes. *Holzforschung*, **52**(2):117–123.

Schwarze, F W M R and Landmesser, H (2000): Preferential degradation of pit membranes within tracheids by the basidiomycete Physisporinus vitreus. *Holzforschung*, **54**(5):461–462, 2000.

Schwarze, F W M R, Landmesser, H, Zgraggen, B, and Heeb, M (2006): Permeability changes in heartwood of Picea abies and Abies alba induced by incubation with Physisporinus vitreus. *Holzforschung*, **60**(4):450–454, 2006.

Schwarze, F W M R, Spycher, M and Fink, S (2008): Superior wood for violins wood decay fungi as a substitute for cold climate. *New Phytologist*, **179**(4):1095–1104.

Schwarze, F W M R, Schubert, M (2009): Enhanced uptake of wood modification agents in ‘bioincised’ wood. *The international Research Group on Wood Protection 40th Annual Meeting, Beijing*, China.

Sirviö, J and Kärenlampi, P (1998): Pit membranes natural irregularities in softwood fibers. *Wood and Fiber Science*, **30**(1), 27–39.

Stührk C, Fuhr M, Schwarze, FWMR, Schubert M, (2010): Analyzing hyphal growth of the `bioincising´ fungus Physisporinus vitreus with light-, confocal laser scanning- and, Synchrotron X-ray tomographic microscopy. *Paper prepared for the 41th Annual Meeting, Biarritz,* France.

Sudbery, P and Court, H (2007): *Polarised growth in fungi*. The Mycota, **8**(6), 137–166, Springer-Verlag Berlin.