

LOSS OF STRENGTH IN BIOLOGICALLY DEGRADED THERMALLY MODIFIED WOOD

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The durability of thermally modified (TM) and untreated (UT) mini-stakes exposed to in-ground contact was compared by modulus of elasticity (MOE) and mass loss with decay type using microscopy. Results showed a strong correlation between MOE and soft rot decay for UT stakes over a 30 month exposure period. For TM stakes, the correlation between MOE and decay rate (soft rot/bacteria) was not as strong. Loss of MOE of the TM stakes is suggested to be accentuated by the extensive micro-checking produced in the TM wood tracheids during the original heat treatment. The micro-checks are thought to expand during the winter season due to water expansion during freezing, thereby leading to weakening of the wood in addition to the decay caused by soft rot and bacteria. Using molecular methods, *Phialophora hoffmannii* was identified as the main fungus causing soft rot decay.

Keywords: *Bacteria; Field test; Light microscopy; MOE; Soft rot; Thermally modified wood*

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INTRODUCTION

Wood strength loss is closely related to the degradation of hemicellulose components and can be divided into early and late stages where the hemicellulose composition changes. In early stages of wood decay, hemicellulose side-chains such as arabinose and galactose are degraded, while in late stages, the main-chain hemicelluloses of mannose and xylose are mineralized (Esteves and Pereira 2009). Previously, Winandy and Morrell (1993) reported a correlation between wood hemicellulose composition and strength properties. In other studies, it was further shown that wood decay can be predicted by changes in modulus of elasticity (MOE) (Curling *et al.* 2002; Nicholas *et al.* 1991; Henningsson *et al.* 1975; Nicholas and Crawford 2003; Wilcox, 1978; Ruddick 1986; Winandy and Morrell 1993). MOE is the strength property that is least affected in thermally modified (TM) wood and, therefore, represents an interesting parameter that can be used for repeated measurements on the same specimen (*i.e.* stake) to reflect the time course of wood decay.

The term soft rot was first coined by Savoy (1954) to describe a type of wood decay initiated by fungi imperfecti and Ascomycetes in moist (*e.g.*, ground contact) and aquatic environments. Soft rot decay is divided into two groups, type 1 and type 2, where type 1 results in the development of cavities in the secondary walls of wood fibres (primarily the S2 layer), and type 2 results in the erosion of the wood cell wall outward from the cell lumen (Daniel 2003).

When studying soft rot decay type 1 in cross sections under the microscope, the first visible cavities normally appear as holes in the S2 layer of latewood fibres. As the process of deterioration proceeds further, cavities are formed until the only remaining parts of the cell wall are the middle lamella regions and the S3 layer (when present). Soft rot cavities are initiated by very thin hyphae, so-called bore hole hyphae, which penetrate into the fibre wall from hyphae growing into the cell lumen. The bore holes produced by soft rot fungi never enlarge in the later stages, but remain narrow in contrast to that produced by Basidiomycete fungi. Some of the bore hyphae also penetrate the cell lumens of adjacent fibres, while others penetrate within the S2 cell wall, causing T-branching/L-bending, and they orientate themselves along the direction of the cellulose microfibrils. Here they cause the characteristic and destructive cavities that reduce the strength of the fibres and wood (Savoy 1954; Nilsson 1973; Daniel and Nilsson 1988, 1989, 1998; Eaton and Hale 1993). Soft rot type 2 is rare in softwoods and results in uniform thinning of the cell wall from the cell lumen (Daniel and Nilsson 1998). This proceeds until only the middle lamella remains. Cell wall erosion is also frequently produced by simultaneous white rot decay fungi, but here the fungi also erode softwood fibres as well as the middle lamella between cells. In hardwoods it is difficult to differentiate between erosion decay caused by soft rot or by white rot fungi (Eaton and Hale 1993). Some common soft rot fungi genera found in decaying wood include *Chaetomium*, *Humicola*, and *Phialophora* (*Lecythophora*).

In this work we have studied the correlation between wood decay and MOE of thermally modified wood to determine if this correlation can be used to predict the degradation rate. A further aim of the study was to identify the major fungal species causing degradation in the soft rot test field in Ultuna, Sweden by means of DNA profiles.

EXPERIMENTAL

Field Description

Ultuna test field ($59^{\circ} 49' N$ and $17^{\circ} 40' E$) for testing of wood protection formulations and treatments is located in close proximity to the Department of Forest Products at SLU Uppsala. The test field provides a clay soil environment with an annual precipitation of 530 mm. The prevailing type of decay is soft rot and bacteria which provides a service life of 2 to 3 years (Edlund 1998) for standard stakes of untreated Scots pine according to EN 252 (1989). The water holding capacity of the soil from the Ultuna test field is approximately 50% (m/m) (Edlund 1998).

Materials

Wood samples of Scots pine sapwood (*Pinus sylvestris* L.) and thermally modified (TM) Scots pine wood (Thermowood D) were produced in dimensions $8 \times 20 \times 200$ mm (along the grain), so called mini-stakes. The production technology of Thermowood D consists of a treatment in steam at a temperature of $212^{\circ} C$. The experiment comprised 30 untreated and 30 thermally modified (TM) mini-stakes, which

were inserted in the soil to approximately half of their length. The exposure started in June, 2009.

Modulus of Elasticity and Mass Loss

The mini-stakes exposed in the Ultuna test field were analyzed to reveal changes in MOE. For determination of MOE, a universal testing machine (Shimadzu AG-X 50 KN) was used. The MOE was measured according to the ISO 3349 standard under the recommendations suggested by Stephan *et al.* (1996). The measurements were carried out after 4, 12, 18, 24, and 30 months of exposure. The difference between the MOE of the mini-stakes prior to the exposure in the test field and after defined exposure intervals was calculated as a percentage of the initial modulus. Mass loss of the mini-stakes was determined twice after 18 and 30 months of exposure as a percentage decrease of the initial mass.

Microscopy

The middle part of each mini-stake was cut into thin slices, approximately 10 to 15 μm thick sections (Fig. 1) using a Microm microtome (HM 350, Microm, Germany). Cross sections were stained using 1% w/v safranin and longitudinal sections with 1% w/v aniline blue (*i.e.* to detect fungal hyphae and bacteria) in lactophenol cotton blue. Sections were analysed using a Leica DMLB light microscope and images recorded digitally with a Leica DC 300 CCD camera at a magnification of 315x. Images were later used for reconstruction of the whole mini-stake section (8 \times 20 mm) using Adobe Photoshop CS2. As a compliment, detailed images of interesting areas of the cross sections were made at various magnifications (*i.e.* 2520x and 3969x).

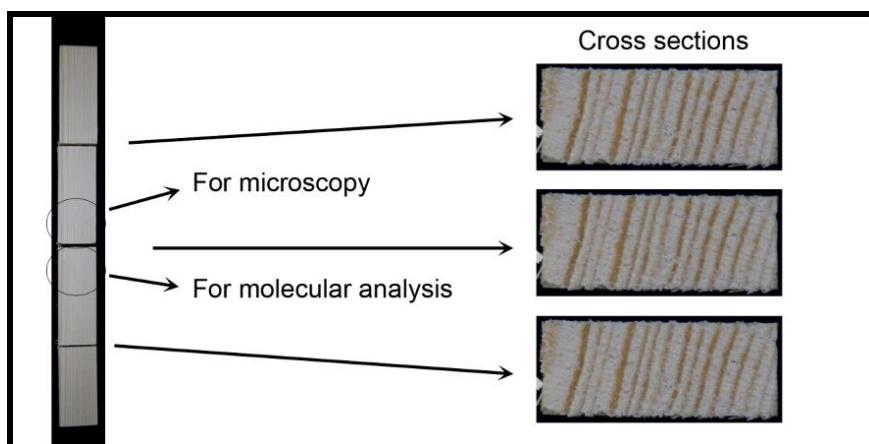


Fig. 1. Experimental design of mini-stakes. The stakes were 20 cm long and at every fifth cm, a cross-cut was made using a circular saw. Images of cross sections were made and studied by light microscopy for signs of wood decay type using whole section overviews. One part of the middle section was also used for molecular analysis.

As an indication of the extent of decay, each cross section of the mini-stakes was assessed on a coarse scale of 0 to 3, where 0 means perfectly sound, 1 indicates rot around the edges (< 1 mm), 2 represents a cross section that has more than 1 mm rot in

the edges and/or is spread along rays, and 3 is given when the sample is totally affected by rot.

Molecular Methods

Samples from the middle part of the mini-stakes (Fig. 1) were prepared for molecular analysis by milling the sample to a fine wood powder using a 1 mm screen Cyclone grinder (Cyclotech 1093, sample mill). Samples were frozen in a -20°C freezer directly after milling until further analysis.

Before extracting gDNA, the wood powder was further homogenised using a hand-held homogenizer (IKA T10). Extraction of gDNA was done using 2% w/v cetyltrimethylammonium bromide (CTAB), and polymerase chain reaction (PCR) run as described in Råberg *et al.* (2005), with the exception of using a WVR Mastermix. The primer pairs were ITS 1F-FAM and ITS 4-HEX for Terminal Restriction Fragment Length Polymorphism (TRFLP) profiles. TRFLP profiles were based on enzyme digestion by Taq I and Cfo I (both Sigma-Aldrich). Samples were purified using isopropanol and sent for profile analysis at Uppsala Genome Centre. Profile data were analyzed in the software Peak Performance 1.0 (Applied Biosystems) and exported to Microsoft Excel for further analysis.

RESULTS AND DISCUSSION

Results show that MOE decreased with time for both untreated and TM mini-stakes excluding the cold months (*ca.* 4 to 5 months/year) (Figs. 2 and 3).

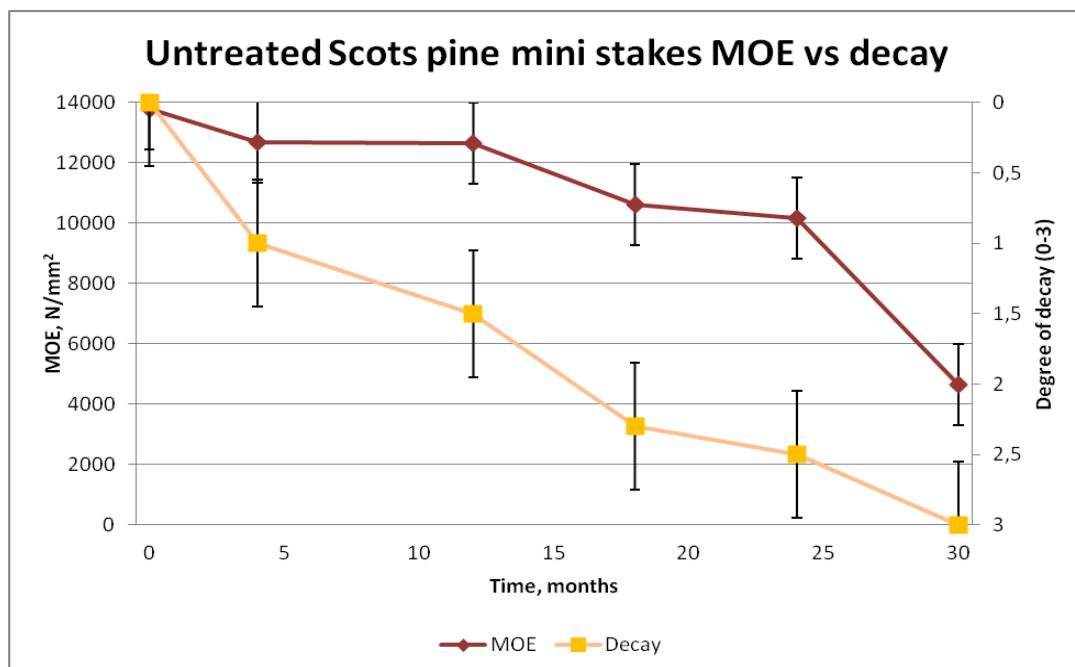


Fig. 2. Correlation of MOE with wood decay using untreated mini-stakes. The red line indicates decrease of MOE (left, y-axis N/mm²). The yellow line indicates degree of decay (grade 0-3, right y-axis). Vertical bars represent standard errors.

During the cold months, the loss of MOE declined (Fig. 2) reflecting the time between months 4 to 12 and 18 to 24. Decay rates of the exposed mini-stakes showed a similar profile as the MOE loss (Fig. 2). Light microscopy revealed soft rot decay in the untreated mini-stakes of Scots pine and bacteria attack of the TM mini-stakes as the main form of decay. By using DNA profiles, the imperfect soft rot fungus *Phialophora* spp. was detected and identified, thus confirming the main decay type in the mini-stakes.

Table 1. Summary of Decay, MOE, and Mass Loss for Untreated (UT) and Thermally Modified (TM) Mini-Stakes after 0, 18, and 30 Months Exposure

Exposure time	Decay		MOE		Mass loss	
	Graded 0 to 3		Loss in percent		Loss in percent	
Months	UT	TM	UT	TM	UT	TM
0	0	0	0	0	0	0
18	2.3	0	23	12	6.3	2.4
30	3	1	67	26	19.2	10.3

Measurements of MOE were performed after 4, 12, 18, and 30 months of exposure. It is obvious that little decrease of MOE took place during the cold months of the year (November-May) for the untreated mini-stakes, while a constant declining trend was observed in the TM mini-stakes (Fig. 3). This observation can possibly be explained by the presence of prominent micro-checks in the secondary cell walls of the tracheids that permit the absorption of higher amounts of free water, and thus, some destruction caused by its freezing (Fig. 5). The MOE of untreated wood was almost unchanged during the winter period. All untreated mini-stakes showed an even decrease of MOE (see Fig. 3). The first TM stake achieved failure after 12 months exposure, while none of the untreated mini-stakes failed (Fig. 4). After 18 and 24 months, the number of failed TM mini-stakes increased to three, while only one untreated mini-stake was rejected as failure on both occasions. However, six TM and 15 untreated mini-stakes were rejected as failure after 30 months exposure (Fig. 4). The initial MOE of TM mini-stakes was lower, but this should not be compared to the untreated mini-stakes, since the wood was not from the same origin. The variation in the results increased with the duration of exposure. The change in mass of the exposed mini-stakes in Ultuna were negligible at the two sampling occasions (4 and 12 months). After 18 months of exposure, the untreated mini-stakes had an average mass loss of 6.3%, while the TM mini-stakes showed only 2.4% average mass loss (Table 1). As concluded in an earlier study (Curling *et al.* 2002), mass loss often poorly predicts the decrease of mechanical properties expressed by MOE, particularly during the beginning of exposure. After 30 months exposure, the mass loss of untreated and TM mini-stakes rose to 19.2% and 10.3%, respectively. Although the mass loss of the TM mini-stakes was lower than that of the untreated mini-stakes, it contributed to a significant loss of MOE (26% after 30 months exposure). The decrease

can be attributed not only to the increasing biological (soft rot/bacteria; see below) decay, but also to inherited weakening of the wood cell wall introduced by the chemical changes of the structural compounds caused by heat (Sivonen *et al.* 2002; Tjeerdsma and Militz 2005), and/or micro- and nano-checks typical for the secondary cell walls of heat treated wood (Terziev and Daniel 2002; Johansson and Moren 2006). One hypothesis is that the micro-checks expand during time of exposure due to the action of ice in winter.

The TM stakes demonstrated significantly lower mass loss, decrease of MOE, and decreased number of failed mini-stakes as compared to the untreated stakes after 30 months of field exposure. At the same time, the untreated mini-stakes had lost 67% of MOE and half of the mini-stakes had failed. These results are in line with the decay characteristics of the field; *i.e.* the average service life of untreated standard stakes (according to EN 252) is estimated as 2 to 3 years.

A distinction should be made between presence of a fungus and its activity. Nilsson and Edlund (1995) have reported that the presence of a decay form fungus/bacteria does not provide any information about its activity. The soil in Ultuna is rich in soft rot fungi and bacteria but is poor in nutrients. The soft rot fungi are abundant but can be more active if the soil moisture is kept optimal, *i.e.* close to the soil water holding capacity. This is shown when soil from Ultuna is used in prENV 807 laboratory tests. Scots pine mini-stakes have approximately 6% mass loss after 4 months when exposed in the laboratory test, while it takes 18 months to achieve a similar mass loss when the stakes are exposed in the field. However, the decrease in MOE was 37% after 4 months exposure in the laboratory test (Ali *et al.* 2011) and 23% in the present field test. This exemplifies the eventual difficulties incurred when comparing laboratory and field test performance, which is important for service life prediction.

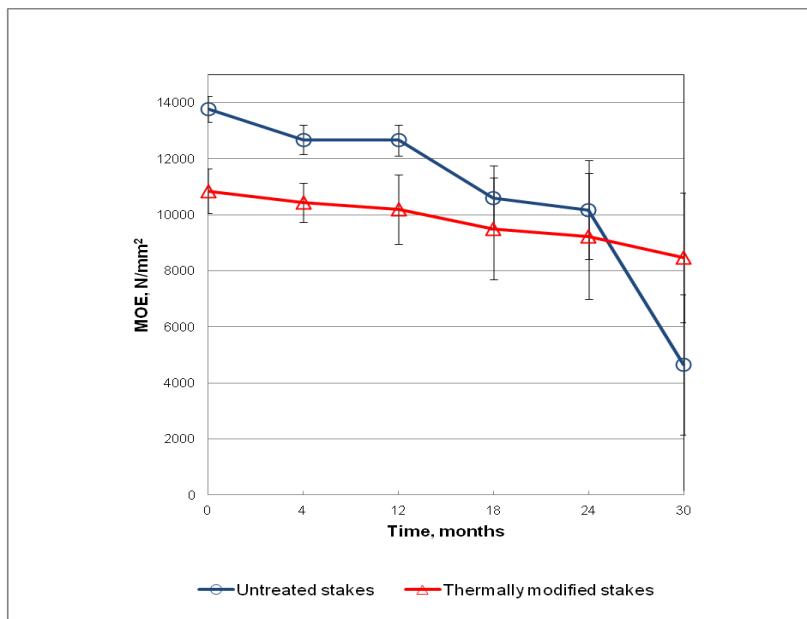


Fig. 3. Decrease of MOE in untreated and thermally modified mini-stakes after 30 months exposure in Ultuna field expressed in N/mm². Vertical bars represent standard deviations.

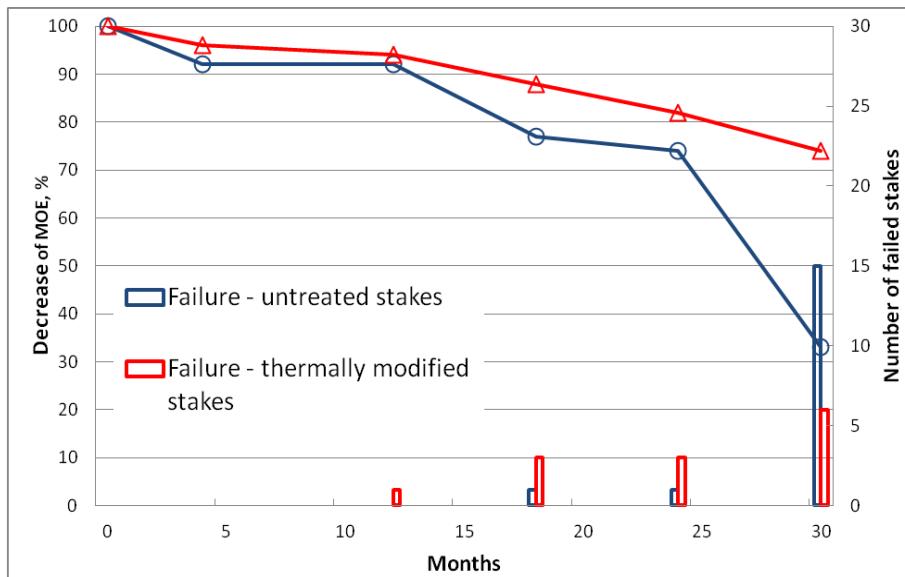


Fig. 4. Decrease of MOE in untreated and thermally modified mini-stakes after 30 months exposure in Ultuna field expressed as percentage. Bars represent the number of failed stakes.

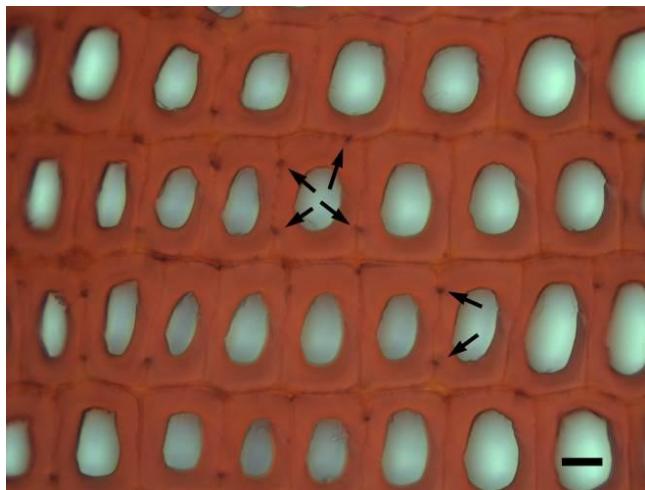


Fig. 5. Cross section of unexposed thermally treated wood. Arrows indicate a repetitive pattern of small cracks present in the S₁ cell wall of the majority of tracheids. Bar 10 μm .

Microscopy

Cross sections of the mini-stakes showed soft rot attack of both TM and untreated mini-stakes and bacterial attack of TM wood after 30 months exposure. The untreated mini-stakes were heavily degraded by soft rot type 1 (Figs. 6 and 7) with cross sections graded as 3 (*i.e.* totally degraded). In TM mini-stakes, however, only minor degradation by soft rot had taken place with a grading of 1 (Table 1). The limited soft rot probably allowed for the bacterial attack to be detected more easily in the TM mini-stakes, but not in the untreated mini-stakes. Another reason may be that the cell walls in the untreated mini-stakes are degraded to such an extent that only the S₁ and S₃ layers were observed together with the middle lamella regions. A third explanation may be the loss of

hemicelluloses during TM treatment. Soft rot fungi prefer cellulose and hemicelluloses, and since the crystallinity of the cellulose has increased during thermal modification, it may have a negative effect on soft rot activity.

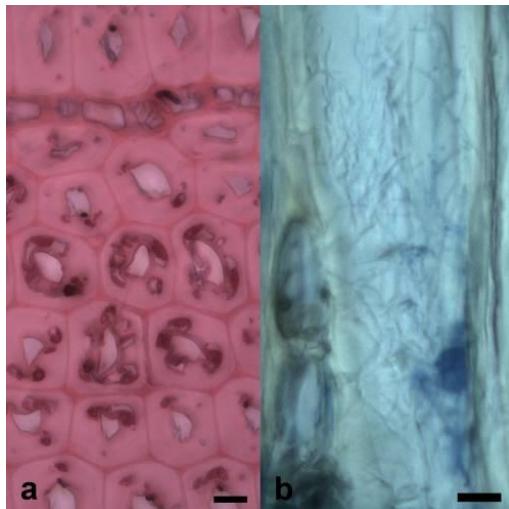


Fig. 6. (A) Soft rot type I cavities are shown (cross section) (B) and tunnelling bacteria attack (longitudinal section). Bars: 10 μm .

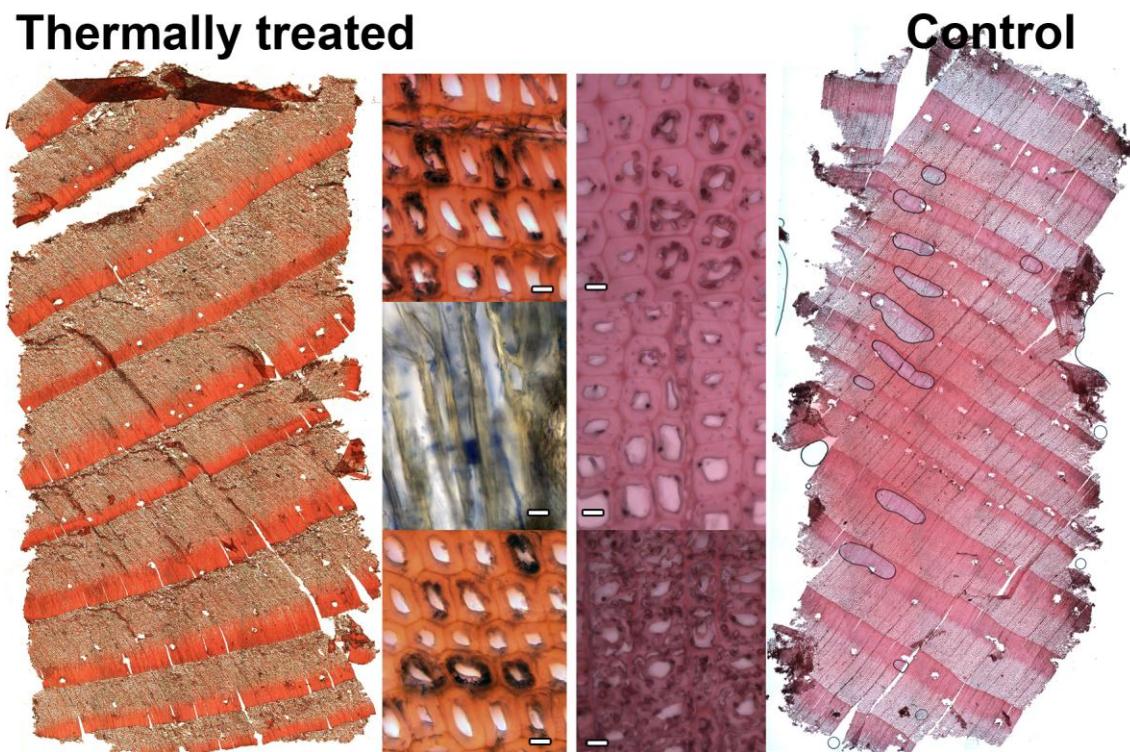


Fig. 7. Cross section (8 x 20 mm) of TM wood exposed 30 months (left). Three areas at higher magnification showing soft rot (upper and lower) and bacteria (middle). Untreated sample (right), at higher magnification of various degrees of soft rot. Typically such cross sections showed no cavity free areas. Bars: 10 μm .

By studying reconstructed whole cross sections (*i.e.* Fig. 9) of the mini-stakes, it became clear that there was a gradient of decay rate for soft rot from the external edges to the centre of the mini-stakes. This makes it very difficult to grade the decay according to the method of Henningsson *et al.* (1975) (Fig. 8). The same stake may show all rates of decay depending on where along the gradient the rating is made (see Fig. 9). Therefore, we adopted a coarse grading system (see material and methods) ranging from 0 to 3 to give an overall indication on the extent of decay in a cross section.

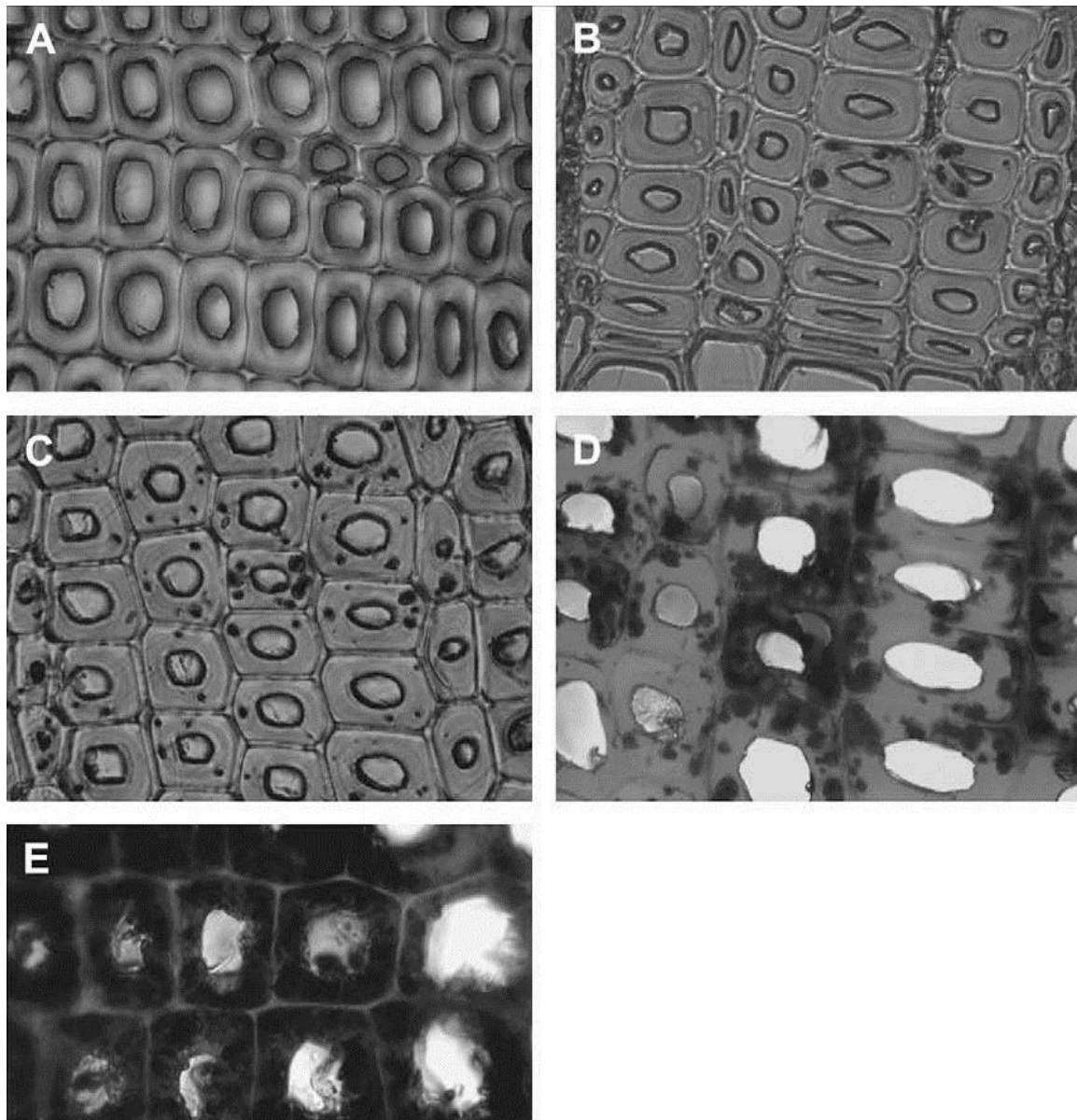


Fig. 8. Grading of soft rot according to Henningsson *et al.* (1975) for soft woods: (A) 0 – no cavities, (B) 1 – few scattered cavities, (C) 2 – numerous cavities in most cells, (D) 3 – cavities abound, often fused together and (E) 4 – total or almost total disintegration of secondary wall.

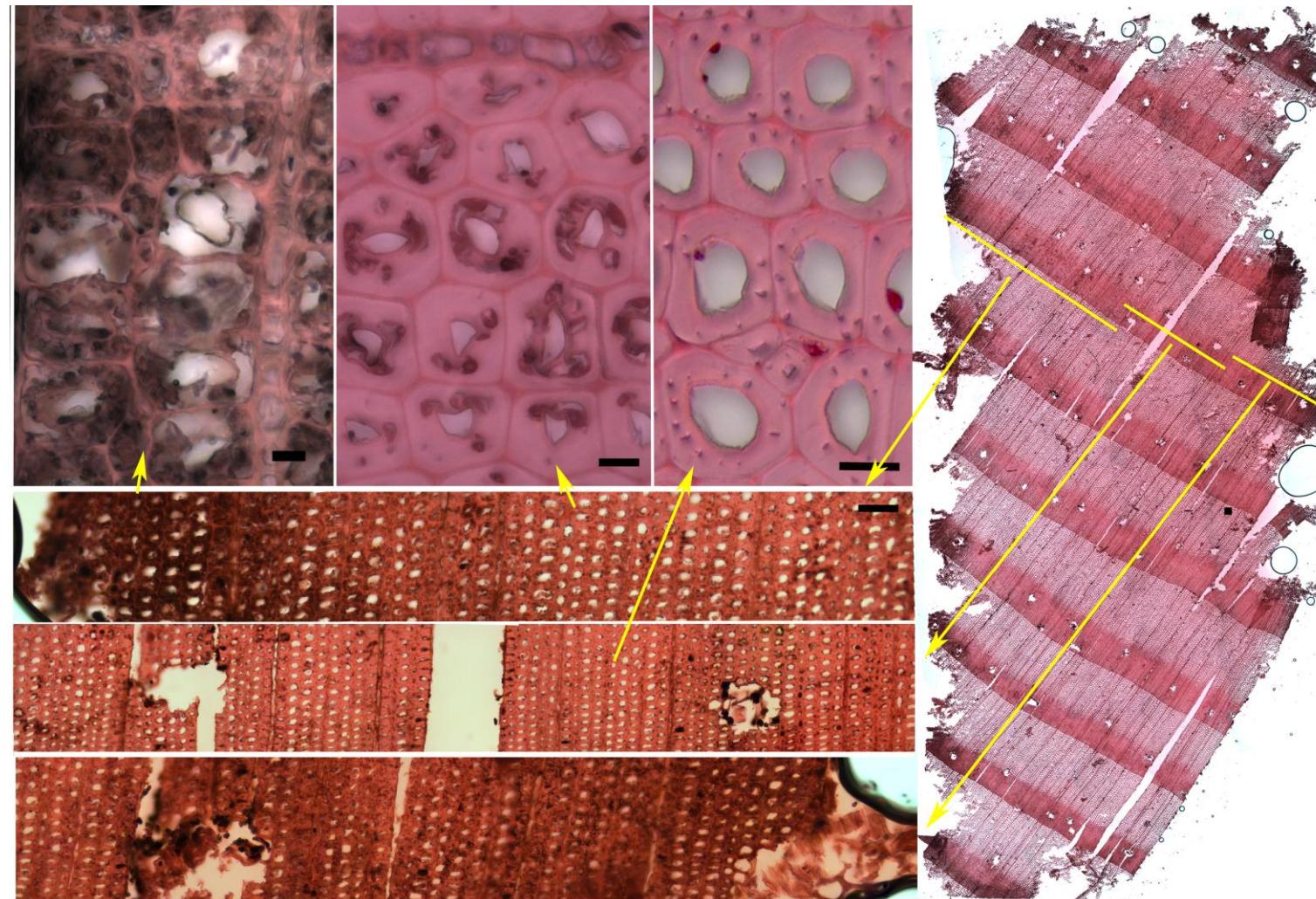


Fig. 9. Graded rot. On the right, the entire cross section 8 x 20 mm is shown with yellow lines that highlight three areas. These areas are shown at higher magnification in the left lower part of the figure. The left higher part of the figure shows high magnification images from the lower left part of the figure, highlighted with yellow arrows from their origins.

The cross section rating was then compared with the MOE measurements (Fig. 2). This showed that the MOE and decay rate was correlated for the untreated mini-stakes. Since the TM mini-stakes did not develop severe soft rot decay, no comparison of soft rot decay and MOE was possible.

In TM mini-stakes, the extent of soft rot was rather poor, with a grading of 1 (Table 1). This is why it is difficult to explain the decrease of MOE in the TM mini-stakes shown in Figs. 3 and 4. By studying cross and longitudinal sections by microscopy, it was evident that the TM mini-stakes showed considerable tunnelling by bacterial attack (Fig. 6). When comparing untreated and TM treated mini-stakes over time and loss of MOE, they resemble each other (Fig. 4), excluding the last sample point. Comparing the course of decay, on the other hand, shows a gap between the untreated and the TM treated mini-stakes. At 30 months exposure, the untreated mini-stakes had a grading of 3, indicating that the whole section was affected by rot. TM mini-stakes showed a more modest soft rot attack grading of 1, indicating that less than 1 mm of the outer part of the cross section was affected. Therefore, the loss in MOE presumably reflects the course of decay contributed by a combination of soft rot, bacteria, and changes induced through micro-checking of the cell walls in the original treatment of the mini-stakes and their modification over time.

Fungal Identification

DNA profiling for fungi performed on the mini-stakes after 12, 18, and 30 months exposure showed various profiles (Fig. 10) from where the soft rot fungus *Phialophora hoffmanni* was identified. This is consistent with the decay pattern for this species which was also isolated earlier at the Ultuna test field (Råberg *et al.* 2009). *Phialophora hoffmanni* is a well known soft rot fungus that acts in terrestrial ground contact and can cause soft rot decay with severe mass loss (Nilsson 1973).

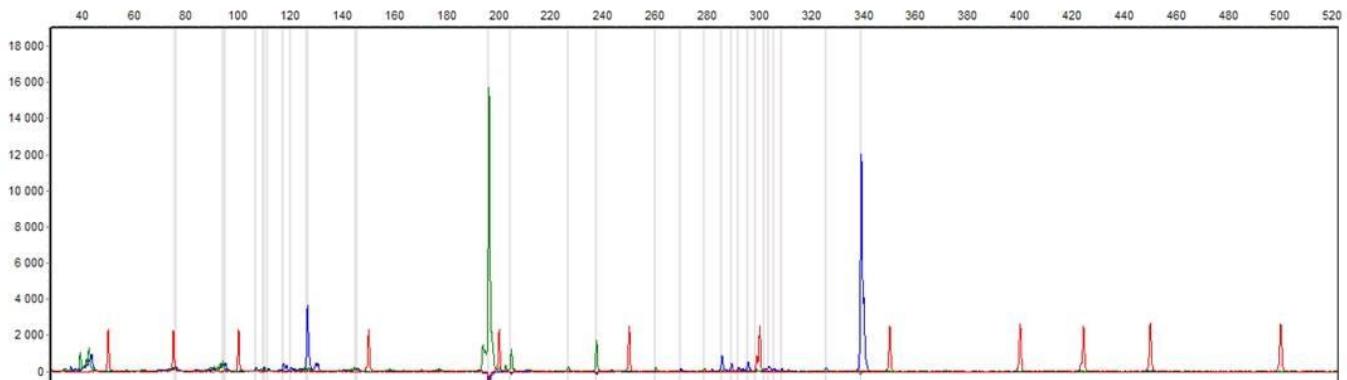


Fig. 10. Typical DNA profile. On the horizontal axis is the length in bases and the vertical axis show the intensity of the fluorophore. The red bars are the ladder, and the blue and green bars indicate the DNA profile of the sample.

CONCLUSIONS

1. The decrease of MOE for the TM and untreated mini-stakes after 30 months exposure in the field at Ultuna follows different patterns. TM mini-stakes follow a

linear decrease of MOE, while the untreated controls exhibit an abrupt loss of MOE after 24 months exposure. Apparently, the TM mini-stakes are more durable compared to the untreated mini-stakes for the period of 30 months. It is clear that soft rot decay causes a stiffness decrease of the untreated mini-stakes. For the TM stakes, the explanation is more complicated and includes not only soft rot and bacteria decay, but also inherited mechanical micro-checks in the tracheal cell walls caused by the initial heat treatment. Most probably the stiffness of TM wood is decreased by expansion of ice in the micro-checks in the winter months. This can also partly explain that the number of failed TM mini-stakes is greater than that of the untreated mini-stakes after only 18 months of exposure.

2. There is a stronger correlation between MOE and decay for untreated than for the TM mini-stakes, and MOE can be used as a predictor of decay for untreated mini-stakes of Scots pine. For the TM mini-stakes, the correlation between MOE and decay is more complicated, reflecting both biological (soft rot/bacteria) decay and physical changes induced during the heat treatment (*i.e.* micro-checks) and their indirect effects during exposure.
3. Results further confirm that MOE is a very sensitive method that allows one to follow changes in strength for the same mini-stakes over time. Together with mass loss, this provides unique information on the expected service life of a treatment. The examination of mini-stake cross sections and the use of microscopy scaling provide complementary information on the type of decay involved, which is not available by MOE. In addition, microscopy can be focused on the central regions of the stakes where maximum and localized decay is expected at in-ground contact.
4. Soft rot is reduced in TM mini-stakes compared to untreated mini-stakes of Scots pine. TM mini-stakes were additionally attacked by wood degrading bacteria.
5. By using DNA profiles, *Phialophora hoffmannii* was detected and identified, thus confirming its presence as one of the main soft rot fungi in the untreated mini-stakes.

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