THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Decay factors in termite in-ground monitoring stations

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Decay factors in termite in-ground monitoring stations

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Abstract

Subterranean termites are serious pests of wood in service in much of the world. One of the most common techniques for monitoring and controlling termites is the use of in-ground monitoring stations which comprise a wood or other cellulosic material monitor (cellulosic matrix) and/or a termiticide bait held in a open plastic holder so facilitates examination and the termite access. Wood and other cellulosic substrates are subjected not only to termite attack, but also to fungal decay, which may interfere both with usefulness of the monitoring stations and with termite attraction.

Decay susceptibility of commercial monitoring stations was assessed over one year in the field. Variables evaluated were: mass loss and moisture content of cellulosic matrix, termite presence, type of cellulosic matrix (cellulose powder, *Pinus* spp., *Populus* spp., *Pinus pinaster*, *Hevea brasiliensis*, cardboard), monitoring station model (Advance™, FirstLine™, Terminate™, LNEC) and a wood borate-based treatment.

A multivariate analysis (RDA) was performed, resulting in 78.8% of mass losses data variability being explained by the decay factors considered in this analysis. Five factors were considered significant ($P<0.002$): moisture content, cellulose powder and type of monitoring stations (namely FirstLine™, Advance™ and LNEC). Advance™ stations were used with different cellulosic matrices: cellulose + *Populus* spp. and *H. brasiliensis* (treated and not treated). Although cellulose powder had very low mass loss and fungi attack, termites were not attracted to this substrate. *H. brasiliensis* Advance™ stations were attacked by termites. FirstLine™ stations showed no mass loss; however, these traps were attacked by termites and moulds. LNEC baits, using *P. pinaster*, had low mass losses although termites' presence was low. The use of wood borate-based treatment was not considered significant for decay resistance in this study; it was noticed that termites did not seem to avoid this fungicide treatments at the levels used.

Monitoring stations design must be done carefully for the achievement of good results in termite monitoring and control with in-ground termite baiting systems. The replacement of substrate after wetting and fungal decay may be necessary. Cellulosic matrices' decay resistance should be considered and evaluated in the field, including the search for adequate fungicides. The type of cellulosic matrix must also be chosen according to termites' preferences.

Keywords: subterranean termites; fungal decay, monitoring stations design; cellulosic matrix

Introduction

Subterranean termites have cryptic habits, living mostly in soil, becoming serious pests when their foraging activities extend into man-made structures (Su and Scheffrahn 2000). Subterranean termite control measures rely mainly on chemical methods, but environmental concerns are leading to an increase of restrictions in the application of pesticides. In-ground monitoring stations comprise a plastic holder and a cellulosic matrix (wood or other cellulose source) that is replaced by bait (active ingredient added to the cellulosic matrix or to the station) when termites are detected. This method is advantageous for termite control, because it uses small quantities of active ingredients which are insect specific (Evans and Gleeson 2006, Verma et al. 2009). However, it is considered expensive and time-consuming due to the need for monitoring and replacement of the baits (Su and Scheffrahn 2000).

A decay resistant cellulosic matrix would allow the optimization of monitoring stations, extending the time between visits, with the advantage of being more suitable for termite installation because it is less disturbed (Woodrow et al. 2008). Termite baiting control may require high durability cellulosic matrices or baits, because control programmes may be effective after two to 15 months (Su et al. 2002, Cabrera and Thoms 2006). A long-term monitoring program would also require a durable cellulose matrix.
Monitoring stations enhancement should include features as cellulosic matrix quality and quantity, termite feeding preferences, bait design, and need for inspection and replacement (Evans and Gleeson 2006).

The objective of this work was to investigate the decay factors involved in cellulose matrix deterioration and the features that would lead to monitoring stations optimization.

Materials and methods

Termite monitoring systems were installed in February 2010 during 12 months in LNEC garden areas, in Lisbon, Portugal. Termite monitoring systems assessed were:

1. Prescription Treatment® brand Advance™, Whitmire Micro-Gen Research Laboratories, Inc. (now BASF): a plastic holder containing both a set of two aspen (*Populus* sp.) wood pieces (Advance aspen) and powder cellulose (Advance cellulose) in a plastic holder.
2. FirstLine: a plastic holder containing two wood pieces of *Pinus* sp. attached to the plastic lid (FirstLine pinewood).
3. Spectracide Terminate®, Spectrum Brands: corrugated cardboard and string attached to a spring-loaded activity indicator within a plastic holder (Terminate cardboard).
4. Advance plastic holder with: a) untreated rubberwood (*Hevea brasiliensis*) (Adv. untreated rubberwood); b) rubberwood submitted to a full cell treatment with borate (Adv. full cell rubberwood); c) rubberwood submitted to a spot treatment with borate (Adv. spot rubberwood).
5. LNEC monitoring system: a plastic perforated tube with three pieces of untreated *Pinus pinaster* (LNEC *Pinus pinaster*).
6.

Five replicates of each monitoring systems were installed, except for LNEC (n=7). Moisture content was measured weekly, until 6 months and once per month thereafter. Mass loss was calculated at the end of the trials, after oven-drying cellulosic matrices at

103 ºC for 24 hours. Termite activity was observed during the moisture content measurements. Meteorological data of the study area was collected within a meteorological station.

A multivariate redundancy analysis (RDA) followed by a stepwise regression were performed to investigate the importance of the variables measured: moisture content, baiting model (Advance™, FirstLine™, Terminate™, LNEC), wood species/cellulose substrate (cellulose powder, *Pinus* spp., *Populus* spp. *Pinus pinaster*, *Hevea brasiliensis*, cardboard), termites presence and type of borate based treatment, influence cellulosic matrix mass loss values. Statistical significance of the canonical axes was evaluated by Monte Carlo permutation test ($p<0.05$). The analysis was performed in CANOCO 4.0 software (Ter Braak and Smilauer, 2002).

Results

Average daily temperatures ranged from 11.4 to 23.5 ºC; average daily relative humidity ranged from 46 to 95% (Figures 1). Rainfall increased in winter months as expected (Figure 2).

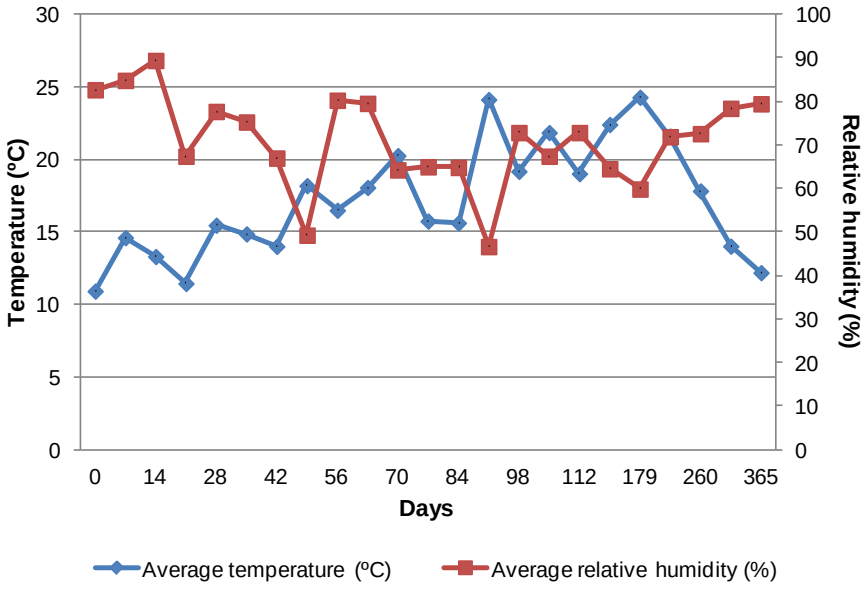

Figure 1. Average temperature (ºC) and average relative humidity (%) during monitoring period for LNEC, Lisbon.

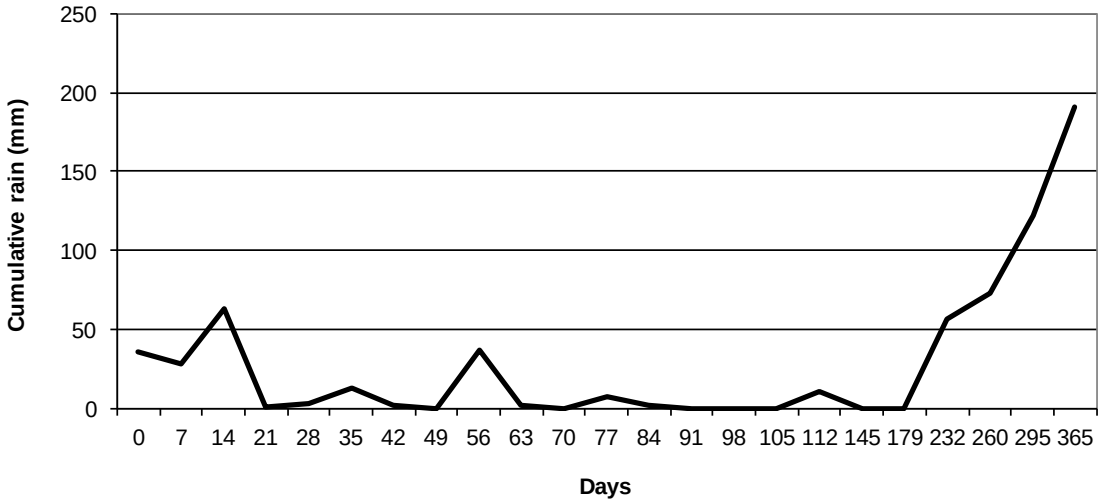

Figure 2. Cumulative rain (mm) during monitoring period for LNEC, Lisbon.

Terminate monitoring stations with cardboard as the cellulosic matrix showed a faster and higher water uptake comparing with other monitoring stations tested, being above 100% moisture content almost all year (Figure 3). The only other monitoring stations which reached 100% moisture content in the end of the test were Advance (aspen), with *Populus* sp. as cellulosic matrix. Rubberwood matrices, with or without treatment, showed low moisture content values during the 12 months in comparison with the other matrices evaluated, even with high rain volumes.

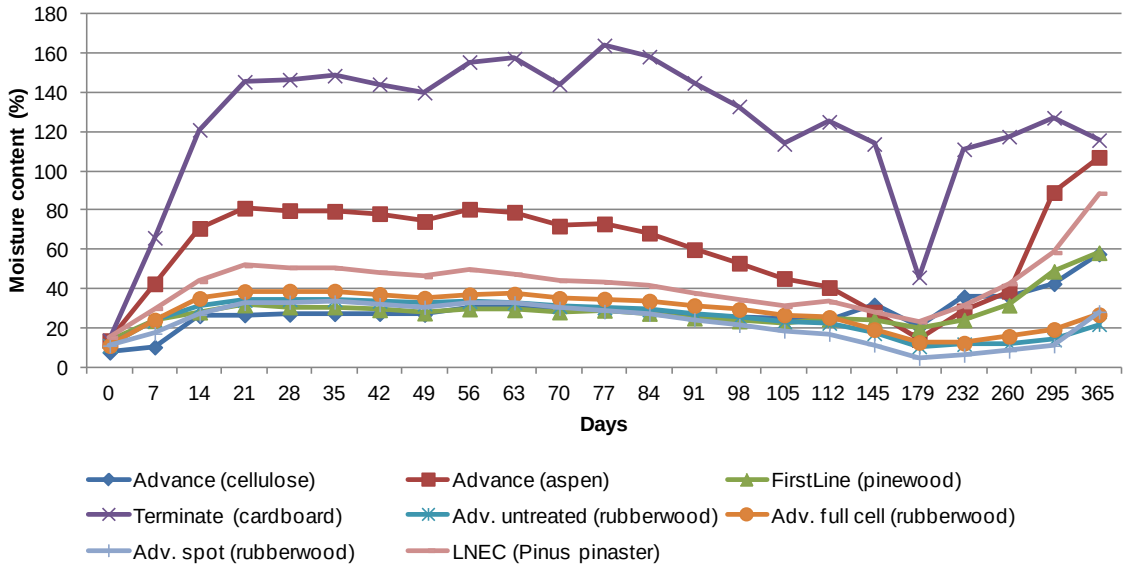

Figure 3. Average moisture content (%) of cellulosic matrices/baits of termite monitoring stations: Advance (cellulose), Advance (aspen), FirstLine (pinewood), Terminate (cardboard), Advance - untreated (Adv. untreated rubberwood), Advance - full cell treatment (Adv. full cell rubberwood), Advance - spot treatment (Adv. spot rubberwood) and LNEC (*Pinus pinaster*).

The Advance stations with cellulose powder as the matrix had negligible mass variation (0.1%). FirstLine monitoring stations showed no mass loss in the end of the field trials, opposing to Terminate monitoring stations (cardboard) which reached high mass losses (three out of five stations above 50% of mass loss); LNEC monitoring stations (*Pinus pinaster*) had low mass loss values, while treated (Adv. full cell and spot rubberwood) and untreated (Adv. untreated rubberwood) rubberwood showed similar mass losses (Figure 4).

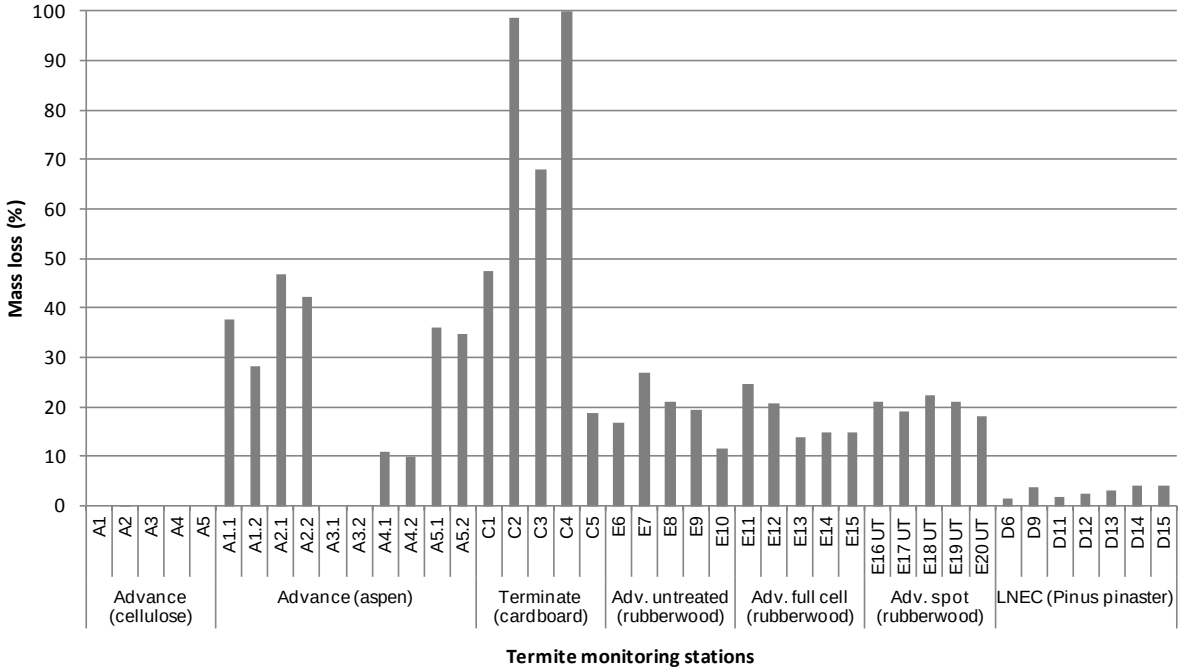

Figure 4. Mass loss (%) of cellulosic matrices/baits of termite monitoring stations: Advance (cellulose), Advance (aspen), FirstLine (pinewood), Terminate (cardboard), Advance - untreated (Adv. untreated rubberwood), Advance - full cell treatment (Adv. full cell rubberwood), Advance - spot treatment (Adv. spot rubberwood) and LNEC (*Pinus pinaster*). FirstLine (pinewood) had no mass loss.

Termites were not detected in Advance (powder cellulose + aspen) monitoring stations, while in FirstLine and Advance with full cell treated rubberwood monitoring stations they were often detected (Table 1).

Table 1. Termite presence (n=1) in termite monitoring stations during monitoring period. FirstLine (pinewood), Terminate (cardboard), Advance - untreated (Adv. untreated rubberwood), Advance - full cell treatment (Adv. full cell rubberwood), Advance - spot treatment (Adv. spot rubberwood) and LNEC (*Pinus pinaster*). Termites were not detected in Advance monitoring stations with cellulose powder + aspen.

		day 0	day 7	day 14	day 21	day 28	day 35	day 42	day 49	day 56	day 63	day 70	day 77	day 84	day 91	day 98	day 105	day 112	day 145	day 179	day 232	day 260	day 295	day 355
FirstLine (pinewood)	B1																						1	1
	B2																							
	B3																				1	1	1	
	B4											1	1	1	1							1	1	
	B5																							
Terminate (cardboard)	C1																				1			1
	C2																							
	C3																							
	C4													1	1	1	1	1						
	C5																							
Adv. untreated (rubberwood)	E6		1																					1
	E7																							
	E8																							
	E9										1			1										
	E10																							
Adv. full cell (rubberwood)	E11				1	1	1	1		1	1	1												
	E12																							
	E13																							
	E14											1			1	1								
	E15																							
Adv. spot (rubberwood)	E16 UT		1		1		1																	1
	E17 UT																							
	E18 UT																							
	E19 UT																							
	E20 UT																							
LNEC (*Pinus pinaster*)	D6																							
	D9					1																		
	D11																							
	D12																							
	D13																							
	D14																							
	D15																						1	

Decay factor considered in this analysis explained much (78.8%) of the mass losses data variability. Five factors were considered significant (P<0.002): cellulose powder (F=737.69), moisture content (F=100.43) and type of bait, namely LNEC (F=590.81), FirstLine™ (F=351.65) and Advance™ (F=267.73) (Figure 5).

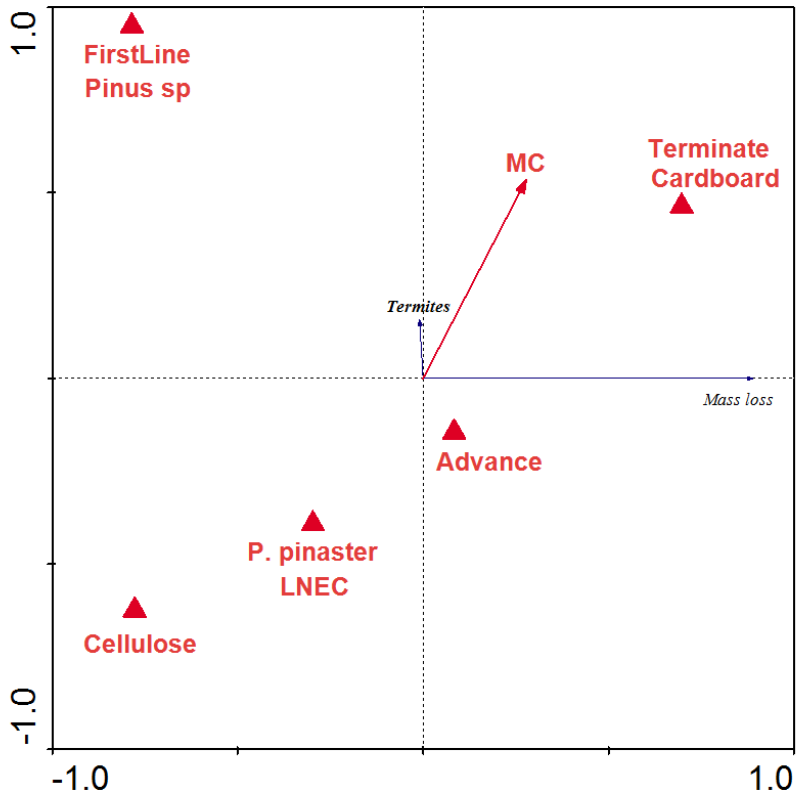

Figure 5. RDA biplot for termites' presence and mass loss of cellulosic matrix with moisture content (MC), Terminate (cardboard), Firstline (*Pinus* sp.), LNEC (*Pinus pinaster*), Advance and Cellulose powder as significant variables (P<0.002).

Mass loss seemed to be associated with the monitoring station cellulosic matrix, namely cardboard. Moisture content was higher in cardboard, which may have led to high decay mass loss values. Cellulose powder was resistant to water uptake,.

Discussion

As expected, moisture content was a significant variable. Moisture content controls chemical and biological degradation, and affects the physical and mechanical properties of wood (Baeza and Freer 2001). Subterranean termites prefer to feed on high moisture content food resources, while moulds, fungi and other biological degradation agents are active when wood moisture content is above 20% (Nakayama et al. 2005).

Tendencies in different cellulosic matrices moisture content variation curves seemed to be related to rainfall. Terminate monitoring stations' average moisture content raised above 100% after 21 days of testing. This rapid wetting was associated with degradation of the cardboard monitors. At the end of the study, mass loss values balanced between 19% and 100%. Termites where detected once in the monitoring station with total mass loss, but no termites were detected in the monitoring station which reached 98% mass loss. Other

decay factors, besides termite presence, must be involved in the severe mass loss observed in some cardboard monitoring stations, as the chemical and physical degradation of the material or the presence of other biological degradation agents, as fungi. In this study, cardboard showed to be very sensitive to degradation in the field, and thus may not be suitable for longer-term studies, since the rapid wetting and weak resistance to decay factors implies short times between visits for manual inspection in order to substitute the cellulosic matrix. Less frequent disturbance of monitoring stations is advisable since subterranean termites seem to be negatively affected by disturbance (Woodrow et al. 2008), although Gautam and Henderson (2012) suggest that termites return to the food resource after disturbance. Bait design is also important to evaluate; cardboard may increase the available surface area (Evans and Gleeson 2006).

Cellulose powder showed a good performance in terms of water uptake, although in the last 100 days the moisture content increased, due to the increase in rain and maybe because the plastic cage around the cellulose powder does not allow for easy air circulation. Mass loss was null for all cellulose powder monitors except one, although some fungal attack was observed. Termites were not detected in these monitoring stations, which also included aspen wood monitors. Subterranean termites feeding preferences seem to be based on wood species, either native or exotic (Arango et al. 2008, Duarte et al. 2011); however, wood species were not varied in this test. *P. pinaster* mass loss in the LNEC stations was low (<5%) and water uptake was below 50% during most of the year, except for the last 100 days, when raining level increased. The dimensions of these monitors were smaller than the other monitors used, which may be part of the explanation for the low number of termite presences detected in LNEC monitoring stations.

FirstLine monitoring stations mass loss calculations may be explained by their design, since it was composed by two wood blocks glued to each other and attached to the monitoring station lid. This made correct weighting of wood samples difficult and may have led to a bias in calculations.

Advance plastic cages were used for two types of monitoring stations: with cellulose powder and aspen, and with treated or untreated rubberwood as cellulosic matrices. The response of these two types of monitoring stations to decay factors was different, for example, aspen showed a high water uptake while rubberwood monitors maintained their average moisture content values below 40%. Termite visits were not detected in cellulose powder + aspen monitors, while for rubberwood monitors their presence was observed. Rubberwood was considered, together with maritime pine, a preferred food source by subterranean termites, as suggested by laboratory studies (Duarte et al. 2011). Rubberwood could be a good choice for monitoring stations for subterranean termites since it showed a good resistance to decay factors, and was preferred by subterranean termites when alternative food sources were available, which is a key factor for a good monitor matrix (Jones 1993, Evans and Gleeson 2006, Lenz et al. 2009).

The use of borate based treatments in order to prevent decay was not considered significant in this study, because the performance of treated and untreated rubberwood monitors was similar regarding mass loss and moisture content values. However, termite presence was more consistent in full cell treated rubberwood monitors. Low toxicity borate treatments did not caused repellence to termites, as reported by other authors (Campore and Grace 2007, Gentz and Grace, 2008).

Resistance to decay factors, termites feeding preferences and monitoring stations design are key features for the optimization of monitoring stations, together with active ingredients and the knowledge about termites foraging behaviour (Su and Scheffrahn 1988, Nakayama et al. 2005, Lenz et al 2009). These features may need to be adjusted to termite species, geographical and meteorological characteristics (Cornelius and Osbrink 2010).

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References

Arango, R., Green III, F., Hintz, K., Lebow, P. and Miller, R. 2008. Natural durability of tropical and native woods against termite damage by *Reticulitermes flavipes* (Kollar). International Biodeterioration & Biodegradation. 57: 146-150.

Baeza, J. and Freer, J. 2001. Chemical characterization of wood and its components. In Hon, D. and Shiraishi, N. Wood and Cellulosic Chemistry. 2nd edition. pp275-384. Marcel Dekker, Inc. New York. United States of America.

Cabrera, B. and Thoms, E. 2006. Versatility of baits containing noviflumuron for control of structural infestations of Formosan subterranean termites (Isoptera: Rhinotermitidae). Florida Entomologist. 89(1): 20-31.

Campora, C. and Grace, J. 2007. Behavioral response of the Formosan subterranean termite to borate-treated wood. Proceedings of the Hawaiian Entomological Society. 39: 127-137.

Cornelius, M. and Osbrink, W. 2010. Effect of soil type and moisture availability on the foraging behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology. 103(3): 799-807.

Duarte, S., Ricart, M. and Nunes, L. 2011. Preferências alimentares de *Reticulitermes grassei* (Clément) (Isoptera: Rhinotermitidae) relativamente a diferentes espécies de madeira. CIMAD11 - 1º Congresso Ibero-LatinoAmericano da Madeira na Construção, 7-9/06/2011. Coimbra. Portugal.

Evans, T. and Gleeson, P. 2006. The effect of bait design on bait consumption in termites (Isoptera: Rhinotermitidae). Bulletin of Entomological Research. 96: 85-90.

Gautam, B. and Henderson, G. 2012. Escape behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in response to disturbance. Journal of Insect Behavior. 25: 70-79.

Gentz, M. and Grace, J. 2008. The response and recovery of the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) from sublethal boron exposures. International Journal of Pest Management. 55(1): 63-67.

Jones, S. 1993. Effect of a decay fungus on subterranean termite (Isoptera: Rhinotermitidae) response to bait toxicant treated wood. In Wildey, K. and Robinson, H. (eds.) Proceedings of the First International Conference on Urban Pests. Pp 199-206.

Lenz, M., Kard, B., Evans, T., Mauldin, J., Etheridge, J. and Abbey, H. 2009. Differential use of identical food resources by *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) in two types of habitats. Environmental Entomology. 38(1): 35-42.

Nakayama, T., Yoshimura, T. and Imamura, Y. 2005. Feeding activities of *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) as affected by moisture content of wood. Journal of Wood Science. 51: 60-65.

Su, N. and Scheffrahn, R. 1988. Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiology. 14(2): 353-359.

Su, N. and Scheffrahn, R. 2000. Termites as pests of buildings. In Abe, T., Bignell, D. and Higashi, M. (Eds.) Termites: Evolution, Sociality, Symbioses, Ecology. Pp 437-453. Kluwer Academic Publishers. Dordrecht. Netherlands.

Su, N., Ban, P. and Scheffrahn, R. 2002. Control of subterranean termite populations at San Cristóbal and El Morro, San Juan National Historic Site. Journal of Cultural Heritage. 3: 217-225.

TerBraak, C. J. F, Smilauer, P., 2002. CANOCO reference manual and user's guide to Canoco for Windows: software for canonical community ordination (version 4.5). Microcomputer Power. Ithaca, New York. 352 pp.

Verma, M., Sharma, S. and Prasad, R. 2009. Biological alternatives for termite control: a review. International Biodeterioration & Biodegradation. 63: 959-972.

Woodrow, R., Shelton, T., Oshiro, R., Grace, J. and Wagner, T. 2008. Effects of disturbance-induced trauma on foraging by subterranean termites (Isoptera: Rhinotermitidae). Sociobiology. 52(1): 107-118.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Assessment of decay risk of airborne wood-decay fungi

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Assessment of decay risk of airborne wood-decay fungi

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ABSTRACT

The decay risk of airborne wood-decay fungi was investigated by using an air sampler. Japanese cedar disks measuring about 8 cm in diameter and 3 mm in thickness with moisture content at about 100 % were placed in a "BIOSAMP" air sampler and exposed to 1000 liters of air. Air sampling was carried out from June to September at the same sampling site in Tsukuba, Japan. The exposed disks were then incubated for 16-week in a damp container kept at 26 ± 2°C. During the incubation period, wood mass loss ranged from -15 mg to 807 mg with a mean mass loss of 244 mg. Factors affecting mass loss were explored. Wood moisture content and ratio of heartwood area proved to be significant factors. In addition, five weather factors were found to influence mean mass loss. Disks that were sampled on a cloudy day showed significantly higher mean mass loss compared to those sampled on a shiny day.

Filamentous fungi grown on the disks during 16-week incubation were subcultured to investigate the relationship between the taxa of airborne fungi and the decay risk. The subcultured fungi were isolated and DNA extracted from each isolate was amplified with the primers ITS4/ITS5. The DNA sequences of the amplified products were determined and compared to the sequence data of GenBank to determine the species or genus according to a BLAST search. This search revealed that the isolate consisted of 5 major taxa, namely *Bjerkandera* sp., *Phanerochaete* sp. (A), *Phanerochaete* sp. (B), *Polyporales* sp. *Polyporus arcularius*, and 6 minor ones. Statistical analysis revealed that the disks attached by *Phanerochaete* spp. or *Polyporales* sp. showed higher mean mass loss. It is concluded that, under these experimental conditions, related species of *P. sordida* play an important role in increasing the decay risk caused by airborne wood-decay fungi.

Keywords: decay risk; airborne fungi; air-sampler; identification; white-rot

1. INTRODUCTION

Wooden structures such as guardrails, noise barriers and bridges can be considered as a carbon sink (Kobayashi 2005, Tsunetsugu and Tonosaki 2010, Kayo *et al*. 2011). However, they are also a source of carbon dioxide emissions. Therefore, it is very important to prolong the service life of wooden structures and delay carbon dioxide gas emissions. One possible method for retarding gas emissions is through preservative treatments (Deroubaix 2008), and many investigative studies on preservatives and preservative treatments have been conducted (Hwang *et al*. 2007, Miyauchi *et al*. 2009, Momohara *et al*. 2009).

On the other hand, research on the factors that accelerate carbon dioxide gas emission from wooden structures is also important, and has revealed that one of the most important biological factors for accelerating emissions is wood-decay fungi (Hunt and Garratt 1967, Zabel and

Morrell 1992). Many studies have been conducted to investigate wood-decay fungi and the decay phenomenon, and have revealed that the majority of wood-decay fungi are classified as Basidiomycota (Zabel and Morrell 1992), which form basidia that produce basidiospores. Basidiospores released from the basidia are transferred to wood by wind, water and insects (Kramer 1982, Agrios 1997). Fungal hyphae grow from the basidiospores and degrade the wood cell-wall components with enzymes and/or low-molecular-weight compounds (Agrios 1997, Eriksson *et al*. 1990). Therefore, it was assumed that the decay risk of wood used for aboveground application might be affected by the number of living spores attached to the wood surface and the ability of their hyphae to degrade the wood-cell-wall components.

The decay risk for aboveground applications was investigated through various methods, such as the lap joint test, L-joint test and double-layer test (Råberg *et al*. 2005). However, since these studies required that wood samples be exposed to outdoor air for several months or years, the estimated decay risk was actually the mean decay risk for the entire exposure period. Attempts were also made to trap wood-decay fungi by using potato dextrose agar plates (Toyoumi *et al*. 2009). However, these experiments looked not efficient for estimating decay risk because additional experiments were required to evaluate the wood-decay activity of the trapped fungi.

To overcome these problems, a novel method was devised utilizing a wood disk and an air sampler. The method has several advantages for estimating decay risk in a certain amount of air during a specific period. In this paper, we explain the process of estimating the decay risk, and discuss factors affecting the decay risk.

2. EXPERIMENTAL METHODS

2.1 Sampling and incubation procedure

Two hundred five Japanese cedar (*Cryptomeria japonica*) disks about 3 mm thick were prepared from logs about 8 cm in diameter. The disks were dried at 60°C for 1 day and then weighed to determine their initial mass (M0). Their thickness, diameter and heartwood diameter were measured. Disks were randomly placed in individual Petri dishes, and then placed in individual sealed bags for gas sterilization. Gas sterilization was carried out in an ethylene oxide gas sterilizer (Model YS-A-C64E, Yuyama Co. Ltd., Osaka, Japan) according to the product manual. Each disk was kept in its sterilized sealed bag until use. Some of the Petri dishes were weighed to determine their mean mass (Mp).

A Petri dish containing a Japanese cedar disk was taken out from a sterilized bag just before air sampling. About 6 ml of sterilized deionized water was aseptically added to the disk. The damp disk with the plate was weighed (M1) to calculate initial moisture content of the disk according to the following equation:

$$\textit{Initial moisture content}\ (\%) = \{(M1\text{-}Mp)\text{-}M0\} \div M0 \times 100 \qquad (1)$$

All air sampling was carried out at about 100 cm in height using a “BIOSAMP MBS-1000” air sampler (Midori Anzen Co. Ltd., Tokyo) at the same place on the grounds of the Forestry and Forest Products Research Institute in Tsukuba, Japan. Each air sampling was run for 10 minutes between 09:00 and 17:00 on a shiny day or cloudy day, but not on a rainy day, from June to November in 2008. The sampling procedure followed that described in the product manual except for replacing the agar medium for the damp Japanese cedar disk. Each disk exposed to 1000 liters of air was kept in a plastic plate and incubated in a damp container for 16-week at 26 ± 2 °C. In addition to these disks, four control disks without exposure to 1000 liters of air were also incubated in the container for the same period.

After the 16-week incubation period, filamentous fungi growing on the disk surface was collected and transferred to plates containing potato dextrose agar medium (DifcoTM Potato Dextrose Agar) with 100 ppm tetracycline-HCl, 50 ppm of benzothiazol and 0.05 % guaiacol.

Each disk was then dried at 60°C for 1 day, and weighed to determine its dry mass after incubation (M2). Percentage of mass loss was calculated according to the following equation:

$$Mass\ loss\ (\%) = (M2 - M0) \div M0 \times 100 \quad (2)$$

2.2 Identification of wood-decay fungi

The filamentous fungi transferred onto the plates were subcultured repeatedly until visual examination confirmed the absence of any contamination. The isolated fungi were incubated on cellophane membranes placed on potato dextrose agar plates at 25°C for 1–2 week. The cultural appearances were then checked in order to select fungal strains for further studies in accordance with the following rules:

1. Compare the culture morphology of all the strains isolated from each Japanese cedar disk.
2. Select all strains showing different cultural appearances.
3. Select one strain from all of the strains showing the same cultural appearance.

The mycelia of the selected strains were harvested from each plate by scratching the surface of the membranes with a sterilized spatula and placing them in 1.5-ml tubes. The mycelia were frozen at -80°C for more than 30 min and lyophilized. The freeze-dried mycelia were then ground to a fine powder using a sterile pipette tip.

DNA was extracted from the powder using a DNeasy extraction kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol.

The primers ITS4/ITS5 were used to amplify a portion of the ITS region (White *et al*. 1990). Each 20-µl reaction mixture contained 10 ng of template DNA (or no DNA template for a negative control), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of each primer, 2.5 mM of each dNTP, and 0.5 U Takara Ex Taq (Takara, Tokyo, Japan). The PCR condition was as follows: 94°C for 1 min, 30 cycles at 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min respectively. The PCR products were purified with MicroSpin Columns and Sephacryl S-300 (GE Healthcare, Piscataway, NJ, USA) and sequenced bidirectionally with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) to ensure accuracy. Sequences were assembled and manually edited using Bioedit and Chromas Pro (Technelysium Pty Ltd, Australia). The sequences excluding 18S and 28S ribosomal RNA region were clustered into the operational taxonomy units (OTUs) that shared 99 % or greater sequence similarity using clustalX (Jeanmougin *et al*. 1998). All sequences of representative OTUs were subjected to similarity searches against those deposited in GenBank using Blastn (Basic Local Alignment Search Tool). One representative sequence of each OTUs was deposited in GenBank (Accession No. AB638337-AB638347). When there was no exact match for a given sequence, the phylogenetic affinity of the sequence was also considered.

2.3 Collection of weather data

Weather data was collected by a C-CR1000 weather monitoring system (CLIMATEC Inc., Tokyo) set at about 300 m southwest of the sampling point.

3. RESULTS AND DISCUSSION

3.1 Advantage of using wood disks as a fungal medium

As mentioned in the Introduction, several methods were developed to estimate the decay risk for aboveground applications (Råberg *et al*. 2005). However, the decay risk estimated by these methods was the averaged decay risk through the entire testing period. Therefore, a new method was needed to estimate the decay risk in a certain amount of air during a specific period.

Detection of airborne fungi and identification of spore species using an air sampler have been well investigated in the indoor air of homes, hospitals and so on (Nardoni *et al*. 2005, Rainer *et al*. 2000). The main targets of these studies were molds, which are classified mainly as

Ascomycota and "Zygomycota". In this study, we aimed to estimate the decay risk from airborne wood-decay fungi, which are mainly classified as Basidiomycota, in 1000 liters of air by using an air sampler.
In a preliminary study, we first tried to count the number of spores of airborne wood-decay fungi in outdoor air by trapping them on a potato dextrose agar plate containing antimicrobial agents (Doi and Fujii 2007, Nagano 2001). Medium with antimicrobial agents was reportedly successfully used for counting the number of mold colonies growing on the plate (Miyazaki *et al*. 2006). However, in our preliminary study, no antimicrobial agents could be found that would allow the growth of Basidiomycota, such as *Trametes versicolor* and *Fomitopsis palustris*, and inhibit the growth of all other microorganisms, especially molds, namely *Aspergillus niger*, *Penicillium funiculosum*, *Aureobasidium pullulans*, *Gliocladium virens* and *Rhizopus oryzae* (data not shown). The preliminary study suggested that counting the number of colonies of airborne wood-decay fungi on PDA medium without interference of mold growth would be difficult. Therefore, we abandoned the use of PDA medium, and tried to adapt Japanese cedar disks as substrates that would allow the growth of wood-decay fungi and inhibit the growth of mold.
There is another merit to use Japanese cedar disks as a substrate for wood-decay fungi. Using wood disks eliminates the need for additional experiments to estimate decay activity. Wood-decay activity can be directly estimated by measuring mass loss, which reflects not only the number of living fungal spores in the sampled air but also the wood-decay activity of these fungi. Therefore, we decided to estimate the decay risk directly by air sampling of 1000 liters of air, followed by investigating the mass loss of Japanese cedar disks during the 16-week incubation period.

3.2 Appearance of wood disk and mass loss of exposed wood disk and

Typical appearance of the Japanese cedar disk after the test is shown in Fig. 1. The disk was exposed to 1000 liters of air followed by 16-week incubation period. When the sampled air did not contain spores of wood-decay fungi, the fungi did not come out on the disk surface during the incubation period. Of course, there would still be many other types of airborne fungi such as mold. Therefore, traces of mold growth were observed on the disk as black spots (Fig. 1, upper side). On the other hand, when the sampled air contained spores of wood-decay fungi, the spores attached themselves to the disk surface, and then started growing, assimilating wood components and other micro-organisms (Käärik 1974). Consequently, fungal hyphae elongated beyond the black spots and partially covered the disk surface (Fig. 1, lower side). These results indicate that Japanese cedar disks are a good substrate for screening wood-decay fungi from molds.

Fig. 1 Appearance of Japanese cedar disk after exposure to 1000 liters of air, followed by 16-week incubation

3.2 Wood disk properties affecting decay risk

Figure 2 shows importance of heartwood area on mass loss derived by air-borne wood-decay fungi. The plot of mass loss (%) against the percentage of heartwood area indicates that the mass loss decreased with an increase of heartwood area. Correlation coefficient between mass loss and percentage of heartwood area was low, but the *p*-value clearly indicates a significant correlation between them. It was revealed by laboratory decay tests and field tests that Japanese cedar heartwood is more durable than its sapwood (Momohara *et al*. 2001, Matsuoka *et al*. 1970). The results of this study showing that the percentage of heartwood area affected mass loss can be explained by the difference in durability between heartwood and sapwood of Japanese cedar.

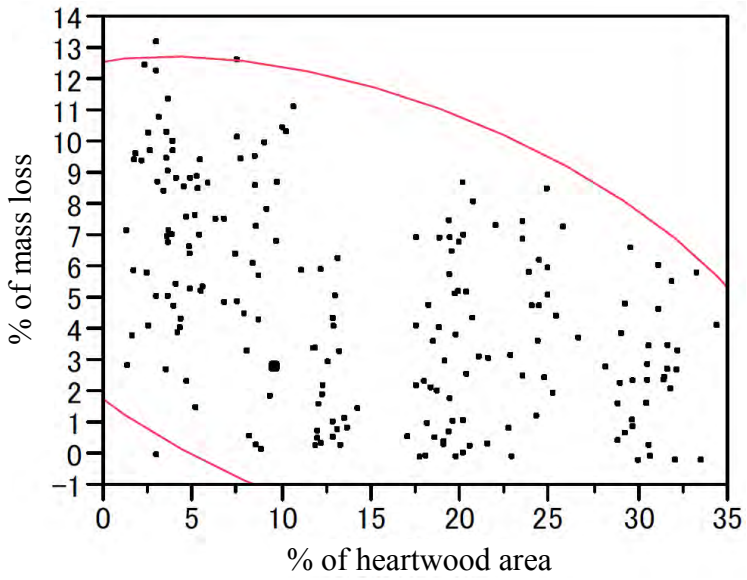

Fig. 2 Relationship between heartwood area and mass loss

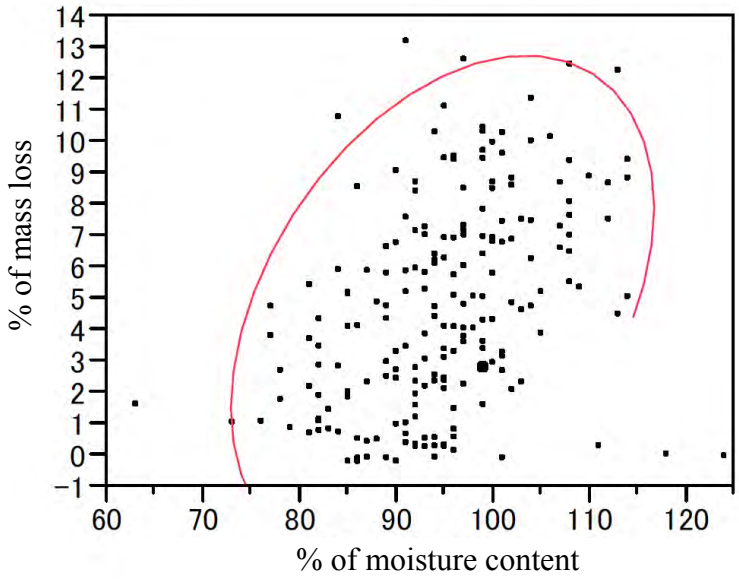

Fig. 3 Relationship between moisture content and mass loss

Another factor significantly affecting % of mass loss was moisture content of the disk during the air-sampling period. An increase of initial moisture content resulted in an increase of mass loss (%) in the case of initial moisture contents ranging from 60% to 125% (Fig. 3). Statistical analysis revealed a correlation between moisture content and mass loss. Increase in initial

moisture content probably resulted in an increase in moisture content during the incubation period. Therefore, the relationship observed in Fig. 3 may be attributed to the integral effect of high moisture content during both the sampling period and incubation period. Our next task is to estimate the effect of individual moisture content during the sampling period and an incubation period on mass loss caused by airborne decaying fungi.

3.3 Weather factors affecting decay risk

The effect of weather factors such as temperature on the % of mass loss of the Japanese cedar disks was also investigated. In order to estimate the contribution of each weather factor on mass loss (%), correlation coefficients and *p*-values were calculated between mass loss and each weather factor (Table 1). As shown in the table, relative humidity during the sampling period and five weather factors, i.e., mean relative humidity, amount of solar radiation, minimum relative humidity, daily range of temperature and daily range of relative humidity on sampling day, significantly affected mass loss. In contrast, temperature and atmospheric pressure during the sampling period, and mean, maximum and minimum temperature and atmospheric pressure as well as maximum relative humidity on the sampling day proved to have no effect on mass loss. The number of weather factors significantly affecting mass loss decreased from 5 to 4 with an increase in duration from the sampling time from 0 day to 1 day.

Table 1 Correlation between weather factor and mass loss of Japanese cedar disk

Weather factor	On the sampling day		One day before	
	Correlation coefficient	*p* -value	Correlation coefficient	*p* -value
Temperature at sampling period (ºC)	0.005	0.942	-	-
Relative humidity at sampling period (%)	**0.320**	**<0.001**	-	-
Atmospheric pressure at sampling period (hPa)	-0.014	0.846	-	-
Mean temperature (ºC)	0.079	0.267	0.093	0.191
Mean relative humidity (%)	**0.363**	**<0.001**	**0.281**	**<0.001**
Mean atmospheric pressure (hPa)	-0.031	0.658	0.000	0.995
Mean wind speed (m/s)	0.035	0.620	0.100	0.159
Amount of solar radiation (MJ/m2)	**-0.724**	**0.001**	-0.138	0.051
Sunshine duration (hrs)	-0.084	0.238	-0.148	0.036
Maximum temperature (ºC)	-0.014	0.842	0.057	0.416
Minimum temperature (ºC)	0.129	0.068	0.135	0.055
Maximum relative humidity (%)	-0.148	0.036	0.046	0.519
Minimum relative humidity (%)	**0.360**	**<0.001**	**0.226**	**0.001**
Maximum atmospheric pressure (hPa)	-0.018	0.795	-0.009	0.900
Minimum atmospheric pressure (hPa)	-0.044	0.535	0.002	0.973
Daily range of temperature (ºC)	**-0.336**	**<0.001**	-0.182	0.010
Daily range of relative humidity (%)	**-0.369**	**<0.001**	**-0.222**	**0.002**
Daily range of atmospheric pressure (hPa)	0.063	0.375	-0.022	0.751
Precipitation (mm)	0.138	0.051	**0.195**	**0.006**

After confirming that 6 weather factors on the sampling day affected the decay risk, the contribution of each significant weather factor was estimated in detail using the data from table 1. Among the 6 weather factors (Table 1), relative humidity during the sampling period and mean and minimum values on the sampling day showed a positive correlation coefficient. In other words, decay risk from airborne wood-decay fungi increased with an increase in those factors. In contrast, the decay risk of airborne fungi decreased with an increase in the amount of solar radiation, daily range of temperature and daily range of relative humidity because the correlation coefficient between these three factors and mass loss was negative. Considering these factors on

the decay risk, it is speculated that the decay risk caused by airborne wood-decay fungi was high on cloudy day. Relative humidity is commonly higher on cloudy days compared to shiny days. As solar radiation is shaded by clouds on cloudy days, any increase in temperature during the daytime is less than that on sunny days, which probably results in a smaller daily range of relative humidity. Statistical analysis revealed that there was a correlation between the daily range of relative humidity and that of temperature.

To investigate the above speculation that decay risk is likely to be higher on cloudy days, mean mass loss of the disks on cloudy days and shiny days were compared. As shown in Fig. 4, the decay risk looked higher on the cloudy day compared to the shiny day. Student's t-test using data on mass loss during the 16-week incubation period also reveals that the cloudy day's air induced a significantly higher mean mass loss on the damp disk compared to that of the shiny day. The relationship between the release of basidiospores and the weather factors have been well examined in phytopathogenic fungi (Haard and Kramer 1970, McCracken 1978). These studies also revealed that an increase in relative humidity increased the release of spores. In addition to these findings, this study revealed that the decay risk is significantly affected not by maximum relative humidity but by minimum relative humidity.

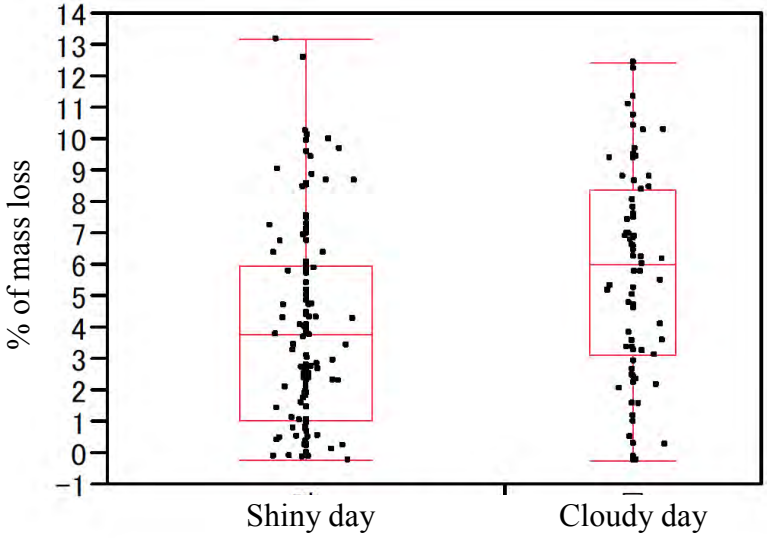

Fig. 4 Comparison of decay risk between shiny day and cloudy day

Comparing the correlation between mass loss and weather factors, the correlation coefficient and *p*-value both appear to decrease with an increase in duration from the sampling period. For example, correlation coefficient of mean relative humidity decreased from 0.363 to 0.281. These facts indicate that the influence of weather factors diminished with an increase in duration from the sampling period. However, there is one exception. Precipitation positively affected mass loss only on the day before the sampling. It was reported that rainfall prompts phytopathogenic fungi to produce and release their spores (Rockett and Kramer 1974). Wood-decay fungi are also prompted to release their basidiospores as shown by the phytopathogenic fungi.

3.4 Decay fungi identified from wood disks

The subcultured strains were first screened by cultural appearance as mentioned in Materials and Methods, and 119 strains were finally selected from 110 Japanese cedar disks.

The genus and/or species of all selected strains were investigated by BLAST searches of the GenBank using DNA sequences amplified with ITS4/ITS5 (White *et al*. 1990). The result of the BLAST searches indicated that all selected strains were divided into 11 identical taxa, namely *Bjerkandera* sp., *Ceriporia* sp., *Irpex* sp., *Phanerochaete* sp. (A), *Phanerochaete* sp. (B), *Phanerochaete* sp. (C), *Polyporales* sp., *Polyporus arcularius*, *Sistotreme* sp., *Trametes hirusuta*

and *Trametes versicolor*. Except for *Polyporus arcularius* and *Trametes* spp., 8 taxa could not be determined because there are many species with similar DNA sequences comparable to that showing the nearest match. The taxa observed in this study are similar to those found in decayed wood in above-ground applications (Wilcox and Dietz 1997, Kim *et al*. 2005).
The identified taxa firstly had their decay types determined, namely white-rot or brown-rot fungi, by a literature survey, and suggests all of them except for *Polyporales* sp. are white-rot fungi. In addition, *Polyporales* sp. is probably a white-rot fungus because the DNA sequence of the fungus shows the nearest match to that of the white-rot fungus, *Flavodon flavus*.

3.5 Relationship between taxa and decay risk

The involvement of the identified taxa in the mass loss of Japanese cedar disks was also investigated. Prior to the investigation, the efficacy of sampling and isolation of airborne wood-decay fungi was checked. We identified 11 taxa from 110 disks, despite using 201 disks for the sampling and found mycelium on 149 disks. Presumably, part of the important fungi may be lost during the subculturing processes on a PDA medium containing biocides. To check this hypothesis, mass losses of the Japanese cedar disks were compared between the disks from which the identified taxa were selected and those not selected. The mean mass loss of the disks from which the taxa were identified was about twice as larger as that of the disks from which no taxa were isolated. Statistical analysis using T-test revealed a significant difference between these two groups. In conclusion, important taxa that influence decay risk can be successfully identified in this experimental procedure even though some unimportant fungi might be missed during the subculturing processes. Therefore, further study was focused on the relationship between the mass losses of the Japanese cedar disks and the identified taxa.
The relation between the mean mass losses and the 5 major taxa and minor fungi is shown in Fig. 5. The Tukey–Kramer test reveals that the major taxa can be divided into three groups from mass loss cause by the fungi: the first group consists of *Phanerochate* sp. (A), *Polyporales* sp. and *Phanerochaete* sp. (B); the second consists of *Phanerochaete* sp. (B) and minor fungi that were observed in few disks; and the third consists of *Bjerkandera* sp., *Polyporus arcularius* and the minor fungi. In other words, the three fungi in the first group are considered to have the highest decay activity.
As shown in Table 3, the DNA sequences of the ITS region of *Phanerochaete* sp. (A) and (B) are very similar to that of *Phanerochaete sordida*, which has been well-known as a species distributed worldwide. Because of its abundance and decay activity (Fig. 5), it is concluded that related species of *P. sordida* play an important role in increasing the decay risk caused by airborne wood-decay fungi under these experimental conditions.
It is concluded that a combination of the air-sampling on wood disk and the following incubation and DNA sequence analysis discussed here is a useful method to reveal fungi contributing to wood-decay in above-ground applications and the relationships between such contributing fungi and other conditions such as weather factors (Momohara *et al*. 2010, Momohara *et al*. 2012).

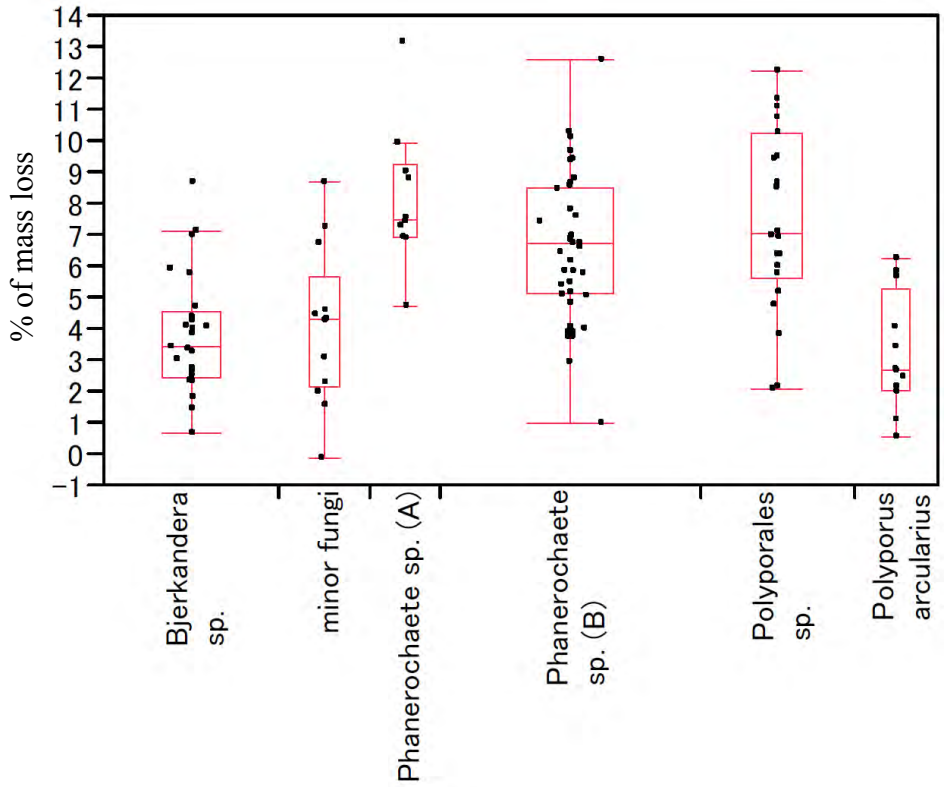

Fig. 5 Relationship between identified taxa and decay risk

4. ACKNOWLEDGEMENT

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5. REFERENCES

Agrios GN (1997): Wood Rots and Decays Caused by Basidiomycetes, In *Plant Pathology (4th edition)*, pp. 399-406, Academic Press, San Diego

Deroubaix G (2008): Wood protection, a tool for climate change mitigation?, *IRG/WP*, 08-50257

Doi S, Fujii Y (2007): Precise diagnosis of decay (in Japanese). In *Committee on diagnosis of housing bio-deterioration held in JWPA* (ed) Diagnosis manual of decay and termite damage in house. pp. 135-145

Eriksson KEL, Blanchette RA, Ander P (1990): Morphological Aspects of Wood Degradation by Fungi and Bacteria. In *Microbial and Enzymatic Degradation of Wood and Wood Components*, pp. 1-87, Springer-Verlag, Berlin

Haard RT, Kramer CL (1970): Periodicity of spore discharge in the Hymenomycetes. *Mycologia*, **62**, 1145-1169

Hunt GM, Garratt GA (1967): Agencies of wood deterioration. In *Wood Preservation*, pp. 21-46, McGraw-Hill, New York

Hwang WJ, Kartal SN, Imamura Y, Tsunoda K, Shinoda Katsumi (2007): Comparative effectiveness of two alkylammonium compounds as wood preservatives. *J Wood Sci*, **53**, 332-338

Jeanmougin F, Tompson JD, Gouy M, Higgins DG, Gibson TJ (1998): Multiple sequence alignment with Clustal X. *Trend Biochem Sci*, **23**, 403-405

Käärik A (1974): Decomposition of wood. In *Biology of plant litter decomposition*. pp 129-174, Academic press, New York

Kayo C, Hashimoto S, Numata A, Hamada M (2011): Reductions in greenhouse gas emissions by using wood to protect against soil liquefaction. *J Wood Sci*, **57**, 234-240

Kim G-H, Lim Y, Song Y-S, Kim J-J (2005): Decay fungi from playground wood products in service using 288 rONA sequence analysis. *Holzforschung*, **59**, 459-466

Kobayashi N (2005) Wood resources and climate change. In *Proceedings of the International Symposium on Wood Science and Technology*, Yokohama, 1O01

Kramer CL (1982) Production, release and dispersal of basidiospores. In *Decomposer basidiomycetes: their biology and ecology* (ed) Frankland JC, Hedger JN, Swift MJ, pp. 33-49, Cambridge University Press, Cambridge

Matsuoka S, Amemiya S, Shoji Y, Inoue M, Abe H, Naito K (1970): Stake test at Asakawa Experiment Forest (3) – The natural durability of some woods (in Japanese). *Bull Gov For Exp Sta*, **232**, 109-135

McCracken FI (1978): Spore release of some decay fungi of southern hardwoods. *Can J Bot*, **56**, 426-431

Miyauchi T, Mori M, Imamura Y (2009): Leaching characteristics of homologues of benzalkonium chloride from wood treated with ammoniacal copper quaternary wood preservative. *J Wood Sci*, **54**, 225-232

Miyazaki K, Yamashita K, Kawabata Y, Nitta T (2006): The study on the component of medium for researching on air borne fungi in mushroom cultivation houses (in Japanese). *Kyusyu J For Res*, **59**, 275-276

Momohara I, Yamamoto K (2001): Evaluation of wood durability by laboratory test - A new equation to predict wood durability. *IRG/WP*, 01-20230

Momohara I, Saito S, Ohmura W, Kiguchi M (2009): Effect of drying method as a pre-treatment on CUAZ preservative impregnation in Japanese cedar log. *J Wood Sci*, **55**, 441-445

Momohara I, Ota Y, Nishimura T (2010): Assessment of decay risk of airborne wood-decay fungi. *J Wood Sci*, **56**, 250-255

Momohara I, Ota Y, Sotome K, Nishimura T (2012): Assessment of decay risk of airborne wood-decay fungi II: relation between isolated fungi and decay risk. *J Wood Sci*, **58**, 174-179

Nagano Y (2001): Fungus cellar testing as an evaluation method for performance of treated timber in ground contact. *IRG/WP*, 01-20227

Nardoni S, Mancianti F, Sgorbini M, Taccini F, Corazza M (2005): Identification and seasonal distribution of airborne fungi in three horse stables in Italy. *Mycopathologia*, **160**, 29-34

Råberg U, Edlund ML, Terziev N, Land CJ (2005): Testing and evaluation of natural durability of wood in above ground conditions in Europe – an overview. *J Wood Sci*, **51**, 429-440

Rainer J, Peintner U, Pöder R (2000): Biodiversity and concentration of airborne fungi in a hospital environment. *Mycopathologia*, **149**, 87-97

Rockett TR, Kramer CL (1974): Periodicity and total spore production by lignicolous basidiomycetes. *Mycologia*, **66**, 817-829

Toyoumi A, Horisawa S, Yoshimura T, Doi S, Imamura Y (2009): The effect of different foundation systems against fungal flora in the crawl space of a new wooden Japanese house. *IRG/WP*, 09-10700

Tsunetsugu Y, Tonosaki M (2010): Quantitative estimation of carbon removal effects due to wood utilization up to 2050 in Japan: effects from carbon storage and substitution of fossil fuels by harvested wood products. *J Wood Sci*, **56**, 339-344

White TJ, Bruns T, Lee S, Taylor J (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and application* (ed) Innis MA, Gelfand GH, Sninsky JJ, White TJ, pp. 315-322, Academic Press, San Diego

Wilcox W, Dietz M (1997): Fungi causing above-ground Wood Decay in Structures in California. *Wood Fiber Sci*, **29**, 291-298

Zabel RA, Morrell JJ (1992): Wood Deterioration Agents, In *Wood Microbiology – Decay and Its Prevention*. pp. 21-51, Academic Press, California

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Classifying white rot decay resistance of some hardwoods from Sarawak and Peninsular Malaysia and correlations with their tropical in-ground durability

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Classifying white rot decay resistance of some hardwoods from Sarawak and Peninsular Malaysia and correlations with their tropical in-ground durability

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ABSTRACT

White rot wood decay under Malaysian (humid tropical) terrestrial conditions pose more serious threats to the in-ground service life of hardwoods than other common fungal decay types. A study is made on decay resistance variation for a total combined list of 30 Peninsular Malaysian and Sarawak timber species (plus 6 exotic reference temperate commercial woods for comparison) using the laboratory soil-block decay test method of ASTM D 2017, challenged with a representative virulent Malaysian white rot Basidiomycete *Pycnoporus sanguineus*. Results showed that *Hevea brasiliensis* (rubberwood) suffered the most severe wood decay with average percentage mass loss of 43.9%, and regarded as non-durable. On the other scale, there was expectedly negligible decay of the most durable species *Eusideroxylon zwageri* (belian) heartwood with mean mass loss of only 0.7 %. The remaining species varies between non-durability and high decay durability, but mainly moderately durable on the American ASTM 2017 and European EN350-1 decay resistance classification scales. The decay test findings were weakly correlated with recent Malaysian stake test results. Comparative variation of the white rot decay resistance among the timber species will augment the existing pool of information on wood quality classifications of some tropical timbers that are currently sought by the international timber trade, as well as detecting promising relatively decay resistant lesser-utilised species, that the international forest products trade may also be inclined to utilize in addition to the traditional commercial Malaysian species that are now in limited supplies.

Keywords: Decay resistance, natural durability, decay test, durability classification, ASTM D 2017, white rot, *Pycnoporus sanguineus,* Malaysian timbers

INTRODUCTION

Tropical timber species contributed a lot to our economic growth as we provide timber sources to both the local and world markets. However, timbers are at risk to attack by microorganisms, for instance by wood decaying fungi and wood destroying termites, marine borers and other insects (Stirling 2009, Wong *et al* 2005). Hence natural durability is an important factor to analyze wood quality (Yamamoto & Hong 1994) of species to be applied in particular regions. In more temperate climes durability as expressed by decay resistance guides the end-use of a wood, while in tropical climes the additional threats of termites cannot be avoided when selecting suitable wood products. Decay resistance of wood has been shown to be associated with the amount of extractives content in the heartwood (Scheffer & Cowling 1966, Wong *et al* 1983, 1984, Yamamoto & Hong 1994). The heartwood of some tropical hardwoods can exhibit high natural durability because it has low moisture content, lower diffusion rate and blocking cell cavities and nano-porous walls by extractives, and perhaps infiltration of tyloses in vessels (Panshin & de Zeeuw 1980). Decay resistance could also be influenced by wood density (eg. Wong *et al* 1983, Wong & Ling 2009), and in tropical species the influence of wood density is largely due to extractives contents (Haygreen & Bowyer, 1996, Ona *et al*. 1997).

To offset declining quantities of popular commercial Malaysian/Sarawak species where their natural durability is recognized (Ling & Wong 2007), studies on the decay durability of the relatively abundant lesser-utilised species are urgently needed. Information on decay resistance of such woods would be particularly significant for woods exposed to cold temperate climes where fungal decay severity usually dictates the natural durability of a species. A comparison of 2 well established laboratory decay resistance classification methods (ASTM, EN) is also timely in order to improve the durability classification of Malaysian hardwoods against white rot decay, as hardwoods are generally more susceptible to white rot than brown rot fungi (Scheffer 1964). This paper highlights the results of a laboratory evaluation of white rot decay resistance variations among 36 timbers, according to accepted international decay test methodology and their differing durability rating criteria, and the results compared with the invaluable in-ground decay durability data of wood species from Sarawak (Wong & Ling 2009).

MATERIALS AND METHODS

Wood material

A total of 36 wood species combining of Malaysian timber and temperate commercial wood species were collected from the plantation forest, sawmills, wood collections in Sarawak Forestry Corporation, and Wood Biodeterioration and Protection Laboratory of Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. The wood materials obtained from the forests came from mature trees of 1.3 m above ground (DBH). The tree age (wood age) samplings were not considered as the intention of the project was to provide broad inter-species comparisons from a wide range of timber species, and that the project was constrained by time and resources of a postgraduate (Master) research degree. A list of wood species selected for study is shown in **Table 1**.

Table 1: List of timber species studied

No.	Origin	Local Name	Botanical Name	Family	Heartwood
1	Sar	Acacia	*Acacia mangium*	Fabaceae	√
2	PM	Hoop pine	*Araucaria cunninghamii*	Araucariaceae	?
3	DK	European beech	*Fagus sylvatica*	Fagaceae	x
4	PM	Kedondong	*Canarium* spp.	Burseraceae	√
5	PM	Ubah	*Eugenia* spp.	Myrtaceae	√
6	Sar	Kelempayan	*Neolamarckia cadamba*	Rubiaceae	x
7	PM	Kempas	*Koompassia malaccensis*	Fabaceae	√
8	PM	Keruing	*Dipterocarpus* spp.	Dipterocarpaceae	√
9	Sar	Medang lui kasar	*Alseodaphne insignis*	Lauraceae	√
10	PM	Mempening	*Lithocarpus* spp.	Lauraceae	√
11	PM	Pauh Kijang	*Irvingia malayana*	Simoroubaceae	√
12	PM	Perah	*Elateriospermum tapos*	Euphorbiaceae	√
13	Sar	Rubberwood	*Hevea brasiliensis*	Euphorbiaceae	x
14	DK	Scots Pine	*Pinus sylvestris*	Pinaceae	x
15	PM	Sena, Angsana	*Pterocarpus indicus*	Fabaceae	x
16	Sar	Senumpul	*Ryparosa hullettii*	Flacourtiaceae	x
17	Sar	Terbulan	*Endospermum malaccensis*	Euphorbiaceae	x
18	Sar	Engkabang jantong	*Shorea macrophylla*	Dipterocarpaceae	x
19	NZ	Radiata pine	*Pinus radiata*	Pinaceae	x
20	Aus	Mountain ash	*Eucalyptus regnans*	Myrtaceae	√
21	Sar	Kapur bukit	*Dryobalanops beccarii*	Dipterocarpaceae	√
22	Sar	Tampoi	*Baccaurea polyneura*	Euphorbiaceae	√
23	Sar	Plajau	*Pentaspadon motleyi*	Anacardiaceae	√
24	Sar	Meranti merah	*Shorea carapae*	Dipterocarpaceae	√
25	Sar	Selangan batu kuning	*Shorea flava*	Dipterocarpaceae	√
26	Sar	Asem embang	*Mangifera pajang*	Anacardiaceae	√
27	Sar	Mergasing	*Mesua macrantha*	Guttiferae	√
28	Sar	Lun siput daun besar	*Shorea iliasii*	Dipterocarpaceae	√
29	Sar	Bindang	*Agathis borneensis*	Araucariaceae	√
30	Myr	Teak	*Tectona grandis*	Verbenaceae	√
31	US	Douglas Fir	*Pseudotsuga menziesii*	Pinaceae	√
32	PM	Bitis	*Madhuca utilis*	Sapotaceae	√
33	Sar	Mata ulat	*Kokoona littoralis*	Dipterocarpaceae	√
34	Sar	Belian	*Eusideroxylon zwageri*	Lauraceae	√
35	DK	European Oak	*Quercus robur*	Fagaceae	√
36	Sar	Sepetir	*Sindora* spp.	Fabaceae	√

Notes: Origin**:** Sar (Sarawak), PM (Peninsular Malaysia), US (United States), Myr (Myanmar), Aus (Australia), DK (Denmark) and NZ (New Zealand)

Decay testing procedures

Six replicates of wood blocks for each 36 timber species were weighed oven dry (105°C) and subjected to decay using the soil- block method according to the general method of ASTM D 2017 (ASTM 1981, Wong *et al* 1983, Wong 2006, Venas & Wong 2008) by using white rot fungus (*Pycnoporus sanguineus)*. Each 300-ml screw-capped glass jar, containing 100 ml moist garden soil with 130 % of its water holding

capacity and layered with 2% malt solution soaked filter papers as feeder strips was inoculated with 14-day-old mycelium of *P. sanguineus.* Decay susceptibility of the wood blocks was expressed in percent (%) mass loss of before-test oven dry (105^{o}C) weight after 16 weeks incubation period, which was the duration taken for the most decay susceptible rubberwood inspection blocks to reach about 60% mass loss as a basis to terminate the decay test. The EN decay resistance index (x) was computed as the ratio between the average corrected mass loss of test species and the average mass loss of the most decay-susceptible species as a reference (EN 1994).

Classification of natural durability

The timber species were classified into four natural durability classes as shown in **Table 2** according to ASTM D2017 and EN350-1 classification scale.

Table 2: Classes of natural durability of wood to fungal attack using laboratory tests based on ASTM D- 2017 and EN350- 1

Durability class	Description	Average mass loss (%) **ASTM D-2017**	Description	Resistance Index (x) **EN 350-1**
1	Highly resistant	0 - 10	Very durable	$x \leq 0.15$
2	Resistant	11- 24	Durable	$x > 0.15$ but ≤ 0.30
3	Moderately resistant	25- 44	Moderately durable	$x > 0.30$ but ≤ 0.60
4	Slightly resistant/ Non resistant	Above 45	Slightly durable/ not durable	$x > 0.60$

Notes: x = Average corrected mass loss of test specimens / Average mass loss of most decay-susceptible species

RESULTS AND DISCUSSIONS

The percentage mass loss of overall timber species combining the Malaysian and temperate timber species ranged from 0.7 % to 43.9 % when exposed to white rot, *P. sanguineus* as summarized in **Table 4**. As shown in **Table 3**, there were significant differences in percent mass losses of 36 wood species (F-value: 113.2, sig. 0.05) when the timber were subjected to decay by *P. sanguineus*. Most severe decay mass loss of 43.9 % occurred in rubberwood (*Hevea brasiliensis*). In addition, mata ulat (*Kokoona littoralis*) and kelempayan (*Neolamarckia cadamba*), also had relatively high percentage mass loss of 39.5 % and 34.4 % respectively. These species can be regarded as moderately resistant.

However, negligible decay rate was observed in belian wood (*Eusideroxylon zwageri*) with the percentage mass loss of only 0.7 % therefore can be classified as very durable. The remaining timber species; teak (*Tectona grandis*, mass loss 1.8%), bitis (*Madhuca utilis*, 1.2%), sepetir (*Sindora* spp., 5.2%), bindang (*Agathis borneensi*s, 1.4%), lun siput daun besar (*Shorea iliasii*, 1.9%), mergasing (*Mesua macrantha*, 1.7%), selangan batu kuning (*Shorea flava*, 0.8%), meranti merah (*Shorea carapace*, 1.9%), kapur bukit (*Dryobalanops beccarii*, 3.2%), tampoi (*Baccaurea polyneura*,

5.5%), plajau (*Pentaspadon motley*, 4.0%), engkabang jantong (*Shorea macrophylla*, 6.8%), senumpul (*Ryparosa hullettii*, 0.9%), pauh kijang (*Irvingia malayana*, 7.5%), mempening (*Lithocarpus* spp., 6.5%), kempas (*Koompassia malaccensis*, 1.6%) and ubah (*Eugenia* sp., 6.3%) were also listed in high resistance class against the white rot fungus.

Other timber species classified as resistant to the attack by *P. sanguineus* were acacia (*Acacia mangium*, 16.7%), hoop pine (*Araucaria cunninghamii*, 13.3%), medang lui kasar (*Alseodaphne insignis*, 11.3%), perah (*Elateriospermum tapos*, 12.4%), sena (*Pterocarpus indicus*, 15.5%), terbulan (*Endospermum malaccensis*, 12.6%) and asam embang (*Mangifera pajang*, 11.9%).

Temperate wood species such as European beech (*Fagus sylvatica*, 10%), Scots pine (*Pinus sylvetris*, 6.3%), radiata pine (*Pinus radiate*, 1.6%), mountain ash (*Eucalyptus regnans*, 10.4%), European oak (*Quercus* robur, 9.5%) and Douglas fir (*Pseudotsuga menziesii*, 4%) were considered to have high resistance to the white rot fungus.

Table 3: ANOVA for mass loss of the 36 timber species subjected to decay induced by *Pynoporus sanguineus*

Source	**DF**	**Seq SS**	**Adj MS**	**F**	**P**
Species	35	22388.10	639.66	113.2	0.000*
Error	180	1017.50	5.65		
Total	215	23405.60			

*) Significant different at 5% level

Table 4: Mass loss (%) and white rot decay resistance classes of 36 timber species according to ASTM D2017 and EN350-1 evaluation criteria

Local name	**Scientific name**	**Mass loss (%)**	**Resistance Index (*x*)**	**Resistance class**	
				ASTM	**EN**
Acacia	*Acacia mangium*	16.7 *o* (2.7)	0.38	2	3
Hoop pine	*Araucaria cunninghamii*	13.3 *lm* (1.7)	0.30	2	2
European beech	*Fagus sylvatica*	10.0 *hijk* (0.7)	0.23	1	2
Kedondong	*Canarium* spp.	9.2 *fghij* (0.6)	0.21	1	2
Ubah	*Eugenia* spp.	6.3 *e* (1.6)	0.14	1	1
Kelempayan	*Neolamarckia cadamba*	34.4 *p* (5.7)	0.78	3	4
Kempas	*Koompassia malaccensis*	1.6 *bc* (0.7)	0.04	1	1
Keruing	*Dipterocarpus* spp.	9.1 *fghi* (0.8)	0.21	1	2
Medang lui kasar	*Alseodaphne insignis*	11.3 *ijkl* (2.8)	0.26	2	2

Mempening	*Lithocarpus* spp.	6.5 *ef* (2.4)	0.15	1	1
Pauh Kijang	*Irvingia malayana*	7.5 *efgh* (1.4)	0.17	1	2
Perah	*Elateriospermum tapos*	12.4 *kl* (1.8)	0.28	2	2
Rubberwood	*Hevea brasiliensis*	43.9 *r* (5.7)	1.00	3	4
Scots Pine	*Pinus sylvestris*	6.3 *e* (1.5)	0.14	1	1
Sena, Angsana	*Pterocarpus indicus*	15.5 *mn* (5.6)	0.35	2	3
Senumpul	*Ryparosa hullettii*	0.9 *b* (0.4)	0.02	1	1
Terbulan	*Endospermum malaccensis*	12.6 *kl* (3.9)	0.29	2	2
Engkabang jantong	*Shorea macrophylla*	6.8 *efg* (1.1)	0.15	1	1
Radiata pine	*Pinus radiata*	1.6 *bc* (0.1)	0.04	1	1
Mountain ash	*Eucalyptus regnans*	10.4 *ijkl* (2.9)	0.24	1	2
Kapur bukit	*Dryobalanops beccarii*	3.2 *bcd* (1.2)	0.07	1	1
Tampoi	*Baccaurea polyneura*	5.5 *de* (2.4)	0.13	1	1
Plajau	*Pentaspadon motleyi*	4.0 *cd* (1.7)	0.09	1	1
Meranti merah	*Shorea carapae*	1.9 *bc* (0.5)	0.04	1	1
Selangan batu kuning	*Shorea flava*	0.8 *b* (0.2)	0.02	1	1
Asem embang	*Mangifera pajang*	11.9 *jkl* (0.8)	0.27	2	2
Mergasing	*Mesua macrantha*	1.7 *bc* (0.5)	0.04	1	1
Lun siput daun besar	*Shorea iliasii*	1.9 *bc* (0.5)	0.04	1	1
Bindang	*Agathis borneensis*	1.4 *bc* (0.2)	0.03	1	1
Teak	*Tectona grandis*	1.8 *bc* (0.4)	0.04	1	1
Douglas Fir	*Pseudotsuga menziesii*	4.0 *cd* (0.5)	0.09	1	1
Bitis	*Madhuca utilis*	1.2 *b* (0.4)	0.03	1	1

Mata ulat	*Kokoona littoralis*	39.5 *q* (5.3)	0.90	3	4
Belian	*Eusideroxylon zwageri*	0.7 *a* (0.1)	0.01	1	1
European Oak	*Quercus robur*	9.5 *ghij* (1.8)	0.22	1	2
Sepetir	*Sindora* spp.	5.2 *de* (1.9)	0.12	1	1

The mean value sharing the same italized letter does not differ significantly (at 5% level)
Standard deviation value for each mean is shown in parentheses
Mean value (replication= 6) compared using LSD of 2.7

Table 5: Natural durability (decay resistance) classifications of Sarawak and Peninsular Malaysian timbers according to ASTM D2017, EN 350-1 and Sarawak stake test data (Wong & Ling 2009)

Classification scale	**Local names**			
	Class 1 (Very durable)	**Class 2 (Durable)**	**Class 3 (Moderately durable)**	**Class 4 (Non-durable)**
Present work ASTM D2017 classification	European beech, Kedondong, Ubah, Kempas, keruing, Mempening, Pauh kijang, Scots pine, Senumpul, Engkabang jantong, Radiate pine, Mountain ash, Kapur bukit, Tampoi, Plajau, Meranti merah, Selangan batu kuning, Mergasing, Lun siput daun besar, Bindang, Teak, Douglas fir, Bitis, Belian, European oak, Sepetir	Acacia, Hoop pine, Medang lui kasar, Perah, Sena, Terbulan, Asam embang	Kelempayan, Rubberwood, Mata ulat	-
Present work EN 350-1 classification	Ubah, Kempas, Mempening, Scots pine, Senumpul, Engkabang jantong, Radiata pine, Kapur bukit, Tampoi, Plajau,	Hoop pine, European beech, Kedondong, Keruing, Medang lui kasar, Pauh kijang,	Acacia, Sena	Kelempayan, Rubberwood, Mata ulat

	Meranti merah, Selangan batu kuning, Mergasing, Lun siput daun besar, Bindang, Teak, Douglas fir, Bitis, Belian, Sepetir	Perah, Terbulan, Mountain ash, Asam embang, European oak		
Tropical stake test - Wong and Ling (2009) ASTM D1758 classification	Belian, *selangan batu	*Kapur, *Medang, *Selangan batu, Teak	*Kapur, *Keruing, *Lun (yellow meranti), Plajau	Acacia, Asam embang, Bindang, *Engkabang, Kelampayan, *Keruing, *Lun (yellow meranti), Sepetir, Ubah, Mergasing, Perah, Dark red meranti, Light red meranti

*: Different species belonging to particular genera

The evaluation of the in-ground durability of 132 Sarawak hardwood species by Wong & Ling (2009) was established by testing the wood to a combined termites and decay using the method of ASTM D-1758 where among all the species, *E. zwageri* (belian) was only reduced to mean rating 7 (moderate biodeterioration) after 26 years exposure to test site whereas many other species were destroyed after 5- 15 years. Hence belian was reaffirmed as very durable (Class 1) together with several hardwood species of penyau (*Upuna borneensis*), selangan batu (species of *Shorea*) and rhu ronang (*Gymnostoma nobile*). Several other different timber species of meranti, keruing, selangan batu, and other medium and light hardwoods showed variations in natural durability as shown in **Table 5**. Data of tropical Malaysian hardwoods by Yamamoto and Hong (1994) also had reported five class of natural durability when cengal (*Neobalonocarpus heimii*), giam (*Hopea* sp.), resak (*Vatica* sp.), rengas (Family Anacardiaceae) to have high decay resistance but nevertheless the species actually varies from highly to moderately resistant. Timber species of other heavy, medium and light hardwood as shown in **Table 5** were reported to vary from durable to perishable. The remaining wood species studied namely; rubberwood (*Hevea brasiliensis*), ramin (*Gonystylus* sp.), perupok (*Lophopetalum* sp.) and jelutong (*Dyera costulata*) were perishable (Class 5). The present results on white rot

durability of Malaysian hardwoods are especially useful to temperate applications where termite problems are minimal or absent, and also since unprotected utilized hardwoods are known to have far greater incidence of white rot than brown rot (Scheffer 1964, Wong *et al* 2005).

Of interest, the high white rot resistance in all the softwoods examined (bindang, Scots pine, Hoop pine, Douglas fir and radiata pine) can be misleading about the overall decay resistance of these softwoods, although this further supports general findings of fallen softwoods being not preferred by white rot fungi (Scheffer 1964, Wong 2006), Essentially softwoods (and hardwoods) need to be evaluated against brown rot resistance. Also, the correspondingly high white rot decay resistance of kempas supports a previous study (Seehann 1973, Wong 1988), making it yet another promising hardwood for temperate region applications. Indeed those tropical hardwoods in the Classes 1 and 2 durability groups including those lesser-utilized Malaysian species present in abundance could find markets in temperate climes outdoors on the basis of durability (and known good wood strength). The planted species *Acacia mangium* seems particularly promising as the wood resource is harvested from sustainable supplies. Here the Class 2 (Durable) to Class 3 (Moderately durable) decay resistance found on this species (**Table 5)** planted widely in Sarawak and Sabah, Malaysia, may eventually help endorse the acacia heartwood (the species has a typical narrow sapwood ring only) as among the new generation relatively durable species (besides teak heartwood) produced in tropical plantation forestry that find competitive market niches in the temperate regions as aboveground outdoor wood products.

Table 6. Correlations of durability classifications between test stake, ASTM D 2017 and EN 350- 1

Durability classification scale	Correlation Coefficient (r)	
	Stake test	ASTM D 2017
ASTM D2017	0.241	-
EN 350-1	0.311	0.925 *

(*) - correlation coefficient significant at 5% level; n=19

Overall the criteria for durability classification apparently varies between regions that use tropical wood resources, and the decay test results for white rot resistance clearly indicate that a careful selection of classification criteria is needed to satisfy questions about decay resistance of particular species under certain exposure conditions (**Table 5**). Clearly durability classifications from in-ground stake tests (Wong & Ling 2009), where decay pressures were reportedly prevalent and complex (while termites are also present here as well as other decay fungi/types), were more hazardous than the laboratory test findings using representative virulent decay fungi (present work) because many species from Sarawak stake tests were found to be of about one-fold lesser durability ratings than that of laboratory decay test results. Therefore such tropical stake test results do not appear to mimic the laboratory decay test results, hence no correlations existed between the 2 test methodologies (**Table 6**) where only 19 wood species were exposed to both the field and laboratory tests as shown in **Table 5**. When deciding between the ASTM resistance classification criteria and that

of the EN resistance index classification, the latter is perhaps a preferred choice since the decay resistance of a species is matched with the most decay susceptible species (ie. rubberwood) to harmonise the profile of decay resistance value across all the species evaluated. The EN resistance index could have wide international appeal for the forest products industry. In addition, the challenge is also to find mathematical measures of transposing durability test results from timber producing region to the timber importing region so that service life predictions of the durability of the study wood species can be estimated when used in the latter region, and this has garnered much attention lately (Van den Bulcke *et al* 2010).

It has been a long term challenge for wood quality specialists worldwide to ascertain which among the wood properties among various species, are important determinants of natural durability, in this case decay resistance, where the role of heartwood extractives feature prominently (Scheffer & Cowling 1966, Wong *et al* 2005, Kim *et al* 2006). Among tropical woods, Kim *et al*. 2006 revealed that the heartwood of the known naturally durable cengal (*Neobalonocarpus heimii*) had high amount of extractives as well as lignin which assist in the high degree of soft rot decay resistance of this species. It is previously shown that the white rot decay resistance in Australian, Malaysian and African hardwood timbers are due to the presence of polyphenolic compounds which are largely soluble in methanol (Wong *et al* 1983, 1984, Yamamoto & Hong 1994, Antwi- Basiako & Pitman 2009) whereas wood density exercised little influence on the decay resistance of the African woods studied. Some studies (Huang *et al.* 2004) reported that the extractives removed by using methanol reduced decay resistance of the wood to white rot fungi, but not brown rot fungi, thus supporting a growing body of evidence that methanolic heartwood extractives seem to be potent to decay fungi or being largely responsible for decay resistance (Wong *et al* 1983, 1984, Yamamoto & Hong 1994). Results of a correlation analysis to demonstrate that extractives contents (hot water- and methanol-soluble contents) as an indicator of white rot decay resistance among the wood species examined in this study would be reported in future.

Elsewhere however, evidence of the role of wood density on decay durability of the low-durability low density hardwood species appears compelling (Wong *et al* (1983, 1984), and for many durable tropical species, this is manifested by high heartwood extractives contents (Haygreen & Bowyer, 1996, Ona *et al*. 1997, Wong et al 2005, Wong & Ling 2009). For example, Wong and Ling (2009) also reported the in-ground natural durability of Sarawak timbers obtained by field stakes test where decay pressures prevailed showed that the natural durability is influenced by wood density where species with high density have relatively higher resistance to biological degradation. Results of a correlation analysis to explore the role of wood density and other anatomical features of wood on white rot decay resistance among the wood species examined in the present study would also be reported in future.

CONCLUSIONS

The white rot decay resistance between several hardwood and a few softwood species is emphasized in this paper. This finding shows that timber species of *E. zwageri* is an important potential and commercial timber resource due to the high durability. Conversely, species such as *H. brasiliensis* with low durability is very much

susceptible to decay fungi which greatly decreased service life. This study found that, 26 timber species were regarded as highly resistant (Class 1), 7 timber species were resistant (Class 2) and 3 species were classified as moderately resistant (Class 3) according to ASTM classification scale. However, 20 species were classified as very durable (Class 1), 11 species were durable (Class 2), 2 species were moderately durable (Class 3) and 3 species were classified as non-durable (Class 4) to white rot based on the EN resistance index classification scale. There is no correlation between the laboratory decay test and Sarawak stake test durability classification scales suggesting that additional biotic factors are operative in the field. The EN decay resistance index appears to be a much preferred criteria for wood durability classification.

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REFERENCES

Antwi- Basiako, C. and Pitman, A.J. (2009). Influence of density on the durabilities of three Ghanaian timbers. *Journal of Science and Technology*. 29(2), 36- 45.

ASTM D2017 (1981). American Society for Testing and Materials. Accelerated Laboratory Test of Natural Decay Test of Woods. ASTM D- 2017- 71. pp 612- 618.

ASTM D1758 (1996). Standard test method of evaluating wood preservatives by field tests with stakes. D1758 (approved April 10, 1996). In, 1996 *Annual Book of ASTM Standards*, Vol 04-10. The American Society for Testing of Materials.

EN 350-1 (1994). Durability of wood and wood based products - Natural durability of solid wood - part 1: Guide to principles testing and classification of the natural durability of wood. European Standards.

Haygreen, J.G. and Bowyer, J.L. (1996). *Forest Products and Wood Science: An Introduction. Third Edition.* United States, America: Iowa State University Press.

Huang, Z., Maher, K. and Amartey, S. (2004). Effects of heart wood extractives in Dahoma (*Piptadeniastrum africanum*) on decay resistance to white- and brown- rot fungi. *International Research Group on Wood Protection Document* No: IRG/WP 04-10536, 10pp.

Kim, Y.S., Singh, A.P., Wong, A.H.H., Eom, T.J., and Lee, K.H. (2006). Micromorphological and chemical characteristics of cengal (*Neobalanocarpus heimii*) heartwood decayed by soft rot. *Korean Journal of Wood Science and Technology* 34(2), 68-77.

Ling, W.C. and Wong, A.H.H. (2007). An overview of the raw material quality and demand for the wood products industry in Sarawak, with insights for future planted forests. *Proceedings of Conference on Natural Resources in the*

Tropics, 6 – 8 June 2006, Kuching, Sarawak, Published by Universiti Malaysia Sarawak, pp. 259-268.

Ona, T., Sonoda, T., Ito, K. and Shibata, M. (1997). Relationship between various extracted basic densities and wood chemical components in *Eucalyptus camaldulensis*. *Journal of Wood Science and Technology* 31(3), 205-216.

Panshin, A.J. and de Zeeuw, C. (1980). *Textbook of Wood Technology*. Fourth Edition. New York: McGraw- Hill, Inc. 722 pp.

Scheffer, T.C. (1964). Biological observations of significance for improved preservative treatment. *Holzforschung* 18, 88-94.

Scheffer, T.C. and Cowling, E.B. (1966). Natural resistance of wood to microbial deterioration. *Annual Review of Phytopathology* 4, 147-170.

Seehann, G. 1973: Zur natürlichen Dauerhaftigkeit von Kempas und Keruing gegenüber holzzerstörenden Pilzen. *Holz Roh-Werkstoff* 31, 269-272.

Stirling, R. (2009). Natural durability classification systems used around the world. *International Research Group on Wood Protection Document* No: IRG/WP 09-10694, 10pp.

Van den Bulcke, J., Wong, A.H.H, Ling, W.C, Kim, Y.S. and Van Acker, J. (2010). Improved analysis of field test data related to service life prediction of tropical wood species. *International Research Group on Wood Protection Document* No. IRG/WP 10-20458.

Venas, T.M. and Wong, A.H.H. (2008). Feasibility study on three furfurylated non-durable tropical wood species evaluated for resistance to brown, white and soft rot fungi. *International Research Group on Wood Protection Document* No: IRG/WP 08-40395, 9pp.

Wong, A.H.H. 1988: Natural decay resistance of kempas (Koompassia malaccensis) with included phloem against soft rot fungi: a laboratory evaluation. *J. Trop. For. Sci.* (2), 162-169.

Wong, A.H.H. (2006). Laboratory evaluation of soft rot resistance of non-durable lesser-known Malaysian hardwoods. *International Research Group on Wood Protection Document* No: IRG/WP 06-10582,10 pp.

Wong, A. H.H., Wilkes, J. and Heather, W.A. (1983). Influence of wood density and extractives content on the decay resistance of the heartwood of *Eucalyptus delegatensis* R. T Baker. *Journal of the Institute of Wood Science* 9, 261- 263.

Wong, A.H.H., Wilkes, J. and Heather, W.A. (1984). Inter- and intra- incremental variation in basic density, extractives content and decay resistance in *Eucalyptus delegatensis*. *Material und Organismen* 19, 141- 148.

Wong, A.H.H. and Ling, W.C. (2009). Natural durability variations of Malaysian timber from Sarawak after 26 years exposure by stake test. *International Research Group on Wood Protection Document* No: IRG/WP 09-10704, 26pp.

Wong, AHH; Kim, YS; Singh, AP; Ling, WC (2005). Natural durability of tropical species with emphasis on Malaysian hardwoods – variations and prospects. *The International Research Group on Wood Protection Document* No: IRG/WP 05-10568.

Yamamoto, K. and Hong, L.T. (1994). A Laboratory Method for Predicting the Durability of Tropical Hardwoods. *JARQ*. 28(4):2, 68- 275.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 Biology

Succession after Fire of Fungal Fruiting Bodies in Mediterranean *Pinus pinaster* Stands in Spain

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Succession after Fire of Fungal Fruiting Bodies in Mediterranean *Pinus pinaster* Stands in Spain

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ABSTRACT

In this study we present the results of a 4-year survey aimed at describing the succession of fungal communities following fire in a Mediterranean ecosystem in Northwest Spain, dominated by *Pinus pinaster* Ait. After a large wildfire in 2002, six 2 x 50 m study plots were established in burned and unburned areas corresponding to early and late succession stages. During the autumn seasons from 2003 to 2006, fruiting bodies were collected and identified. We also collected information about dry and fresh weight, the saprotrophic or mycorrhizal status and the edibility of every species. During the four-years sampling, a total of 115 fungal taxa were collected (85 in the late stage and 60 in the early stage) from which only 30 appeared along the whole succession. Mycorrhizal population not only increased the number of species from early to late stage but also shifted in composition. After fire, pyrophytic species such as *Pholiota carbonaria, Peziza violacea, Rhizopogon luteolus* and *Rhizopogon sp.* appeared. The effect of fire on fungal fruiting body's production was opposite depending on the saprotrophic or mycorrhizal status of the species: mycorrhizal decreased 6-fold, while saprotrophic increased 4-fold. Production of edible species was negatively affected by fire, decreasing significantly the potential of rural populations to harvest marketable mushrooms. The provided results can be useful to forest managers for optimization of management and harvest of these increasingly appreciated non-wood resources. Management may also prevent or alleviate stand-replacing wildfire in these Mediterranean forests.

Keywords: fungal community succession, fungal production, fire, Mediterranean ecosystem

1. INTRODUCTION

Pinus pinaster Ait. is a pyrophytic species widely distributed in the Mediterranean region which is able to form fungal associations with a high number of different fungal species. Its serotinous cones release the seeds due to the heat generated during a forest fire. Then, seeds germinate and grow rapidly as a selection pressure from fire (Dahlberg, 2002). However, pines need obligatory mycorrhizal association and the presence of active fungal propagules in a soil after fire is crucial for survival and later regeneration of seedlings (Dahlberg et al., 2001).

Pre-fire fungal communities are largely eradicated by fires and, subsequently, post-fire fungal succession is initiated. The pioneer fungal species are developed in the absence of spores typically produced during the early post fire season. Their source is usually the spore bank in the soil that has built up over time since previous fire (Claridge et al., 2009). Secondary succession beginning after fire depends on multiple factors such as initial plant species composition, fire intensity, seed bank availability, and ability of soil microbial communities to recover (Hart et al., 2005). Thus, fungi play a fundamental role in the recovery of damaged plants or dead vegetation replacement, in soil stabilization and habitat restoration (Claridge et al., 2009). Moreover, edible fungi represent an important forest economic resource, occasionally generating higher benefits than timber production (Martín-Pinto et al., 2006). These fungi also play an important succession

development role in the regeneration of *P. pinaster* stands after wildfire (Milne, 2002). Therefore, the aim of this study is to analyse fungal community succession following wildfire in a Mediterranean vegetation type dominated by *P. pinaster* in Northwest Spain.

2. EXPERIMENTAL METHODS

2.1 Study site

The analysis was carried in a Mediterranean ecosystem dominated by *P. pinaster* where a large wildfire burned 1.427 hectares in August 2002. This site is located at 750-780 m.a.s.l. in the northwest of Zamora province (NW Spain). The region has a sub-Mediterranean climate with 3 months of dry season in the summer, a mean annual rainfall of 700-750 mm and mean temperatures ranging from 14.5-15ºC. The soil is classified as Inceptisol suborder Xerept (Soil Survey Staff, 2010).

2.2 Sampling

Fruitig bodies were collected between October and December from 2003 to 2006 in twelve randomly selected *P. pinaster* stands. The study consisted of two treatments: early and late stage fungal communities after fire. Six plots of 2 x 50 m per each treatment were established. In the early stage treatment (burned area), plot distribution was designed to avoid direct interactions with roots from the adjacent unburned forested zones. Fungi were weekly collected as described by Ohenoja (1984). Following Bonet et al. (2004), sampling was carried out on Fridays to reduce error due to mushroom removals by recreational weekend collectors. Fruiting bodies were completely removed facilitating species identification and diminishing disturbance in the production calculation. Fruiting bodies were stored in the laboratory at 4ºC and processed within 24 hours after collection for identification, fresh and dry weight measurements.

2.3 Statistical analysis

An early stage period (1-4 year-old forest stands), and a late stage period (51-54 year-old stands) after fire were established. Fresh and dry weights, edibility and life strategies were analysed. Data were subjected to a Repeated Measures ANOVA analysis and means were compared by LSD Fisher Tests ($P<0.05$). STATISTICA ’08 Edition software was used for the analysis.

3. RESULTS AND DISCUSSION

3.1 Early/late stage comparison

Although the study plots are located in extremely poor, degraded and stony soils, 115 fungal taxa were collected during the four-year sampling period (Table 1) (complete list of species can be found in Gassibe et al., 2011). This result reveals a very high fungal richness, compared to the 49 taxa found by Oria-de-Rueda et al. (2010), where 50-year old reforested *P. pinaster* areas in degraded soils in Northwest Spain were studied. Even though the analysed *P. pinaster* reforested areas are located in very poor and stony soils, the high number of collected species could be due to the siliceous conditions where a high number of fungal species is frequently collected. In addition, *Cistus ladanifer* understory may favour high fungal richness conditions. These shrubs act as a bridge for many ectomycorrhizal fungi (Oria-de-Rueda et al., 2008).

Adverse effects of fire on the number of fungal species in the early stage were observed. Previous studies confirmed the decrease of many fungal species after fire (Salerni and Perini, 2004; Martín-Pinto et al., 2006). This suggests that vegetation burning affects spore production and infection potential of some fungal species (Vilarino and Arines, 1991). Subsequent loss of topsoil by erosion also reduces infectivity of fungal propagules (Rashid et al., 1997).

Table 1: Number of taxa collected from *P. pinaster* plots, classified into species functional groups.

	Mycorrhizal		Saprotrophic		Edible		Total	
Year	E	L	E	L	E	L	E	L
2003	5	21	8	11	4	17	13	32
2004	6	22	9	19	2	13	15	41
2005	9	6	12	19	2	6	21	25
2006	14	23	20	27	7	17	34	50
Total E/L	27	40	33	45	12	23	60	85
TOTAL	56		59		30		115	

E: early stage; L: late stage; (data source: Gassibe et al. 2011)

We found an overall functional groups approximated to 1:1 ratio between mycorrhizal and saprotrophic fungal species whereas previous studies reported higher percentages of mycorrhizal species (88% reported by Bonet et al. (2004)). The relatively high proportion of saprotrophic compared to mycorrhizal fungi observed in the late stage could be due to the presence of high amounts of organic matter in the forests, since decomposition rates are particularly low in the Mediterranean ecosystems, where no coincidence of high precipitation levels and high temperatures occurs (Oria-de-Rueda et al., 2010).

Post-fire species such as *Pholiota carbonaria, Peziza violacea, Rhizopogon luteolus* and *Rhizopogon* sp. were harvested in *Pinus* early stage plots. Other well-known pioneer fungal species were collected in the early stage treatment, such as *Laccaria amethystina, L. laccata*, *Mycena* spp., *Gymnopilus spectabilis* and *G. picreus*. Although *Lactarius deliciosus* or *Suillus bellini* are considered pioneer species, they were not present in the early stage treatment (1-4 years following fire). This result is in agreement with Fernández-Toirán et al. (2006) who found *L. deliciosus* to be a dominant species in 11-20-years old stand.

3.2 Production according to functional groups and edibility

High total fresh weight production (372.4 kg fw ha^{-1}) was harvested. Lower results were reported by Bonet et al., (2004) in *P. sylvestris* natural stands (60.6 kg fw ha^{-1}). The high average fresh weight observed in the late stage plots (209.95 kg fw ha^{-1}fresh weight) could have resulted from the quick growth rates obtained for this host species. Yields in the early stage treatment (162.45 kg fw ha^{-1}) were significantly lower than those observed later. The negative effect of fire on fungal production has been reported in previous studies (Cairney and Bastias, 2007).

The effect of fire on fungal fruiting bodies production was opposite depending on the saprotrophic or mycorrhizal status of the species: mycorrhizal decreased 6-fold, while saprotrophic increased 4-fold (Fig.1). This result can be explained as ectomycorrhizal fungi require more energy from the host plant and have slower growth rates and therefore, are less competitive after fire events (Torres and Honrubia, 1997), whereas spore germination of certain saprotrophic post-fire fungi is stimulated by heat (Dahlberg, 2002). Furthermore, saprotrophic growth pattern allows some of these fungal species to survive short periods of live-host absence by persisting on the dead host root systems or other organic matter (Bonello et al., 1998).

Regarding edible taxa, mushroom harvesting can be an important source of rural income, in some cases generating higher revenues than timber production (Oria-de-Rueda et al., 2010). In this study, edible taxa represented 26% of the total taxa but comprised 42.55% (158.36 kg fw ha^{-1}) of total fresh weight yield. Fresh weight production for edible species was also strongly affected by fire decreasing significantly from 32.64 kg fw ha^{-1} in the late stage to 0.34 kg fw ha^{-1} in the early stage. In the study region, *Pinus* ecosystems provide a habitat for high production of fungi including species in demand, such as *Hygrophorus gliocyclus, H. agasthomus, Suillus bellini, Tricholoma portentosum* and *Lactarius deliciosus.*

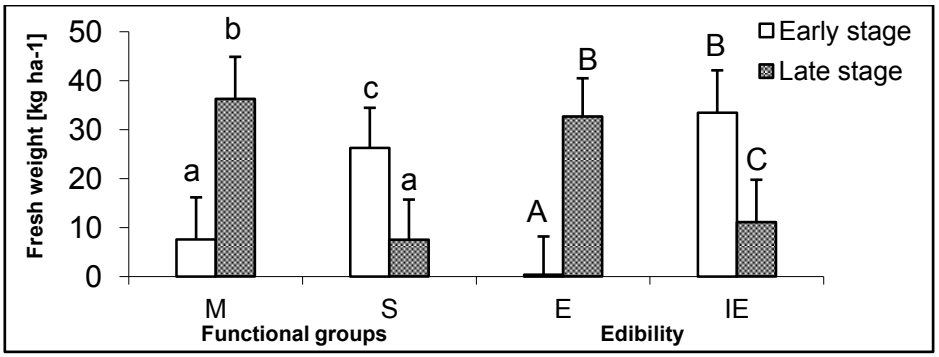

Figure 1. Fruiting bodies production [kg ha^{-1}]. M: Mycorrhizal, S: Saprotrophic, E: Edible, IE: Inedible. Mean results ± standard error of the mean. Independent comparisons were carried out within functional groups and edibility. Values with the same letter are not significantly different (Gassibe et al., 2011).

4. CONCLUSIONS

In conclusion, very high fungal richness was found in the *P. pinaster* studied forests, characterized by extremely poor and degraded soils. Following fire, fungal succession can be observed. Thus, post-fire, super-pioneer, pioneer and late stage species were found. After fire, mycorrhizal and saprotrophic fungal productions were differently affected. In this way, mycorrhizal mushroom yields increased from early to late stage and a contrary trend was found for saprotrophic taxa. Finally, edible species production was affected by fire decreasing tremendously rural population incomes in this marginal region. Adequate management in these forest stands will improve tree health and vigour, which is likely to increase the production of mycorrhizal fungi. Management may also prevent or alleviate stand-replacing wildfire in these Mediterranean forests.
Further information can be found in Gassibe et al. (2011).

5. REFERENCES

Bonello, P, Bruns, T D, Gardes, M (1998): Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens. New Phytologist* **138**, 533-542.

Bonet, J A, Fischer, C R, Colinas, C (2004): The relationship between forest age and aspect on the production of sporocarps of ectomycorrhizal fungi in *Pinus sylvestris* forests of the central Pyrenees. *Forest Ecology and Management* **203**, 157-175.

Cairney, J W G, Bastias, B A, (2007): Influences of fire on forest soil fungal communities. *Canadian Journal of Forest Research* **37**, 207-215.

Claridge, A W, Trappe, J M, Hansen, K (2009): Do fungi have a role as soil stabilizers and remediators after forest fire? *Forest Ecology and Management* **257**, 1063-1069.

Dahlberg, A (2002): Effects of fire on ectomycorrhizal fungi in Fennoscandian boreal forests. *Silva Fennica* **36**, 69-80.

Dahlberg, A, Schimmel, J, Taylor, A F S, Johannesson, H (2001): Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. *Biological Conservation* **100**, 151–161.

Fernández-Toirán, L M, Ágreda, T, Olano, J M (2006): Stand age and sampling year effect on the fungal fruit body community in *Pinus pinaster* forests in central Spain. *Canadian Journal of Botany,* **84**, 1249-1258.

Gassibe, P V, Fabero, R F, Hernández-Rodríguez, M, Oria-de-Rueda, J A, Martín-Pinto, P (2011): Fungal community succession following wildfire in a Mediterranean vegetation type dominated by *Pinus pinaster* in Northwest Spain. *Forest Ecology and Management,* **262**, 655-662.

Hart, S C, DeLuca, T H, Newman, G S, MacKenzie, M D, Boyle, S I (2005): Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *Forest Ecology and Management*, **220**, 166-184.

Martín-Pinto, P, Vaquerizo, H, Peñalver, F, Olaizola, J, Oria-de-Rueda, J A (2006): Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by *Cistus ladanifer* and *Pinus pinaster* in Spain. *Forest Ecology and Management*, **225**, 296-305.

Milne, J M, (2002): Post-fire colonization of *Cistus creticus* L. seedlings by ectomycorrhizal fungi in Aleppo pine forests in central Greece. PhD Thesis, Univesity of Edimburg.

Ohenoja, E, (1984): Fruit body production of larger fungi in Finland. 1. Introduction to the study in 1976-1978. *Annales Botanici Fennici* **21**, 349-355.

Oria-de-Rueda, J A, Hernández-Rodríguez, M, Martín-Pinto, P, Pando, V, Olaizola, J (2010): Could artificial reforestations provide as much production and diversity of fungal species as natural forest stands in marginal Mediterranean areas? *Forest Ecology and Management* **260**, 171-180.

Oria-de-Rueda, J A, Martín-Pinto, P, Olaizola, J (2008): Bolete productivity of cistaceous scrublands in northwestern Spain. *Economic Botany* **62**, 323-330.

Rashid, A, Ahmed, T, Ayub, N, Khan, A G (1997): Effect of forest fire on number, viability and post-fire re-establishment of arbuscular mycorrhizae. *Mycorrhiza* **7**, 217-220.

Salerni, E, Perini, C (2004): Experimental study for increasing productivity of *Boletus edulis* s.l. in Italy. *Forest Ecology and Management* **201**, 161-170.

Soil Survey Staff, (2010): *Keys to Soil Taxonomy*, 11th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Torres, P, Honrubia, M (1997): Changes and effects of a natural fire on ectomycorrhizal inoculum potential of soil in a *Pinus halepensis* forest. *Forest Ecology and Management* **96**, 189-196.

Vilarino, A, Arines, J (1991): Numbers and viability of vesicular-arbuscular fungal propagules in field soil samples after wildfire. *Soil Biology and Biochemistry* **23**, 1083-1087.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Recommendations for handling of mold infestation of wooden artifacts

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ABSTRACT

The presentation gives an overview of the handling of wooden artifacts with mold infestation. The causes of mold deterioration are demonstrated by investigations in a church, a historic library and a monastery in Germany. Investigated infested wooden artifacts were altars, sculptures, picture frames and sarcophagi. Important investigation methods including indoor climate measurement, material and air sampling, microscopy, lab investigations and determination of the mold species are described. The most typical mold species are discussed. Whereas various species of *Penicillium* are typical fungi, found after water injuries (condensation, leakage, flood), these fungi were not often found in wooden artifacts. Instead, for example, *Trichoderma* sp. and *Aureobasidium* sp. were frequently detected on the materials and caused discoloration. Contemporary measures for disinfection and protection of both wooden artifacts and the health of the restorers are shown by examples. Problems are discussed and possibilities for the removal of molds and material preservation by chemicals are shown.

Keywords: mold, discoloration, disinfection, protection

1. INTRODUCTION

Damages by molds at wooden artifacts are always associated with high moisture of the material and the air. So, churches and museums in medieval buildings are affected frequently because of the adverse climatic conditions caused by the architecture in connection with sporadic heating and insufficient ventilation. Occasionally, many people at church services and other events introduce moisture which may condensate on cold surfaces of walls and artifacts under some circumstances. In addition, natural events or catastrophes such as flood, fire water damage or burst pipes can lead to mold growth. Especially in the latter cases, prompt action and good cooperation between professionals are essential to prevent the expansion of the damage at the best. Because of the health risk of molds in general, an infestation cannot be tolerated. Furthermore, mold fungi can cause changes on the material like discoloration or damage of the paint by metabolism products, particularly in connection with bacteria within a biofilm (Ranalli *et al.* 2009).

For the choice of appropriate measures, there must be considered not only the aspects of cleaning and disinfection, healthy risks by mold fungi for staff, craftsmen, restorers as well as for users and visitors, but also how to prevent a new decay in the future. For the right strategy, it is important to know the occurring species, the dimensions of the affected areas, the technical possibilities, and last but not least the kind of affected substrate due to its different susceptibility to mold infestation (Weiss *et al.* 2004 and 2006, Scheiding *et al.* 2006). In the last years, a rapid mold infestation especially of recently restored wooden artifacts was observed. This phenomenon was proved within

a research project of IHD, funded by the German Federal Ministry of Economic Affairs and Technology. Furthermore, increasing mold problems occurred at organs, independent of type and year of construction.

2. INVESTIGATED PLACES

2.1 Zwickau Cathedral (Saxony, Germany)

The Zwickau Cathedral was built as a Romanic one-nave church in 1180 and rebuilt to a three-nave hall church in the style of later Gothic between the 15th and 16th century. A century later, the steeple, which was destroyed by a lightning, was replaced by a Baroque style steeple with bells. The church is famous for its organ, which was installed in 1966 as one of the biggest in Germany (6,000 pipes). Furthermore, there are some important sacral wooden art treasures as the pietà of Peter Breuer (1507), the winged altar of Michael Wohlgemut (1479), or the “Sacred grave” (1507), carved by Heffner (figure 1). Since the nineties of the last century, the cathedral is restored continuously (http://www.zwickau2000.de). The cathedral was affected by several flooding events in the past, leading to mold infestation, which was caused as well by adverse climatic conditions in some parts, as in niches with insufficient ventilation. Organ components of soft wood, carvings and coated components of kneelers, altars and buildings are affected.

Figure 1: Sacred grave

2.2 Duchess Anna Amalia Library in Weimar (Thuringia, Germany)

This history-charged building with the famous Rococo-Hall from about 1760 is a part of the Unesco World Heritage. A fire in September 2004 destroyed some parts of the building, a lot of precious books from the 16th to the 18th century and 35 paintings. The damages not only resulted directly from the fire, but also from the abundance of fire water, which penetrated the walls and the numerous wooden built-in components as stairs, balustrades and panelling (figure 2). Drying measures with heating elements started promptly after fire extinction and took more than seven months to achieve an acceptable moisture content of the brick wall. Nevertheless, an extensive mold infestation on coated panels and wooden artifacts could not be prevented. Infestation of

materials by mold and microbial burden of indoor air by germs were investigated and monitored in the progress of restoration work (Plaschkies 2007).

Figure 2: Rococo hall during the restoration in 2006

2.3 St. Marienthal Cistercian Convent Ostritz (Saxony, Germany)

The convent is situated in the very eastern part of Germany, closed to the Polish border. It was built in Bohemian baroque style after the great fire of 1683, but nuns have already been living there since 1234 (http://www.kloster-marienthal.de*)*. The very impressive, completely preserved convent is today hosting also the "International Encounter Centre St. Marienthal". In August 2010, there was a huge flood in that region which caused devastating damages at the buildings which are located directly on the Neisse riverside. As a result of the submergence, a heavy mold infestation occurred. Evacuation of infested sacral wooden artifacts, cleaning and drying measures where arranged immediately. The church with wooden panelling is exceedingly affected (figure 3).

The restoration will last some years; the monitoring of the situation regarding mold infestation is performed by the authors.

Figure 2: Church in the St. Marienthal Convent, removed panels

3. METHODS

3.1 Determination of the airborne germ concentration

The sampling of airborne germs was performed with the instrument type MBASS 30 (Holbach), which consists of a carrier for 90 mm petri dishes and an integrated pump for sampling defined air volumes. By this method, the spores are directly deposed on a nutrient agar; usually malt agar as a universal medium for fungi and DG 18 agar (dichloran-glycerin) for xerophile fungi are used. Other media are to be chosen for special issues, for instance to gather bacteria as well. After sampling, the plates were transported to the lab and incubated at 26-28 °C for several days. The grown fungi were determined as colony forming units (cfu) and related to one m³ air. Furthermore, the mold species were determined for a qualitative evaluation of the situation. According to DIN ISO 16000 parts 17-19, several samples were taken from every sampling point.

3.2 Sampling of surfaces

It is essential to estimate the molds which infest the different materials in the objects. Therefore, an adhesive tape put on the surface can show a reliable image of the current condition. The tape can be stained with cotton blue or other dyestuffs for fungi and observed at the microscope directly.
Sometimes it can be useful to make an impression to the nutrient agar, for example if many particles from the coating or the building material adhere on the tape and so structures of microorganisms cannot be detected. The fungi on the nutrition medium can be determined after the incubation.

3.3 Determination of mold species

The mold species were determined by both macromorphological characteristics, like colour and colony shape on nutrient agar, and micromorphologic characteristics, like shape and size of the spores, conidiophores and fruiting bodies. For species determination, identification keys (Samson et al. 2010, Hoog et al. 2000) and DNA-sequencing (in some cases) were used.

3.4 Test of the susceptibility of restoration materials

For the choice of appropriate restoration materials it is important to know their susceptibility to a mold infestation. This property is preferably determined within a lab test. Herefore, the company standard AA-20-25 was developed by IHD, basing on EN 60068-2-10. An example for the restoration in the church of the above mentioned convent is described subsequently. The uncoated backside of the wall panelling made of spruce wood was heavily affected by mold. In contrast, some areas, which were coated with a historic red paint, were less infested. This paint was analysed and reproduced originally by a restorer. To evaluate its susceptibility, a laboratory test was performed together with some other eligible coats. The coats were applied to specimens of spruce and specimens of obeche (obeche or Abachi is a light-brown African hardwood). As test fungi, *Aspergillus versicolor, Eurotium herbariorum, Trichoderma viride* and *Alternaria alternate* were used, since these species occurred in the church. At first, a spore suspension was prepared. Fungi were cultivated on malt agar tubes for two weeks. Then, the spores were harvested, washed with sterile deionised water with a detergent and mixed to same parts. The end concentration was 10^4 spores/ml. The suspension was sprayed onto the unsterile specimens and incubated up to 4 weeks at 28 °C and > 95 % relative humidity. The evaluation was performed by visual estimation of the extent of the infested areas.

4. RESULTS

4.1 Mold in the air and on surfaces

As expected, the bioburden of the indoor air was different at several measuring times for different locations (Tab. 1). Recommendations for further proceeding were deduced from current conditions and determined values. When high values were detected, specific measures had to be continued, like separation of affected areas from clean areas, or the efficiency of cleaning and disinfection had to be checked. However, low values do not indicate automatically the absence of a health risk. Despite of some heavy mold infestation on material surfaces found in the Cathedral Zwickau, not always an adequate, high spore concentration in the indoor air was determined.

Table 1: Concentration of viable germs in the air

Number of sampling	Viable airborne germ concentration [cfu/m³]		
	St. Marienthal Convent[a] (Convent church)	Anna Amalia Library[b] (Rococo hall)	Zwickau Cathedral[c]
1	October 2010: 2000	May 2006: 5000	June 2004: < 100
2	March 2011: 200	May 2006: 800	March 2005: < 100
3	September 2011: > 6000	July 2006: 2200	-
4	March 2012: 1000	September 2007: 400	-

[a] *Restoration ongoing in 2012* [b] *Restoration finished in September 2007*
[c] *Restoration in sections during ongoing normal use of the buildings*

The investigations in the church nave of the St. Marienthal Convent are still going on. As in the previous investigations, the predominant mold species are *Penicillium* (e. g. *P. chrysogenum*), *Aspergillus* (e. g. *A. glaucus, A. versicolor*) and *Cladosporium* species. Figure 4 shows typical petri dishes after incubation. Furthermore, there are indications for the occurrence of actinobacteria; this fact must be pursued. During the first investigations, *Trichoderma* sp., *Fusarium* spp., *Epicoccum* sp., *Alternaria* sp. and *Mucor* sp were detected on the wooden surfaces, particularly on spruce panels, whereas oak panels were not infested. In later samplings on the gallery, species of the *Aspergillus glaucus* group on black painted frames were found (Fig. 5).

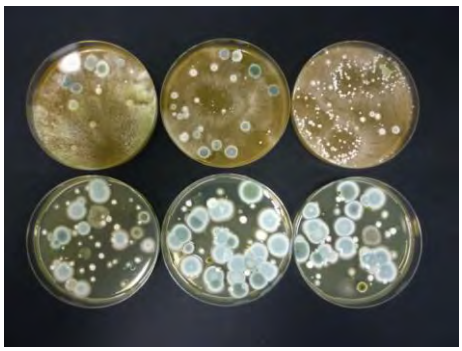

Figure 4: Air sampling September 2011: petri dishes after 6 days of incubation (above: malt extract agar, below: DG18 agar)

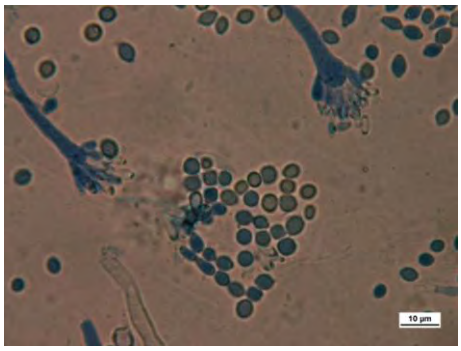

Figure 5: Conidiophores and conidiospores of a species of the *Aspergillus glaucus* group (600-times magnified)

On uncoated wooden surfaces in the Rococo Hall of Anna Amalia Library, the fungus *Trichoderma viride* was mainly found. Furthermore, *Rhizopus oryzae*, *Mucor plumbeus* and several *Penicillium* species were detected. The most frequent fungi in the air were *Penicillium* species, first of all *Penicillium chrysogenum* (figure 6).

Figure 6: Extensive infestation by *Trichoderma viride* on a beam in the Rococo Hall before restoration

Heavy infestation on wooden artifacts of the Zwickau Cathedral was found on surfaces painted with black casein tempera, e. g. on frames of the altar and on parts of the kneelers (fig. 7). Mold infestation was also found on some organ compartments made of softwood and on glue lines of the "Sacred grave". Different mold species were determined on material surfaces and in the air: *Alternaria alternata, Aspergillus versicolor, A. flavus, Eurotium herbariorum* (*Aspergillus glaucus* group)*, Botrytis cinerea, Chaetomium funicula, C. globosum, Cladosporium herbarum, Penicillium chrysogenum, P. expansum* and *P. glabrum.*
In all places, mold fungi on wooden surfaces caused discolorations, which mostly appeared as dark spots. The climate conditions – changes between high and low temperature and humidity – were not appropriate to the storage of wooden artifacts (details see Scheiding et al. 2006). Thus, swelling and shrinking of wood can cause also cracks in the coat or in the whole wooden artifacts.

Figure 7: Extensive mold infestation on black casein tempera (Zwickau Cathedral, kneeler of widow Bose)

4.2 Susceptibility test of panel coatings

The test series were performed with spruce and with obeche specimens. All tested materials were grown by mold within the test time, but there were differences between the two test series (Table 2). In the spruce test series, an infestation on the coatings did not appear before 3 weeks. The coats with borax casein and borax bone glue were infested clearly less than the coats with fish glue and bone glue without borax. The best result was obtained for borax casein. In contrast, in the obeche test series, extensive mold growth was recorded already after two weeks, and the advantage of borax casein was not observed (figure 8). Obviously, the intensity of infestation was stronger in the obeche test series because of the higher susceptibility of obeche in comparison with spruce; that fact was already found in earlier investigations (Weiss et al. 2006).

Table 2: Results of the fungus test

	Rating of the infested surface[a]			
	2 weeks	**3 weeks**	**4 weeks**	**6 weeks**
Borax casein as base coat / semi-oil/ borax casein tempera as top coat				
test on spruce	0	1	1	2
test on obeche	3	3	3	3
Borax bone glue				
test on spruce	0	2	3	3
Borax bone glue as base coat/semi-oil/ borax-bone-glue-tempera as top coat				
test on spruce	0	1	3	3
test on obeche	3	3	3	3
Borax fish glue				
test on spruce	0	2	3	3
Reference pine sapwood: infestation on > 50 % of the surface, visible by naked eyes				

[a] *rating scheme*

0 No infestation
1 Infestation microscopically visible (50-times magnified)
2 Infestation weaker than on reference specimens
3 Infestation equal to or stronger than on reference specimens

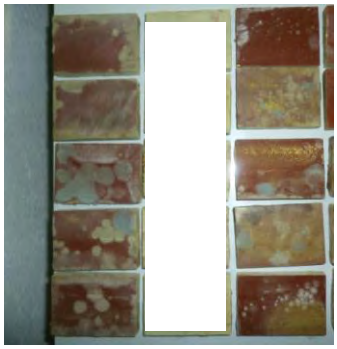

Figure 8: Susceptibility test against mold: Obeche specimens after two weeks incubation (left: borax casein, right: borax bone glue)

4.3 Cleaning and disinfection

The following measures were generally performed with the infested wooden artifacts: Within a dry cleaning as a first step, little pieces were brushed and bigger areas vacuum-cleaned. Then, the cleaned areas were disinfected, either with 70 per cent isopropanol or with a commercial mold remover, basing on quaternary ammonium compounds. In some cases, infested areas were cleaned by a professional company with a special vacuum washing procedure, combined with simultaneous disinfection with quaternary ammonium compounds. Special care was bestowed on coated artifacts which were expected to be susceptible to chemicals. To prevent a re-infection, all cleaned, disinfected and restored artifacts were protected from the infested area if possible, either they were stored in the restorers workshop or covered with an appropriate plastic sheet. During this work, it was observed that cellulosic compresses, applied on walls for demineralisation, can increase the moisture and provide an additional nutrient source for mold and bacteria.

In case the infested components were without historic value, susceptible wood species were exchanged by more durable ones. Some artifacts contain natural oils from earlier treatments. These oils effect as nutrients and thus can stimulate mold infestation. The oil-immersed artifacts should be removed, if possible.

5. CONCLUSIONS AND RECOMMENDATIONS

Investigations of mold infestation at wooden artifacts showed that the same fungi species appear, regardless of place or reason for higher moisture content (flood, condensation).

The susceptibility of materials to mold differs widely. A laboratory test with defined conditions can be an important tool to determine that. But it must be noticed that it is often difficult to estimate the conditions in service.

In each case, wooden artifacts with mold infestation shall be investigated carefully before taking actions. Various intensities of decay can indicate various susceptibilities. For example, panels of oak heartwood and the historic red coat on the spruce panel backsides were grown weakly, compared to uncoated spruce wood. Thus, the reproduction and application of the historic paint can provide some mold protection. Beside mold infestation, other damages like cracks have to be taken into consideration. It should be noticed, that fillers or glues for crack restoration can be a nutrient source for mold fungi.

Infested artifacts or components must be separated or removed from the infested area. For cleaning and disinfection it is useful to start with a dry cleaning to remove spores, dust and pollutions; a brush or – preferably – a vacuum cleaner with (spore-tight filters!)

can be used. An appropriate agent for the following disinfection is 70 per cent isopropanol. Sometimes, the application of a water solution with quaternary ammonium compounds can be useful. The treatment of larger areas – more than 0.5 m² – should be operated by a professional company. It is important for the restorer to know the surrounding conditions. If the relative humidity of the air is higher than 65 %, the place may be insufficiently ventilated, and both dust settlement and mold infestation can be expected. Additionally, the changing indoor climate conditions and different properties and susceptibilities of materials and coatings against mold must be taken into consideration. To predict mold infestation, models for wood (Hukka 1999) or other materials (Viitanen and Ojanen 2007) are available. Under unfavourable conditions, the application of antifungal agents could be an option. Several commercial products with certified antimicrobial efficacy are available. In each case, the suitability for the concrete material should be checked, and the recommendations for application and health safety must be respected. If so called “alternative” agents are used, like thymol or eugenol, only a time-limited efficacy can be expected due to their high volatility.
To evaluate the damage by mold, generally a large experience is necessary. A problem is the insufficient knowledge of the microbial burden in historic buildings. Therefore it is worthwhile to include all available information in the evaluation.

6. REFERENCES

Hoog, de G S , Guarro, J, Gené, J, Figueras, M J (2000): *Atlas of Clinical Fungi*. 2nd edition. Centraalbureau voor Schimmelcultures, Utrecht.

Hukka, A, Viitanen, H (1999): A mathematical model of mould growth on wooden material. *Wood Science and Technology 33(6)*, p. 475-485.

Plaschkies, K (2007): Baubiologische Untersuchungen in der Herzogin-Anna-Amalia-Bibliothek Weimar. *2. Mykologisches Kolloquium*, 15th/16th/11/2007, Dresden

Ranalli, G, Zanardini, E, Sorlini, C (2009): Biodeterioration – Including Cultural Heritage, *Encyclopedia of Microbiology (Third Edition),* p. 191–205.

Samson R A, Houbraken, J, Frisvad, J C, Thrane U, Andersen B (2010). *Food and Indoor fungi.* CBS Laboratory Manual series 2, Utrecht.

Scheiding, W, Plaschkies, K, Weiß, B (2006): Mykologische, holzschutztechnische und bauklimatische Untersuchungen an schimmelpilzbefallenen Einrichtungs- und Kunstgegenständen aus Holz in Kirchen. *Holztechnologie 47*, p. 45-50.

Viitanen, H, Ojanen, T (2007): Improved model to predict mould growth in building materials. Thermal Performance of the Exterior Envelopes of Whole Buildings. X-Proceedings CD. 2nd-7th/12/2007 Clearwater Beach, USA.

Weiß, B, Plaschkies, K, Scheiding, W, (2004): Schimmelpilzbefall in Kirchen und an sakralen Kunstwerken. *Restauro 2/2004*, p. 135-140

Weiß, B, Plaschkies, K, Scheiding, W (2006): Schimmelpilzbefall in Kirchen und an sakralem Kunstgut. Ein Projekt zur Untersuchung der Ursachen – Teil 2. *Restauro 6/2006*, p. 376-383.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Effect of growth rate and radial position on the natural durability of Douglas-fir

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ABSTRACT

In terms of natural durability, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) fame owns to the favourable characteristics of centuries-old trees harvested in old-growth North American forests. The properties of wood coming from plantations harvested between 50 and 100 years-old are likely to be different. In such stands, plantation density and thinning intensity may have a large impact on the trees growth rate. Since this parameter is known to affect some properties of the wood, it was decided to assess to what extent an increase in Douglas fir growth rate affects the natural durability of its wood. This issue is indeed poorly documented in the scientific literature.

This parameter was evaluated on standardized heartwood specimens taken from 60 trees originating from 10 stands in Wallonia (Southern Belgium). In all these stands, the average girth of the trees ranged between 140 and 160cm, whilst their age (from plantation) ranged from 38 to 66 years old: These stands are thus representative of very contrasted silvicultural management practices. In terms of individual growth, the *Mean Ring Width* of the trees ranges between ca 3 and 7mm. Globally, 600 tests specimens were taken from two radial positions in the heartwood of each tree. Half of the specimens were taken in the heartwood at the border of the sapwood; the other half encloses the 20 years old ring, counting from the pith. The mass losses caused by the wood decaying fungus *Poria placenta* were assessed according to Cen/ts 15083-1 (2005).The natural durability of the wood is discussed as affected by sites, trees, radial positions in tree and tree growth rate.

Key words: Douglas-fir, natural durability, growth rate, radial position.

INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) wood is known for its noticeable natural durability (e.g. Kennedy 1956, Highley 1995) combined with excellent physico-mechanical properties (Jayawickrama *et al*. 2009). This fame owns to the favourable characteristics of centuries-old trees harvested in old-growth North American forests. However, the characteristics of the present resource are different than those of the "historical Douglas fir resource".
Focusing on exotic plantations (i.e. outside North-America), the resource consists in smaller and younger trees, with generally a faster growth rate in their young age, as early competition is assumed to be lower in plantations than in naturally regenerated stands. In Central Europe for instance, the largest sawmills, who absorb the widest proportion of the forest production, need logs with standard dimensions. Because of technical and productivity requirements, the girth at breast height of the logs sought by these industries mainly ranges between 125 to 190 cm (or diameter at breast height - $dbh_{1.5m}$- of 40cm and 60cm); Some sawmills even report optimal $dbh_{1.5m}$ around 40-45cm, i.e. trees with a log volume ranging from 1 to 2.5m³ (Leroy-Terquem 2009). Hence, the final cut of the stands occurs most generally 50 years (40 at the earliest) to 100 years after the plantation, depending on the silvicultural management practices (initial plant density, thinning intensity and schedule). From a financial point of view, Lejeune *et al.* (2000) also demonstrated that it is in the interest of forest owners to exploit even-aged plantations very early (around 45 y.o.), whereas longer revolutions (60 or 75 y.o.) lead to lower financial yields. Lower climatic hazard risks are also associated with shorter revolutions. Obviously, these considerations ignore environmental issues or the sustainability of the production in the long term: They are however the only taken into account by some forest owners.
In West European Douglas-fir plantations, *mean ring width* (MRW) of the trees – calculated on the whole tree radius –, may range from 2mm (125cm girth at 100y.o.) to 7mm (190cm girth at 40y.o.). Practically however, the MRW observed on Douglas-fir grown in Wallonia (Southern Belgium) is scarcely lower than 3mm and generally do not exceed 6mm. The range of tree MRW is thus very wide, and the range of MRW which can be observed on lumber is still wider, as a consequence of the ring width variability observed from pith to bark (e.g. Jozsa and Middleton 1994).
Natural durability may vary according to a lot of factors: fungus, tree species, genetic origin, site, between- and within-tree differences. The MRW is known to influence some physical and mechanical properties of Douglas-fir wood (Nepveu and Blachon 1989) and of other species (Zhang 1995, Zobel and Van Buitenen 1989). Conversely, the impact of growth rates on wood natural durability is poorly documented in literature. It also seems to vary according to the species: Narrower ring width seems to positively influence natural durability of European Larch (Jacques *et al.* 2002); wider rings have a favourable impact on the natural durability of oak (Humar *et al.* 2008); Taylor *et al.* (2006) report that "*increasing the growth rate of young western red cedar trees through silvicultural treatments is not expected to affect natural durability*". Besides, because he found that some extractives involved in western red cedar natural durability increases from the pith to outer heartwood, Nault (1988) suggests that "*products made from the wood of younger trees will be less resistant to decay than similar products made from the wood of old trees*".

To the best of our knowledge, few studies were dedicated to the impact of tree age and growth rate on Douglas-fir natural durability, and the results are not always fully consistent. In that way, whereas Taylor *et al.* (2003) consider "*silvicultural treatments that affect growth rate may affect wood durability in Douglas-fir*", Gartner *et al.* (1999) suggest that "*young* (Douglas-fir) *stands may be robust with respect to the effect of silvicultural regimes on heartwood durability*". The

latter authors however analysed relatively young trees (34y.o.) which are somewhat younger than trees harvested at the final cut.

Therefore, it has been decided to assess, on mature trees, to what extent natural durability of Douglas fir is influenced by their growth rate. As the latter is strongly linked to silviculture, this research should help forest owners to manage their stands in a way enabling the production of a material with the most advantageous characteristics. Subsequently, the influence of site, tree and radial position has also been analysed.

EXPERIMENTAL METHODS

The experimental material originates from 10 stands distributed all over Wallonia – Southern Belgium. At the time of the cutting (in 2007), all the stands had an average girth comprised between 140 and 160 cm, whilst their age (from plantation) ranged from 38 to 66 years old. In each stand, six trees were cut, belonging to the girth (at 1.5m) categories [120cm; 129cm], [130cm; 139cm], until [170cm; 179cm]. The trees were semi-randomly chosen; avoiding however injured or bowed trees, border trees, etc. All these trees belonged to mature stands.
As a consequence of the sampling methodology, the stands are representative of very contrasted silvicultural management practices (although few information is available concerning the latter, at least for some stands): the MRW of the trees, calculated on disks located 2m-high in the standing tree, ranges from 3mm to 7mm (Fig. 1).

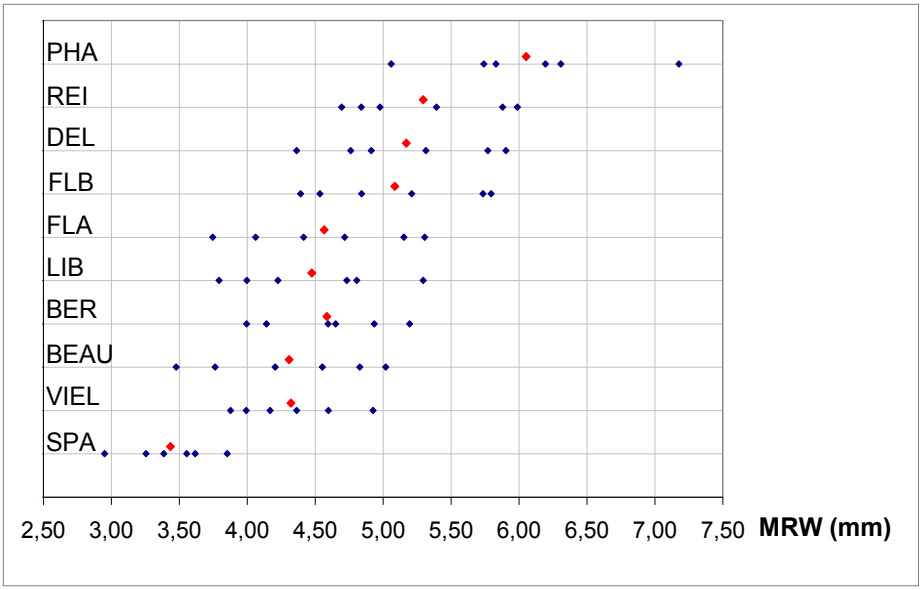

Fig. 1. Individual *mean ring width* (MRW) of the 60 trees (blue dots), according to the stand they come from. Based on the 6 individual values, an average MRW has been computed for each stand (red squares).

In the 2m long butt log of the 60 trees, an 85 mm-thick central plank containing the pith was cut perpendicularly to maximum radius. A section 60cm long (from 0 to 0.6m in the standing tree) was then cut off and longitudinally sawn, from the pith, in two half-planks. Depending on the trees, two or four 30mm-thick battens were sawn out of both half-planks (Fig. 2). Battens "**D**" are centred on the 20th ring and battens "**C**" were taken at the limit of the sapwood, both in the mature heartwood. Depending on tree age and size, the cambial age of some battens C and D were very similar, or even equal, as shown on figure 2 (tree PB11).

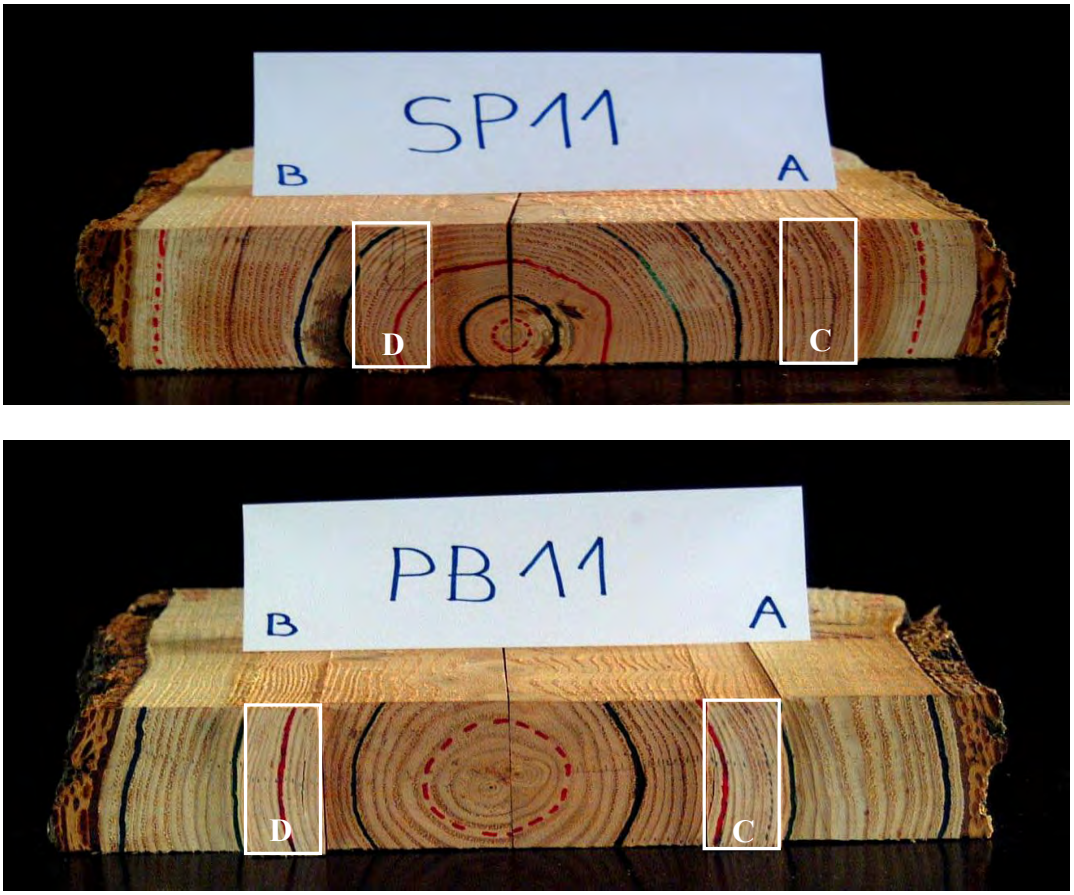

Fig. 2. Location of the battens in a central plank (85mm thick). Tree SP11 is more than 60 years old whilst tree PB11 is around 40y.o.

A preliminary test enabled to identify the most aggressive fungus between *Poria placenta* CTB 863A and *Coniophora puteana* BAM Ebw.15, according to the requirements of CEN/TS 15083-1 (2005). So this is the most aggressive fungus, *Poria placenta*, which was used for the tests reported in the present paper.

Table 1. Number and distribution of the samples

Variables	n	Description/Remark
Site	10	
Tree(site)	6	Numbered from 1 to 6
Position(tree)	2	C (at the border of the sapwood) / D (20 y.o. ring)
Fungus	1	*Poria placenta*
Replicates	5	
Total test specimens	600	6 unsuitable specimens (waterlogging, contamination by micro-ogranisms)
Total test specimens for analyse	594	
Total of reference test specimens (Pine)	18	

For each "tree-radial position" combination, five replicates were used to assess fungus aggressively (Table 1). The initial total number of test specimens is thus 600, but 6 specimens were discarded because of waterlogging or mould contamination.

All the test specimens (50 × 25 × 15 mm³) and reference test specimens were conditioned to 12% of moisture content and then weighed (M_1). The initial theoretical oven-dry mass (M_i) of the test

specimens was then given using the mean moisture content (MC) measured on another group of test specimens oven-dried at 103°C to constant mass:

$$M_i = \frac{(100 \times M_1)}{(100 + \mathrm{MC})} \quad \text{(g)} \qquad (1)$$

The test specimens and reference test specimens were then placed in Kölle flasks exposed to *Poria placenta* CTB 863 A for 16 weeks in a controlled environment (20°C, 70% RH, darkness). At the end of the period of exposure, the mycelium adhering to the test blocks was removed and the blocks were dried at 103°C to constant mass (M_f). The mass loss (%), used to evaluate the natural durability (decay resistance) of wood, was calculated for each test specimen using:

$$\text{Mass loss} = 100 \times \frac{(M_i - M_f)}{M_i} \quad (\%) \qquad (2)$$

The tentative classification adopted from Annex D of CEN/TS 15083-1 (2005) defines five durability classes (class 1, very durable, to class 5, not durable) based on the median mass loss caused by the most aggressive fungal species on the test specimens.

An ANCOVA model of analysis of partially hierarchical variance with 3 classification factors with co-variable density was used to account for the variation of natural durability. The site and tree(site) factors were assumed to be random, and the fixed factor was the radial position in the tree (C and D). The statistical analysis was carried out using Minitab 13.31 software at a statistical significance level of 5% (p<0.05) on the 594 values of mass loss of the test specimens. Results are also evaluated by regression analysis.

RESULTS AND DISCUSSION

Table 3 presents the mean and median values of the mass losses (%) caused by *Poria placenta* according to site and radial position. The samples bordering the sapwood (radial position C) and those enclosing the 20 years old ring counting from the pith (radial position D) are considered separately and together (radial position C and D), for each site and all the sites. Depending on tree age and size, cambial age of some battens C and D were very similar, or even equal. Therefore, in order to evidence a possible age or position effect, this table only takes into account C and D couples differing from at least 10 years (total of 378 test specimens).

Table. 3. Mean and median mass losses (%) caused by *Poria placenta* and classification according to Cen/Ts 15083-1 (based on median) for both studied radial positions (n=378).

	Radial position C (close to sapwood)				Radial position D (20 years)				Radial position C and D			
Site	Mean	Median	Class	n	Mean	Median	Class	n	Mean	Median	Class	n
SPA	22.2	21.3	4	30	23.0	21.9	4	30	22.6	21.6	4	60
BEAU	17.5	18.2	4	30	20.0	19.6	4	30	18.7	19.2	4	60
VIEL	20.6	20.7	4	25	22.8	22.6	4	25	21.7	21.7	4	50
LIB	15.3	16.2	4	15	22.6	22.1	4	15	19.0	18.5	4	30
FLA	18.0	17.8	4	20	20.3	19.5	4	20	19.2	18.7	4	40
BER	17.4	18.1	4	25	18.9	18.8	4	25	19.5	18.9	4	50
FLB	19.0	19.6	4	20	21.1	21.1	4	20	20.1	19.8	4	40
DEL	20.2	20.1	4	15	22.4	21.0	4	15	21.3	20.2	4	30
REI	15.7	17.4	4	10	18.1	18.6	4	8	16.8	18.0	4	18
All sites	18.8	18.5	4	190	21.2	20.3	4	188	20.0	19.5	4	378

Regardless to position and to site, the mean/median mass losses are respectively 20% and 19.5%. Van Acker *et al*. (2003) report the natural durability class of Douglas fir obtained in five European research laboratories: Among the five mean/median mass losses resulting from *P. placenta* decaying action, three are similar to our values (Table 3). The mass losses observed in the present study are lower than those observed in the laboratory tests presented in Scheffer and Englerth (1952), Englerth and Scheffer (1955) and Akhter and Hale (2002a), but they are higher than those reported from the field or non-pure culture tests in the same studies. Based on the results of field tests, Douglas fir is considered as "moderately durable to slightly durable" – class 3-4 – in EN 350-2 (1994). Van Acker *et al.* 2003 highlight that whereas laboratory tests only involve Basidiomycetes, soft rot fungi may be present in the soil and attack the wood in field tests: They suggest this may lead to a poor correlation between Lab and field tests. Several authors (Akhter and Hale 2002a; Flæte *et al.* 2009) also point out that a variety of micro-organisms may interact in non-pure culture tests, making their outcome less reproducible and "universal".

Concerning the impact of radial position, mean mass loss appears slightly higher in the inner heartwood than at the border of the sapwood. This difference (only 2% in average, excepted for LIB) is however not highlighted by CEN/TS 15083 (2005) classification: Class 4 (slightly durable) is obtained whatever the site or radial position.

Considering the 37 trees (378 tests specimens) for which battens C are more than 10 years older that battens D, Fig. 3 presents the mean mass loss differences between battens C and D, according to their age differences.

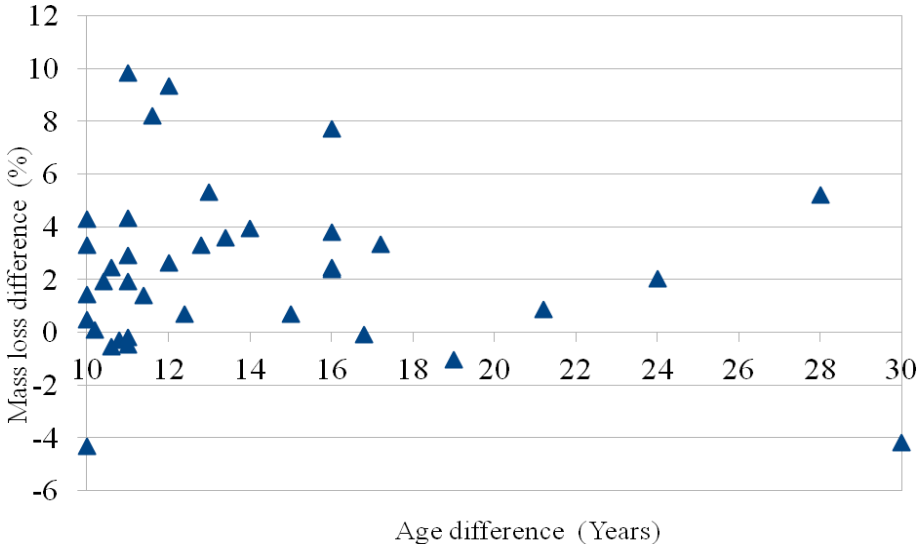

Fig.3. Difference in mean mass losses between radial position D and C, according to the age difference between these radial positions in the tree.

For most of these trees, the difference in mean mass loss between both radial positions is less than 5%, and it never exceeds 10%. In a few cases, the mean mass loss in position C is higher than that observed in D (Fig. 3: values <0). It therefore appears that the maturing of the wood inside tree (i.e. age of the wood counted from the cambium) has a limited effect on its natural durability.

Table 4 shows the results of the analysis of covariance (ANCOVA) carried out to assess how, with equal density, natural durability is influenced by the three factors: site, tree(site) and radial position.

Table. 4. ANCOVA of effects of site, tree(site) and radial position on natural durability with equal density (co-variable).

Source	Df	SS	MS	F	P	statistical significance
Density	1	1152,222	133,704	15,73	**0,000**	***
site	8	1075,721	133,528	2,84	**0,028**	*
Tree(site)	29	904,763	30,896	3,64	**0,000**	***
Position	1	70,069	48,195	3,00	0,102	NS
Site x Position	8	211,476	26,435	3,11	**0,002**	**
Error	330	2804,672	8,499			
Total	377	6218,924				

***, **, *, NS: significance at 0.1%, 1%, 5% and not significant.

It appears that density has a significant effect on mass loss. Indeed, the correlation between these factors is significant for both position C and D (fig.4): An increase in density induces a slight decrease in mass loss and therefore a better decay resistance. A similar influence of density on durability was reported by Akhter and Hale (2002a). However, as reported by several authors, density does not appreciably affect decay resistance and it can certainly not be concluded to a relationship comparable to that well-known between density and mechanical properties of wood (Roosen 1951). Scheffer and Morell (1998) argue that the significant relationship observed between mass loss and density is rather due to a relatively high extractive content (chemical factors inhibiting or slowing down fungi development) of which the accumulation can locally increase the density of the wood.

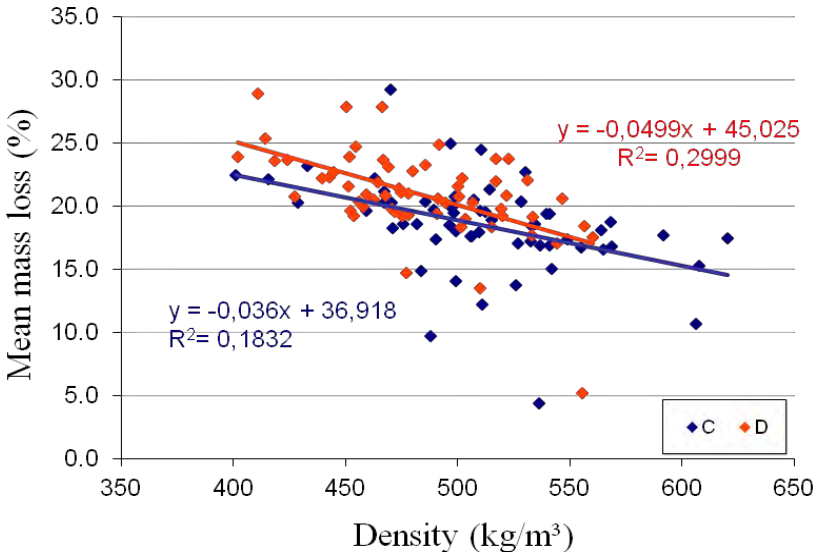

Fig.4. Mean mass loss (%) according to mean density (kg/m³) of batten C and D for the 60 trees

Statistical analysis shows significant and very highly significant differences respectively between sites and trees (within sites). Amusant *et al.* (2004) suggest that environmental effect is also an important factor, but the difference observed amongst trees should be largely genetic. Moreover, Akhter and Hale (2002a) report highly significant variation in natural durability

between four seed origins of Douglas-fir. Chemicals which make the wood decay-resistant were also shown to vary in quantity and/or quality according to the genetic origin of the tree (Scheffer and Hopp 1949, Akhter and Hale 2002b). The genetic origin of our selected stands is not known, and so cannot be analysed in our study. Furthermore, the difference in age and in growth rate between trees cannot explain these observations, as no relation between this parameters and the mean mass loss could be established (Fig. 5). Similarly, in their study on Sessile oak, Guilley *et al.* (2005) didn't find clear significant relationship between radial growth rate and age of trees on the one hand and mass losses on the other hand.

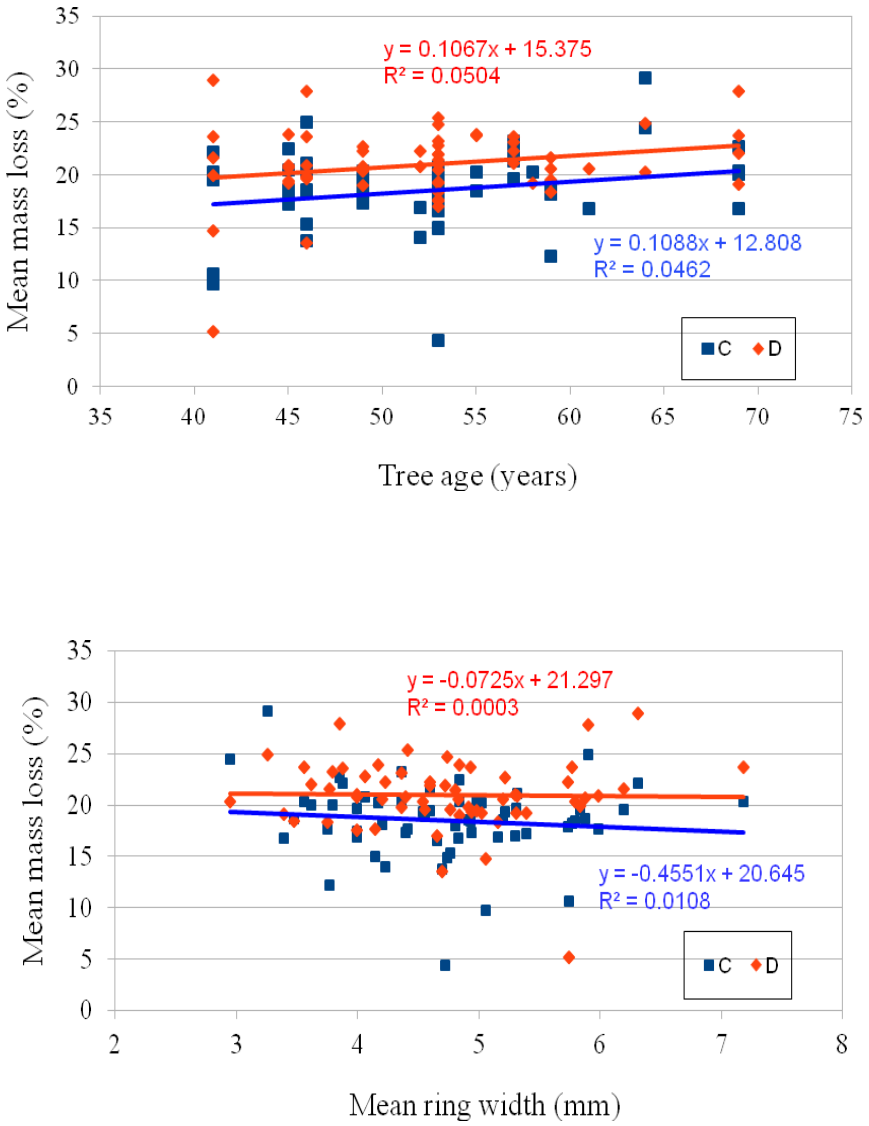

Fig.5. Mean mass loss (%) according to tree age (on the top) and mean ring width of tree (at the bottom), for radial position C and D (60 trees).

Once the impact of density is neutralized, the slight variations of mass loss observed in Table 3 between radial positions C and D do not appear significant in the statistical analysis (table 4). These results are consistent with those of Gartner *et al.* (1999) who found no relationship between heartwood durability and the position (radial and vertical) in the bole of Douglas-fir.

Also, no trend in the radial distribution of extractives was found in Douglas fir cross sections (Campbell *et al.* 1965).

The relationship between mean mass loss and mean ring width at the tree level is not significant (Fig.6).

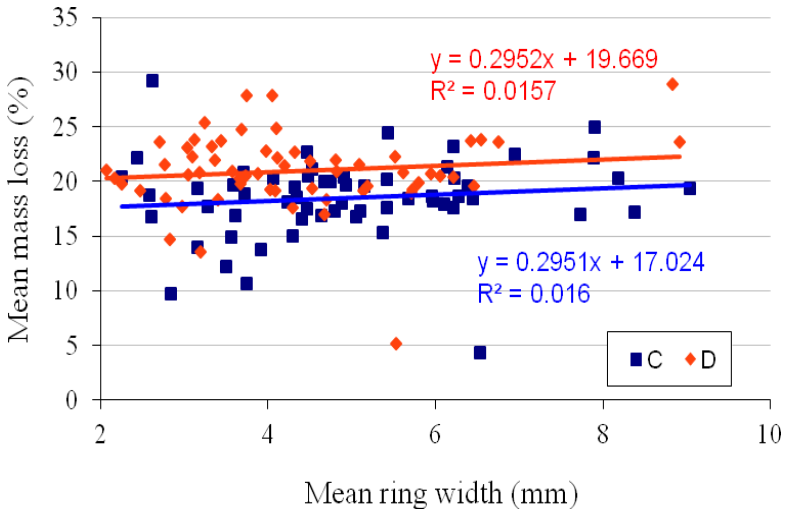

Fig.6. Mean mass loss (%) according to mean ring width (mm) of batten C and D for the 60 trees.

In their investigations on spruce and pine, Rydell *et al.* (2005) did not found either significant correlation between annual ring width and durability. On the other hand, Gardner and Barton (1960) report that there can be considerable differences in extractives content between Douglas-fir trees; the heartwood from large, slow-grown trees is usually darker in colour and contains more extractives than those fast-grown trees with younger wood. Maybe the difference observed in natural durability of those trees (fast and slow-grown) could be found in the proportion of early- and latewood, as suggested by Björkman (1944): It can be more difficult for fungi to penetrate into denser latewood.
In our study, mean density of test specimens is inversely related to their mean ring width: This relationship is significant for test specimens in position D (and the p value is 0.055 for position C). However, the relationship between wood density and growth rate is much debated, especially for Douglas-fir (Akhter and Hale 2002a).

CONCLUSIONS

Our study showed that natural durability of Belgian grown Douglas-fir is somewhat less favourable than reported in EN 350-2 for European Douglas-fir. The mass losses observed in our trials are however consistent with those reported in Van Acker *et al.* (2003) from Laboratory tests performed on European grown Douglas-fir, and they are even lower than those reported for North-American (Scheffer and Englerth 1952; Englerth and Scheffer 1955) and British (Akhter and Hale 2002a) Douglas-fir. If laboratory tests may be suitable to assess the impact of a given factor (e.g. growth rate) on wood natural durability, their outcome should be carefully considered and should not tarnish the fame of a species which demonstrated its interest through centuries of use. We thus think the class 4 observed here is not alarming and should not question the

suitability of Douglas-fir in its conventional structural uses or cladding, provided that the wood is properly installed and that the sapwood is purged.

Natural durability of wood depends on several factors (site, tree, position within tree) and poor literature is available concerning the effect of growth rate. A general pattern in softwood is that wood with narrow annual ring width is more durable, because of greater density (Sjömar 1988, Björkman 1944). However, in our study, no correlation was found between mean mass loss and mean ring width of test specimens. Differences observed in durability cannot be explained by ring widths, but other factors influence wood durability, such as density, genetic origin and certainly extractive content, often reported in the literature as one of the most important factor. Regarding the forest manager point of view, the absence of impact of growth rate and the negligible impact of radial position in the heartwood are encouraging. If the management of Douglas fir is modified in the future, only the impact of silviculture on physico-mechanical properties should be taken into account

REFERENCES

Akhter, S., Hale, M D C (2002a): Variation in natural durability of British grown Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Part I: Effect of density and growth rate. IRG/WP 02-10445. Proceedings of the 33rd Annual Meeting of the International Research Group on Wood Preservation (IRG), Cardiff, Wales, 12-17 May 2002.

Akhter, S., Hale, M D (2002b): Variation in natural durability of British grown Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). Part II: Effect of extractive contents and taxifolin. IRG/WP 02-10446. Proceedings of the 33rd Annual Meeting of the International Research Group on Wood Preservation (IRG), Cardiff, Wales, 12-17 May 2002.

Amusant, N, Beauchêne, J, Fournier, M (2004) : Intraspecific variability of Wapa courbatil (*Eperua grandiflora*) against *Antrodia* sp. And *Coriolus versicolor*: effect of radial and height position in the stem. IRG/WP 04-10531. Proceedings of the 35d Annual Meeting of the International Research Group on Wood Preservation (IRG), Ljubljana, Slovenia, 6-10 Juin 2004.

Björkman, E (1944): Om röthärdigheten hos lärkvirke. Norrlands Skogsvårdförbunds Tidskrift nr 1: 18-45 *In* Bergstedt, A, Lyck, C (2007): Larch wood – a literature review. Forest and Landscape working papers no. 23-2007. Forest and Landscape Denmark.

Campbell, J R, Swan E P, Wilson J W (1965): Comparaison of wood and growth zone resinous extracts in Douglas-fir. Pulp and Paper Magazine Canada 66: T-248, T-252 *In* Hillis, W E (1987): *Heartwood and tree exudates*. Springer series in wood science, Germany: 268p.

CEN/TS 15083-1 (2005): Durability of wood and wood-based products – Determination of the natural durability of solid wood against wood-destroying fungi, test methods – Part 1 : Basidiomycetes. European Commitee For Standardization, Brussels, 20p.

EN 350-2 (1994): Durabilité du bois et des matériaux dérivés du bois - Durabilité naturelle du bois massif - Partie 2 : Guide de la durabilité naturelle du bois et de l'imprégnabilité d'essences de bois choisies pour leur importance en Europe. Bruxelles, Institut belge de Normalisation (IBN), 40p.

Englerth, G H, Scheffer, T C (1955): Tests of decay resistance of four western pole species. *Journal of Forestry*, **53**(8), 556-561.

Flæte, P O, Evans, F G, Alfredsen, G (2009): Natural durability of different wood species - results after 5 years testing in ground contact. *In:* Bergstedt, A (2009): Proceedings of the 5th meeting of the Nordic-Baltic Network in Wood Material Science and Engineering (WSE), pp. 65-70. October 1-2, 2009, Copenhagen, Denmark. Forest and Landscape Denmark, Copenhagen.

Gardner, J A F, Barton, G M (1960): The distribution of dihydroquercetin in Douglas-fir and western larch. *Forest Product Journal,* **10**, 171-173.

Gartner, B L, Morrell, J J, Freitag, C M, Spicer, R (1999): Heartwood decay resistance by vertical and radial position in Douglas-fir trees from a young stand. *Canadian Journal of Forest Research*, **29**, 1993-1996.

Guilley, E, Charpentier, J P, Ayadi, N, Snakkers, G, Nepveu, G, Charrier, B (2005): Decay resistance against Coriolus versicolor in Sessile oak (Quercus petraea Liebl.) : analysis of the between-tree variability and correlations with extractives, tree growth and other basic properties. *Wood Science and Technologi,* **38**, 539-554.

Highley, T L (1995): Comparative durability of untreated wood in use above ground. lnternational Biodeterioration and Biodegradation **35**(4): 409-419. Online : http://www.fpl.fs.fed.us/documnts/pdf1995/highl95a.pdf . Accessed on May 6th 2012.

Humar, M, Fabčič, B, Zupančič, M, Pohleven, F, Oven, P (2008): Influence of xylem growth-ring width and wood density on durability of oak heartwood, **62**(4), 368-371.

Jacques, D, Curnel, Y, Jourez, B, Pâques, L (2002) : Among and within provenance variability for decay resistance of Larch wood to fungi. International Symposium : Improvement of Larch (Larix sp.) for better growth, stem form and wood quality, 16-21 September 2002, Gap, France.

Jayawickrama, K J S, Ye, T Z, Gupta, R, Cherry, M L (2009): Including Wood Stiffness in Tree Improvement of Coastal Douglas-fir in the US Pacific Northwest: A Literature Review and Synthesis. Research contribution 50, Forest Research Laboratory, Oregon State University, Corvallis. 95 pp.

Jozsa, L A, Middleton, G R (1994): A discussion of wood quality attributes and their practical implications. Forintek Canada Corp., Vancouver, B.C., Canada. Special Publication, 34. 42 pp. Accessed on April 24th 2012 : http://www.laszlojozsa.com/documents/SP-34.pdf

Kennedy, R W (1956): Fungicidal toxicity of certain extraneous components of Douglas-fir heartwood. *Forest Products Journal*, **6**(2), 80-84.

Lejeune, P, Hébert, J, Rondeux, J (2000) : Croissance et rentabilité des plantations de douglas en Région wallonne. *Silva Belgica*, **107**(1), 6-15.

Leroy-Terquem, E. (2009) : Utilisations du douglas et attentes des acheteurs. *La Forêt privée*, **306**, 43-47.

Nault, J (1988): Radial distribution of thujaplicins in old growth and second growth western red cedar (Thuja plicata Donn). *Wood Science and Technology*, **22**(1), 73-80.

Nepveu, G, Blachon, J L (1989): Largeur de cerne et aptitude à l'usage en structure de quelques conifères : Douglas, Pin sylvestre, Pin maritime, Epicéa de Sitka, Epicéa commun, Sapin pectiné. *Revue Forestière Française*, **XLI**(6), 497-506.

Roosen, P (1951) : Les tests d'appréciation de la durabilité naturelle ou artificielle du bois. *Bulletin de la Société Royale Forestière de Belgique*, **10**, 393-415.

Rydell, A, Bergström, M, Elowson, T (2005): Mass loss and moisture dynamics of Scots pine (Pinus sylvestris) exposed outdoors above ground in Sweden. *Holzforschung,* **59**, 183-189.

Scheffer, T C, Englerth, G H (1952): Decay resistance of second-growth Douglas-fir. *Journal of Forestry*, **50**, 439-442.

Scheffer, T C, Hopp, H (1949): Decay resistance of black locust heartwood. Technical Bulletin 984. USDA Forest Service, Washington, D.C. 37p.

Scheffer, T C., Morrell, J J (1998): Natural durability of wood : a worldwide checklist of species. Forest Research Laboratory, Oregon State University. Research Contribution 22, 58p.

Sjomar, P (1988) Building technology and the art of carpentry. Chalmers University of Technology, School of Architecture, Gothenburg, Sweden. (In Swedish) *In* Bergström, M (2005): Above ground durability of Swedish softwood. Thesis for degree of Doctor of Technology, Växjö University, Sweden.

Taylor, A M, Gartner, B L, Morrell, J J (2003): Co-incident variations in growth rate and heartwood extractive concentration in Douglas-fir. *Forest Ecology and Management*, **186**, 257–260.

Taylor, A M, Gartner, B L, Morrell, J J (2006): Western redcedar extractives: Is there a role for the silviculturist? *Forest Product Journal*, **56**(3), 58-63.

Van Acker, J, Stevens, M, Carey, J, Sierra-Alvarez, R, Militz, H, Le Bayon, I, Kleist, G, Peek, R D (2003): Biological durability of wood in relation to end-use. Part 1. Towards a European standard for laboratory testing of the biological durability of wood. *Holz als Roh und Werkstoff*, **61**, 35-45.

Zhang, S Y (1995): Effect of growth rate on wood specific gravity and selected mechanical properties in individual species from distinct wood categories. *Wood Science and Technology*, **29**, 451-465.

Zobel B J, Van Buijtenen, J P (1989): *Wood variation. Its causes and control*. Springer-Verlag, Berlin, Germany.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 Biology

Sustainable Mycological Alternatives in Natural forest and Conifer plantations in México

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Sustainable Mycological Alternatives in Natural forest and Conifer plantations in México

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Abstract

Concepts of mycoforests, mycosylviculture and their relationship to education, production and sustainable management of fungi in forests in México are analyzed. These concepts may be applied in Mexican protected areas, parks and forestry rural communities and improve socio-economic conditions. Two decades ago commerce of wild edible mushroom in the world was relatively small; mushroom industries were selling their products in a rather informal way. At the end of the 80´s important changes in mushroom commerce occurred; and it became organized in activities such as mushroom picking, cleaning, processing and packing and selling to retailers. Mushrooms prices may depend on factors such as: size, freshness, color, abundance, appearance, flavor, texture and familiarity of sellers and buyers with the species. Currently natural forests and forest plantations where mushrooms grow produce an important income in some European countries. Those countries have multifunctional forest practices integrating mushrooms into sustainable forest management. Mexicans living in most rural forestry conditions are used to picking and eating wild edible mushrooms every year. Natural forests in protected areas and national parks are ideal places for implementation of mycosilviculture and mycopark projects, mushroom courses may be offered to park officers and people living in rural communities inside parks and protected areas. These activities will serve to educate people and generate yearly income for them and these activities should be conducted in keeping with current laws to achieve sustainable management and conservation. Forest management programs and mushroom harvesting practices for commercial and home purposes use should be regulated to ensure sustainability. Thus, mushrooms pickers should buy mushroom picking permits, price to accord to their activities. Money obtained from these permits can be reinvested in forest and edible mushroom management that focuses on multifunctional conservation practices. Countries already applying some degree of mycosilviculture practices to mycoparks or truffle culture include France, Italy, Spain, Portugal, United States of America, New Zealand, Australia, Argentina, Chile, Israel and Morocco. Every year these countries produce significant income from wild edible mushrooms.

Keywords: sustainable mycology, education, mycoforests, mycosylviculture, mycoparks, México.

Introduction

Due to its biodiversity México is considered as one of the mega diverse countries of the world. Mushrooms are very abundant in the world (Hawksworth, 1991) and approximately 7,000 species of fungi have been studied from Mexico so far and the number of edible wild mushrooms EWM species may vary from one region to another (Guzmán, 1998; García & Garza, 2001). Diversity, richness and naming of edible wild mushroom species (EWM) may vary from one ecoregion, vegetation type and cultural region to another (Guzmán, 1998; Hawksworth, 1991). Edible wild mushrooms are thus

an important component of forestry ecosystems in México and have been used for centuries by native people and currently their fruiting bodies are studied with multiple purposes (García & Carrillo, 2011; González *et al.,* 2009). EWM contribute to forests health and productivity and yearly generate incomes of ca. 1.6 and 3 billion dollars around the world (Boa, 2004; Lopez *et al.,* 2009; Pilz *et al*., 2003). Productivity of EWM may occurr in forest ecosytems either pure or mixed natural or natural or man made and it might be due to the high diversity of ecological conditions and multiple possible associations to hosts species. Edible wild mushrooms are important for people leaving in rural communities of México since every year they eat and sell some species in order to have money in the "mushroom season" (Garza & Carrillo, 2011).

Ecological regulations

Regarding ecological conservation initiatives and laws México has different federal agencies which are focused on natural resources biodiversity of organisms as The National Commission for the study of Biodiversity (CONABIO), The National Commission for Natural Protected Areas (CONANP), The National Forestry Commission (CONAFOR) and the National Institute of Ecology (INE). There are several Ecological Regulation Norms for conservation of plants, animals and fungi and species are placed in different categories accordying to their status for their conservation and protection.

Mushrooms and people

Conifer and oak-pine forests in México produce abundant edible species and *Amanita caesarea, A. rubescens, Boletus pinophilus, B. edulis (sensu lato), Lactarius deliciosus, L. indigo, Russula delica, Cantharellus cibarius, Hypomyces lactifluorum, Bovista cyathiformis, Lycoperdon perlatum, L. pyriforme, Craterellus lateritius, Agaricus campestris* and *A. bitorquis, Pleurotus dryinus and Hericium erinaceus amongst other species.* Thornscrubs have some edible species such as *Boletus rubellus, Cantharellus cibarius, Lycoperdon perlatum, Agaricus campestris, Phlebopus portentosus, Pleurotus dryinus. Tropical and subtropical forest may produce some of the later species as well as some lignicolus species such as: Pleurotus spp.*

People living near the mountains generally prefer *Amanita caesarea, A. rubecens, Hypomyces lactifluorum, Agaricus campestris, Agaricus bitorquis, Boletus sff. edulis, B. pinophilus, Lactarius indigo, Lactarius deliciosus, Ramaria botrytis. Cantharellus cibarius, Lycoperdon perlatum, L. pyriforme, Armillaria mellea, Tricholoma magnivelare, Tricholoma terreum, and Hericium erinaceus* amongst many other edible species. Most people likes eating fresh mushrooms and infrequently they dry them or keep them in brine or salty water to be used during the year.

Growth conditions

Site conditions for growth of vegetation, biodiversity, plant age, soil type, rain cycles, temperatures and general weather conditions may vary considerably from one region and forest type to another and all these conditions together play an important role in edible wild mushrooms productivity. They are produced annually and some conifer and oak-pine forests are particulary interesting as they may produce a good amount of species, local peolpe may collect and eat edible mushrooms in season but there are still some states in which people never would eat a wild edible mushroom as they think that they are all poisonous or hallucinogenic. Thus we can mention the states of Nuevo León, Coahuila and Tamaulipas as mycofobic wheras most states from the center of the country are mycophilous. Interesting to mention that in the state of Querétaro there

is a record of people collecting and eating fresh *Mycenastrum corium* (García *et al*., 1998).

Aims

To make a review of the mycological panorama in México and to focus on the possibility of applying wild edible mushroom sustainable management thorugh the implamentation of mycoforest, mycotourism and mycosilviculture projects in order to improve socialeconomical income in rural communities keeping with current ecological laws in México.

Results

We offer an overview of the activities that we have carried out in México in the last 10 years regarding use and management of edible wild mushooms (see pictures). Promotion of a new vision on EWM culture and thier sustainable management through mycoforests including mycotourist and mycosilvicultural activities is already taking palces in some places in México with different degrees of success. Thus mycology related projects and thier activities might be used to achieve forests land conservation to a certain extent and have a positive socioeconomical result for people living nearby forests. The later because non timber forest products including EWM represent new alternatives which can generate an income in rural communities. Thus people can obtain employment at any of the different stages involved in these projects, and land owners, their families, neighbours and prople from other regions can work picking and procesing mushrooms. Promotion of mushroom cultural practices can be made thorugh courses on basic mushroom taxonomy for tourist, accomodation and restaurant facilities together with excelent gastronomic dishes using delicious edible wild mushrooms can also improve land owners economy. All activities should be integrated in the legal framework in each region in order to achive conservation and to improve economy. Trasability of the mushrooms aquired is important in order to avoid any health problems and to get people into business paying local taxes which can be applied for local mushroom promotion. Wild edible mushrooms either fresh, processed, dried or thier subproducts in different presentations may represent new ways to manage a small business locally and if local people are well organized they can become a big and interesting company based on mycoforests, mycotourism and mycosilviculture practices using sustainable management and conservation.

Threats to mushrooms productivity

Most forest ecosystems in México have threats for their sustainable management and that of wild edible mushrooms due mainly to forest fires which are a real threat every year, cattle, intensive or ilegal cutting of trees. Edible wild mushrooms can also be threatened by intensive collection of fruiting bodies e.g. *Tricholoma magrnivelare, Cantharellus cibarius, C. lateritius, Boletus edulis, B. pinophilus, Amanita caesarea, Lactarius delicious, Morchella esculenta and Ramaria spp.* in different states from the central region of México and all other edible species are recollected for local use and people that collect and sell mushrooms are known as hongueros which means mushroom people.

Activities

Forest land owners are now taking care of their forests and they now know that forest can be very productive regarding wild edible mushrooms and they also know that forest conservation allows a good production od mushooms every year and in some cases land owners do not allow people to come into their land without permission. They now

know that cattle can be very damaging for forests and mushrooms and in as much as possible they keep cattle away from the main areas were edible mushrooms grow yearly. They also know that leaving a certain amount of the total production of edible mushrooms in the forest is a good practice in order to get fruiting bodies every year. Further development is still on its way in order to improve people participation in the community as to start a small production enterprise. Plantations using inoculated pine seedlings with edible forest mushrooms smashed in water solutions have been made in several locations.

Conclusion

Mycoforests, mycotourist and mycosilviculture activities might become an important part of future multifunctional activities in forestry areas in México. Mushrooms are already important for many people living in rural communities as they either eat or sell wild edible mushrooms every year. These mushrooms are very important and produce an extra income for some people living in nearby forest communities. Lack of knowledge or traditional believes may sometimes limit the use of some edible species in some regions as people eat only what they know and identify by tradition as edible species and they do not want to eat other species unless someone eats them first without showing any health problems. Better understanding of the organization process required for good management of wild edible mushroom species including e.g. recollection, selling, purchase processing and conversion into new produtcs e.g. Truffled white wine. (Garibay, R. *et al.,* 2009; Trappe & Claride, 2010). Promotion events such as a mushroom town fair which considered divulgation of edible species, academic talks, gastronomy, art and sport activities for people of all ages are good ejemples of mushroom related activities.

Edible forest mushroom are abundant in Mexican forests and mixed oak-pine and oak forests have a high diversity of species (García & Garza, 2001; Guzmán, 1998). Edible mushrooms are also abundant in forests from the North and Central part of México but still little sustainable management practices are carried out (Garza *et al*., 2010, Quiñónez *et al.,* 2009). Mexican ecological agencies can be responsible for these new developments and generate socioeconomical development thorugh the mushroom related projects proposed.

Strategies:

1.- Promoting micoforests, mycoturist and mycosilviculture projects and education focused on mushroom organized activities and their socioeconomical importance as well as their relation to sustainable management and conservation.
2.- Promoting forest conservation practices in order to achive good edible wild mushroom productions and viceversa.
3.- Educating local and government people regarding mushroom importance in order to generate new alternatives of business in their lands always in the framework of current laws.
4.- Educating people for cleaning of forests in order to avoid forest fires and avoid all different threats for forest management and conservation of biodiversity.
5.- Generate new alternatives to improve socioeconomical income in mountain forestry zones during the mushroom season.

Bibliography

1) Boa, E. 2004. Wild edible fungi A global overview of their use and importance to people, Non-wood forest products 17, FAO, Rome (2004).

2) Garibay, R., J. Cordova, J. Cifuentes, R. Valenzuela, A. Estrada and A. Kong. 2009. Integrating wild mushrooms use into a model of sustainable management for indigenous community forests, For. Ecol. And Manag. (258): 122-131.

3) García, J., D. Pedraza, C. I. Silva, R. L. Andrade y J. Castillo. 1998. *Hongos del Estado de Querétaro.1a.* Edición. Universidad Autónoma de Querétaro, Facultad de Ciencias Forestales, 263 p.

4) García, J., y F. Garza. 2001. Conocimiento de los hongos de la Familia Boletaceae de México, Revista Ciencia UANL Vol. 4 (3): 336-343.

5) Garza, F., Quinónez M., Chacón, V., Garza, L., Carrillo, Q., and Cázares, E. (2010) Sustainable management of edible forest mushrooms in chihuahua, México. Actes du Colloque International des Champignons forestiers Comestibles a Potentiel Commercial. Univesrite Labal, Quebec, Canada. Pp. 105-118

6) Garza, F., y Carrillo, A. (2011). Panoramas micológicos futuros ante el embate del cambio climático en los Bosques del Norte de México. Simposio internacional gestión forestal del recurso micológico. Organizado por el Centro de Investigación Forestal Valonsadero de la Junta de Castilla y León. Valladolid 9-11 de Junio.

7) González, P, Garza, L., Salinas, M.C., Vera, L., Garza, F., Ramírez, X., Torres, O. 2009. Producción de metabolitos antimicrobianos, alcaloides y taninos por Suillus lakei *in vitro,* Revista Ciencia UANL, vol. XII., (1): 62-70.

8) Guzmán, G. 1998. Inventorying the fungi of México*,* Biodiversity and Conservation 7: 369-384. HAMMOND, P. M. 1992. *Species inventory,* pages 17-39 in: Global Biodiversity. B. Groombridge, ed.Chapman and Hall, London.

9) Hawksworth, D. L. 1991. The fungal dimension of biodiversity: Magnitude, significance, and conservation, Mycol. Res. 95:641-655.

10) Pilz, D., Molina, R., Danell, E., Waring, R., Rose, C., Alexander, S., Luoma, D., Kromack, K., Lefebre, C. (2003). Silvimushrooms: Predicting edible ectomycorrhizal mushroom productivity. Proceedings form the world compatibility iniciative workshop, No. 21: 199-207.

11) Quiñónez, M., S. Anguiano, V. Chacón, A. Hernández, F. Garza and J. Marmolejo. 2009. *Conocimiento y uso de los hongos macromicetos comestibles de la Alta Sierra Tarahumara, Chihuahua,* X Congreso Nacional de Micología, 20-24 Septiembre, Guadalajara, Jalisco, México, 181 p.

12) Trappe, J., & Claride, A.W. (2010). The hidden life of truffles: not just for gurrmands truffles play essential role in the health of ecosystems. Scientific American: 78-84.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 2 **Test Methodology and Assessment**

Analysis of decay progress anisotropy by X-ray computer tomography

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Analysis of decay progress anisotropy by X-ray computer tomography

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ABSTRACT

Wood has a serious handicap as structural materials that it is vulnerable to decay by fungi. A weather exposed bridge has a possibility of an abrupt fall down, and wooden houses with heavily decayed structural members are easily destroyed by large earthquakes. To avoid such damages, a new predicting method on the decay progress in timber structures, and also its strength losing behavior along with time, is required. We are now preparing a computer simulation method that enables above mentioned prediction, but basic data on wood decay process is lacking.
So, we carried out forced decay tests on small prismatic specimens with three anisotropic directions L, R and T in the length direction to clarify the decay developing velocity in the wood. Spruce was used as specimen, and both white-rot and brown-rot fungi were applied. At each projected term, decay progress was stopped, and the precise three dimensional density distributions in the specimen was measured using X-ray CT equipment.
As a result, the velocity of decay progress in longitudinal direction was the highest by the both used fungi. The variability of decay progress in the specimen was observed on the CT images, especially by white-rot fungi. This variability was seemed to be caused by the moisture contents distribution. In the radial and tangential direction specimens, the apparent weight loss was sporadically occurred by brown-rot fungi, that is in one third of the specimens per each exposure time group. From CT images, many cracks and shrinkage were seen in the vicinity of the cultural media by the dried tested pieces.

Keywords: decay anisotropy, X-ray, computer tomography (CT).

1. INTRODUCTION

Wood has a serious handicap as structural materials that it is vulnerable to decay by fungi. A weather exposed bridge has a possibility of an abrupt fall down (Karube, 2001), and wooden houses with heavily decayed structural members are easily destroyed by large earthquakes (Doi, 1995). To avoid such damages, a new predicting method on the decay progress in timber structures, and also its strength losing behavior along with time, is required. We are now preparing a computer simulation method that enables above mentioned prediction. This method can simulate very simple case as shown in Fig. 1. This figure demonstrates the decay progress in the longitudinal direction and the fall down by its own weight of a partially decayed beam.

To go to next step, we are preparing to extend this simulation method into three dimensional one, but basic data on wood decay process is lacking. For this purpose, it is indispensable to reveal the decay progress anisotropy in wood, then the X-ray Computer Tomography (CT) method is employed. With X-ray CT, it is possible to measure the three dimensional changes of density distribution of wood nondestructively. Using this method, Petutschnigg showed the possibility to detect the decay by taking account of the moisture contents (2002). Using X-ray CT, Jan Van den Bulcke et al. (2009) demonstrated that hyphae in the decayed wood could be observed.

We carried out forced decay tests using both white-rot and brown-rot fungi on small prismatic specimens with three anisotropic directions L, R and T in the length direction to clarify the decay developing velocity in the wood. At each projected term, decay progress was stopped, and the precise three dimensional density distributions in the specimen was measured using X-ray CT equipment. This paper is an expanded version of our previous article (Maeda et al., 2011).

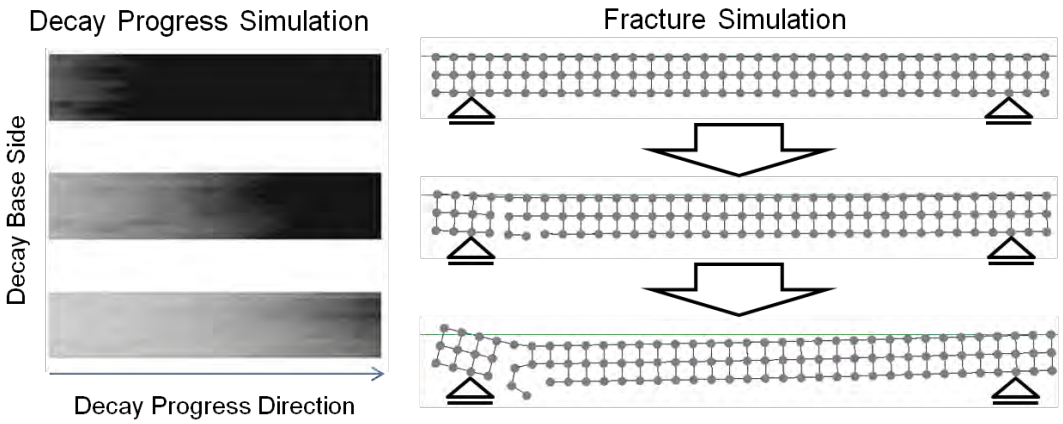

Figure 1: The simulation model.

2. MATERIALS AND METHODS

Three types of spruce specimens (*Picea Sitchensis*) were prepared from one log (Table 1). After measurement of the dry weight of the specimens (dried 2 days at 60 ˚C), epoxy resin was applied to four side-wall of prismatic test specimen (Fig.2) to prevent the fungal invasion. Then the specimens were oven dried at 60 ˚C until the weight become constant (about 1 week) to measure the weight of applied epoxy resin. One-third specimens were scanned before exposure to fungi, using X-ray CT scanner SKYSCAN 1174. Scanned condition was an average tube voltage of 50 kV, a tube current of 800 μA and an exposure time of 1,600 ms per image. A rotation step size of 1˚ and voxels size of approximately 30 μm × 30 μm × 30 μm were used. As about 3 cm height of the specimen could be taken per 1 time scan, to scan most part of the specimen, 3 times scan were carried out per one test specimen.

After the scanning of the nondecayed specimens, all specimens were sterilized using an ethylene oxide gas sterilizer and exposed to *Fomitopsis palustris* (brown-rot) or *Trametes versicolor* (white-rot), which were defined by Japanese Indusirial Standards (JIS) Z 2101 (2009). The fungus were cultured in several cultural bottles on ager medium containing 2% agar, 1% malt extract, and 0.5% peptone for 1 week in a cultivation room (26±2ºC, RH >70%), then specimens were put on the fungal medium. For the specimens exposed to brown-rot fungus, a plastic mesh sheet was put between the specimen and the fungal medium to prevent the specimens from absorbing too much water (Fig.2). After the exposure treatment, agar and hypha were removed from test specimens and scaning for all test specimens were carried out before and after oven dry treatment (at 60 ˚C until the weight becomes constant).

The grayscale value distributions of scanning images were converted to density distribution using linear relationship between them. The bright area of the images has high density. Five

wood species (Balsa, Hinoki, Teak, Oak and Indian Ebony) were employed as density phantoms (0.13~1.1 g/cm^3) and those oven dried phantoms were scanned before the test specimens scan.

Table 1: Test material matrix

Decay progress direction	Fungi invasion plane	Specimen size (mm)	Oven dried density (g/cm^3)	Sample number (n)
Longitudinal	RT	20 × 20 × 100(L)	0.46 ~ 0.56	72
Radial	LT	20 × 20 × 100(R)	0.45 ~ 0.51	72
Tangential	LR	20 × 20 × 100(T)	0.45 ~ 0.48	66

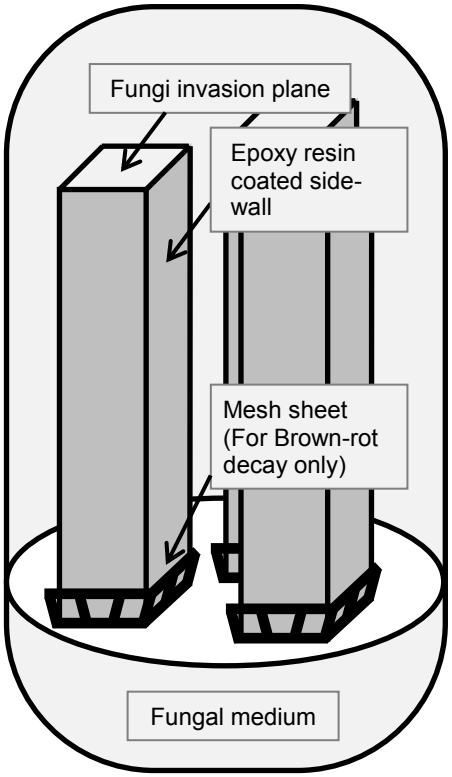

Figure 2: Schematic diagram of the decay tests.

3. RESULTS AND DISCUSSION

3.1 Forced decay test

Regardless of used decay fungus, weights of the decay tested samples in longitudinal direction were decreased constantly with time (Fig.3). Specimens exposed to brown rot fungi were decayed faster than that by white rot fungi. This can be explained by the difference of the fungi and the test condition. It is well-kown that brown rot fungi can decay soft wood faster than white rot fungi. The presence or absense of plastic mesh sheet caused the difference of the moisture contents. The average moisture content of white rot decayed specimen were about 100% and this condition is tough to decay progress (Mizumoto, 1964) in contrast to the 50% moisture content of brown rot decayed one.

In other directons with brown rot, apparent weight loss was occured by only one or two specimens per each exposure time. Vicinity of specimens exposured for more than 40 days were attacked by fungi in most cases. The moisture contents of them were about 10 ~ 30 %.
Any visual sign of decay couldn't be seen in the samples exposed to white rot in radial or tangential direction and mass of them weren't decreased.

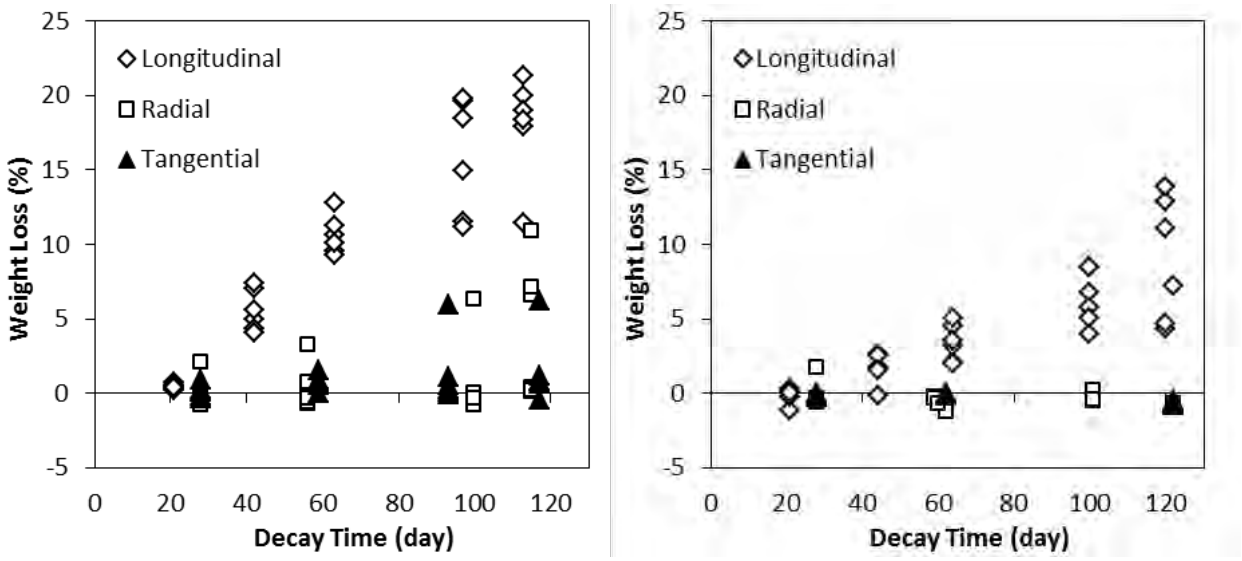

Figure 3: Weight loss change. (Left: Exposed to brown-rot fungi, Right: Exposed to white-rot fungi.)

3.2 X-ray CT images of samples

3.2.1 Decay progress in longitudinal direction (brown rot fungi)

Figure 4 shows X-ray CT images of decay tested specimen in longitudinal direction for 153 days. The shrinkage can be seen at all area of the specimen and that of the vicinity of the fungal medium is harder than that of the opposite end. The cracks were generated along the annual ring boundaries at first. The cracks along the radial direction can be seen at the fungal base side of the specimen. The density of the specimen was also decreased.
The density distribution of this specimen is shown in Fig. 5. In this figure, the correction of the shrinkage was done. The density decreased in late wood, but in early wood, that was no clear tendency. Since total mass of this plane decreased, this difference was considered to be caused by the difference of the shrinkage between late wood and early wood.

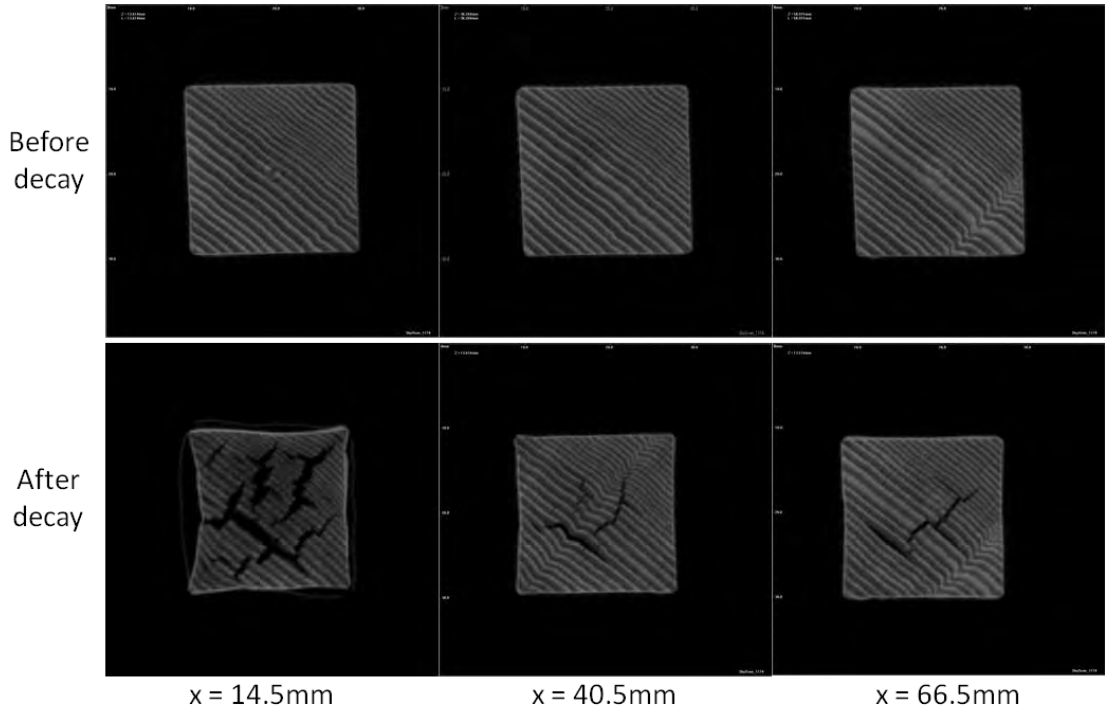

Figure 4: The X-ray CT images of the decayed sample in longitudinal direction for 153 days (Weight loss = 20%). (x: distance from the fungal medium)

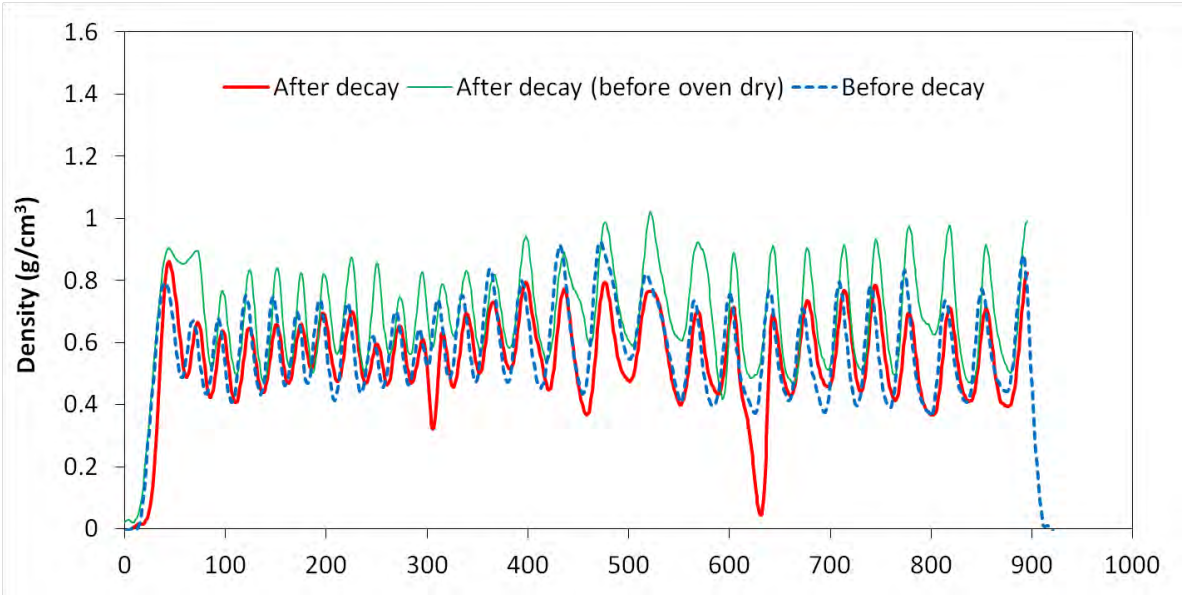

Figure 5: The density distribution of the decayed sample in longitudinal direction for 153 days. (Weight loss = 20%, x=66.5mm, the correction of the shrinkage was done.)

3.2.2 Decay progress in radial direction (brown rot fungi)

X-ray CT images of a decay tested sample in radial direction were shown in Fig. 6. The longitudinal shrinkage observed region was about 10 mm and the tangential shrinkage was about 30 mm from the cultural medium. In that region, the density was decreased and the pattern of the annual ring became unclear.

Figure 8 shows 3D CT images of this sample. It was seen that cracks propagated along the L, R, T direction respectively (Fig.8b). From Fig.8c, severe decay progress was seen in both latewood and earlywood at the vicinity of the fungi invasion plane.

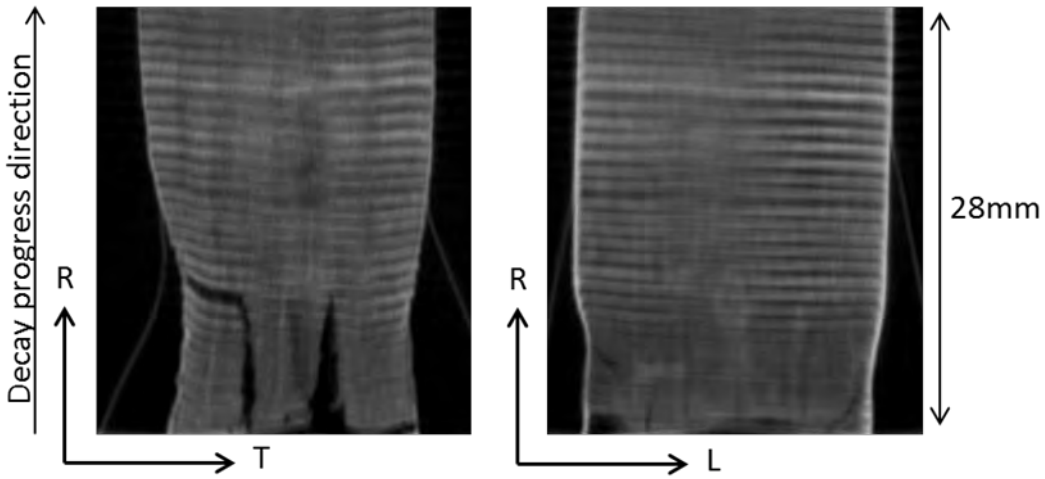

Figure 6: The X-ray CT images of the decayed sample in radial direction for 115 days.
(Weight loss = 11%)

3.2.3 Decay progress in tangential direction (brown rot fungi)

In tangential direction, decayed advanced samples were infected at the interspace between the epoxy resin and the end grain. In Fig. 7, the X-ray CT images of the early stage decay sample were shown. The cracks were progressed about 2 mm along annual ring boundary. In this case, the apparent density loss and shrinkage weren't observed.

Figure 9 shows 3D CT images of this sample. The shrinkage was seen only in the vicinity of the fungi invasion plane. From Fig. 9c, clear decay progress from the fungi invasion plane wasn't seen.

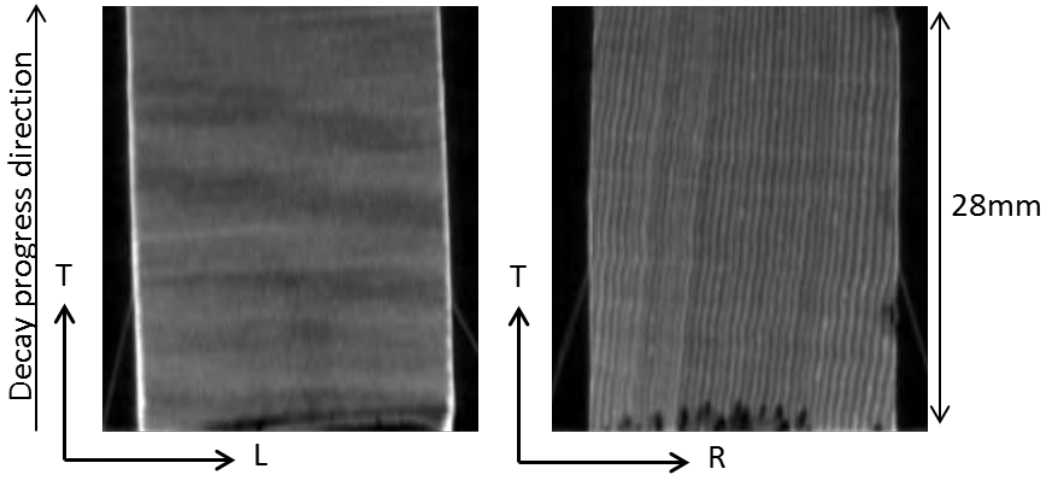

Figure 7: The X-ray CT images of the decayed sample in tangential direction for 117 days.
(Weight loss = 1.2%)

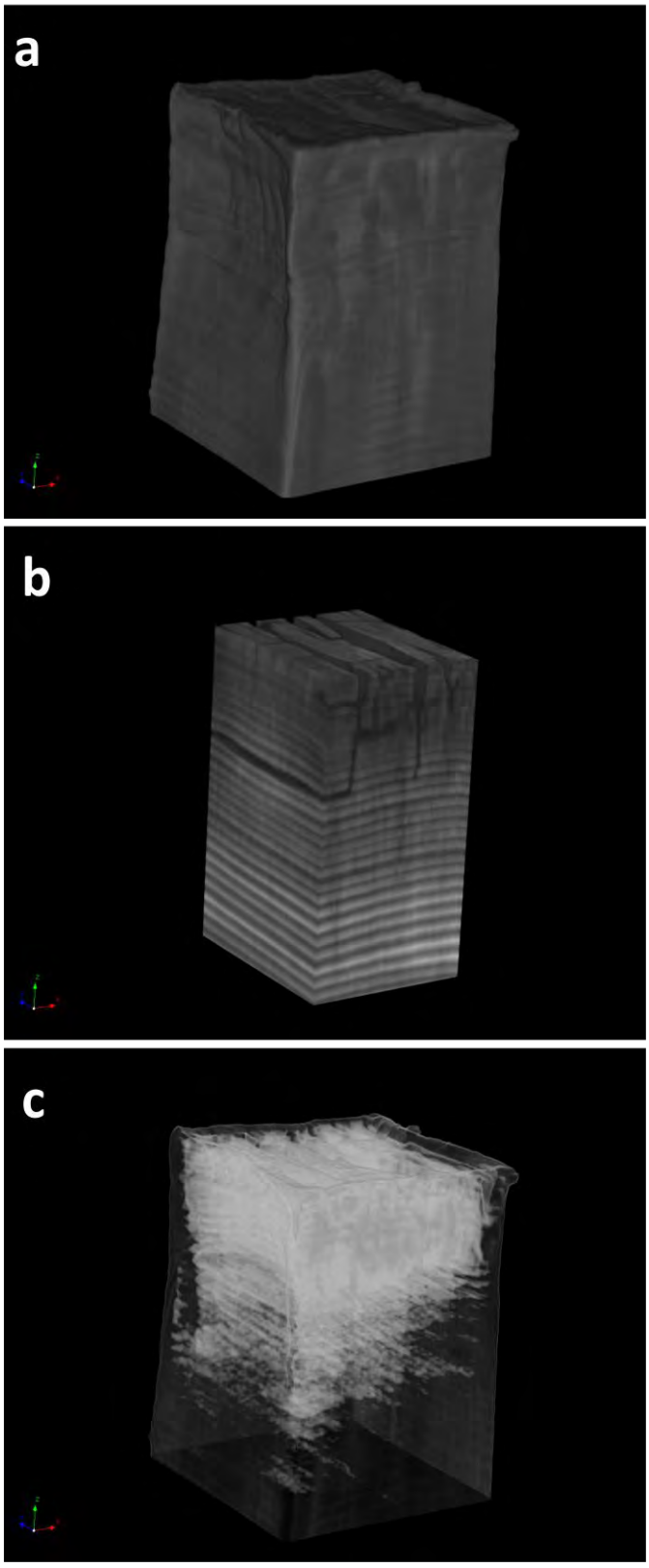

Figure 8: The 3D CT images of the decayed sample in radial direction for 115 days.
(Weight loss =11%)
(a: surface rendering, b: surface removed image, c: extracted low density part.)

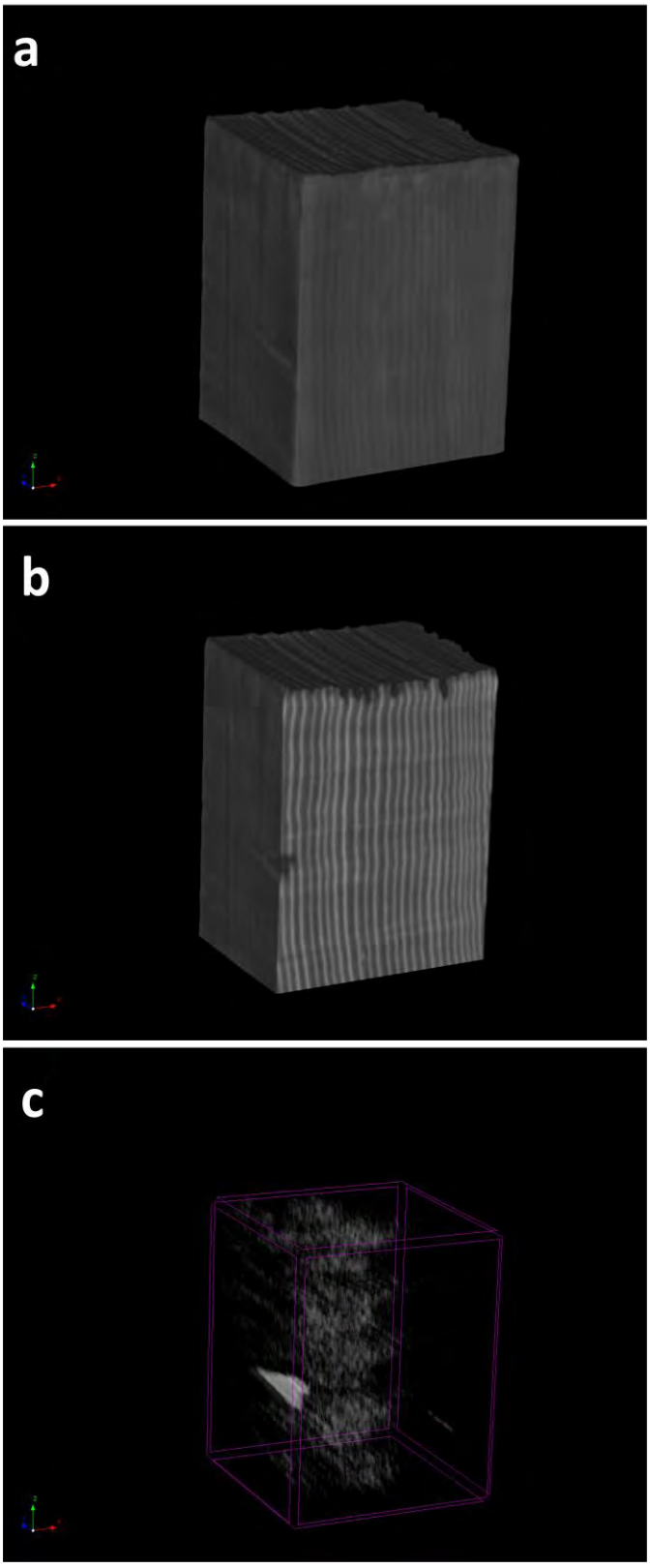

Figure 9: The 3D CT images of the decayed sample in radial direction for 117 days.
(Weight loss = 1.2%)
(a, b, c: see Fig. 8.)

3.2.4 Decay progress in longitudinal direction (white rot fungi)

Figure 10 shows X-ray CT images of decay tested specimen in longitudinal direction for 157 days. In the samples exposed to white rot fungi, no crack caused by the decay was observed. The shrinkage was smaller than that exposed to brown rot. The decreased area could be seen at all pictures.

The density distribution of this specimen is shown in Fig. 11. The density of the left side of Fig. 11 decreased in both late wood and early wood. In the right side, the density decrease was smaller than that of the left half and no density decrease can be observed in the center. In right side and center, the density before oven dry treatment was very high and indistinguishable between late wood and early wood. This indicates that this decay progress difference was caused by the difference of the moisture content.

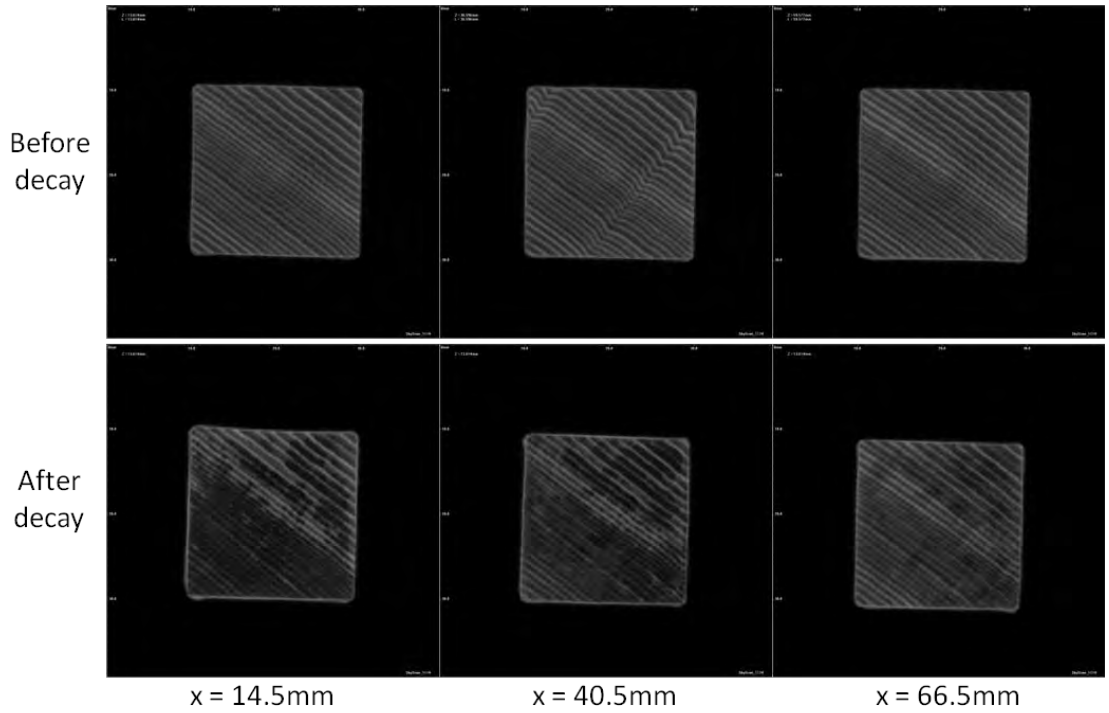

Figure 10: The X-ray CT images of the white-rot decayed sample in longitudinal direction for 157 days (Weight loss = 22%). (x: distance from the fungal medium)

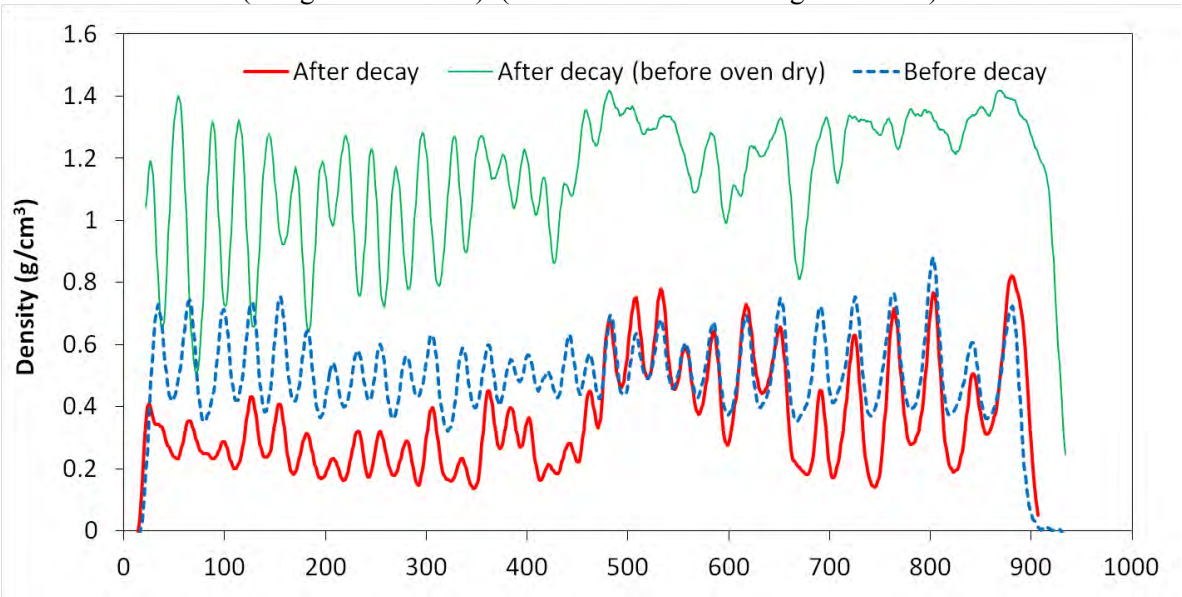

Figure 11: The density distribution of the decayed sample in longitudinal direction for 157 days. (Weight loss = 22%, x=14.5mm, the correction of the shrinkage was done.)

4. SUMMARY

The forced decay tests for three orthotropic directions were carried out to reveal the anisotropy of the decay progress in wood. Weight loss of the decay tested samples in longitudinal direction increased constantly regardless used fungi and they progressed faster than that in other directions. The X-ray CT images brought much information in detail. Regardless of the decay progress direction, cracks were observed along the radial and tangential direction. The shrinkage in the tangential direction was harder than that in the longitudinal direction. The density loss was observed widely and severe in late wood.

5. REFERENCES

Doi T, Miyano M, Lu H, Kitamoto H (1995): The damage of wooden houses attacked by the 1995 Hanshin-Awaji earthquake. Research report of AIJ Kinki branch, 35, 221-224. ISSN 1345-6660

Van den Bulcke J, Matthieu B, Van Acker J, Van Hoorebeke L (2009): Three-Dimensional X-Ray Imaging and Analysis of Fungi on and in Wood, Microscopy Microanalysis, 15, 395–402. ISSN 1431-9276

Japanese Industrial Standards (2009): Methods of tests for wood, JIS Z-2101. Japanese Standards Association, Tokyo

Karube M, Miyatake A, Suzuki K, Kato H (2001): Report on the collapse of bridge made with Bongossi. Journal of architecture and building science, 12, 89-92. ISSN 1341-9463

Maeda K; Ohta M; Momohara I (2011): Analysis of decay anisotropy by X-ray computer tomography, Pro Lingo, 7(4), 18-25.

Mizumoto S (1964): Relation of Moisture Content of Wood and Relative Humidity in an Atmosphere to the Decay of Japanese Red Pine Wood, Due to Attack of Four Species of Gloeophyllum. Journal of the Japanese Forestry Society. 46, 9-13. ISSN 0021-485X

Petutschnigg A. J, Flach M, Katz H (2002): Decay recognition for spruce in CT-Images. Holz als Roh- und Werkstoff, 2002, 60, 219–223. ISSN 0018-3768

IRG/WP 12-20502

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 2 **Test methodology and assessment**

Field-testing of Norway Spruce Claddings with Monitoring of Moisture Content, Material Temperature and Microclimate

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Field-testing of Norway Spruce Cladding with Monitoring of Moisture Content, Material Temperature and Microclimate

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ABSTRACT

The physical function of a cladding is to protect the interior construction. Under normal circumstances the performance requirements can be met for a very long time, meaning that the technical service life of a wooden cladding can be very long. Since the cladding is a major part of the facade, it also has visual requirements that may define the aesthetic service life, and often it is much shorter than the technical service life. The visual changes that occur during weathering may be colour changes, abrasion or wear, blistering, flaking, and even cracks in the wood or coating, but more often growth of mould and blue stain fungi.

A field test with claddings was established in southern Norway to study the variation in moisture content in the cladding, the material temperature and the ambient microclimate. The aim of the field test is to provide data that can be used to estimate aesthetic service life of claddings based on material properties, surface treatments, and climate. One of the primary objectives is to identify conditions that are critical for establishment and development of mould and blue stain fungi. The field test will also be used for preference studies in order to identify critical levels of visual changes.

Claddings were made of Norway spruce (*Picea abies* (L.)) from two sites with different growth conditions. It includes both heartwood and sapwood, and both juvenile and mature wood. Selected boards were crosscut into four pieces, of which three were treated with different paints, and one was left untreated. The samples are exposed in an open environment, facing either north or south. Relative humidity and temperature is measured in air close to surfaces, and wood temperature and moisture content are measured in the claddings. Moisture content is calculated by measuring direct current resistance across grain, and corrected for temperature. Mould growth and blue stain fungi, as well as mechanical changes on the surface of the claddings were evaluated visually according to the EN 927-3 standard. Results from the first year are presented. It shows differences in moisture content and material temperatures depending on colour and exposure, whereas the differences in mould growth are minor so far.

Keywords: Norway spruce, cladding, coating, moulds, blue stain fungi, field test

1. INTRODUCTION

Wooden claddings in façades are common in Norway, and Norway spruce (*Picea abies*) is the most frequently used species. In order to maintain or even strengthen this position it is necessary to provide documentation of service life for wooden façades since this is a requirement in the EU Construction Products Directive (CPD 1988). The technical service life of a cladding can be very long, but since the cladding is a major part of the façade, it also has visual requirements that may define the aesthetic service life, which often is shorter than the technical service life. The visual changes that occur during weathering can be colour changes, abrasion or wear, blistering,

flaking, and even cracks in the wood or coating, but more often growth of mould and blue stain fungi. The available methods and procedures only take technical service life into account, and methods for estimating the aesthetical service life are missing.

A Norwegian project targeting the aesthetical service life of wooden cladding and decking is carried out, and the overall objective is to improve the carbon footprint of modern construction through extending service life of innovative wood building products. The project is divided into four work packages:

1. Analyse the effects of meso- and microclimate on variation in wood moisture content and temperature, and the development of discolouring fungi on cladding and decking.
2. Evaluate aesthetic service life both with quantitative methods and with respect to user preferences.
3. Compare service life of improved wooden cladding products with a set of reference products.
4. Establish an outdoor test site for cladding and decking products.

This presentation describes a field test with synchronous monitoring of microclimate, wood moisture content and temperature in Norway spruce claddings in southern Norway. The aim of the field test is to provide data that can be used to estimate aesthetic service life of cladding based on material properties, surface treatments, and climate. One of the primary objectives is to identify conditions that are critical for establishment and development of discolouring fungi. Together with visual evaluation, the data provided by the monitoring system can for instance be used to calculate dose/response functions for establishment and growth of discolouring fungi. Combined with studies of user preferences, this can be used to calculate aesthetical service life of wooden claddings in different environments.

2. EXPERIMENTAL METHODS

2.1 Material

Norway spruce was sampled from two origins in Sweden; one high productive site in southern Sweden, and one low-productive site further north (Table 1). The aim of the sampling was to obtain differences in growth conditions and large variation in wood properties.

Table 1. Geographical data and forest inventory data of the origins.

Origin	Latitude, N	Longitude, E	Altitude [m]	Site index, H40[a]	Age [years]
Tönnersjöheden	56.7066	13.1140	140	G23	70
Siljansfors	60.8852	14.3454	300	G11	150

[a] *Dominant height at 40 years age.*

Butt logs were sawn, kiln-dried, and resawn into 19 by 98 mm boards. Claddings were procured both from inner heartwood, outer heartwood and sapwood, and it included both juvenile and mature wood. The boards were crosscut into four pieces, of which three were treated with different coating systems and one was left untreated. The coating systems were similar, but differed as such; 1) red coloured with fungicide, 2) white coloured with fungicide, and 3) white coloured without fungicide. The coated samples were applied with one layer of a fully pigmented waterborne alkyd emulsion primer and two layers of a waterborne acrylic topcoat on the top face and the sides. Primer and topcoat were applied by spraying in an automatic industrially setup.

2.2 Test setup and data recording

The panels were installed vertically and exposed facing both north and south on a test house in an open environment in Ås, Norway from November 2010. The experiment also includes a test house in a partially shadowed environment (Gobakken & Vestøl 2012), but only data from the test house in the open environment is reported in this paper.

Relative humidity and air temperature are measured in air close to surfaces of the claddings, and wood temperature and moisture content are measured at different depths (1 mm, 3 mm and 6 mm from the surface) in claddings. Moisture content is calculated according to Samuelsson (1993) based on measuring resistance across grain with direct current between electrodes 25 mm apart. Electrodes are inserted in predrilled holes from the backside and fastened with conductive glue. Material temperature is measured with thermocouples at the same depths, and it is used for adjusting the recorded moisture content (Samuelsson 1993). Each measurement is recorded every half an hour.

The mould coverage was evaluated according to EN 927-3 (2006) in September 2011. Gobakken and Vestøl (2012) have reported the results, and only a brief presentation is given here. This presentation will present results on moisture content and material temperatures recorded in 2011. The results are limited to the recordings 3 mm from the surface, and the presented values are means of several pairs of electrode in each combination of coating and exposure. It is also limited to uncoated claddings and claddings coated with red paint with fungicide and white paint with fungicide, while the claddings coated with white paint without fungicide are excluded. The statistical calculations were performed in JMP (SAS Institute Inc 2010).

3. RESULTS AND DISCUSSION

3.1 Moisture content

The measuring system provides time series of moisture content, material temperature, and relative humidity and temperature in ambient air. In Figure 1 the moisture content in claddings with different coating and different exposure are compared. It shows lower moisture content in claddings with red coating, particularly on walls facing south. A red coloured surface absorbs more heat than a white one, and this contributes to the lower moisture content.

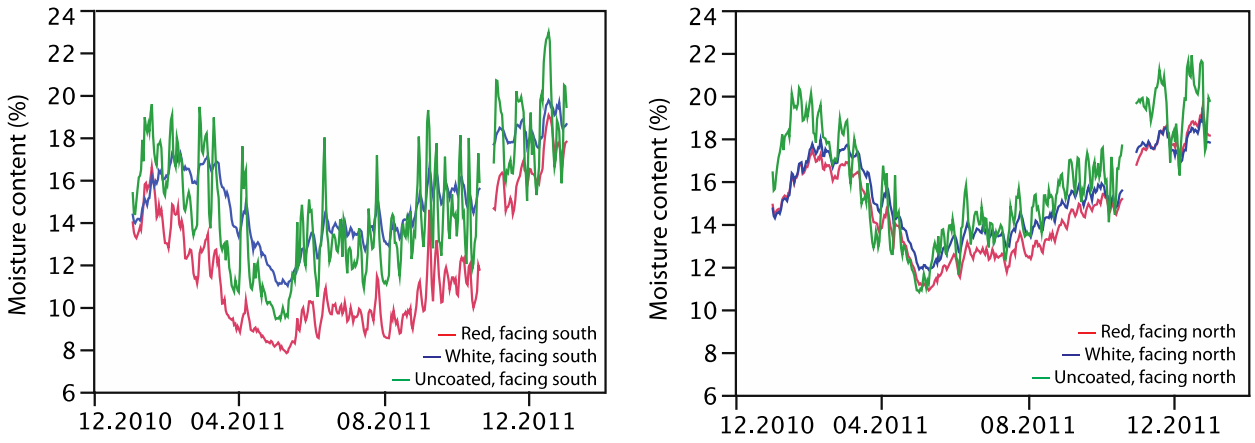

Figure 1. Daily average of moisture content in claddings facing south (to the left) and those facing north (to the right).

The annual mean moisture contents in claddings facing south was 11.7% for those with red coating, 15.1% for those with white coating, and 14.7% for the uncoated. Corresponding values for claddings facing north was 14.7% for those with red coating, 15.3% for those with white coating, and 16.0% for the uncoated. Except for white claddings, claddings facing south had larger fluctuations in moisture content than those facing north. Uncoated claddings had larger variations than the coated ones, but the red-coated claddings facing south had almost as large variation as the uncoated ones.

Figure 2 shows the distributions of moisture content in claddings with different coating and different exposure. Combined with records of mould growth and deterioration, such data can be used in dose-response calculations. The darker green distributions in Figure 2 show the moisture content in periods when air temperature is above 5˚C and relative humidity is above 80%. This is considered as critical conditions for mould growth (Gobakken 2009), since germination and colonization occurs for the majority of the mould species when relative humidity is above 80-85% (Adan 1994, Viitanen 1994, Viitanen 1996), and the lower limit for fungal colonization is generally about 0-5˚C. These conditions occurred slightly more in front of claddings facing north than those facing south.

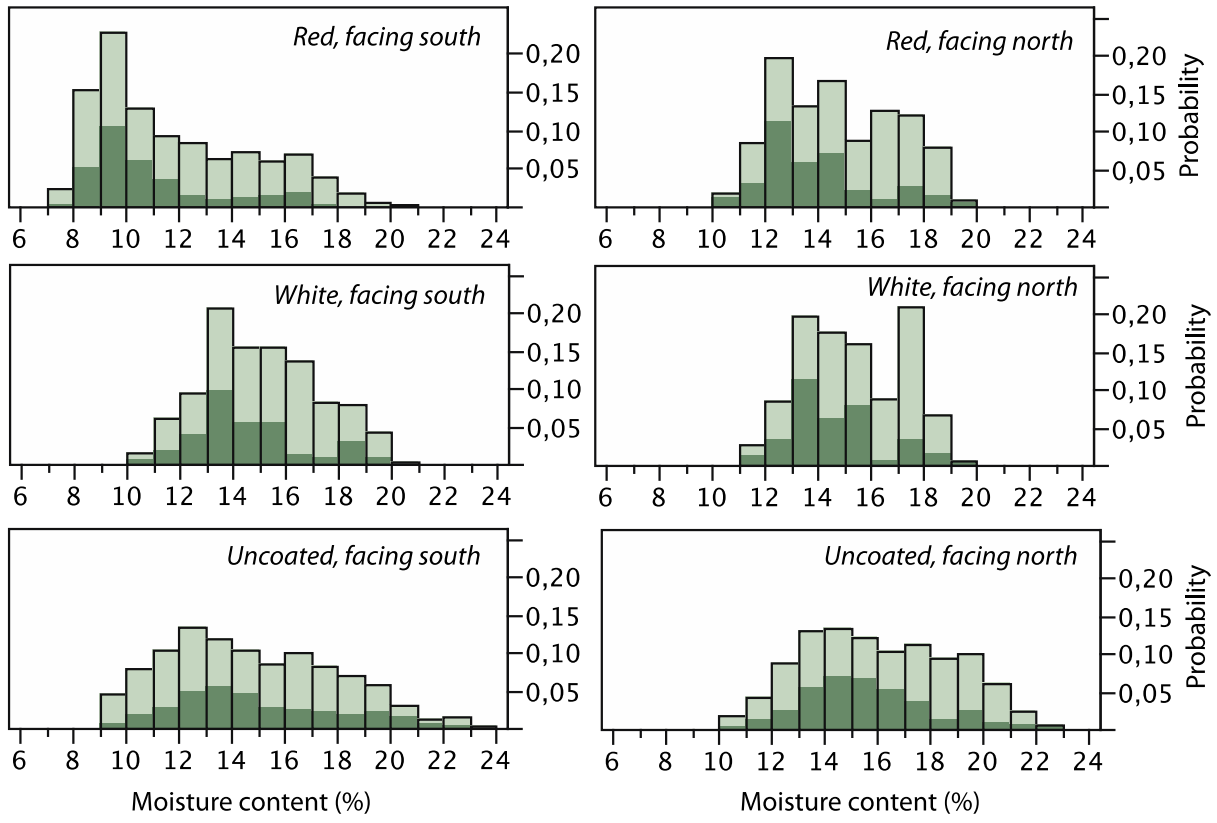

Figure 2. Distributions of moisture content in 2011. The dark green parts show the distribution occurring when ambient air has temperature above 5 ˚C and relative humidity above 80%.

3.2 Material temperature

The material temperatures are recorded mainly for calculation of moisture content, since it has an effect on the relation between resistance and moisture content. The formulas used are those presented by Samuelsson (1993). Some observations of unexpected moisture increase during daytime on sunny days indicate that the moisture corrections used are not accurate, and this has

to be investigated. The system provides raw data on resistance, and it is possible to apply any available formula for calculating moisture content from resistance and temperature.

Figure 3 shows time series of material temperatures in the claddings in 2011. It shows that the red-coated surfaces absorb more heat than the white and the uncoated ones, and they have higher mean temperatures. Especially the peaks in temperature are higher in claddings with red coating. Data on temperature variations during a day shows that the red surfaces facing south can experience quite large range of temperature. This may induce mechanical stress in the paint, and eventually cracking. Together with records of mechanical damages to the paint these data can be used in estimation of service life of different coatings under different exposures and microclimates.

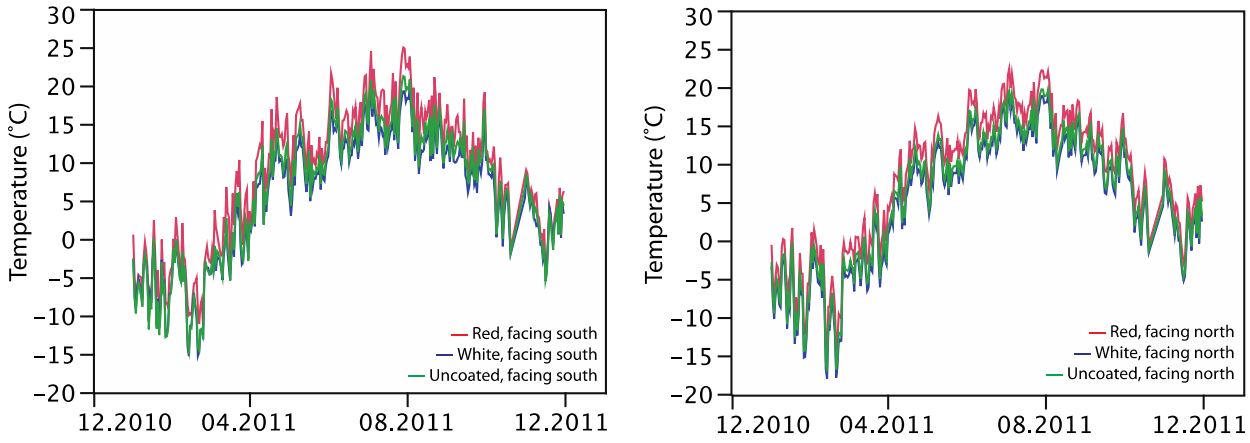

Figure 3. Daily average of material temperatures in claddings facing south (to the left) and those facing north (to the right).

3.3 Mould growth

The mould ratings after 10 months exposure were reported by Gobakken and Vestøl (2012). In general, the ratings were low (0 and 1) due to the relatively short exposure time, but some of the uncoated specimens showed higher ratings (2 and 3). Claddings facing south had somewhat higher mould rating than those facing north, and it was explained as an effect of UV-degradation, making the surface more susceptible for growth of mould and blue stain fungi (Gobakken & Vestøl 2011).

4. CONCLUSIONS

The field test will be a useful tool in studies of performance and aesthetic service life of wooden claddings. The combination of data provided will be used to estimate aesthetic service life of wooden claddings. It can for instance be used to quantify critical conditions for establishment and development of mould and blue stain fungi, and it can be used to calculate doses, in terms of time with critical conditions.

5. REFERENCES

Adan, O C G (1994): On the fungal defacement of interior finishes. *PhD Thesis, Eindhoven University of Technology, Eidenhoven, The Netherlands*.

Gobakken, L R (2009): Surface mould growth on painted and unpainted wood; — Influencing factors, modeling and aesthetic service life. *PhD Thesis, Norwegian University of Life Sciences, Ås, Norway.*

Gobakken, L R, Vestøl, G I (2012): Effects of microclimate, wood temperature and surface colour on fungal disfigurement on wooden claddings. *The International Research Group on Wood Preservation*, Doc. No. IRG/WP/12-20490.

CPD (1988): Council Directive 89/106/EEC of 21 December 1988 on the approximation of laws, regulations and administrative provisionsof the Member States relating to construction products. *Official Journal of the EuropeanCommunities No L 40/12 of 1989-02-11.*

EN 927-3 (2006): Paint and varnishes. Coating materials and coating systems for exterior wood. Part 3: Natural weathering test.

Samuelsson, A (1993): Calibration curves for resistance-type moisture meters. Paper presented at 3rd IUFRO International Wood Drying Conference, Vienna, August 18-21, 1992. Trätek Rapport I 9302012, Trätek, Sweden.

SAS Institute Inc. (2010): JMP® 9 User Guide. SAS Institute Inc, Cary, NC.

Viitanen, H (1994): Factors affecting the development of biodeterioration in wooden construction. *Materials and Structures*, 27: 483-493.

Viitanen, H (1996): Factors affecting the development of mould and brown rot decay in wooden material and wooden structures. Effect of humidity, temperature and exposure time. *Dissertation. Department of Forest Products. The Swedish University of Agricultural Sciences.* Uppsala.

IRG/WP 12-20503

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 2 **Test methodology and assessment**

Mould growth on wood-based materials – a simulated in-service study

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Mould growth on wood-based materials – a simulated in-service study

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ABSTRACT

Ten different wood-based materials including preservative-treated wood, fire retardant-treated wood, modified wood, WPCs and untreated references of pine sapwood and spruce were placed in three different environments (an attic and two crawl spaces) for a period of 26 months. Mould growth was analysed at five to seven month intervals in an effort to map the growth development. The relative humidity and temperature were logged continuously.

The results obtained from testing in the two crawl spaces generally corresponded well with a previous laboratory study. None of the materials tested could completely withstand mould growth during the 26 months' exposure time. Most promising results were obtained with the preservative-treated WPC and the least promising with the modified wood materials. For the latter, poor mould resistance is of major concern.

Keywords: mould, crawl space, attic, preservative-treated wood, fire retardant treated wood, modified wood, WPC

1. INTRODUCTION

This study is part of a series of tests to investigate the mould resistance of different wood materials and is a follow-up of a laboratory study presented at the IRG Annual Meeting in 2010 (Johansson *et al* 2010).

The purpose of this study was to assess the performance of the same materials tested in the laboratory against mould growth in simulated in-service conditions. Thus, test samples were placed in an attic and in two crawl spaces with different climatic conditions. Compared to the laboratory study relative humidity (RH) and temperature in these environments fluctuated and the mould resistance of the different materials was tested for a longer period.

2. MATERIALS AND METHODS

2.1 Materials

The materials tested are specified in Table 1, i.e. the same materials tested in the laboratory study (Johansson *et al* 2010). The sample size was 50 x 100 mm.

Table 1. Material tested in the study.

Material	Description	Comment
Untreated wood (references)		
Pine (*Pinus sylvestris*) sapwood	Planed	
Spruce (*Picea abies*)	Planed	
Preservative-treated wood	**Active ingredients**	
Celcure AC 800	Copper, benzalkoniumchloride	Treated according to Nordic class AB, i.e. use class 3 (above ground); purchased by SWPA from timber yards.
Tanalith E-7	Copper, propiconazole, tebuconazole	
Wolmanit CX-8	Copper, boron, bis-(N-cyclohexyldiazenium-dioxy-) (HDO)	
Fire retardant-treated wood		
Dricon	Planed *P sylvestris* treated with Dricon, fire-retardant system by Arch Chemicals	Treatment carried out by Woodsafe AB
Modified wood		
Acetylated pine (*P sylvestris*)	Acetyl content 22-23%	Prepared at SPs pilot plant
Furfurylated pine (*P sylvestris*)	WPG approximately 35%	Submitted by Kebony ASA
Thermally treated pine (*P sylvestris*)	The thermal process had a maximum temperature of 212 °C for duration of one hour.	Thermal treatment carried out by Scandinavian Finewood AB
WPC		
WPC untreated	~50% M/M *P Sylvestris* fibres (untreated) ~50% M/M polypropylene	
WPC preservative-treated	~50% m/m *P sylvestris* fibres treated with a isothiazolone-based solution to a retention of approximately 700 ppm, giving a retention of approximately 350 ppm in the WPC ~50% m/m polypropylene	Preservative formulation submitted by Viance LLC

2.2 Test environments

Two single-family houses, built in 1997 and 2007, and situated in the Borås-Gothenburg area in south-western Sweden were selected for the study. Samples were exposed in both the attic and the crawl space of the older house but only in the crawl space of the younger house.

The test samples were placed in stainless steel spring clips mounted on aluminium strips on the blind floor in the crawl spaces and on roof trusses in the attic (Figure 1). The samples could easily be dismantled from the clips prior to analysis of microbial growth.

Figure 1. Test pieces and the data logger mounted on the blind floor.

2.3 Measurements of temperature and humidity

The relative humidity and temperature at each test-site were registered every fourth hour by data loggers with internal sensors (Testo 177-H1 and Testo 177 H2). These were placed close to the specimens to ensure that measured conditions matched those the specimens were exposed to. One logger was placed at each test-site.

The data loggers were calibrated after the test periods but the data presented in this report is raw data and not adjusted. It should be regarded as a description of prevailing climate conditions in the different environments. The adjusted relative humidity is expected to be higher than presented, whereas the temperature is expected to be more or less the same.

2.4 Assessment of mould growth

The test samples were analysed with regard to mould growth at intervals of five to seven months. At each inspection they were removed from the racks and the surface that had been exposed to the open air in the attic and crawl spaces was studied under the microscope at 10–40x magnification, making it possible to detect mould growth not visible to the naked eye. The mould growth was rated according to the scale in Table 2.

Table 2. Rating scale for assessment of mould growth.

Rating	Description
0	No fungal growth
1	Initial fungal growth consisting of scattered hyphae on the surface
2	Still scattered growth, but more apparent than in 1. Conidiophores may have started to develop.
3	Patchy distributed heavy growth. Hyphae with developed conidiophores.
4	Heavy growth over the entire surface

3. RESULT AND DISCUSSION

3.1 Relative humidity and temperature in the attic

The relative humidity and temperature in the attic during the exposure period is shown in Figure 2.

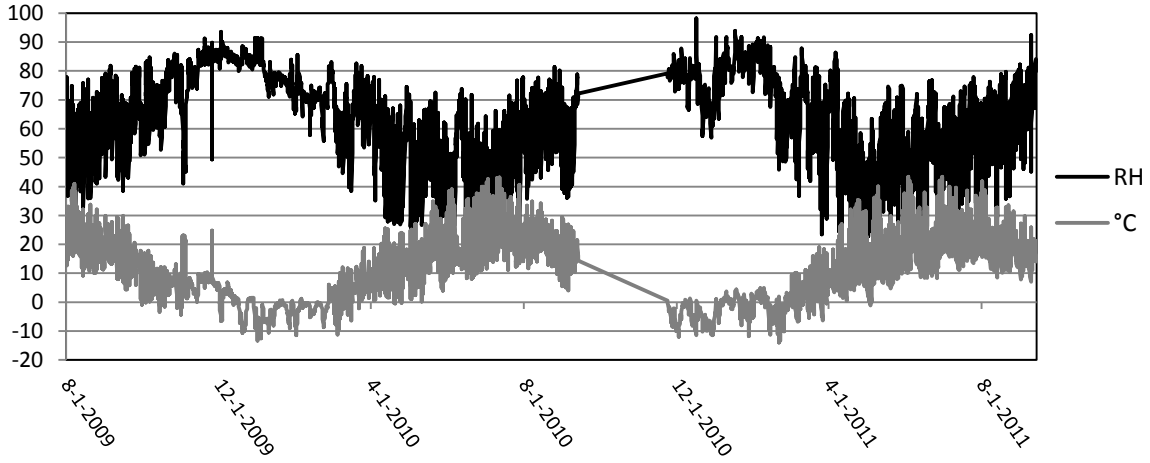

Figure 2. Climate data from the attic.

The attic can be characterized as dry and relatively hot in the summer and more humid in the winter but at the same time cold which reduces the potential for mould growth.

3.2 Relative humidity and temperature in the crawl spaces

Climate data for the two crawl spaces are shown in Figures 3 and 4. Crawl space no. 2 had higher moisture levels than crawl space no. 1. Both crawl spaces can be characterized as warm and humid in the summer and early autumn. This is common for naturally ventilated crawl spaces (Bok *et al* 2009).

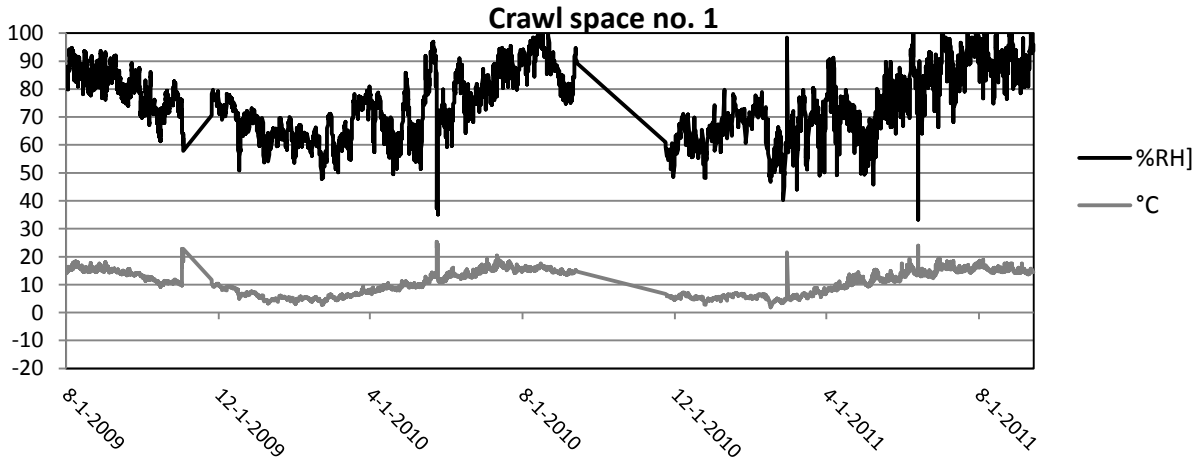

Figure 3. Climate data from crawl space no. 1.

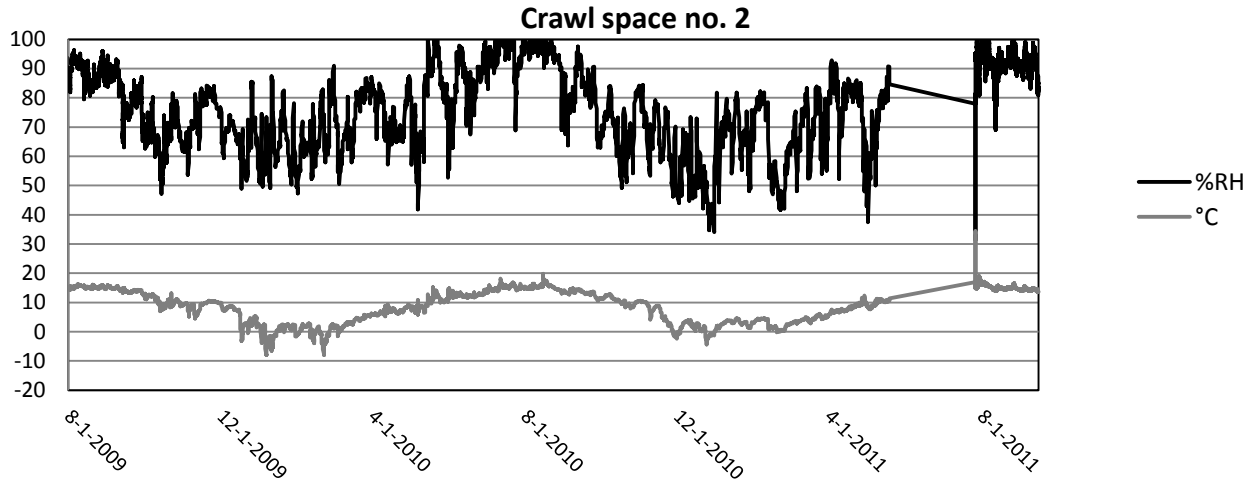

Figure 4. Climate data from crawl space no. 2.

3.3 Mould growth in the attic

No mould growth was detected on any of the test samples at any inspection. Thus, the environment in the attic did not support mould growth and this can be illustrated in Figure 5. The curve shows the critical moisture level at different temperatures. The dots represent one measuring point of relative humidity and temperature. The total numbers of measurements above the calculated critical moisture level were relatively few and scattered in time and therefore no mould growth was established.

The result corresponds well with recent results from a study on critical moisture levels on different materials (Johansson *et al* 2012). The result also corresponds well with practice. Most attics actually have a climate which does not support mould growth.

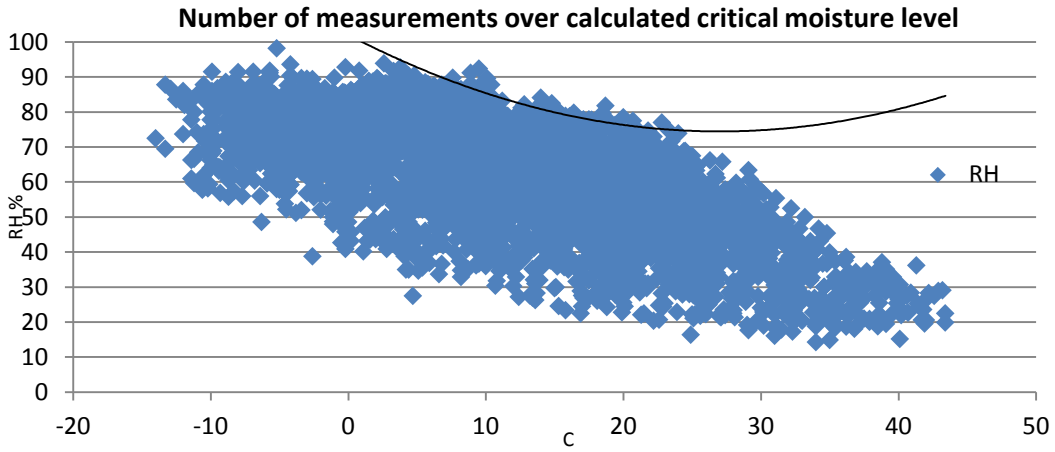

Figure 5. Monitored relative humidity and temperature in the attic in relation to calculated lowest critical moisture level.

3.4 Mould growth in the crawl spaces

Mould growth was supported in both the crawl spaces, and the median of the ratings is presented for the different materials in Figures 6-11.

The spruce references and untreated WPCs in crawl space no. 2 were assessed to have a lesser degree of mould growth at the last inspection (Figure 11). The estimation of the mould growth is always subjective and can therefore differ between separate occasions. A small difference in estimated growth can result in a noticeable difference in the median value.

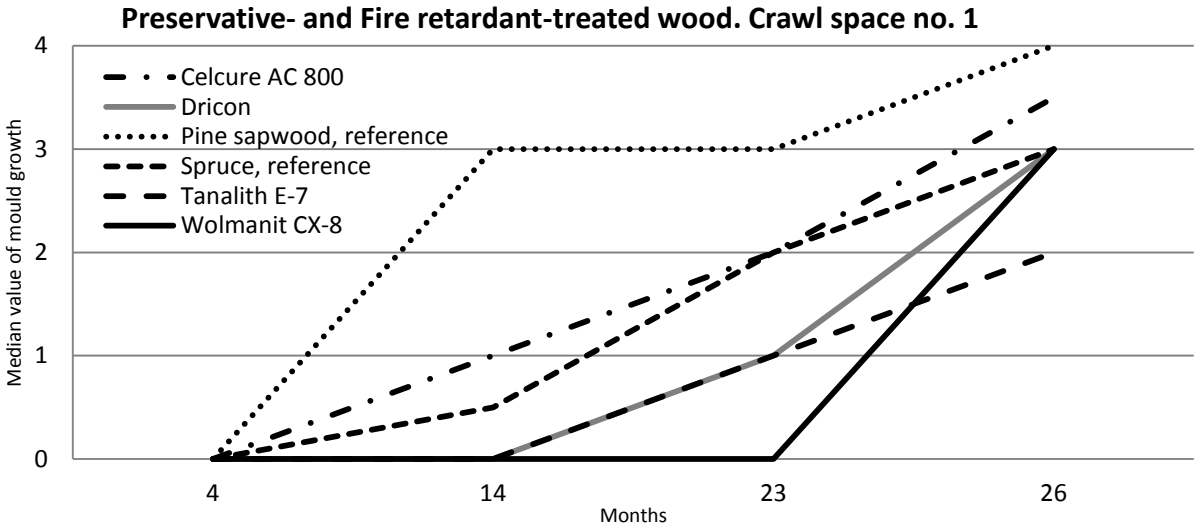

Figure 6. Median value of the mould growth on preservative-treated and fire retardant-treated wood in crawl space no. 1.

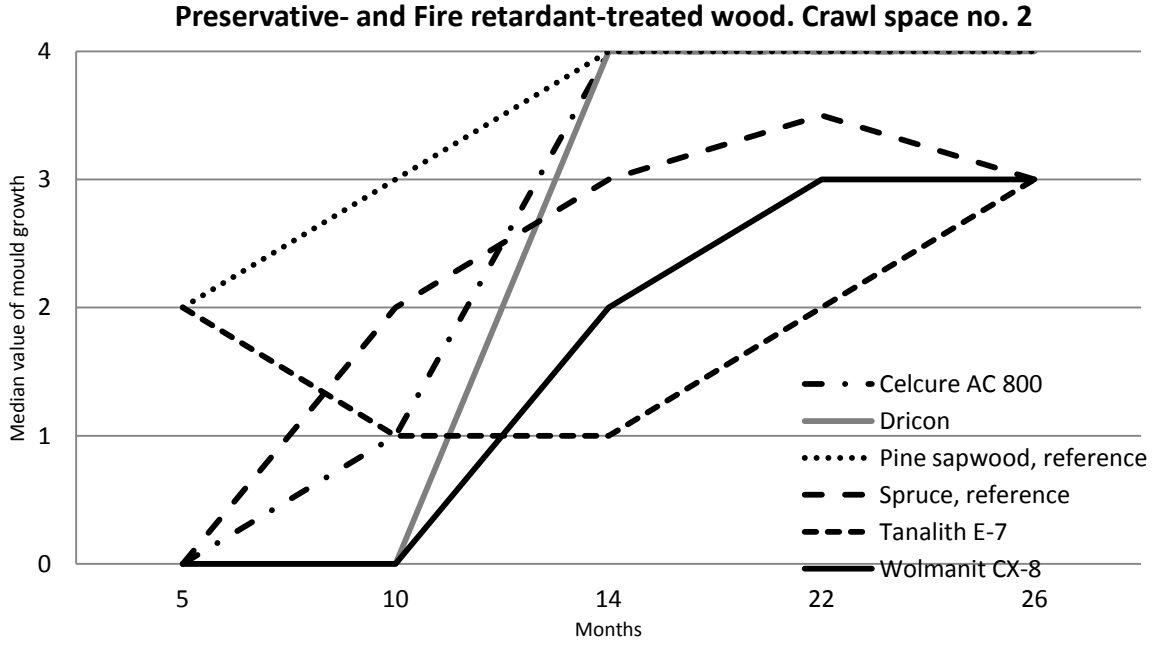

Figure 7. Median value of the mould growth on preservative-treated and fire retardant-treated wood in crawl space no. 2.

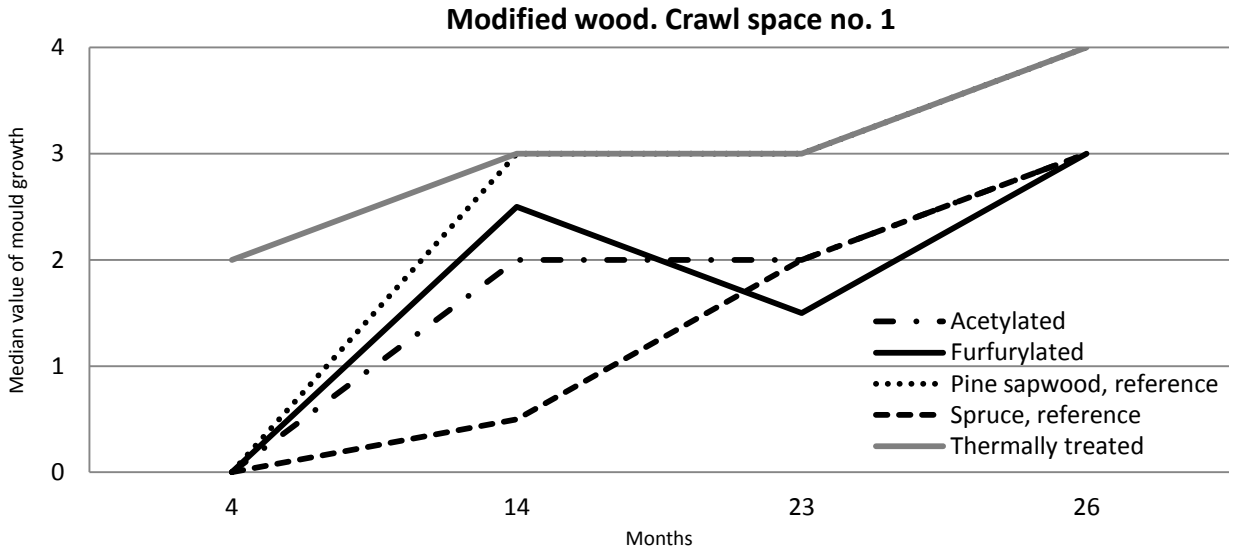

Figure 8. Median value of the mould growth on the modified wood in crawl space no. 1.

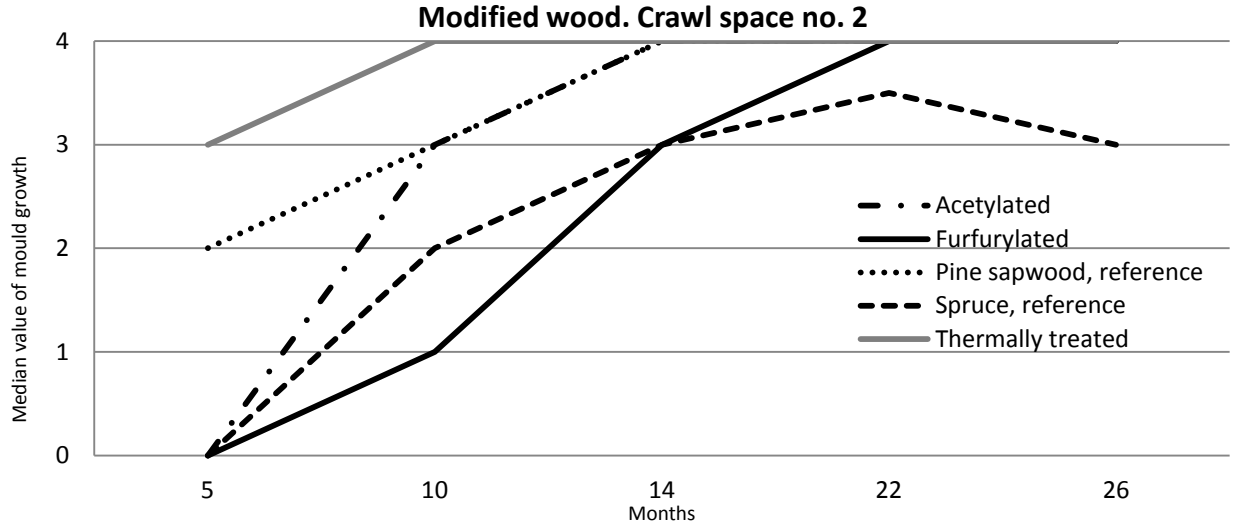

Figure 9. Median value of the mould growth on the modified wood in crawl space no 2.

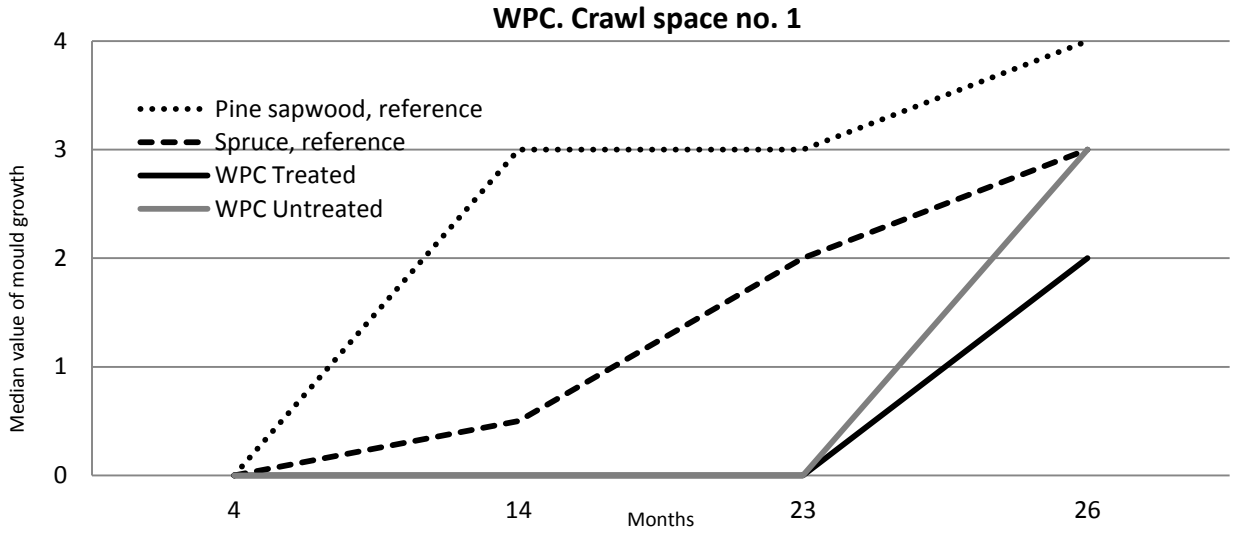

Figure 10. Median value of the mould growth on the WPCs in crawl space no 1.

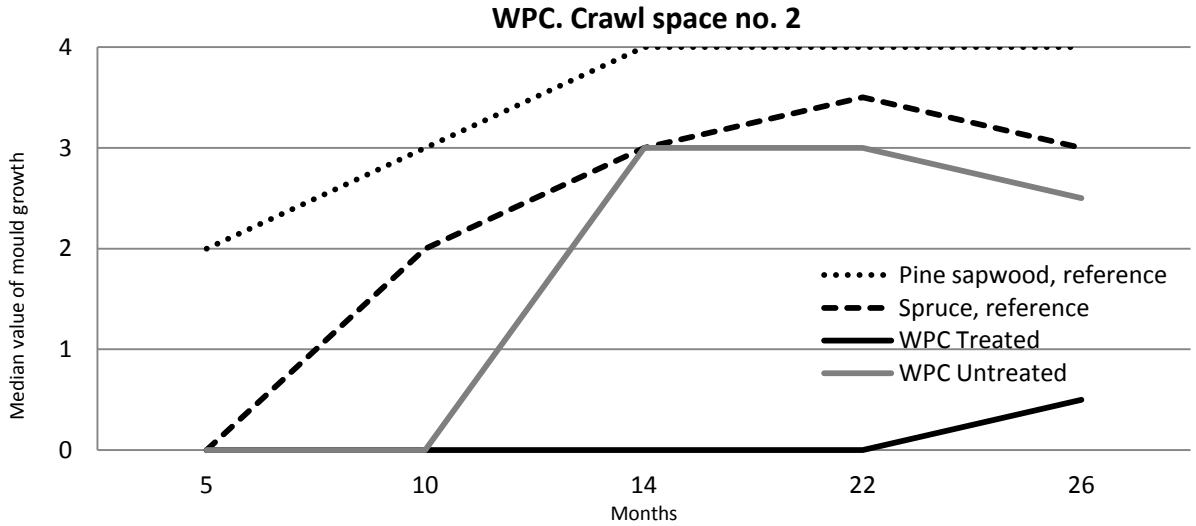

Figure 11. Median value of the mould growth on the WPCs in crawl space no. 2.

When considering Figures 6-11 one can conclude that the result for each of the two crawl spaces corresponds fairly well. The somewhat higher moisture load in crawl space no. 2 clearly enhances early mould growth on all materials and on thermally modified wood in particular.

No significant differences with respect to susceptibility for mould growth between different types of preservative-treated or modified wood materials can be observed at the end of the exposure. None of these materials has performed better than the spruce reference. The preservative-treated WPC is the only material that has performed better than the spruce reference throughout the test.

4. CONCLUSIONS

The following conclusions can be drawn from this study:

- The results obtained from testing in the two crawl spaces in general correspond well with the previous laboratory study.

- None of the materials tested could completely withstand mould growth during the 26 months' exposure time.

- The most promising results were obtained with the preservative-treated WPC and the least promising with the modified wood materials. For the latter, poor mould resistance is of major concern.

Further evaluation of test data from the laboratory and in-service study will be carried out in order to improve the knowledge of the different materials resistance to mould growth.

5. ACKNOWLEDGEMENT

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6. REFERENCES

Bok, G, Hallenberg, N, Åberg, O. 2009. Mass occurrence of *Penicillium corylophilum* in crawl spaces, south Sweden. *Building and Environment*, **44,** 2413-2417.

Johansson, P, Jermer, J. 2010. Mould growth on wood-based materials – a comparative study. *Proceedings IRG Annual Meeting,* IRG/WP 10-20455, 8 pp.

Johanson, P, Ekstrand-Tobin, A, Svensson, T. 2012. Mould growth on building materials under different climatic conditions: determining the critical moisture level. International Biodeterioration and Biodegradation. In press.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 3 **Wood Protecting Chemicals**

Triazole – based ionic liquids to protect of lignocellulosic materials against fungi

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Triazole – based ionic liquids to protect of lignocellulosic materials against fungi

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ABSTRACT

In presented paper we examined on biotic properties of novel structure of tebuconazole derivatieves: tebuconazole hydrochloride, allyltebuconazole chloride, methyltebuconazole iodide, tebuconazole dihydrocitrate. Our investigation against wood-degrading fungi were contained also the didecyldimethylammonium 3-aminotriazolate as well as didecyldimethylammonium nitrate(V) with tebuconazole or with (tebuconazole + propiconazole). In order to confirm the structure of the new ionic liquids analyses using thin layer chromatography and proton and carbon spectra of nuclear magnetic resonance were carried out, as well as elementary analyses CHNO.

The most active compound against brown and white rot fungi were precursor of ionic liquids-tebuconazole hydrochloride . The fungistatic dose (ED_{50}) for *Coniophora puteana* reached 0.1 ppm, the fungitoxic dose (ED_{100}) - 5 ppm and lethal dose (LD) - 5 ppm. The fungicidal value of didecyldimethylammonium nitrate(V) with tebuconazole for *Coniophora puteana* were < 0.73kg/m^3 , for *Trametes versicolor* ranged from 0.81 kg/m^3 to 1.76 kg/m^3 The investigation of protic triazole-based ionic liquids demonstrated the strongest action against blue stain and mould fungi. The growth of mycelium on the surface of wood samples was inhibited in the amount of application 15 g/m^2. The penetration depth into Scots pine wood *Pinus sylvestris* L. of didecydimethyl-ammonium nitrate(V) including the tebuconazole was reached 6.2 mm.

Key words: ionic liquids, triazole, fungal activity, wood, protection

1. INTRODUCTION

Ionic liquids (ILs) represent a new group of compounds consisting of a cation and an anion with tend to be non-volatile substances in room temperature. Structure modification of ILs makes it possible to obtain compounds of new physico-chemical properties, deep of penetration into wood and higher effectiveness of action against microorganisms.

Technological advances in organic synthesis made it possible to obtain multifunctional compounds in the form of ionic liquids (ILs) with melting temperatures below 100^{o}C, as a result of the replacement of a chloride anion of quaternary ammonium or bis-ammonium salts by a tetrafluoroborates, nitrate, propionate, bis[(trifluoromethyl)-sulphonyl)]imide [Tf_2N] or other anions (Petkovič et al. 2010).

These substances whose ammonium or bis-ammonium cation has a long alkyl chain of C_8 to C_{12} length, apart from their effectiveness of biocidal action, also possess very good penetration properties, which is extremely important when protecting solid wood. The first publication

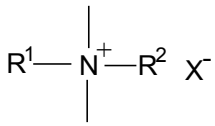
concerning ionic liquid application in timber conservation appeared in 2004 (Pernak et al.) 3-alkoxymethyl-1-methylimidazolium tetrafluoroborates and hexafluorophos-phates showed very high antifungal activity against *Basidiomycotina* and *Deuteromycotina* comparable with commercial didecyldimethylammonium and benzalkonium chlorides used as wood protection agents. The impact of other anions, in particular, of lactates ($_{DL}$-lactate and $_{L}$-lactate), acesulfamates and saccharinates on ILs antimicrobial and antifungal activity described by Cybulski et al.(2008), Hough-Troutman et al. (2009), Pernak et al. (2005) allowed their utilization as environmentally-friendly disinfecting agents and wood protecting preparations.
Studies of other structures of ionic liquids performed by Zabielska-Matejuk et al. (2008), showed their activity against soft-rot fungi and a possibility of sorption on the soil.
Another important property of ionic liquids is their biodegradability in water and soil environments (Harjani et al. 2009, Petkovič et al. 2010, Grabińska-Sota 2010, 2011, Ford et al. 2010. Continued search for new wood protection agents encouraged authors of this study to undertake studies of azoles active against wood destroying fungi, in particular, derivatives of 1,2,4-triazole, having structure of quaternary triazolium salts. 1,2,3-triazolium –based ILs with [Tf_2N] and iodide as anions were prepared by Nulwala et al.(2011). A phenyl or a propyl group was attached at the "4" position of the triazolium ring.
Recognised compounds from the group of azoles known as: tebuconazole, propiconazole, etaconazole, azaconazole are applied as pesticides in plant protection (accepted for commercial used in the European Union) as well as in wood protecting preparations due to their low threshold values against a wide spectrum of microorganisms. Dissolution of tebuconazole in biologically active ionic liquids should increase the effectiveness of their protective action, whereas new quaternary 1,2,4-triazole structure can act as functional organic compounds of very strong biocidal activity.
Wood Technology Institute together with the Chemical Technology Department at the Poznań University of Technology has developed new ionic liquids based biocides. These include so far not described originally protic ILs - 1,2,4-triazole derivatives. These compounds have many attractive properties possess a unique structure, even more excellent antifungal activity.
This paper presents a new group of aprotic and protic ILs derived from 1,2,4-triazole-based cations or anion, as well as the mixture of ammonium ionic liquids including tebuconazole or (tebuconazole + propiconazole). The aim of the investigations was to determine the influence of cation and anion structures of triazolium-based ILs on the biological activity. For comparison a fungistatic and fungicidal action against wood degrading fungi of ionic liquids with environmentally-frendly cation of natural origin obtained from plant (natural coconut oil), and didecyldimethylammonium with nitrite or nitrate anion are presented. In addition, a spectrum of biological activity of the new group of ILs against wood degrading fungi and *Deuteromycotina* imperfecti fungi causing blue stain as well as mould growth of coniferous wood was examined. The aim of this study was to determine of penetration capabilities of the mixture of ILs including 1,2,4-triazole derivatives. The binding of triazole-base IL with Scots pine wood was presented.

2. MATERIALS AND METHODS

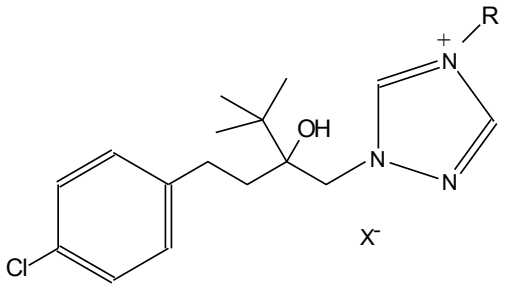
Chemicals

A novel structure of 1,2,4-triazole derivatives, as well as the mixture of didecyldimethylammonium nitrate (or nitrite) with tebuconazole or with (tebuconazole + propiconazole) were selected in this study.
The structure of the compounds are presented in Scheme 1 and Table 1. All chemicals were prepared at the Poznań University of Technology by the team headed by Prof. J. Pernak. Purity of compounds was 97-99%. The rest was water. The structure of examined ILs was confirmed performing ^{1}H NMR and ^{13}C NMR spectra with the Varian Model XL 300

spectrometer. NMR spectra and elemental analyses were performed at the A. Mickiewicz University, Poznań All hydrophobic chemicals were soluble in 2-propanol or in mixture 2-propanol/water.



X=NO_3, NO_2,3-aminotriazolate
R^1= alkyl C_{10}, C_{12}, coco(C_8-C_{14}) R^2=$CH_2C_6H_5$, alkyl C_1, C_{10}



X= Cl,I, H_2citrate R=H, alkyl C_1, allyl (C_3H_5)

Scheme 1 Structures of the prepared compounds

Didecyldimethylammonium nitrite ([DDA][NO_2]): ^{1}H NMR (DMSO-d_6) δ ppm = 3.25 (m, 4H), 3.00 (s, 6H), 1.62 (m, 4H), 1.26(m, 28H), 0.85 (t, J = 7 Hz, 6H); ^{13}C NMR 62.7, 49.8, 31.2, 28.8, 28.7, 28.6, 25.7, 22.0, 21.6, 13.8. Elemental analysis: Found; C 71.12, H 13.15, N 7.43. Calc. for C_{22} H_{48} N_2O_2 (372.6): C 70.71, H 12.98, N 7,52%.

Table 1. Investigated ionic liquids

Symbol	Active subctance content (%)	Description	Type of ILs
[DDA][NO_2]	99.0	didecyldimethylammonium nitrite*	aprotic ILs
[DDA][NO_3]	98.0	didecyldimethylammonium nitrate*	
[Arq C35][NO_3]	95.0	cocotrimethylammonium nitrate (alkyl obtained from coco nut oil C_8-C_{14})	
[DDA][3AT]	97.5	didecyldimethylammonium 3-aminotriazolate	
[BA][3AT]	97.0	benzalkonium 3-aminotriazolate	
[Allyl Teb][Cl]	98.0	allyltebuconazole chloride*	
[Methyl Teb][I]	98.0	methyltebuconazole iodide*	
[Teb H][H2 citrate]	98.0	tebuconazole dihydrocitrate	protic IL
[DDA][NO_3](15%T)	97.5	didecyldimethylammonium nitrate* + tebuconazole	mixture of biocides
[DDA][NO_2](15%T)	97.5	didecyldimethylammonium nitrite* + tebuconazole	
[DDA][NO_3](7.5%P+7.5%T)	97.5	didecyldimethylammonium nitrate* + propiconazole + tebuconazole	
[DDA][NO_2](7.5%P+7.5%T)	97.5	didecyldimethylammonium nitrite* + propiconazole + tebuconazole	
[Teb H][Cl]	98.0	tebuconazole hydrochloride	precursor of ionic liquids
Tebuconazole	99.0	tebuconazole	biocide

* hydrophobic ILs soluble in 2-propanol

Methods

Agar-plate test

The activity of ionic liquid with organic and inorganic anions was assessed using the agar dilution test described by Ważny and Thornton (1986). The fungal growth rates were measured in 90 mm diameter Petri dishes. Appropriate concentrations of the ionic liquids were studied in a geometric progression from 0.1 to 5000 ppm. A stock solution of each concentration was produced in sterile malt agar (1.5% agar and 4% malt-extract). Three replicate plates of each concentration from each salts were centrally inoculated with a 5-mm diameter disc taken from the submargin of 10-day-old cultures of the described test fungus. The plates were incubated at 22 ±1^{o}C in darkness. The duration of the test was determined either by waiting for complete plate coverage, or until 6 days for white-rot fungus *Trametes versicolor* strain CTB 863 A and 12 days for brow-rot fungus *Coniophora puteana* strain BAM 15 and blue stain fungus *Sclerophoma pihtyophila* strain S 231. If there was no growth on the preservative-containing agar after 12 days, the inoculum was removed and transferred to a fresh malt-agar plate to determine fungal viability. As evaluation criteria, the author adopted the ED_{50} and ED_{100} (effective dose) parameters, i.e. the effective concentration inhibiting the growth of mycelium in 50% or 100% in relation to the fungus grown on the medium without a fungicide, as well as the LD (lethal dose), i.e. the concentration causing mycelium death. The above-mentioned concentrations were expressed in parts per million.

Fungicidal activity of ionic liquids against *Basidiomycotina*

The fungicidals activity of studied ionic liquids was determined on Scots pine (*Pinus sylvestris* L.) and beech (*Fagus sylvatica* L.) wood using standardized method according to PN-EN 113:2002 and screening agar-block method described by Zabielska-Matejuk (1997).

Fungicidal activity of ionic liquids against mould fungi

The resistance to mould fungi was tested using a method based on the instruction of the Building Research Institute Instruction No 355/98 (1998). The test specimens of Scots pine sapwood *Pinus sylvestris* L. were of the dimensions of 40 x 40 x 4 mm (L x T x R). Before the exposure to fungi, the wood samples were conditioned in KBF 720 chamber at 20^{o}C and 65% relative humidity , until the moisture content of. 12±1% was obtained. Next, on the surface of wood were applied 10, 15 and 25 g m^{-2} of investigated ammonium and triazole derivatives, presented in table 1. After seasoning, samples were exposed for 4 weeks to the action of a suspension mixture of pure cultures (at a concentration of 10 -23 x10^{6} cfu cm^{-3}) of the following fungi: *Aspergillus niger* v. Tieghem, *Penicillum funiculosum* Thom, *Pecilomyces varioti* Bainier, *Trichoderma viride* Persoon ex Fries, *Alternaria tenuis* Link ex Fries or to the action of a pure culture of fungus *Chaetomium globosum* Kunze. The growth of mycelium on the surface samples was measured after 4 weeks of incubation, at the temperature 27±1^{o}C and 90% relativity humidity, using the following scale:

0- no growth of fungi on the sample, visible under the microscope,
1- trace growth of fungi on a sample, hardly visible with the naked eye but well visible under the microscope or growth limited to the edge of a sample, visible with the naked eye,
2- growth of the fungi on a sample , visible with the naked eye, but less than 15% of the surface is covered with fungus,
3- over 15% of the surface is covered with fungi visible with the naked eye.

Fungicidal activity of ionic liquids against blue stain in service

The resistance to blue stain fungi was tested using a method according to PN-EN 152-1:2011. Samples of Scots pine (*Pinus sylvestris* L.) sapwood, measuring 110 x 40 x 10 mm (L x T x R) were conditioned in KBF 720 chamber at 20^{o}C and 65% relative humidity, until the moisture content of. 12±1% was obtained. After protecting the heads of the samples, on the surface of wood were applied investigated ionic liquids (50g m^{-2}-ground coat of [DDA][NO_3](15%T) and 150g m^{-2}-finish coat of [DDA][NO_3] with a pigment of iron oxides) and a Sadolin Base as reference coating (150g m^{-2}). The samples were exposed to the action of weather conditions (ageing process) within 6 months since March up to the October. After ageing process the blue stain of the upper and bottom surface of the samples were evaluated. Samples without visible growth of fungi were exposed to the action of blue stain *Sclerophoma pithyophila* and *Aureobasidium pullulans*(de Bary)Arnaud strain P 268 in laboratory conditions. A suspension of mixture of spores at a concentration 44-56 x10^{6}cfu cm^{-3} was prepared.

Determination of penetration depth

Samples of Scots pine (*Pinus sylvestris* L.) sapwood were used, at a density of 480-540 kg m^{-3} and number of growth rings 5-8 per 1 cm, measuring 50 x 50 x 20 mm (one of the longer edges had to be parallel to the fibers and the annual growth increment rings visible in cross section had to be positioned against the edge at an angle of 45±10^{o}) and conditioned to a moisture content of 12 ±1%. On the 50 x 50 mm^{2} wood surface, 0.5 g of investigated salts were applied. Then, the samples were conditioned in the dishes over a saturated solution of ammonium nitrate at 20 ± 2^{o}C for 7 days. Subsequently, the samples were cut perpendicularly to fibers by means of a cross cut saw, and were sprayed carefully on the cross-section surface with bromophenol blue indicators (giving a typical blue color on contact with IL). The range of blue section were marked with a sharp pencil, which permitted determination of the penetration depth of IL.

Determination of leaching

The applied procedure of leaching was suggested in the study of Zhang and Kamdem (2000). Cubes measuring 19 mm were prepared from defect-free sapwood boards of Scots pine *Pinus sylvestris* L. The samples stored in a climatic chamber (Binder KBF 720) maintained at 65% relative humidity (RH) and 20 ^{o}C until they reached an equilibrium moisture content (EMC) of 12 ±1%. The conditioned samples were then pressure-treated with the solution of IL and mixture of IL with polypropylene glycol PPG 425, both dissolved in 2-propanol. The concentrations of the ionic liquids solutions were 1.0 and 1.6% by weight. The treated cubes were subjected to seasoning at room temperature for 3 weeks before further testing. The leaching test was carried out to determine the amount of salts leachable from treated wood. Three treated wood cubes weighing about 10 g, were placed in Erlenmeyer flash and immersed in 100 cm^{3} of distilled water. The flasks were positioned on a horizontal-shaking tray with continuous mild shaking at 150 rpm for 8 days. The ILs content in water and in leached cubes was analyzed by two-phase titration, according to standards for determination of quaternary ammonium compounds in wood, according to procedure CEN/TR 15314:2006.

Spectral analysis – ATR

The ATR spectra analysis was used to identify the bonds of triazole based-ionic liquids to Scots pine wood. The study was performed on the device IFS 66 V / S Bruker. Transmission spectra were recorded in the mid-infrared range 4000-400 cm-1 at a resolution of 2 cm-1.

RESULTS

Fungicidal activity of ionic liquids

Table 2 presents the results of investigations regarding fungistatic (ED_{50}) and fungicidal (ED_{100} and LD) activities of protic and aprotic ammonium- and triazolium ionic liquids with respect to didecyldimethylammonium nitrite and cocotrimethylammonium nitrate as well as to tebukonazole. The ED_{50} value of [DDA[NO_2] for *C. puteana* amounted to 25 ppm, ED_{100} and LD =500 ppm. The introduction into the anion structure of the ionic liquid with a cation [DDA] of 3-aminotriazole ensured strong fungistatic properties: ED_{50} = 25 ppm. Ionic liquids with a cation of natural origin from coconut oil (containing a mixture of hydrocarbons from C_8 to C_{14}) with nitrate anion [Arq C_{35}][NO_3] exhibited stronger fungicidal properties against *S. pithylophila.* Dissolution of 15% tebukonazole as well as of the 7.5% tebuconazole + 7.5% propioconazole mixture in didecyldimethylammonium nitrates and nitrites made it possible to obtain very strong fungistatics, at the level of tebukonazole activity (ED_{50} = 1 ppm). Toxic ED_{100} values of these mixtures for: *S. pithyophila, T. versicolor* and *C. puteana* amounted to 10-50 ppm. Following the performed statistical assessment of the dependence of the growth of test fungi colonies on [DDA][NO_3](15%T) and [DDA][NO_2](15%T) toxic substrates on the concentration, trend functions in the form of y=a-b ln(x) were determined logarithmically. Correlations shown in Figures. 1-2 are characterised by high determination coefficients. In the case of investigations in relation to *C. puteana*, R^2 amounted to, respectively, 0.961-0971 and for *S. pithyophila* - R^2=0.918-0.928. The developed new structures of tebukonazole derivatives exhibited varying biotic activity. Tebuconazole turned out to be biologically most active with the threshold value ED_{50} for *C. puteana* amounting to 0.1-1 ppm and ED_{100} – from 1 to 5 ppm. Introduction into tebuconazole of a methyl substituent (methyltebuconazole iodide) reduced (even up to 10 times) its biotic activity against all test fungi. Effective (ED) and lethal (LD) doses of tebuconazole citrate [Teb H][H_2Cytr] against the blue stain test fungus were at the level of tebuconazole (5 to 50 ppm) but in the case of degrading fungi (*C. puteana* and *T. versicolor*), the fungicidal activity ED_{100} (10 ppm) and LD (50ppm) of this compound was two times lower.
Results of biotic activity obtained using the culture method were corroborated by fungicidal values determined on Scots pine *Pinus sylvestris* L. and beech *Fagus sylvatica* L. woods against *C. puteana* and *T. versicolor* fungi (Table 3). Ionic liquids with a cation obtained from coconut oil and with nitrate and nitrite anions exhibited fungicidal values for *C. puteana* 2.85-4.85 kg m^{-3} and for *T. versicolor* 6.55-10.51 kg m^{-3}. A similar activity against degrading fungi was observed in the case of didecyldimethylammonium 3-aminotriazolate [DDA][3AT]. Similar fungicidal values were obtained in the case of ionic liquids of coconut oil cation. Fungicidal values of didecyldimethylammonium nitrate including 15% of tebuconazole were reduced to the level of < 0.73 kg m^{-3} for *C. puteana* (fungicidal value of didecyldimethylammonium nitrate was at the level of 3.95-6.78 kg m^{-3}) and 0.81-1.76 kg m^{-3} for *T. versicolor*. This confirms reinforcement of the biotic action by tebuconazole. After the leaching test carried out in accordance with the EN 84 procedure, the examined compounds were not found to decrease their effectiveness which confirmed good fixation of didecyldimethylammonium nitrate with tebuconazole in Scots pine wood. Fungicidal values of tebuconazole dihydrocitrate for *T. versicolor* was at the level of 0.86-1.38 kg m^{-3}, while in the case of tebuconazole hydrochloride was reached the amount of <0.2 kg m^{-3}.

Activity against fungi causing wood moulding

Table 4 presents the results of the fungicidal activity of ammonium- and triazole-based ionic liquids against *Ch. globosum* and a mixture of moulds. Ammonium ionic liquids with a nitrite anion were characterised by strong fungitoxic properties. The application in the amount of 15 g m^{-2} caused an insignificant growth of fungi on the surface of Scots pine wood.

Didecyldimethylammonium 3-aminotriazolate effectively inhibited growth of moulds on the surface of treated wood when used in the amount of 10 g m^{-2}, while the effectiveness of tebuconazole dihydrocitrate [Teb H][H_2 Cytr] against mould fungi was lowest. The average degree of *Ch. globosum* coverage on the surface at the application of 25 g m^{-2} was 2.8 – 3.0, whereas when a mixture of mould fungi was used, it ranged from 2.3 – 3.0. Didecyldimethylammonium nitrate with tebukonazole or propiconazole and tebuconazole showed very good effectiveness against *Ch. globusom* even when they were applied at 10 g m^{-2}, while in the case of the mixture of mould fungi, the effective protection was achieved when they were used in the amount of 25 g m^{-2}.

Protective effectiveness of wood treated against blue stain

Table 5 presents the results of blue staining of surface treated pine wood (*Pinus sylvestris* L.) following 6-month aging in field conditions. A slight, local blue stain of less than 2 mm in diameter was observed on wood surface treated with didecyldimethylammonium 3-aminotriazolate and nitrate with the addition of 15% tebuconazole (applied quantity: 50 g m^{-2}). Average degree of surface blue stain reached the level of 0.17 to 1.0. On the underside surface, the samples were observed to be completely covered by blue stain (degree of 3.0). Following 6-week exposure to the action of *A. pullulans* and *S. pithyophila* fungi, the degree of blue stain infestation of the above-mentioned samples underwent slight change to 1.33, while the depth of the area without blue stain ranged from 2.3 to 3.1 mm. When [DDA][NO_3] (15% T) (50 g m^{-2}) was applied as a primer and didecyldimethylammonium nitrate with a pigment in the form of iron oxides (red and yellow) in the amount of 150 g m^{-2} as a surface coat, a very good anti-blue stain protection of wood surface was achieved and the area without blue stain ranged from 6.1 to 6.6 mm. The action effectiveness was better than that of the reference preparation - Sadolin Base (150 g m^{-2}).

Wood penetration

Figure 3 shows the depth of penetration into pine wood of 12% moisture content of five ionic liquids of varying anion and cation structures with respect to 4% NaF solution. Nitrite and nitrate with didecyldimethylammonium cation showed very weak penetration properties. Penetration depth was ranged from 2.4 to 3.8 mm. On the other hand, the addition of 15% tebuconazole or mixture of 7.5% tebuconazole and 7.5% propiconazole resulted in 1.6 fold increase of the penetration into wood of didecyldimethylammonium nitrate. This was associated with reduced viscosity (about of 17%) of this ionic liquid caused by the addition (dissolution) of 1,2,4-triazole derivatives.

Leaching from Scots pine wood

Table 6 presents the results of analysis of water extracts obtained following a cycle of leaching of Scots pine wood treated with ionic liquids, untreated and with the addition of 425 polypropylene glycol. They revealed variable fixation which depended on the structure and hydrophilic properties of ionic liquids. The degree of leaching for hydrophobic didecyldimethylammonium nitrite ranged between 1.71 and 2.83% Water soluble didecyldimethylammonium 3-aminotriazolate formed stable bonds with wood. The degree of leaching, depending on the retention in wood, ranged from 3.56 to 4.02% and was smaller in comparison with the commercial didecyldimethylammonium chloride [DDA][Cl]. The introduction into the impregnation solution of 425 polypropylene polyglycol limited its extraction into water in the case of all the examined compounds.

ATR spectra

Pine veneer treated with didecyldimethylammonium 3-aminotriazolate [DDA] [3AT]

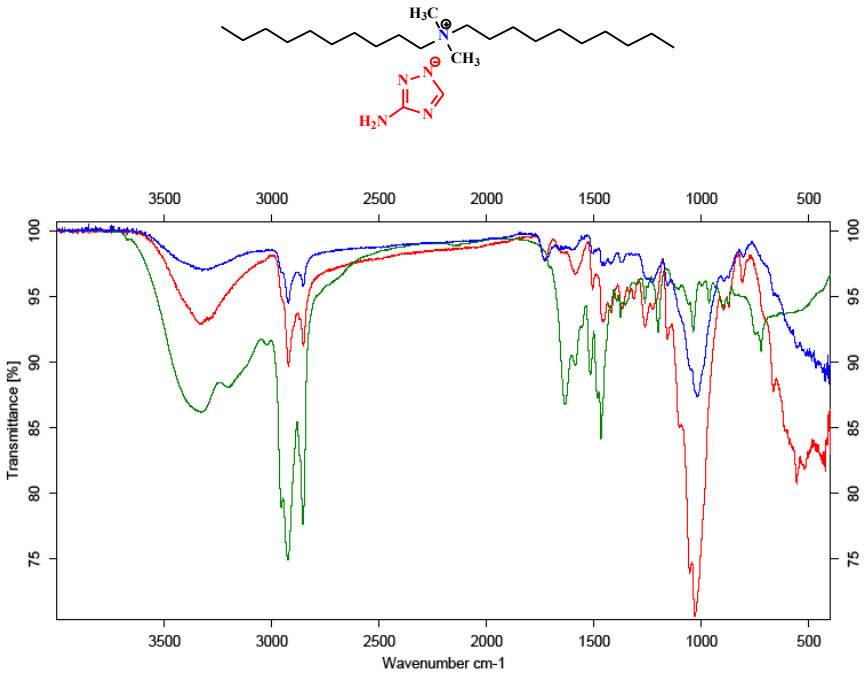


Figure 4. ATR spectra of Scots pine wood: control – blue, treated with [DDA][3AT] – red, [DDA][3AT] – green

The comparison of spectra of control pine and pine treated with [DDA] [3AT] clearly shows that after impregnation the aminotriazolate anion was incorporated into the wood structure due to the increased intensity of the following characteristic bands in the ATR spectrum, which was measured after the treatment of the wood:
1) bands of valence vibrations of amines: $\nu(N–H)_{asymmetric}$ (approx. 3400 cm^{-1}) and $\nu(N–H)_{symmetric}$ (approx. 3326 cm^{-1});
2) band of deformation vibrations δ(N–H) with a maximum at 1588 cm^{-1};
3) band of fan vibrations γ(N–H) with a maximum of approximately 577 cm^{-1};
4) triazolate ring – band of vibrations ν(C=N) with a maximum at 1635 cm^{-1}.

The inclusion of aminotriazolate anion in the wood structure may be inferred from the ATR spectrum on the basis of the drastically increased absorption of the ether bands ν(C–O–C) located at approximately 1030 cm^{-1}. The amino groups bind to ether oxygen atoms of pine, thereby affecting activation of the ν(C–O–C) vibrations in the infrared range. The reduction of the intensity of the band at 1726 cm^{-1} [$\nu(C=O)_{COOH}$] after modification implies binding of pine acidic groups –COOH with the amine groups of the anion component of the ionic liquid (Fig. 4). The increase in the intensity of the ν(C–H) bands in the ATR spectra measured after the treatment also indicates the presence of the $[DDA]^+$ cation closed in the wood structure (bands at 2955, 2925, 2871, and 2834 cm^{-1}). All these changes prove a successful introduction of the ionic liquid to the pine wood structure.

Conclusion

The most active compound against brown and white rot fungi were precursor of ionic liquids-tebuconazole hydrochloride, the fungicidal values for *C. puteana* and *T. versicolor* was reached the amount of < 0.2 kg m^{-3}. In the case of tebuconazole dihydrocitrate the biocidal activity for *T. versicolor* was at the level of 0.86-1.38 kg m^{-3}. Ammonium–based ILs with a cation containing a substituent derived from coconut oil exhibited fungicidal values against *C. puteana* at levels ranging from 2.7 to 4.6 kg m^{-3}. Dissolution of 15% tebuconazole or its mixture with propiconazole in didecylodimethylammonium nitrate or nitrite reinforced the biocidal action of this compound (<0.73 kg m^{-3} for *C. puteana*) and increased the penetration depth into wood up to 6.2 mm. This allowed effective protection of processed wood against blue stain fungi comparable with the reference preparation of Sadolin Base. Quaternary tebuconazole derivatives with methyl and allyl substituents demonstrated a slightly weaker fungicidal action than tebuconazole. Majority of the examined salts was characterised by a strong anti-mould action already when only 15 g m^{-2} were applied onto the surface of pine wood. Hydrophobic ionic liquids did not undergo leaching from treated wood. Water soluble triazole- based ionic liquids with 3-aminotriazolate anion got fixed in treated wood. The ATR spectra analysis of treated Scots pine wood indicated the presence of the cation $[DDA]^+$ and the 3-aminotriazolate anion in the structure of wood. Positive results of investigations of novel functional ionic liquids presented in this study make their potential application in wood protection possible.

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References

CEN/TR 15314:2006 Durability of wood and wood based products-Quantitative determination of quaternary ammonium compounds in wood.

Cybulski J., Wiśniewska A., Kulig-Adamiak A., Lewicka L., Cieniecka-Rosłonkiewicz A., Kita K., Fojutowski A., Nawrot J., Materna K., Pernak J.(2008) Long-Alkyl-Chain Quaternary Ammonium Lactate Based Ionic Liquids. Chem-Eur. J. 14:9305-9211.

Ford L., Harjani J.R., Atefi F., Garcia M. T., Singer R.D**.,** Scammels P.J. (2010): Further studies on the biodegradation of ionic liquids. Green Chemistry 12: 1783-1789.

Grabińska-Sota E. (2010) Effect of quaternary ammonium salts on operations of biological treatment plants. Przemysł Chemiczny 89(11): 1437-1439.

Grabińska-Sota E. (2011) Genotoxicity investigations of nitrite with cation obtained from coconut oil - UmuC Easy CS test. Technical University of Silesia. Typescript (in Polish).

Harjani J.R., Singer R.D., Garcia M.T., Scammels P.J. (2009) Biodegradable pyridinium ionic liquids: design, synthesis and evaluation. Green Chemistry, 11: 83-90.

Hough-Troutman W. L., Śmiglak M., Griffin S., Reichert W. M., Mirskla I., Jodynuis-Liebert J., Adamska T., Nawrot J., Stasiewicz M., Rogers R. D., Pernak J (2009) Ionic liquids with dual biological function: sweet and anti-microbial, hydrophobic quaternary ammonium-based salts. New J. Chem., 33:26-33

ITB Instruction no 355/98 (1998) Protection of the building wood materials against biological corrosion with chemicals. Requirements and investigations. Building Research Institute, Typescript, Warsaw: 1-27.

Nulwala H. B., Tang Ch. N., Kail B. W., Damodaran K., Kaur P., Wickramanayake S., Shi W., Luebke D. R. (2011) Probing the structure-property relationship of regioisomeric ionic liquids with click chemistry. Green Chem. 13, 3345-3349.

Pernak J., Zabielska-Matejuk J., Kropacz A., Foksowicz-Flaczyk J. (2004) Ionic liquids in wood preservation. Holzforschung 58(3):286-291.

Pernak J., Goc I., Fojutowski A. (2005) Protic ionic liquids with organic as wood preservative. Holzforschung , 59:473-475.

Polish standard (2011) PN-EN 152-1 Laboratory method for determining the protective effectiveness of preservative treatment against blue stain in service – Part 1 Brushing procedure.

Polish standard (2000) PN-EN 84 Wood preservatives – Accelerated ageing of treated wood prior to biological testing – Leaching procedure.

Polish standard (2002) PN-EN 113 Wood preservatives – Test method for determining the protective effectiveness against wood destroying basidiomycetes – Determination of the toxic values.
Perkovic M., Seddon K. R., Rebelo L.P.N., Pereira C. S. (2010) Ionic liquids:a pathway to environmental acceptability. Chem. Soc.Rev., View Online 10.1039/C004968A
Ważny J., Thornton I.D. (1986) Comparative testing of strains of the dry rot fungus *Serpula lacrymans* (Schum. Ex Fr.) S.F. Gray II. The action of some wood preservatives in agar media. Holzforschung 40: 383-388.
Zabielska-Matejuk J. (1997): The effect of the shape of miniaturized experimental samples on the rate of wood decomposition, fungicidal value and leaching properties of the preparation of CCB type. Pr. Inst. Techn. Drew. 1997, z. 1/2 (149/150):33-46.
Zabielska-Matejuk J., Stangierska A., Skrzypczak A. (2008) Research of the action of hydrophilic and hydrophobic ionic liquids against soft-rot fungi and processes of their sorption on soil. Drewno, vol 51 no 179: 5-28.
Zhang J., Kamdem D.P. (2000) Interaction of copper-amine with southern pine**:** retention and migration**.** Wood and Fiber Science, 32: 332-339

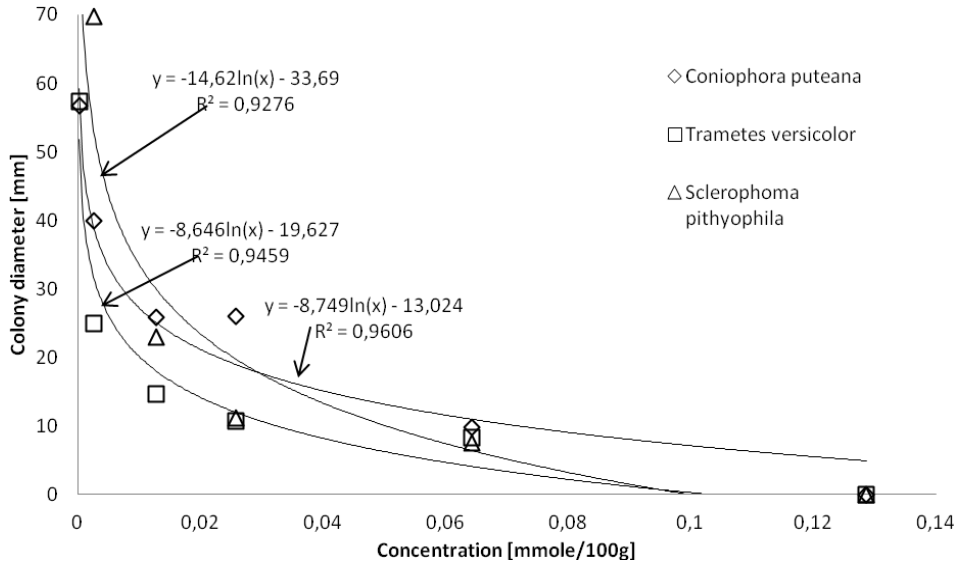

Figure 1 Average growth of tested fungus on malt-agar containing [DDA][NO_3](15%T) of different concentrations

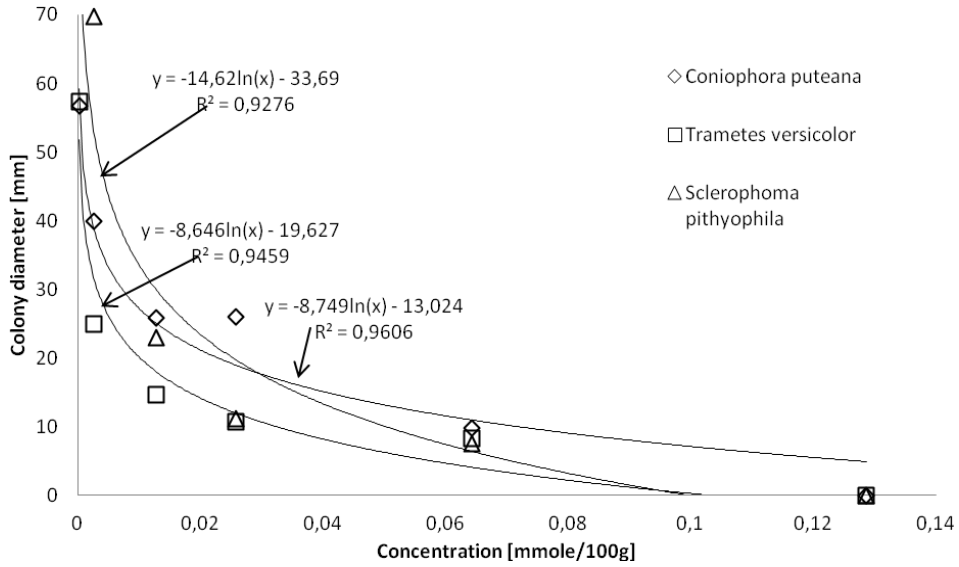

Fig. 2 Average growth of tested fungus on malt-agar containing [DDA][NO_2](15%T) of different concentrations

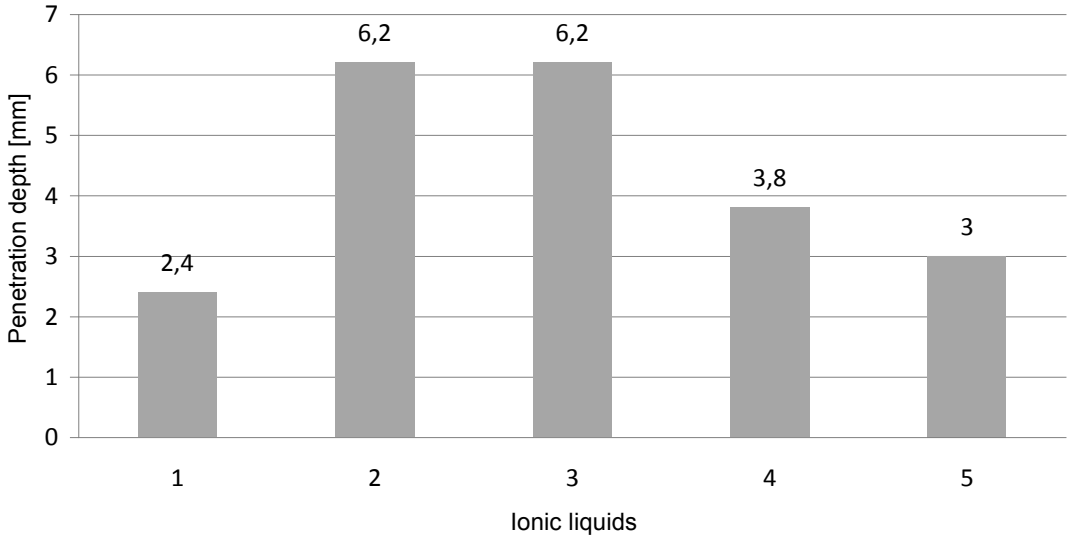

Figure 3 Penetration depth into Scots pine wood of: 1- [DDA][NO_2]; 2-[DDA][NO_3](15%T); 3-[DDA][NO_3](7,5%P+7,5%T); 4 -[DDA][NO_3]; 5- 4% NaF

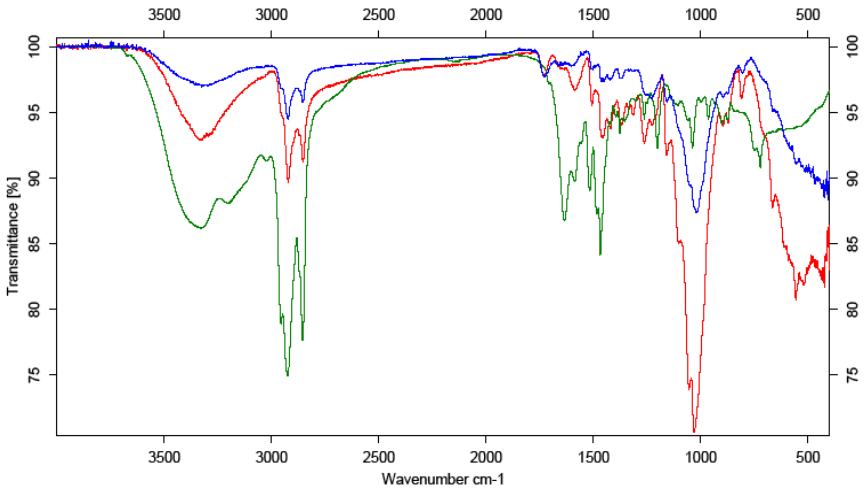

Figure 4 ATR spectra of Scots pine wood: control – blue, treated with [DDA][3AT] – red, [DDA][3AT] – green

Table 2 The toxic values determined with the use of agar-plate method.

Ionic liquid/mixture of biocides	Tested fungi								
	Coniophora puteana			*Trametes versicolor*			*Sclerophoma pithyophila*		
	ED_{50}	ED_{100}	LD	ED_{50}	ED_{100}	LD	ED_{50}	ED_{100}	LD
	ppm								
[DDA][NO_2]	25	500	500	50	250	250	25	500	2500
[Arq C 35][NO_3]	100	1000	2500	50	500	500	<10	50	250
[DDA][NO_3](15%T)	1	50	100	1	25	100	1	10	100
[DDA][NO_2](15%T)	1	5	250	1	25	250	5	50	250
[DDA][NO_3](7.5%P +7.5%T)	1	50	100	1	50	500	1	10	100
[DDA][NO_2](7.5%P +7.5%T)	5	50	50	<0.1	25	250	1	10	100
[Teb H][H_2Citrate]	1	10	50	1	50	250	1	5	50
[Allyl Teb H][Cl]	5	100	100	5	100	500	5	50	500
[Methyl Teb H][I]	750	750	1000	250	500	500	500	500	>500
[DDA][3AT]	25	2500	5000	25	1000	5000	25	500	2500
[BA][3AT]	50	1000	2500	25	100	250	25	100	500
Tebuconazole	1	5	25	1	50	50	1	5	50

Table 3 Fungicidal value of studied ionic liquids against *Coniophora puteana* and *T rametes. versicolor* determined on Scots pine and Beech wood

Ionic liquid / mixture of biocides	Species of wood	Fungus species	Impregnation solution concentration (%)	Fungicidal value according to PN-EN 113 (kg m^{-3})
[DDA][NO_3](15%T)	Scots pine	*C.puteana*	<0.1	<0.73
	Beech	*T. versicolor*	0.1-0.25	0.67 - 1.67
	Scots pine	*C.puteana*	<0.1	<0.72**
[DDA][NO_3](7.5%T+ 7.5%P)	Scots pine	*C.puteana*	<0.1	<0.73
	Beech	*T. versicolor*	0.1 - 0.25	0.68-1.70
	Scots pine	*C.puteana*	<0.1	<0.74**
	Beech	*T. versicolor*	0.25 - 0.4	1.68-2.6**
[DDA][NO_2]	Scots pine	*C.puteana*	>1.0	>6.84
	Beech	*T. versicolor*	1.0-1.6	6.37 - 10.07
[DDA][3AT]	Scots pine	*C. puteana*	0.4 - 0.63	2.85 - 4.85
	Beech	*T.versicolor*	1.0 - 1.6	6.81 - 10.95
[Arq C 35][NO_3]	Scots pine	*C. puteana*	0.4 - 0.63	2.7 - 4.33
	Beech	*T. versicolor*	1.0 - 1.6	6.55 - 10.51
[Teb H][H_2Cytr]	Beech	*T. versicolor*	0.25 - 0.4	0.86 - 1.38*
[Teb H][Cl]	Scots pine	*C. puteana*	<0.04	< 0.19*
	Beech	*T. versicolor*	< 0.04	< 0.21*

*accelerated agar-block method

**results after accelerated ageing – leaching procedure according to PN-EN 84:2000

Table 4 The growth of mould fungi mixture and *Chaetomium globosum* on the surface of Scots pine sapwood, protected with ionic liquids

Ionic liquid/mixture of biocides	*Amount of the ionic liquids applied on the surface of wood (g m^{-2})*					
	10	*15*	*25*	*10*	*15*	*25*
	Average degree of the mould coverage					
	Mixture of mould fungi			*Chaetomium globosum*		
[DDA][NO$_3$](15%T)	*2.5*	*2.67*	*0.58*	*0*	*0*	*0*
[DDA][NO$_3$](7,5%T+7,5%P)	*2.33*	*0.83*	*0.67*	*0.17*	*0*	*0*
[DDA][3AT]	-	*0.7*	*0*	*0*	*0*	*0*
[Teb H][H$_2$Citrate]	*2.33*	*2.67*	*2.33*	*3.0*	*3.0*	*3.0*
[DDA][NO$_2$]	-	*0.3*	*0.2*	-	*0.7*	*0.5*
Control	*3.0*			*3.0*		

Table 5 Blue stain of Scots pine, *Pinus sylvestris* L. treated with ionic liquids after exposition to fungi: *Auroebasidium pullulans* and *Sclerophoma pithyophila* , according to PN-EN 152-1 (investigations after 6 months ageing process)

Ionic liquid/mixture of biocides	Amount of the ionic liquids applied on the surface of wood		Mean degree of blue stain on upper surfaces of the samples (after ageing process)	Mean degree of blue stain on bottom surface of the samples (after ageing process)	Mean degree of blue stain (after laboratory test)	Mean depth of the area without blue stain
	(g/sample)	(gm^{-2})				(mm)
[DDA][3AT]	0.235	50.0	1.0	3.0	1.33	2.3
[DDA][NO_3](15%T) – ground coating	0.236	50.0	0.17	3.0	0.17	3.1
[DDA][NO_3]+4%yellow pigment	0.698	150.0	0.08	3.0	0.17	6.1
+ ground coating [DDA][NO_3](15%T)	0.235	50.0				
[DDA][NO_3]+8%red pigment	0.699	150.0	0.0	3.0	0.0	6.6
+ ground coating [DDA][NO_3](15%T)	0.236	50.0				
Reference coating Sadolin Base	0.698	150.0	1.5	3.0	2.17	6.1
Control	-	-	3.0	3.0	3.0	0.0

Table 6 The leaching of ionic liquids from saturated Scots pine sapwood *Pinus sylvestris* L.

Ionic liquid	Treating solution concentration (%)	IL content in treated wood (kg m^{-3})	Degree of leaching (%)
[DDA][NO_2]	1.0	5.26	1.71
	1.6	8.82	2.83
60%[DDA][NO_2] + 40% PPG 425	1.0	3.34	1.15
	1.6	5.35	1.66
[DDA][3AT]	1.0	7.34	3.56
	1.6	11.77	4.02
60%[DDA][3AT] + 40%PPG 425	1.0	4.34	1.47
	1.6	6.67	1.91
[DDA][Cl]	1.0	5.25	3.65
	1.6	8.51	7.80

IRG/WP 12-30602

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 3 **Wood protecting chemicals**

Hydrogels: a solution to reduce boron leachability without reduction of its biodisponibility to wood decaying fungi?

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ABSTRACT

Products used today for wood protection must fulfill to more and more environmental constraints, such as being of low toxicity in answer to the Biocidal Product Directory, but also to involve waterborne treatments to limit rejection of volatile organic compounds in the atmosphere. Boron preservatives have been described as valuable alternatives for wood protection for non-ground contact applications. Disodium octaborate tetrahydrate (DOT), boric acid and borax are the most widely used boron-based wood preservatives. They possess many advantages such as being colourless, odourless, non corrosive, non flammable, inexpensive, having low vapour pressure and low toxicity for mammals and the environment, but suffer of an important drawback due to their high susceptibility to leaching which limits their use in outdoor applications. Numerous studies have been described to reduce boron leachability involving mainly the use of organic chemicals to reduce boron solubility in water through formation of insoluble or hydrophobic complex. However, complexation reduced boron biodisponibility to fungi, limiting the application of such complex to develop antifungal treatment. The objective of this work was to design supramolecular hydrogels, built on low-molecular-weight amphiphilic molecules and containing boron salts conferring fungicidal properties. Mixing boron with thermoreversible hydrogels allows the formation of a supra molecular network incorporating boron and important amount of water upon gelification of the solution when the temperature decreases. Hydrogels obtained from several amphiphilic peptides, pseudo-peptides or various gelling molecules of the same type were impregnated in pine wood block using vacuum pressure treatment and subjected to leaching. Results indicated that incorporation of boron salts in the hydrogel network, allowed to protect effectively wood from degradation caused by the brown rot fungus *Poria placenta* even after leaching. It was assumed that these hydrogels are able to fill the cell walls and the lumina of the tracheids limiting the leachability of boron salts when the wood is subjected to re-humidification.

Keywords: low-molecular-weight hydrogel, wood preservation, leachability, boron, decay, termite.

1. INTRODUCTION

Boron compounds such as boric acid, borax (disodium tetraborate decahydrate) or TIM-BOR (disodium octaborate tetrahydrate) have proven their efficiency as valuable wood preservatives for non-ground contact applications for many years. Indeed, for more than fifty years boron-based formulations for wood preservation have been proposed. These compounds have shown to be highly toxic to insects like termites and decay fungi. Borates possess many advantages such as being colourless, odourless, non corrosive, non flammable, inexpensive and having low vapour pressure. However, one restriction of their use for timber preservation is their natural solubility in water that leads to the depletion of borates from treated wood under outdoor conditions, particularly in ground-contact, and with loss of biological resistance. This is because, certainly, they are not chemically fixed after treatment and remain mobile. Several alternatives have been investigated to limit boron leachability and improve boron fixation in wood. These alternatives involve the use of water repellents or polymerizable monomers [1-4], formation of insoluble complex [5-7], formation of protein borates [8-10], and formation of organic borates with different polyols like glycol, glycerol, mono glycerides or polyvinyl alcohol [11-15]. However it has been shown that boron biological activity and especially fungicidal activity decreased with its complexation [14, 16-18]. Indeed, borate biological activity was primarily due to tetrahydroxyborate ions $[B(OH)_4]^-$ which are able to form complexes with polyols in wood destroying fungi through extracellular and intracellular substrate sequestration, enzyme inhibition and change in membrane function [16]. It was shown that effect of borate on fungal enzymes as well as on wood decaying fungi was completely negated by the addition of chelators [13,17,18]. Consequently, other alternatives than boron complexation could have interest to avoid this decrease of boron bioavailability. For this reason, we have envisaged to formulate boron-salts in a thermo-sensitive hydrogel allowing wood impregnation with a hot solution of hydrogel and trapping borax in the wood after cooling and gelification of the solution. This type of gel, named *smart gel* because of their sensitivity to stimuli temperature, pH or agitation can be obtained from low-molecular-weight amphiphilic molecules. These supramolecular hydrogels have recently gathered an increased interest due to their wide range of applications in tissue engineering, sensing, drug delivery or water pollution control [19-23]. The gels are made by the self-assembling of low-molecular-weight amphiphilic molecules through non covalent interactions including hydrogen bond, π–π staking and van der Waals forces. Among these hydrogelators, aminoacids-based amphiphiles have been paid much attention in recent past because of their biocompatibility and eco-friendly nature [24-27]. Previous works of the laboratory have shown that hydrogels obtained from amphiphilic peptide derivatives inflate and can absorb a great amount of water [24-25, 28].

We have previously reported results on the use of hydrogelators to reduce boron leachability and the effectiveness of such treatments on the improvement of wood decay durability. Indeed, a previous paper related the synthesis of an amphiphilic pseudopeptide (β-AlaHisC8) and its utilization as hydrogelator with lauric acid was investigated to improve boron retention in wood. [29]. Here, we report new system of hydrogel, obtained from two types of amphiphilic molecules. The first correspond to an amphiphilic tripeptide (GlyGlyPheC10) and the second correspond to a glycerol derivative. The advantage of these new hydrogels comparatively to this used in our previous w.ork was their insensibility to acidic medium.

2. MATERIAL AND METHODS

2.1 Synthesis of hydrogelator

All reactants were purchased from Acros Organics (Noisy le Grand, France) or Sigma-Aldrich Chimie SARL (St Quentin Fallavier, France) or Alfa Aeasar (Johnson Matthey Company, Schiltigheim, France).

General procedure for peptide coupling (between aminoacid or fatty amine):

In 40 mL of CH_3CN, were added 3.77 mmol of Boc-aminoacid, 1eq of BOP (1.67 g, 3.77 mmol), 2 eq of triethylamine (0.76 g, 7.54 mmol) and 1 eq of amino partner (3.77 mmol) (fatty amine or aminoacid). After controlling the pH (7-8), the reaction mixture was maintained under stirring at room temperature for 15 hours. The precipitate was filtrated, washed successively with CH_3CN and diethyl-ether and dried. Recristallisation in CH_3CN give in general a white solid. If no precipitate is observed, CH_3CN was evaporated under reduced pressure and the residue was introduced in ethylacetate and the organic phasis was washed successively with 20 mL of HCl 0.1 N, 20 mL of saturated $NaHCO_3$ and twice with 20 mL of saturated NaCl.

Then, the organic phase was dried on $MgSO_4$ and the solvent was removed under reduced pressure.

Boc deprotection :

Powder was dispersed in anhydrous diethyl-ether and then treated with anhydrous HCl gaz. The mixture was maintained under stirring at room temperature during 18 hours. After evaporation of solvent, the product was a hygroscopic white powder.

Synthesis of glycerol carbamate :

A mixture of glycerol carbonate (2g, 16.93 mmol), one equivalent of tetradecylamine (16,93 mmol), and 5 ml of methanol was heated under stirring at 65°C during 6H. Reaction was monitored by IR (ATR). After evaporation of solvent, the product was a white powder.

2.2 Blocks impregnation

Mini-blocks (20 by 5 mm in cross section by 30 mm along the grain) of Scots pine sapwood *(Pinus sylvestris* L.) were used. 16 replicates were used for each treatment. Samples were oven dried at 103 ºC for 48 hours and weighed to a precision of 0.001g. All treatments were performed using a single vacuum pressure impregnation. Two treatments were performed for hydrogelator agent involving impregnation of hydrogelator alone or with borax (5 %). Treatment solutions containing borax and hydrogelator were heated at 80°C before use to decrease their viscosity and impregnated immediately till hot. 5% borax solution was used as reference solution. Wood specimens were vacuum treated at 5 mbar for 30 min., impregnated with the treatment solutions and kept immersed for 2 hours at atmospheric pressure, and finally reweighed to determine solution uptake. Wood samples kept for 16 hours at ambient laboratory temperature and finally dried at 103 ºC for 48 hours.

2.3 Boron leaching procedure

Leaching was performed according to a procedure adapted from the European standard ENV 1250-2 [30]. Eight samples were immersed in 90 mL distilled water and subjected for six leaching periods of increasing duration under continuous shaking at 20°C. Water was replaced for each leaching period after 1 hour, 2 hours and 4 hours. Samples were then removed and kept air drying for 16 hours. Other leaching periods have been conducted for 8 hours, 16 hours and 48 hours with change of water between each. All leachates were collected and kept for boron analysis.

2.4 Boron analysis

Boron content was analyzed after mineralization of treated or untreated wood blocks subjected or not to leaching. For this purpose, blocks were ground to fine sawdust and dried at 103°C until constant mass. 1 g of sawdust was placed in a 100 mL Erlenmeyer flask and 15 mL of concentrated nitric acid added. The flask was heated at 80°C until reddish-brown fumes stop. Hydrogen peroxide (15 mL) was added drop-wise, and the flask heated at 80°C until total dissolution of organic material. After cooling, the solution is transferred in a 100 mL volumetric flask, rinsed with distilled water and completed to 100 mL. The boron contents was determined using a Varian SpectrAA 220 FS atomic absorption spectrometer with standard solutions comprising between 25 and 1,000 mg L^{-1}.

2.5 Decay test

Sterile culture medium (20 ml), prepared from malt (40 g) and agar (25 g) in distilled water (1 L), was placed in 9 cm Petri dishes, inoculated with a small piece of mycelium of a freshly grown culture of *Poria placenta* and incubated during 2 weeks at 22°C and 70% HR to allow full colonization of the medium by the mycelium. For each treatment, three blocks (two treated and one control) were placed in a Petri dish under sterile conditions and the experiment repeated for times. Incubation was carried out for 16 weeks at 22°C under controlled humidity conditions of 70% RH in a climatic chamber WTB BINDER TYP KBF 240. At the end of the test period (16 weeks), mycelia were removed and all specimens were oven dried to constant mass at 103°C and weighed. Weight loss (WL) was expressed as a percentage of the initial oven-dry weight of wood sample according to the formula:

$WL\ (\%) = 100 \times (m_0 - m_1)/m_0$ where m_0 and m_1 are respectively the initial and final dry mass of wood samples before and after the fungal exposure. Simultaneously with the test series, pine sapwood samples were exposed to *Poria placenta* as virulence controls to validate the test.

2.6. Termite resistance tests

Pine (*Pinus sylvestris*) sapwood of dimensions (30x20x5mm^3, L,R,T) treated, un-leached and leached were used for non choice termite test. Prior to the termite test, each sample was dried at 103°C to get its anhydrous initial weight. For each set of treatment and controls, 3 replicates were tested for their resistance towards *Reticulitermes flavipes* (ex. *santonensis*) termites.

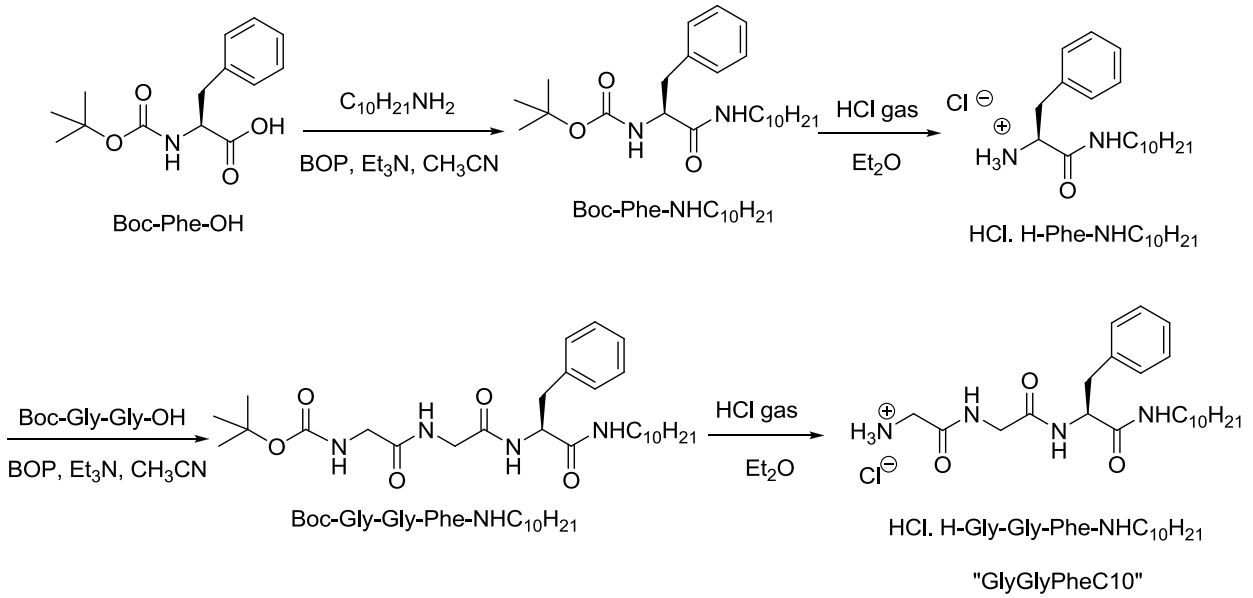
Each sample was introduced in a Petri dish (Ø 9 cm) containing 20g of Fontainebleau sand (sand 4 vol / deionized water 1 vol). The samples were placed on a plastic grid, in order to avaoid waterlogging. Onto the sand, 100 termite workers, 5 nymphs and 5 soldiers were introduced. Pine sapwood controls (30x20x5mm^3) were tested in the same manner.

The test devices were placed in a dark climatic chamber at 27°C, RH > 75%. An observation was done twice a week and the sand was wetted when necessary. After 5 weeks, the samples were taken out from the test devices. Each sample was cleaned from the sand, visually rated according to the EN117 criteria, and the survival rate of the termites is calculated. The samples were also dried at 103°C and their weight loss (as a % of initial weight) was calculated.

3. RESULTS AND DISCUSSION

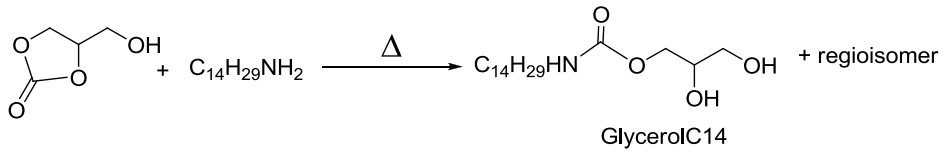
In previous studies, we have shown that appropriately designed pseudo-peptidic surfactants derivated from carnosine (*i.e.* β-AlaHisEO2C14, bearing a β-AlaHis moiety, a diethyleneoxide linker and a C14 fatty alkyl chain) act as efficient hydrogelators [28]. The formation of the hydrogels was consider to be a result of the synergism of the hydrophobic forces, H-bonding between amides and π–π staking between the imidazole groups. We propose here new amphiphilic molecules with gelling properties. The first correspond to an amphiphilic tripeptide, GlyGlyPheC10 and the second to a carbamate derivative of glycerol (glycerolC14). The

synthesis of GlyGlyPheC10 was performed in four steps using classical protection/deprotection and coupling peptidic synthesis procedures. The first step involved the reaction of the fatty amine with the phenylalanine. The carboxylic acid was then activated with BOP to lead rapidly and efficiently Boc-Phe-$NHC_{10}H_{21}$ intermediate. Boc protecting group was removed quantitatively with gaseous hydrochloric acid dissolved in diethyl ether and the resulting amine coupled with Boc-GlyGly-OH activated by BOP. Subsequent deprotection ot the Boc protecting group with gaseous the desired β-AlaHisC8 in its ammonium form (scheme 1).



Scheme 1. Synthesis of amphiphilic tripeptide HCl.H-Gly-Gly-PheC10

The reaction of glycerol carbonate with fatty amine like tetradecylamine is a very easy transformation. A simple mixture of these two compounds in a little quantity of methanol leads to the carbamate in a mixture of region-isomers due to the different way of opening of the carbonate. (Scheme 2)



Scheme 2. Synthesis of amphiphilic glycerol derivative.

The hydrogel was formed by mixing 100 mL of 5% borax solution (pH 10) with a solution of 2 % of GlyGlyPheC10 or 3% of Glycerol C14, which correspond to the minimal concentration of gelification. The dispersion was first heated to 80°C until total solubilization of the reactants, slowly cooled to room temperature and allowed to stand until formation of the hydrogel. These conditions correspond to the best optimized conditions using the minimal gelator agent quantity leading to a gel-to-solution transition taking place between 60 to 70°C.

Formation of hydrogel was visualized by scanning electron microscopy (Figure 1). SEM images indicated the presence of entangled long fibers of more than 10 μm length and up to 1 μm width randomly distributed to form the hydrogel network (Fig.1).

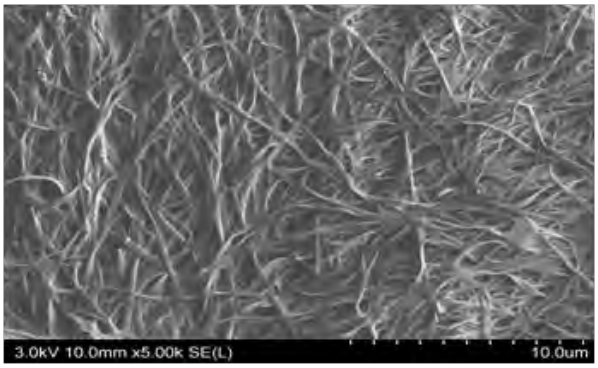


Figure 1. SEM image of the hydrogel derived from GlyGlyPheC10

Hydrogelation mechanism may be explained on the basis of the functional groups present on both molecules that can undergo non-covalent intermolecular interactions like hydrogen bonding, alkyl chains interaction, phenyl groups for π-stacking in the case of GlyGlyPhe. Just after the dissolution at 80°C, both components are dispersed and have no interaction. During cooling to room temperature, intra-molecular interactions appeared leading to self-assemblage into nanofibers forming a three-dimensional network through hydrogen bonding and Van der Waals interactions able to retain water and borate ions formed from borax dissolution [31].
To check the validity of the concept of hydrogels utilization to reduce boron leachability and develop new wood protection treatments, Scots pine sapwood blocks were impregnated with a 5% borax solution (w/w) in the presence or not of hydrogelator. Wood impregnation was performed using classical vacuum pressure. To allow impregnation of solutions containing hydrogelator, these latter ones were heated at 80°C and impregnated till hot. After impregnation, blocks were kept at room temperature, dried at 103°C and subjected to water leaching using several leaching periods of increasing duration under continuous shaking at 20°C. Weight percent gains and boron contents before and after leaching are reported in table 1.

Table 1. Weight percent gain and boron content before and after leaching

Treatment	WPG (%)		Boron content (mg / g dry wood)[a]	
	before leaching	after leaching	before leaching	after leaching
5% Borax	3.0 ± 0.3	-4.4 ± 0.8	8.3	0.73
2% Gel (GlyGlyPheC10)	0.7 ± 0.2	-2.2 ± 0.9	0.67	0.42
3% GlycerolC14	-	-	0.76	0.25
2% Gel (GlyGlyPheC10) / 5% Borax	5.4 ± 0.6	-2.7 ± 0.4	9.5	7
3% Gel (GlycerolC14)/ 5% Borax	5.0 ± 0.6	-1.8 ± 1.3	7.5	6.8
Control	-	-	0.2	-

[a] precision ± 5%

Impregnation of 5% borax solution allows obtaining boron retention of 3.0% corresponding to a boron content of approximately 8.3mg of atomic boron per gram of dry wood. Considering a wood density of 500 kg per cubic meter, this value corresponds to approximately 24 kg of boric acid equivalent (BAE) per cubic meter, which is far above the toxic limit of 1 kg BAE/m^3 set for wood protection under outdoor conditions [17, 31]. Impregnation of gel alone has practically no

effect on the weight percent gain due to small amounts of gelator agents used. Impregnation of gelator agents with borax lead to weight percent gain superior to borax alone. This may be due to the effect of temperature used to perform impregnations in the presence of gelator agents, while impregnation of borax alone was performed at room temperature. After leaching, all WPGs were inferior to zero indicating that most of the impregnated chemicals were leached out during leaching procedure.
Determination of Boron content showed very low values of boron in control and hydrogelator treated blocks. 5% borax treated wood in the presence or not of hydrogelator indicated boron content of approximately 8 to 9 mg of boron per gram of dry wood, which is far above the toxic limit of boron reported in the literature. After leaching, all boron is leached out from borax treated blocks, while quite all boron initially present in wood remains after leaching for blocks treated with borax and hydrogelators.

Treated and untreated, leached or unleached blocks were then exposed to *Poria placenta* during 16 weeks and mass losses due to fungal attack determinated (table 2).

Table 2. Weight losses of Scots pine sapwood blocks exposed to *Poria placenta*

Treatment	WL (%)	
	before leaching	after leaching
5% Borax	0.6 ± 0.2	71.5 ± 4.8
2% Gel (GlyGlyPheC10)	67.2 ± 3	67.2 ± 3.3
3% GlycerolC14	49.4 ± 29.4	50.7 ± 24.8
2% Gel (GlyGlyPheC10) / 5% Borax	0.5 ± 0.3	0.6 ± 0.4
3% Gel (GlycerolC14)/ 5% Borax	0.5 ± 0.2	0.8 ± 0.4
Control	72.0 ± 1.8	

Weight losses recorded after 16 weeks exposure to *Poria placenta* indicated a significant improvement of decay durability of blocks treated with borax in the presence of gelator agents comparatively to controls and blocks treated with borax alone. Indeed, mean weight losses of blocks treated with 5% borax in the presence of gel were inferior to 1 %, while weight losses of control and borax alone treated leached blocks were of 72 and 71.5% respectively. Impregnation of gel alone has no effect on wood durability confirming effect of gel on boron retention and consequently on decay durability. Contrary to results previously described in literature suggesting that complexation reduces biological activity of boron [13,17,18] and similarly to our previous work using hydrogel [29], utilization of these two new hydrogels to reduce boron leachability have no effect on boron bio-disponibility and consequently on it fungicidal activity towards *Poria placenta*.

To assess termites resistance and check the influence of hydrogels on boron retention, leached and unleached samples treated or not with Borax with or without hydrogels were subjected to termite attack using a laboratory test for five weeks. The results are reported in table 3.

Table 3. Survival rate, visual rating and weight losses of Scots pine sapwood blocks exposed to *Reticulitermes flavipes*

Treatement	Survival rate of the termites Workers (W)/Nymphs(N)/Soldiers(S)	Visual rating	Weight loss (%)
5% Borax before leaching	0W/0N/0S	0	2.66
	0W/0N/0S	0	3.16
	0W/0N/0S	0	3.33
5% Borax after leaching	37W/1N/1S	4	8.68
	29W/1N/1S	4	11.14
	18W/1N/1S	4	10.93
3% GlycerolC14 before leaching	63W/3N/1S	4	14.89
	60W/0N/1S	4	11.32
	58W/0N/1S	4	12.64
3% GlycerolC14 after leaching	54W/1N/1S	4	12.48
	45W/0N/2S	4	10.14
	61W/2N/1S	4	13.78
3% Gel (GlycerolC14)/ 5% Borax before leaching	0W/0N/0S	0	2.21
	0W/0N/0S	0	2.86
	0W/0N/0S	0	2.32
3% Gel (GlycerolC14)/ 5% Borax after leaching	0W/2N/0S	4	6.09
	0W/0N/0S	4	3.52
	0W/0N/0S	3	3.08
2% Gel (GlyGlyPheC10) before leaching	59W/2N/1S	4	16.77
	32W/0N/1S	4	9.68
	47W/0N/1S	4	9.10
2% Gel (GlyGlyPheC10) after leaching	64W/1N/1S	4	14.52
	68W/1N/2S	4	17.26
	47W/2N/0S	4	11.55
2% Gel (GlyGlyPheC10) / 5% Borax before leaching	0W/0N/0S	1	4.36
	0W/0N/0S	1	3.99
	0W/0N/0S	1	4.17
2% Gel (GlyGlyPheC10) / 5% Borax after leaching	34W/3N/1S	4	11.20
	12W/2N/1S	4	7.52
	34W/1N/1S	4	10.56

For all the controls, the survival rate of the workers is above 50% and the attack is strong, thus is test is valid. The results for the borax treated samples were rather predictable. When un-leached, the amount of borax (5%) is far above the threshold for termites [31]. On the other hand, when leached, Borax on its own cannot remain in the wood and the attack is strong. Both gels (glycerol and GlyGlyPhe) have no effect on termite attack or survival. When Borax is added to glycerol gel, it is interesting to see that when un-leached, the results are comparable to those obtained with Borax alone, meaning that the gel has no impact on the active ingredient. After leaching, it appears clearly that some Borax is still available and active within the wood. Borax is not a

repellent active ingredient, thus the termites have to ingest some wood before dying. Thus, despite no termite worker have survived, and that the weight loss of the wood is much lower than the one obtained for leached Borax alone or controls, the visual rating appears as average to strong.
Similar trends are observed with GlyGlyPhe gel. For this combination, the visual rating and the weight loss of the samples suggest that (1) the active ingredient is may be less accessible and/or less active, (2) GlyGlyPhe gel retains less boric acid than for the glycerol gel.

4. CONCLUSIONS

The results presented in this study confirm our previous obtained with the amphiphilic dipeptide (β-AlaHisC8) indicating the effectiveness of hydrogels to retain boron in wood allowing development of new strategies to reduce boron leachability from treated wood. Small amounts of hydrogelators, comprised between 2 and 3 %, improve boron retention in wood allowing its protection against the brown rot fungus *Poria placenta* and termites (*Reticulitermes flavipes*) even after leaching, while blocks treated with the same concentration of Borax are strongly degraded after leaching. Nature of hydrogelator influence more or less the obtained results, the glycerol derivative (GlycerolC14) leading to better results than the amphiphilic tripeptide (GlyGlyPheC10). Determination of boron content by atomic absorption before and after leaching confirm the ability of hydrogels to retain Boron in treated wood, most of the boron impregnated in the wood remaining after leaching. According to these results, hydrogels appear as valuable additives to improve boron fixation in wood and develop friendly environmentally wood preservation formulations.

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5. REFERENCES

[1] E. Baysal, S.K. Ozaki, M.K. Yalinkilic (2004): Dimensional stabilization of wood treated with furfuryl alcohol catalysed by borates, *Wood Science and Technology*, 38, 405-415.
[2] S.N. Kartal, T. Yoshimura, Y. Imamura (2009): Modification of wood with Si compounds to limit boron leaching from treated wood and to increase termite decay resistance, *International Biodeterioration and Biodegradation*, 63, 187-190.
[3] A. Temiz, G. Alfredsen, M. Eikenes, , N. Terziev (2008): Decay resistance of wood treated with boric acid and tall oil derivates. *Bioresource Technology* 99, 2102-2106.
[4] D. Mourant, D.Q. Yang, X. Lu, B. Riedl, C. Roy (2009): Copper and boron fixation in wood by pyrolytic resins. *Bioresource Technology*, 100, 1442-1449.
[5] S.N. Kartal, F. Green (2003): Leachability of boron from wood treated with natural and semi-synthetic polymers and calcium precipitating agent, Holz Roh Werkst, 61, 388-389.
[6] S.N. Kartal, Y. Imamura (2004): Effects of N’-N-(1,8-naphthalyl)hydroxylamine (NHA-Na) and hydroxynaphthalimide (NHA-H) on boron leachability and biological degradation of wood, Holz als Roh und Werkstoff, 62, 378-385.
[7] S.N. Kartal, T. Yoshimura, Y. Imamura (2004): Decay and termite resistance of boron-treated and chemically modified wood by *in situ* co-polymerisation of allyl glycidyl ether (AGE)

with methyl methacrylate (MMA), *International Biodeterioration and Biodegradation*, 53, 111-117.
[8] M-F. Thévenon, A. Pizzi, J.P.Haluk (1997): Non-toxic albumin and soja protein borates as ground-contact wood preservatives. *Holz als Roh und Werkstoff*, 55 293-296.
[9] M-F. Thévenon, A. Pizzi, J.P.Haluk (1998): Protein borates as non-toxic, long-term, wide-spectrum, ground-contact wood preservatives. *Holzforschung*, 52, 241-248.
[10] M-F. Thévenon, A. Pizzi (2003): Polyborate ions' influence on the durability of wood treated with non-toxic protein borate preservatives, *Holz als Roh und Werkstoff*, 61, 457-464.
[11] E.D. Gezer, J.H. Michael, J.J. Morrell (1999): Effects of glycol on leachability and efficacy of boron wood preservatives, *Wood and Fiber Science*, 31, 136-142.
[12] E. Toussaint-Dauvergne, P. Souloungangа, P. Gérardin, B. Loubinoux (2000): Glycerol/glyoxal: a new boron fixation system for wood preservation and dimensional stabilization, *Holzforschung*, 54, 123-126.
[13] A. Mohareb, J. Van Acker, M. Stevens (2002): Effect of protective additives on leachability and efficacy of borate treated wood, *IRG/WP 02-30290. International Research Group on Wood Preservation* Stockholm.
[14] A. Mohareb, M-F. Thévenon, E. Wozniak, P. Gérardin, (2010): Effects of monoglycerides on leachability and efficacy of boron wood preservatives against decay and termites, *International Biodeterioration & Biodegradation*, 64, 135-138.
[15] A. Mohareb, M-F. Thévenon, E. Wozniak, P. Gérardin (2011): Effects of polyvinyl alcohol on leachability and efficacy of boron wood preservatives against fungal decay and termites attack, *Wood Science and Technology*, 45 (2) 369-382.
[16] J.D. Lloyd, D.J. Dickinson, R.J. Murphy(1990): The probable mechanisms of action of boric acid and borates as wood preservatives, *IRG/WP 1450.International Research Group on Wood Preservation* Stockholm.
[17] J.D. Lloyd (1998): Borates and their biological applications, *IRG/WP 98-30178. International Research Group on Wood Preservation* Stockholm.
[18] D.N. Obanda, F.T. Shupe, H.M. Barnes (2008): Reducing leaching of boron based wood preservatives - A review of research. *Bioresource Technology*, 99, 7312-7322.
[19] M. Loos, B.L. Feringa, J. H. van Esch (2005): Design and Application of Self-Assembled Low Molecular Weight Hydrogels, *Eur. J. Org. Chem*. 3615–363.
[20] Z. Yanga, B. Xu (2007): Supramolecular hydrogels based on biofunctional nanofibers of self-assembled small molecules, *J. Mater. Chem*., 17, 2385–2393.
[21] T. Sagawa, S. Chowdhury, M. Takafuji, H. Ihara (2006): Self-Assembled nanofibrillar aggregates with amphiphilic and lipophilic molecules, *Macromol. Symp.*, 237 28–38.
[22] N. M. Sangeetha, U. Maitra (2005): Supramolecular gels: Functions and uses, *Chem. Soc. Rev*., 34 821- 836.
[23] J. Steed (2011): Supramolecular gel chemistry: Developments over the last decade, *Chem. Commun.* 47 1379-1383.
[24] A. Pasc, F. Obounou Akong, S. Cosgun, C. Gérardin, Differences between β-Ala and Gly-Gly in the design of amino acids-based hydrogels, Beilstein J. Org. Chem. 6 (2010) 973–977.
[25] P. Gizzi, A. Pasc, N. Dupuy, S. Parant, B. Henry, C. Gérardin (2009): Molecular Tailored Histidine-Based Complexing Surfactants: From Micelles to Hydrogels, *Eur. J. Org. Chem.* 3953-3963.
[26] A. Pal, J. Dey (2011): Water-Induced Physical Gelation of Organic Solvents by N-(n Alkylcarbamoyl)-L-alanine Amphiphiles, *Langmuir*, 27, 3401–3408.
[27] T. Patra, A. Pal, J. Dey (2010): A Smart Supramolecular Hydrogel of N-(4-n-Alkyloxybenzoyl)-L-histidine Exhibiting pH-Modulated Properties, *Langmuir*, 26(11), 7761–7767.

[28] A. Pasc, P. Gizzi, N. Dupuy, S. Parant, B. Henry, C. Gérardin (2009): Microscopic and macroscopic anisotropy in supramolecular hydrogels of histidine-based surfactants, *Tetrahedron Lett.* 50, 6183-6186.
[29] F. Obounou Akong, P. Gérardin, C. Gérardin-Charbonnier (2012): Smart hydrogels from low molecular weight amphiphilic compounds: toward a solution to decrease leachability and increase efficacy of boron preservatives? *IRG/WP* 12-30589, 43th Annual Meeting of the *International Research Group on Wood Protection*, Kuala Lumpur Malaysia.
[30] European committee for standardization (1994): Wood preservatives - methods for measuring losses of active ingredients and other preservative ingredients from treated timber - part 2: laboratory method for obtaining samples for analysis to measure losses by leaching into water or synthetic sea water, *ENV 1250-2*, Brussels.
[31] E.Pezron, A. Richard, F. Lafuma, R. Audebert (1988): Reversible gel formation induced by ion complexation. 1. Borax-galactomannan interactions, Macromolecules, 21(4) 1121-1125
[32] A.J. Drysdale (1994): Boron treatments for the preservation of wood - A review of efficacy data for fungi and termites, *IRG/WP* 94-30037. *International Research Group on Wood Preservation* Stockholm.
[33] W.M. Schoeman, J.D.Lloyd (1998): International standardisation: a hypothetical case study with stand-alone borate wood preservatives, *IRG/WP* 98-20147. *International Research Group on Wood Preservation*, Stockholm.

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Section 3 Wood protecting chemicals

Study of the use of organosolv lignin as bio-preservative of wood

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Study of the use of organosolv lignin as bio-preservative of wood

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ABSTRACT

The service life of wood depends on the species, use and environmental conditions of exposure. The treatment of wood protects it against degradation by xylophagous agents, enhancing the durability of material up to 10 times, and reducing the deforestation around 12.5%. In this way, the use of treatments is necessary because increases the service life of material and protect against xylophagous agents who degrade the wood. The study and development of new preservative products for wood are necessary in order to substitute heavy metals-based preservatives, such as CCA (Chromated Cooper Arsenate) that entails environmental risks. Lignin is considered as a residue in pulp and paper production processes, being mainly used as fuel in the recovery boiler for energy generation. However, more valuable uses of lignin could be developed for several interesting applications such as bio-preservative activity. In this context, the present study aimed to evaluate the properties of eucalyptus organosolv lignin, obtained by using 60% (v/v) ethanol/water solution, in a solid/liquid ratio of 1:10, at 180 ◦C for 90 min. The resulting lignin samples were characterized by different analytical techniques such as FTIR, GPC, and TGA. Furthermore, the antioxidant potential of lignin by ABTS method and the fungicide potential by ASTM D 2017 – 81 were analyzed. The analyzed lignin showed neutralization and inhibition activities (antioxidant capacity). These characteristics demonstrated that lignin could be an excellent wood bio-preservative obtained from renewable sources.

Keywords: wood treatment, wood preservation, wood quality.

1. INTRODUCTION

Wood was one of the first materials used by humanity and remains as a raw material for multiples utilizations, mainly due to availability and intrinsic characteristics.

The useful life of wood depends of species, final utilization and environmental conditions that are exposed. The treated wood is more durable, which reduces the deforestation up to 12.5%. Thus, the utilization of treatments are necessary in order to improve the useful life of wood and, consequently, to protect against biodegradation. However, the wood treatment with toxic compounds, such as CCA (Chromated Copper Arsenate) increases the environmental impacts due to the presence of arsenic. Moreover, the commercialization of products with arsenic is limited, proving the necessity of development of new products for the wood preservation.

Lignin is a chemical substance that along with cellulose forms part of vegetal cell wall and serves as an agent of cellular union. At the same time, the lignin presents a great structural diversity in trees of the same gender, same species or different morphological regions of vegetal. Considering these facts, is indispensable to investigate the lignin structural composition of wood.

The lignin is an impurity of pulp process, which is originated after the pulp bleaching. These residues are used, mainly as fuel in the ovens in order to generate energy and to recuperate inorganic reactive used in the process. In other hand, the bio-protective activity of the lignin against microorganisms and plagues was recently proved, necessitating a noble utilization for this compound.

The lignin acting as a neutralizer and inhibitor of oxidation process due to its aromatic structure and high content of reactive functional groups, which possibly demonstrates its bio-preservative action to wood. Thus, the lignin is an excellent font of new renewable materials.

2. EXPERIMENTAL METHODS

2.1 Conditioning of the raw material

Eucalyptus paniculata wood was used and was obtained from a homogeneous population located in the city of Charqueadas (29º57'17"south and 51º37'31" west), Rio Grande do Sul, Brazil.

2.2 Characterization of the raw material and obtained solid fractions

The characterization of the raw material and the fiber were performed in triplicate and according to standard methods (TAPPI) and bibliographic procedures: moisture content (TAPPI T264 cm-97); holocellulose (Wise *et al*. 1946); α-cellulose and hemicellulose (Rowell 1983); ash content (TAPPI T211 om-93); solubility in hot water (TAPPI 207 om-93); aqueous 1% NaOH soluble matter (TAPPI T212 om-98); ethanol-toluene extractives (TAPPI T204 cm-97) and insoluble lignin (TAPPI T222 om-98).

2.3 Fragmentation processes

The raw material was treated with ethanol in a stirred reactor (4 l) equipped with a temperature control and data acquisition. The experimental conditions was 60% (v/v) ethanol-water solution in a relation liquid/solid 1:10 at 180°C for 90 min. After the reaction time, the solid fraction was separated from the liquid fraction by filtration and the residual liquor was removed (wash with water).

2.4 Liquid fraction characterization

The main physico-chemical properties of the liquid fraction were determined according to standard methods.

The pH was measured with a digital pH meter SELECT "pH-2005". The density was determined by measuring the weight of black liquor in a volume previously weighed and without moisture. The inorganic matter was determined by combustion of samples at 525°C (TAPPI T211 om-93). The organic matter was measured through the difference between total dissolved solids and inorganic matter.

2.5 Lignin recovery from the liquid fraction

The liquid fractions were treated in order to precipitate the dissolved lignin. The liquid ethanol fraction was mixed with two volumes of water and the pH of that solution was lowered to pH 2 with sulfuric acid (72% w/w). Thus, the precipitated lignin was separated by filtration, washed with acidified water and dried with vacuum at 50°C. Finally, lignin samples were characterized by different techniques: infrared spectroscopy (ATR-IR) by direct transmittance at a resolution of 4 cm^{-1} for 32 scans; thermogravimetric analysis (TGA) with a sample exposure between 25 and 800°C; gel permeation chromatography (GPC) and ultraviolet-visible spectrophotometry UV-VIS (Folin method and antioxidant capacity, ABTS described by García *et al*. (2010).

2.6 Hydrothermal treatment of lignin

The lignin obtained previously was treated at high temperatures and pressure during a fixed time (90 min) in presence of catalyst. Thus, the lignin depolymerization was realized, producing a phenolic compounds (oil) and byproducts (residual lignin).

The procedure of separation and extraction of reaction products occurred through precipitation of unconverted lignin. The reaction mix was acidified with hydrochloric acid until pH around 1 in order to precipitate the residual lignin. The solid and liquids products were separated by vacuum filtration.

The filtrated product was extracted with ethyl acetate in order to extract the phenolic monomers produced during the depolymerization of the lignin. The yield of each product was determined by gravimetric method in relation to the initial lignin content.

2.7 Oil composition

The composition of the oil obtained was determined by gas chromatography-mass spectroscopy (GC (7890A)-MS (5975C inert MSD with Triple-Axis Detector) Agilent) equipped with a capillary column HP-5MS ((5%-Phenyl)-methylpolysiloxane, 60 m x 0.25 mm). Moreover, the density (gravimetric method), viscosity (ostwald viscometer method) and inorganic matter (TAPPI T211 om-93) of the oil were measured.

2.8 Wood impregnation

The efficiency of the oil (preservative 1) and the lignin (preservative 2) both with a solution at 1% in water/ketone was measured through of impregnation in the wood samples. For this, two methods of wood impregnation were used. Before the impregnation process, the color and the odor of two preservatives were measured through sensorial techniques.

In the first method, the wood samples were impregnated in a solution of the oil at 1% by vacuum application (0.8 bar) for 90 min. In the second method (empty-cell), the wood samples was submitted a vacuum (0.8 bar) for 30 min. The application of vacuum opened the wood porous and, at the same time, the oil solution at 1% was introduced in the recipient. The oil solution remained under vacuum during 60 min for impregnation of wood samples.

The penetration of oil solution was evaluated by UNE EN351-1 rules through the difference of weight before and after the impregnation of wood samples.

2.9 Characterization of impregnated wood

The characterization of impregnated wood was realized through antifungal activity tests and color variation of the samples.

The antifungal activity (ASTM D2017–81) of oil solution was measured through the accelerate tests methods for evaluation wood biodegradation by *Trametes versicolor* fungus (white-rot) and *Gloeophyllum trabeum* (brown-rot). For this, samples of *Pinus* spp. sapwood with dimensions 2 x 2 x 1 cm were prepared. The weight loss was determined through the difference of samples weight before and after the action of the fungus.

The variation of color was determined with a colorimeter (CR-400 Minolta Chroma Meter) according to the *CIELab* standard. The equipment was adjusted with a D65 light source and observation angle of 2°.

For this, the parameters L^* (lightness), a^* (green-red chromatic coordinate), b^* (blue-yellow chromatic coordinate). Moreover, ΔE (color difference), C^* (chroma) and h^* (hue angle) were measured by Eq. 1, 2 and 3.

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (1)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$h^* = tang^{-1}(b^*/a^*) \quad (3)$$

Where: ΔE = color difference; $\Delta L^{*2}, \Delta a^{*2}$ e Δb^{*2} = lightness, red-green coordinate and blue-yellow coordinate variation; C^* = chroma; h^* = hue angle; a^* = red (+) - green (-) color coordinate; b^* = yellow (+) – blue (-) color coordinate.

3. RESULTS AND DISCUSSION

3.1 Raw Material and fiber characterization

Table 1 shows the chemical analysis of *Eucalyptus paniculata* wood and pulp.

Table 1: Composition of wood and pulp of *Eucalyptus paniculata.*

Analysis (%)	Raw material	Pulp
Ash	0.23 (0.1)	0.27 (0.02)
Ethanol–toluene extractives	2.86 (0.6)	3.88 (0.4)
Solubility in hot water	6.53 (0.6)	6.96 (0.2)
Aqueous 1% NaOH soluble matter	14.97 (0.5)	8.44 (1.2)
Insoluble lignin	36.67 (0.9)	21.23 (2.2)
Holocellulose	52.19 (1.05)	74.56 (0.96)
α-cellulose	43.32 (1.25)	63.02 (0.06)
Hemicellulose	8.87 (0.21)	11.54 (0.94)

Values in parentheses corresponding to the standard deviation.

The lignin content of the pulp was lower than the lignin content of raw material, proving the elimination of the lignin during the delignification process. Thus, the cellulose showed an inversely proportional behaviour to the lignin. The cellulose content of the pulp was higher than the cellulose content of the raw material. This fact also was observed for Alriols (2010).

Moreover, the ash content, extractives content and solubility in hot water presented a high values for the raw material, while aqueous 1% NaOH soluble matter and hemicellulose content were high for the pulp.

3.2 Black liquor characterization

The results of the characterization of black liquor obtained from the organosolv process are shown in the Table 2.

Table 2: Characterization of black liquor.

Analysis	Average
MI_a (%)*	0.37 (0.63)
MO_b(%)*	4.65 (1.11)
MD_c (%)	5.21 (0.56)
pH	3.90 (0.03)
Lignin (%)*	19.63 (0.92)
Density (g/mL)	0.90 (0.0001)

MI_a= inorganic matter; MO_b= organic matter; $MD_{c=}$ dry matter; *= % dry matter (w/w); Values in parentheses corresponding to the standard deviation.

The lignin concentration was 19.63%, similar to found by Serrano *et al*. (2010) and Toledano *et al*. (2010), both studies with different species. However, Cardoso *et al*. (2009) observed lignin concentration between 39 and 42% for the *Eucalyptus grandis* black liquor from Kraft industrial process. This fact could be explained due to relation dissolution/raw material because the present study was a laboratorial extraction (a lower relation dissolution/raw material than the industrial processes).

Moreover, the results of other properties (MI_a, $MO_{b,}$ $MD_{c,}$ pH and density) were similar to found by Serrano *et al*. (2010). Probably, the similar results occurred due to the use of the same extraction process (organosolv), even that the species were different.

3.3 Lignin characterization

ATR-IR spectra of *Eucalyptus paniculata* lignin are shown in Fig. 1.

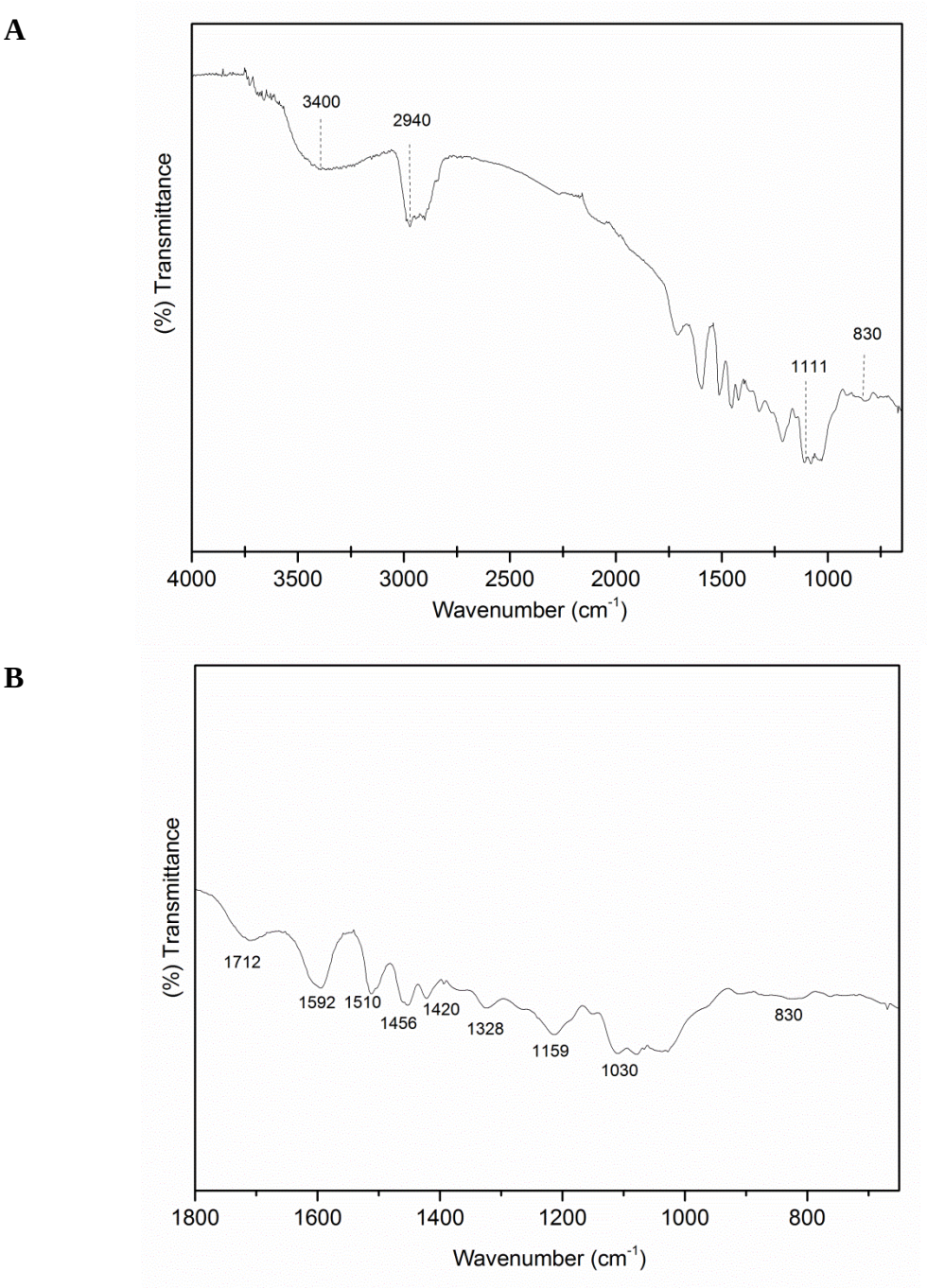

Figure 1: ATR-IR spectra of *Eucalyptus paniculata* lignin. (A) Spectra in the range from 750 to 4000 cm^{-1}; (B) Spectra in the range from 750 to 1800 cm^{-1}

In general, the results were similar to the reported in other study (Tejado *et al*. 2007).

A high absorption band at 3400 cm^{-1} and at 1030 cm^{-1} corresponded to the OH aromatic and aliphatic groups. The bands at 2940 and 1456 cm^{-1} were related to the C-H vibration of CH_2 and CH_3 groups. The range between 1705 and 1715 cm^{-1} was assigned to the unconjugated carbonyl groups.

The lignin showed the presence of bands at 1592, 1512 and 1420 cm^{-1} that were associated to the vibration of aromatic rings of phenylpropane skeletal. Moreover, the spectra showed bands assigned to the syringil (S) and guaiacyl (G): breathing rings of syringil with C-O stretching at 1328 cm^{-1}, aromatic C-H of syringil type in the plane deformation at 1111 cm^{-1}, typical syringil units at 830 cm^{-1} and guaiacyl units at 1268 cm^{-1}.

Fig. 2 showed the thermograms (TG) and its derivatives thermograms (DTG) of *Eucalyptus paniculata* lignin.

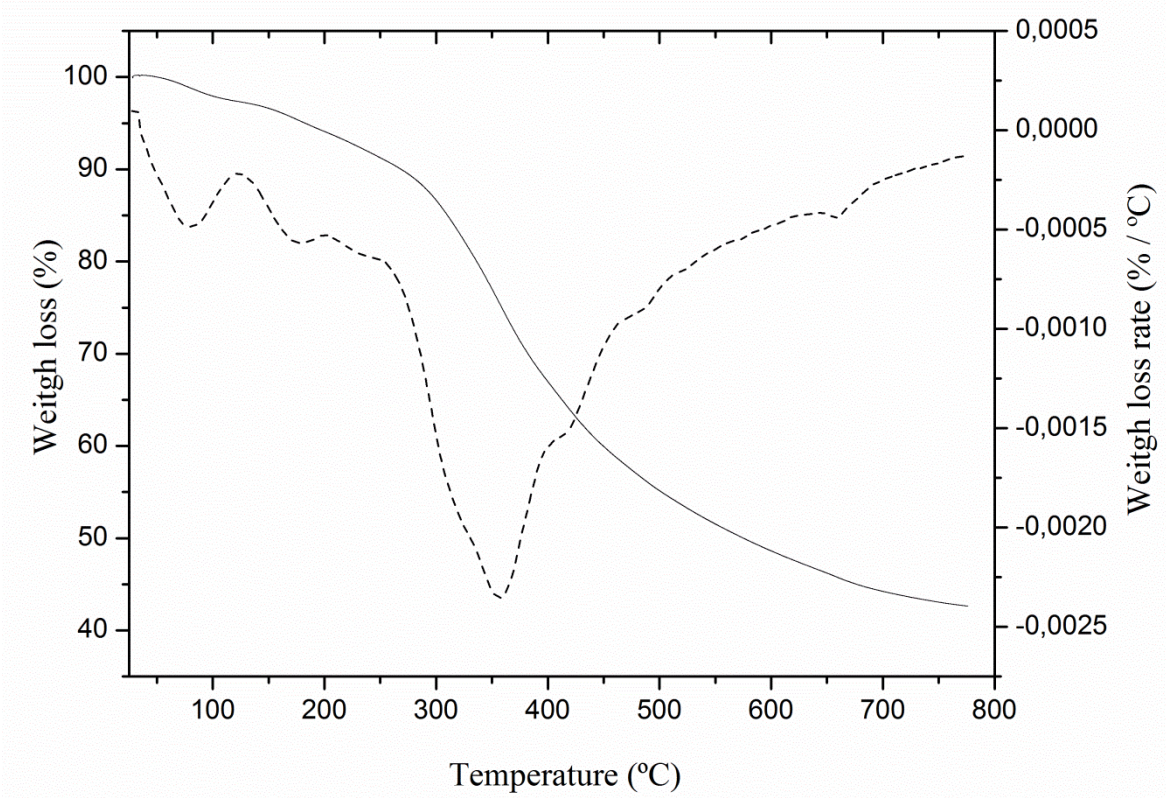

Figure 2: TGA (straight line) and DTG (dotted line) thermograms for the *Eucalyptus paniculata* lignin.

The degradation process was characterized for three ranges. The first range was between 80 and 100°C, that corresponding to the moisture loss. The second range corresponding to the hemicellulose degradation and was at 220-280°C. Finally, the third range was between 280 and 300°C, that corresponding to the lignin degradation.

The lignin presented a high residual content (42.62%). According to Garcia *et al.* (2011), a high residual content means a high thermal stability and a tridimensional structure more complete. However, the high values of residues could indicate presence of inorganic matter, which was very low (1.09%) in the chemical analysis. Thus, the residues percentage could be associated to the phenolic compounds condensation during the thermal degradation.

The results of molecular weight analysis are shown in the Table 3.

Table 3: Average values of molecular weight analysis of *Eucalyptus paniculata* lignin.

Analysis	Mw	Mn	IP	EAG (%) *	Inhibition ABTS (%) **	DTmax (ºC)	Lignin residue (%)
Average	10193	1916	5,32	46,7	94,8	358,18	42,62

Mw= molecular weight; Mn= Molecular number; IP= polydispertivity (Mw/Mn); EAG= gallic acid equivalents DTmax= maximum degradation temperature; *Total phenolic content in the lignin expressed in percentage of content of gallic acid equivalents; **Antioxidant power as a percentage of the ABTS radical reduction related to the lignin solution of 2g/l in DMSO.

The high molecular weight of *Eucalyptus paniculata* lignin could be associated to the higher concentration of contaminants due to the purity degree (75± 2%).

The molecular weight of lignin is related to the quantity of C-C. According to García *et al*. (2012), the lignin with high molecular weight has more guaiacyl units. The lignin with low molecular weight is more soluble and present more capacity to form hydrogen bonds than the lignin with high molecular weight Arantes (2007).

The total phenolic indicated by EAG (gallic acid equivalents) presented better results than observed by García *et al*. (2012) for the *Olea europea* L. specie (olive). Moreover, the antioxidant properties, based in the antioxidant power (percentage of ABTS radical reduction), of lignin sample is in agreement with the results observed in other study (García *et al*. 2010).

3.4 Characterization of oil and lignin of *Eucalyptus paniculata* as a preservative product

Table 4 shows the characterization of phenolic compounds of low molecular weight of *Eucalyptus paniculata* oil (preservative 1). This characterization did not realized for the *Eucalyptus paniculata* lignin (preservative 2) due to the conditions required for the GC-MS equipment.

Table 4: Phenolic compounds presented in the *Eucalyptus paniculata* oil.

Compound	Average content	
	ppm	% (W/W)*
Phenol	38,01	0,39
o-cresol	5,46	0,05
m-p-cresol	16,61	0,17
Guaiacol	10,71	0,11
Catechol	133,28	1,37
4-methylcatechol	15,03	0,15
Syringol	4,32	0,04

ppm= parts-per-million; *Yield in percentage (%) of compounds corresponding to the oil weight (w/w).

The catechol and the phenol were the compounds that presented the high content in the *Eucalyptus paniculata* oil. On the other hand, the syringol and the o-cresol were the lowest compounds verified in the oil analyzed.

The physical and sensorial properties of two preservatives (oil and lignin) are shown in the Table 5.

Table 5: Physical and sensorial properties of two preservatives (oil and lignin).

Analysis	Preservative 1	Preservative 2
pH	5.3	7.0
Viscosity (mm^2/s^2)	0.86	-
Density (g/ml)	0.98 (0.003)	0.87 (0.005)
Color	Yellow	Brown
Odor	Characteristic	Characteristic

Values in parentheses corresponding to the standard deviation.

The pH of preservative 1 (5.3) presented a lower value than the preservative 2 (7). Possibly, it was occurred due to the method of oil (preservative 1) extraction.

The viscosity of the preservative 1 was 0.86. The viscosity of preservative 2 was not determined because the dark color of the product thwarted the measurement by the ostwald method.

Naturally, the density of the preservative 1 (oil) was higher than the preservative 2 (lignin). The viscosity is direct proportionally to the density.

In relation to the sensorial properties, the preservative 1 presented a yellow color, while the preservative 2 showed a dark color (brown). For both preservatives, the odor was shown as characteristic.

3.5 Characterization of impregnated wood

Table 6 shows the results of the impregnation rates for the *Pinus* spp. wood samples.

Table 6: Impregnation rates of two preservatives in the *Pinus* spp. wood samples.

Preservative	Method	Absorption (L/m^3)
1 (oil)	Full-cell	138.66 (25.49)
	Empty-cell	109.66 (16.59)
2 (lignin)	Full-cell	248.92 (30.33)

Values in parentheses corresponding to the standard deviation.

The best impregnation of all the treatments occurred for the preservative 2 (lignin) in the full-cell process. For the preservative 1 (oil), the full-cell method showed higher impregnation rate than the empty-cell method.

The preservative 2 (lignin) presented a lower density than the preservative 1. This fact could justify its high absorption for the wood samples.

The weight losses of impregnated samples after the fungal activity are shown in the Table 7.

Table 7: Weight losses of impregnated samples after the fungal activity.

Treatment	Fungus	Weight loss (%)
Untreated	*Trametes versicolor*	2.83 (1.07)
	Gloeophyllum trabeum	1.35 (0.04)
1	*Trametes versicolor*	0.42 (0.14)
	Gloeophyllum trabeum	0.81 (0.11)
2	*Trametes versicolor*	0.36 (0.59)
	Gloeophyllum trabeum	0.88 (0.16)
3	*Trametes versicolor*	0.66 (0.32)
	Gloeophyllum trabeum	0.24 (0.08)

1= Preservative 1 (oil) in full-cell; 2= Preservative 1 (oil) in empty-cell; 3= Preservative 2 (lignin) in full-cell; Values in parentheses corresponding to the standard deviation.

The treatment 3 (preservative 2 in empty-cell) showed the lowest weight loss for the exposure in *Gloeophyllum trabeum* fungus, while the highest degradation occurred for the untreated samples. The treatments 1 and 2 presented similar weight loss.

In the other hand, the samples exposition to the *Trametes versicolor* demonstrated that the preservative 1 in empty-cell (treatment 2) presented the best resistance to degradation. At the same way that *Gloeophyllum trabeum*, the untreated samples showed the lowest resistance for the *Trametes versicolor* activity.

Thus, these preliminary results showed that the lignin could be used as a wood preservative.

The variations of color of impregnated samples after the fungal activity are shown in the Table 8.

Table 8: The variations of color of impregnated samples after the fungal activity.

Treatment		***L****	***a**'*	***b****	***C****	***h****	**Δ*E***
Untreated	-	78,57	6,21	20,23	21,16	72,94	------
	Trametes versicolor	62,65	10,9	33,80	35,52	72,20	22,14
	Gloeophyllum trabeum	60,37	9,7	30,90	32,40	72,64	19,673
1	-	71,07	9,605	25,615	27,355	69,71	7,87
	Trametes versicolor	49,91	12,76	26,71	29,63	64,45	21,532
	Gloeophyllum trabeum	50,56	10,64	21,95	24,41	63,86	20,27
2	-	70,958	10,373	27,148	29,088	70,958	6,809
	Trametes versicolor	48,19	12,82	25,86	28,88	63,65	24,18
	Gloeophyllum trabeum	52,61	10,64	26,435	28,49	68,06	20,72
3	-	41,84	8,15	14,69	17,22	63,06	35,03
	Trametes versicolor	47,65	9,78	21,305	25,02	65,09	13,19
	Gloeophyllum trabeum	48,83	10,55	22,68	23,46	65,38	10,42

1= Preservative 1 (oil) in full-cell; 2= Preservative 1 (oil) in empty-cell; 3= Preservative 2 (lignin) in full-cell; L*= lightness; a*= red-green chromatic coordinate; b*= blue-yellow chromatic coordinate; ΔE= color difference; C*= chroma; h*= hue angle.

In general, the colorimeter parameters were modified due to fungus activity.

The color of samples after the fungal activity of full-cell method was similar to the empty-cell method for the preservative 1 (oil). In general, the lightness (*L**) decreased around 30%, while the *a** and *b** showed a small variation for both fungus. In the other hand, the samples impregnated with lignin solution at 1% presented a different behaviour, which its color tended to blue.

The higher value of *C**, the better is the proximity of wood with the original color. In this context, the highest values of *C** was verified to the untreated samples attacked by both fungus. The lowest value was observed for the treatment 3 in the samples not attacked by the fungus, probably due to the darkening of samples treated with lignin solution at 1%.

At the same way, the reduction of the *h** was observed for all the impregnated samples in relation to the untreated samples.

A high value of ΔE* indicates a high variation of impregnated samples color in relation to the samples untreated and not exposed to the fungus. Thus, the ΔE* of treatment 3 (lignin solution at 1%) decreased after the fungal exposition, while the treatments 1 and 2 (oil solution at 1%) showed inversely proportional behaviour.

4. CONCLUSIONS

The lignin content of pulp was lower than the raw material, indicating the considerable elimination of the lignin during the organosolv delignification.

The treatment 3 (preservative 2, lignin) showed the high decay resistance to the attack of *Gloeophyllum trabeum*, while the treatment 2 (preservative 1, oil in empty-cell) presented the best decay resistance to the attack of *Trametes versicolor*. However, all the treated samples showed higher decay resistance than the untreated samples.

The colorimetric evaluation showed a high variation for the samples impregnated with lignin solution in relation to the samples impregnated with oil solution. Thus, is necessary to observe the color behaviour of each product due to the esthetic values.

Preliminary studies of the lignin as a wood preservative were possible to perform due to its chemical characteristics.

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6. REFERENCES

Alriols, M G, García, A, Llano-Ponte, R, Labidi, J (2010): Combined organosolv and ultrafiltration lignocellulosic biorefinery process. *Chemical Engineering Journal* **157**(1), 113–120.

American Society for Testing and Materials (ASTM) (1994): Standard method of accelerated laboratory test of natural decay resistance of woods (ASTM D2017-81), 324-328

Arantes, T M (2007): Uso de Soluções Hidrotrópicas na deslignificação do Bagaço de cana de açúcar. *Universidade de São Paulo* (USP), 147p.

Cardoso, M, Oliveira, E D, Passos, M L (2009): Chemical composition and physical properties of black liquors and their effects on liquor recovery operation in Brazilian pulp mills. *Fuel* **88**(4), 756-763.

García, A, Alriols, M G, Labidi, J (2012): Evaluation of the effect of ultrasound on organosolv black liquor from olive tree pruning residues. *Bioresource Technology* **108**, 155–161.

García, A, Alriols, M G, Llano-Ponte, R, Labidi, J (2011): Energy and economic assessment of

soda and organosolv biorefinery processes. *Biomass and Bioenergy* **35**(1), 516–525.

García, A, Toledano, A, Andrés, M A, Labidi, J (2010): Study of the antioxidant capacity of Miscanthus sinensis lignins. *Process Biochemistry*, **45**(6), 935–940.

Rowell, R (1983): The chemistry of solid wood: based on short course and symposium sponsored by the division of cellulose, paper, and textile chemistry. In: *Proceedings of the 185th meeting of the American Chemical Society*, Seattle, Washington, pp. 70-72.

Serrano, L, Egües, I, Alriols M G, Llano-Ponte, R, Labidi, J (2010): *Miscanthus sinensis* fractionation by different reagents. *Chemical Engineering Journal*, **156**(1), 49-55.

Technical Association of the Pulp and Paper Industry (TAPPI) (1993): Drainage time of pulp (T 221 cm-93).

Technical Association of the Pulp and Paper Industry (TAPPI) (1997): Solvent extractives of wood and pulp (T 204 cm-97).

Technical Association of the Pulp and Paper Industry (TAPPI) (1998): One percent sodium hydroxide solubility of wood and pulp (T 212 om-98).

Technical Association of the Pulp and Paper Industry (TAPPI) (1993): Water solubility of wood and pulp (T 207 om-93).

Technical Association of the Pulp and Paper Industry (TAPPI) (1993): Acid-insoluble lignin in wood and pulp (T 222 om-98).

Technical Association of the Pulp and Paper Industry (TAPPI) (1999): Alpha-, beta- and gamma-cellulose in pulp (reaffirmation of T 203 cm-99).

Tejado, A, Peña, C, Labidi, J, Echeverria, J M, Mondragon, I I (2007): Physico-chemical characterization of lignins from different sources for use in phenol-formaldehyde resin synthesis. *Bioresoure Technology* **98**(8), 1655–1663.

Toledano, A, García, A, Mondragon, I, Labidi, J (2010): Lignin separation and fractionation by ultrafiltration. *Separation and Purification Technology* **71**(1) 38-43.

UNE-EN 351-1 (2008): Durability of wood and wood-based products - Preservative-treated solid wood - Part 1: Classification of preservative penetration and retention.. Madrid: AENOR.

Wise, L E, Murphy, M, D'Addieco, D (1946): Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. *Paper Trade Journal*, **122**(2), 35-43.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 3 **Wood protecting chemicals**

Termite resistance of wood impregnated with phenol-formaldehyde (PF) modified boron compounds

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Termite resistance of wood impregnated with phenol-formaldehyde (PF) modified boron compounds

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ABSTRACT

In order to investigate the effect of phenol-formaldehyde (PF) modified boron compounds on termite resistance of two main plantation-grown wood species, namely, Masson pine (*Pinus massoniana* Lamb.) and Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.), laboratory termite tests and field tests were carried out according to AWPA standard E1-97 and AWPC protocols/2007. Different concentrations of boric acid (BA), borax (BX), and disodium octaborate tetrahydrate (DOT) mixed with 20% aqueous solution of PF were used to impregnate the sapwood of both wood species. Formosan termite (*Coptotermes formosanus* Shiraki.) was used as the test termite in laboratory termite tests, while the field tests were carried out in Guangzhou, China. The results showed that the compound treatments of boron compounds with PF could efficiently improve the termite resistance of both wood species, especially the treatments by using BX. According to the laboratory termite tests, the untreated Masson pine and Chinese fir sapwood rated 5 and 4 (seriously attacked) with average weight losses of 29.3% and 43.7%. After treated with PF modified boron compounds with concentrations of 0.5, 1.0, 1.5, and 2.0%, the average weight losses were reduced to lower than 5.9, 5.1, 2.3, 1.2% for Masson pine and 7.6, 5.0, 1.8, 1.1% for Chinese fir, respectively. The performance of Masson pine in field test was much worse than Chinese fir by showing complete digestion by the termites. BX-PF treatment showed the best results in field test by reducing the average weight loss of Masson pine samples from 100% to lower than 6.1% or Chinese fir from 25.6% to lower than 8.6% at a BX concentration lower than 1.0%.

Keywords: wood; PF resin; boron compounds; termite resistance

1. INTRODUCTION

Borates have been used as wood preservatives for a long history due to their effectiveness against many wood-destroying microorganisms and their low toxicity to mammals. However, borates can seldom take effect in outdoor applications up to now because the poor leaching resistance of boron in treated wood. A lot of investigations have been done to come over this drawback of borates by combining various chemicals with borates, which include: (1) some monomers or polymers such as ethylene (Yalinkilic *et al*. 1998, Kartal and Green 2003), methyl methacrylate (MMA) (Baysal *et al.* 2004), polyethylene glycol (PEG) Cui and Kamdem, 1999; (2) some natural substance such as protein and tannins (Thévenon *et al.* 1997, 1998a, 1998b); (3) water repellent (Peylo and Willeitner, 1995a) or some varnish or alcohol ester paint (Peylo and Willeitner, 1995b); Homan and Militz 1995); (4) TMB at vapor phase (Hashim *et al.* 1994a, 1994b, Tomak *et al.* 2008, Baysal and Yalinkilic 2005; (5) inorganic metal-containing compounds such as zinc sulfate and copper sulfate (Furuno *et al*. 2006). These methods can all reduce the boron leaching to different extents but still insufficient to support the application in outdoor especially ground-contact applications.

Phenol-formaldehyde (PF) resin is formed by phenol and aldehyde under certain catalyst. In alkaline circumstance, addition reaction between phenol and aldehyde takes place to generate hydroxymethyl phenol. If the hydroxymethyl phenol stops polymerization before its gelling point, water-soluable resol-type PF will be formed. In 1930s', Stamm et al. first reported the application of PF resin on wood to improve its mechanical properties and dimensional stability. Thereafter, it was found to be able to improve the resistance to microbial attacks (Ryu *et al.*, 1991). Imamura *et al*. (1998) proposed that the improvement on above-mentioned properties was attributed to the deposition of PF molecules in wood cell wall. Furuno *et al.* (2004) used low concentration PF with low molecular weight to impregnate wood and found that PF entered the wood cell wall successfully and improved the decay resistance and dimensional stability. Shams et al. (2004a, b) impregnated wood with low-molecular PF and then compress treated wood, and finally obtain a composite with high density and strength. In our previous studies (Yu *et al.* 2009), we investigated the boron leaching from wood treated by the combination of PF with three types of boron compounds, that is, boric acid (BA), borax (BX) and disodium octaborate tetrahydrate (DOT). We also compared the effect of PF with three different molecular weight levels on boron leaching from DOT/PF treated wood (Liu *et al.* 2012). The results showed that PF could reduce the boron leaching significantly. The higher concentration of PF could fix boron better. Low molecular weight PF was favourable to improve the leaching resistance of boron because of its capability of penetrating into the wood cell wall. Some preliminary results on laboratory decay test and laboratory termite test of PF-boron treated wood showed that the combination of these two treatments was very effective (Yu *et al.* 2009).

In this study, both laboratory and field termite tests of wood treated with PF modified boron compounds were carried out and compared. The results would be able to provide some useful information on wood preservation technologies.

2. EXPERIMENTAL

2.1 Materials

The sapwood of two wood species, namely, Chinese fir (*Cunninghamia lanceolata* Hook.) with a density of 0.341 g/cm^3 and Masson pine (*Pinus massoniana* Lanb.) with a density of 0.523 g/cm^3, was used to make test samples for termite tests. All the samples are characteristic of even width of annual ring and free of visible defects. The samples for laboratory termite test are 25(R)×25(T)×6(L)mm, and the samples for field termite test are 25(R)×25(T)×100(L)mm. The replicates for samples are 6.

The chemical used in this study was all from local chemical companies in China, which included formaldehyde（37-40%), phenol, sodium hydroxide, boric acid (BA), borax (BX) and DOT.

2.2 Laboratory preparation of resol-type PF

Secondary synthesis process was used to prepare three different molecular weight PF solutions with a molar ratio of phenol to formaldehyde at 2.

Preparation of low molecular weight PF: Firstly phenol was melted below 50°C, and then slowly poured in reactor together with NaOH (40 %). After heating up to 45 °C in 10 min, formaldehyde, about four-fifths of the whole was added into reactor for the first time. The reactor needed to be heated up to 80℃ within 20 min and reacted for another 1 h. While

temperature went down to 70℃, the rest formaldehyde was added. Also, the reactor needed to be heated up to 80℃ and reacted for 2 h. After cooling down to below 40℃, the product was poured out and tested with Brookfield at 20℃. The viscosity of obtained PF was 200 mPa.s at 20℃.

2.3 Treatment of wood samples

PF solution with a concentration of 20% was mixed with different types and amount of boron compounds to make a series of compound treating solutions, including 0.5%BA/PF, 1.0%BA/PF, 0.5%BX/PF, 1.0%BX/PF, 0.5BX/PF, 1.0%DOT/PF. For laboratory termite test, another two higher loading levels, namely, 1.5% and 2.0% were also tested. All the concentrations of tested boron compounds were provided in BAE (boric acid equivalent). In order to achieve good penetration, the samples were pressure treated in a full cell process. First, the samples were vacuum treated at 0.01 MPa for 30 min and then the treating solution was let in to completely submerge the samples. After pressurized at 2 MPa for 60 min, samples were taken out and dried in an oven at 60°C for 6 h and further at 110°C for 8 h. Different treating solutions were used to treat both wood species, which would be designated as CF (Chinese fir) and MP (Masson pine) respectively. Besides, 2 control groups (control CF and control MP) were also prepared for comparison.

2.4 Termite tests

2.4.1 Field termite test

The field termite test was carried out according to a modified Australia standard (AWPC protocols/2007). The test site is Guangzhou China, which has a high hazard of termite attack. First, a ditch of 2 m long, 1m wide and 1m deep was dug, put two layers of bark-containing masson pine wood in the ditch, above that put one layer of soil, and then put one layer perforated bricks. The thickness of the bricks is 100 mm. Pine wood was inserted through the holes of bricks to connect with the previous layer of pine in order to attract the termites upward. Then two layers of pine strips or chips were covered on the bricks. The wood samples were put in 6L plastic containers by using masson pine as the feeding source, and then inverted the containers and put on the pine strips or chips, as shown in Fig.1. There are several holes on the bottom of the plastic container. Put the cover on the bottom of the containers, and then bury them with soil and put a plastic cover over them to avoid rain flooding. The test began in November 15th, and then the samples were taken out on July 15th the next year. The total duration for the test was 8 months.

Figure1: Set up of field termite test

2.4.2 Laboratory termite test

Samples were exposed to *Coptotermes formosanus Shiraki* according to the procedure described in the American Wood Preservation Association (AWPA) standard E1-07. First, the specimens was dried to constant weight at 60℃, and then each test sample was placed in one cylindrical glass container (90mm in diameter, 100mm in height) filled with 150g sand and 30ml distilled water. Three test specimens were used for each level of treatment. A total of 360 termite workers and 40 soldiers were introduced into each container. The containers were placed in incubator with temperature setting at 25-28℃ and 75-80% RH. After for 5 weeks, the samples were taken out and cleaned off the debris caused by termite attack, then dried to constant weight in oven at 60℃. The weight loss of specimens due to termite attack was calculated based on the dry weight before and after termite test. The grading of termite attack was referenced to AWPA E1-07.

3. RESULTS AND DISCUSSION

3.1 Weight gain after impregnation

The WPGs were calculated according to the dry weight before and after impregnation. BA is very easy to react with PF, which significantly increase the viscosity of the mixture. Therefore, the combination of 1%BA and PF was not used to treat Masson pine, but we still use it to treat Chinese fir since Chinese fir sapwood shows better penetration than Masson pine. But still we can find a much lower WPG compared to other treating groups. For all treating groups, Chinese fir showed a higher weight gain than Masson pine. Comparing different treating solutions, the difference on WPG is not very obvious for Chinese fir except 1%BA/PF groups considering the rather high SD. For Masson pine, both DOT groups showed higher WPG than BA and BX groups.

Table 1: Weight percent gain (WPG) of samples after impregnation

Treating solution	Masson pine		Chinese fir	
	WPG [%]	SD[%]	WPG [%]	SD [%]
0.5%BA/PF	39.9	8.9	51.6	7.8
1.0%BA/PF	-	-	33.5	9.8
0.5%BX/PF	31.8	3.5	56.3	8.9
1.0%BX/PF	29.1	3.5	55.1	6.1
0.5%DOT/PF	49.3	6.1	60.1	8.5
1.0%DOT/PF	47.8	12.8	52.8	11.9

Note: SD represents standard deviation.

3.2 Field termite test results

Although the test duration was from November to July, but during the first 4 months the activity of termites was very rare. The grading after 8 months exposure was shown in Table 2. Comparing the two wood species, it was very clear that Masson pine without any treatment had a very poor termite resistance by showing a complete digestion by termites. But after different treatments with PF modified boron compounds, the decay resistance was dramatically improved. Most samples are sound after exposure. Chinese fir showed a much better natural termite resistance than Masson pine. After treatments, the termite resistance was further improved. By

comparing the mass losses listed in Table 3, the combination of borax with PF showed the least mass loss for both wood species, suggesting borax might be the optimal boron compounds while considering to be used in combination with PF resin.

Table 2: Grading of untreated and treated Masson pine and Chinese fir wood samples after field termite tests

Treating solution	**Masson pine**						**Chinese fir**					
	1	2	3	4	5	**6**	1	2	3	4	5	**6**
control	0	0	0	0	0	0	7.5	7.5	7.5	7.5	9	9
0.5%BA/PF	9.5	10	8.5	8.5	8.5	10	8.5	9.5	9	10	9.5	8.5
1.0%BA/PF	-	-	-	-	-	-	8.5	8.5	8.5	10	9.5	8.5
0.5%BX/PF	10	10	10	10	10	10	10	10	10	10	10	10
1.0%BX/PF	10	10	10	10	9	10	9.5	9.5	10	9.5	9.5	9.5
0.5%DOT/PF	9	10	10	10	10	9	10	10	10	10	10	10
1.0%DOT/PF	9.5	9.5	9.5	10	10	10	8.5	9	9	8	10	8

Table 3: Mass loss of untreated and treated Masson pine and Chinese fir wood samples after field termite tests

Treating solution	**Masson pine**		**Chinese fir**	
	Mass loss [%]	**SD [%]**	**Mass loss [%]**	**SD[%]**
control	100	0	25.7	14.1
0.5%BA/PF	11.9	2.1	13.8	3.8
1.0%BA/PF	-	-	15.8	7.4
0.5%BX/PF	6.1	0.6	6.2	2.7
1.0%BX/PF	4.1	0.7	8.6	1.6
0.5%DOT/PF	9.6	5.3	12.0	3.4
1.0%DOT/PF	9.4	4.3	13.2	6.8

Note: SD represents standard deviation.

3.3 Laboratory termite test results

Table 4: Grading, termite mortality and mass loss of untreated and treated Masson pine and Chinese fir wood samples after laboratory termite tests

Treating solution	Masson pine			Chinese fir		
	Grading	Termite mortality[%]	Mass loss [%]	Grading	Termite mortality[%]	Mass loss [%]
control	5	20	29.3(8.6)	4	30	43.7(7.9*)
0.5%BA/PF	8	63	5.4(0.6)	8	60	4.2(1.5)
1.0%BA/PF	8	72	4.6(1.2)	8	78	3.4(0.3)
1.5%BA/PF	8	70	2.3(0.5)	9	67	1.8(0.7)
2.0%BA/PF	9	63	1.2(0.1)	9	70	1.1(0.6)
0.5%BX/PF	8	50	4.1(1.0)	8	52	5.5(0.4)
1.0%BX/PF	8	68	5.1(2.9)	9	55	2.8(0.6)
1.5%BX/PF	10	42	0.9(0.1)	10	65	1.0(0.2)
2.0%BX/PF	10	59	0.9(0.1)	10	63	1.0(0.3)
0.5%DOT/PF	8	37	5.9(1.0)	8	33	7.6(0.3)
1.0%DOT/PF	8	50	5.1(1.1)	8	65	5.0(2.2)
1.5%DOT/PF	9	74	1.1(0.1)	10	79	0.7(0.0)
2.0%DOT/PF	10	50	-0.2(0.4)	10	48	-0.4(0.1)

** standard deviation of 6 replicates.*

In laboratory termite tests, we add another 2 loading levels of boron compounds, namely, 1.5% and 2.0%. The laboratory test results are kind of different from those of field test regarding to the two wood species. In laboratory termite test, Masson pine showed lower mass loss than Chinese fir, which may be due to the different test termite species and also because the laboratory test is a no-choice test but the field test is not. The difference between the three boron compounds is insignificant by all showing very promising termite resistance. With the increasing loading level of boron compound, the termite resistance also improved accordingly. When the samples treated with PF and boron compound at a concentration above 1.5%BAE, the mass loss of the samples could be lower than 1.1% for BX and DOT.

4. CONCLUSION

By using compound treating solution of boron compound and 20% low-molecular PF, the termite resistance of Masson pine and Chinese fir could be dramatically improved. Boric acid will increase the viscosity of PF while mixing with it, which would definitely pose difficulty in treatment. Therefore, BX and DOT are recommended boron compounds to be used in combination with PF. Judging from the field termite test results, BX/PF treating groups could provide best termite resistance compared to other treating groups. The results from laboratory termite test and field termite test are very different concerning the wood species, which may be due to the different test termite species and also because the laboratory test is a no-choice test but the field test is not. The drawback of this study is that we did not test the samples only treated with PF as control, so the contribution of PF in termite resistance could not be evaluated. We will further adjust the PF concentration and molecular weight in our further studies.

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5. CONCLUSION

AWPA E 11(2007): Standard method of determining the leachability of wood preservatives.

Baysal, E, Ozaki, S K, Yalinkilic, M K (2004): Dimensional stabilization of wood treated with furfural alcohol catalyzed by borates. *Wood Science Technology*, **38**(6), 405-415.

Baysal, E, Yalinkilic, M K (2005): A new boron impregnation technique of wood by vapor boron of boric acid to reduce leaching boron from wood. *Wood Science Technology*, **39**(3), 187-198.

Cui, W, Kamdem, P (1999) Bioefficiency of Boric acid grafted on to wood. *The 30th Annual Meeting of International Research Group on Wood Preservation*. Rosenheim, Germany, Document No: IRG/WP 99-30202.

Furuno, T, Imamura, Y, Kajita, H (2004): The modification of wood by treatment with low molecular weight phenol-formaldehyde resin: a properties enhancement with neutralized phenolic-resin and resin penetration into wood cell walls. *Wood Science and Technology*, **37**(5), 349–361.

Furuno, T, Wada, F, Yusuf, S (2006): Biological resistance of wood treated with zinc and copper metaborates. *Holzforschung*, **60**(1), 104–109.
Hashim, R, Murphy, R J, Dickinson, D J, Dinwoodie, J M (1994): The mechanical properties of boards treated with vapor boron. *Forest Products Journal*, **44**(10), 73-79.

Homan, W J, Militz, H (1995): Influence of a surface coating on the leachability of boric acid and bifluorides from Spruce wood. *The 26th Annual Meeting of International Research Group on Wood Preservation*, Helsingör, Denmark, Document No: IRG/WP 95-50050.

Imamura, Y, Takahashi, M, Ryu, J Y, Kajita, H (1998): Distribution of Polymers in cell walls and their effect on the decay resistance in wood-plastic composites. *Biocontrol science*, **3**(2):109-112.

Kartal, S N, Green, F (2003): Leachability of boron from wood treated with natural and semi-synthetic polymers and calcium precipitating agent. *Holz als Roh-und Werkstoff*, **61**(5), 388-389.

Liu, R, Cao, J, Xu, W, Li, H (2012): Study on the anti-leaching property of Chinese fir treated with borate modified by phenol-formaldehyde resin, *Wood Research*, **57**(1),111-120.

Peylo, A, Willeitner, H (1995a): The problem of reducing the Leachability of boron by water repellents. *Holzforschung*, **49** (3), 211–216.

Peylo, A, Willeitner, H (1995b): Influence of hydrophobic agents on the leachability of boron. *The 26th Annual Meeting of the International Research Group of Wood Preservation*. Helsingör, Denmark, Document No: IRG/WP/95-30064.

Ryu, J Y, Takahashi, M, Imamura, Y, Sato, T (1991): Biological resistance of Phenol-resin treated wood. *Journal of the Japan Wood Research Society*, **37**(9), 852-858.

Shams, M I, Yano, H, Endou, K (2004a): Compressive of wood impregnated with low molecular weight phenol formaldehyde (PF): resinⅠ: effect of perssing pressure and pressure holding. *Journal of Wood Science*, **50**: 337-342.

Shams, M I, Yano, H (2004b): Compressive of wood impregnated with low molecular weight phenol formaldehyde (PF):resinⅡ: effect of processing parameters. *Journal of Wood Science*, **50**, 343-350.

Thévenon, M F, Pizzi, A, Haluk, J P (1997): Non-toxic albumin and soja protein borates as ground-contact wood preservatives. *Holz als Roh- und Werkstoff*, **55**, 293-296.

Thévenon, M F, Pizzi, A, Haluk, J P (1998a): Protein borates as non-toxic, long-term, wide-spectrum, ground-contact wood preservatives. *Holzforschung*, **52**(3), 241-248.

Thévenon, M F, Pizzi, A, Haluk, J P (1998b): One-step tannin fixation of non-toxic protein borates wood preservatives. *Holz als Roh-und Werkstoff*, **56**: 90.

Tomak, E D, Yıldız, Ü C, Yıldız, S, Pesman, E (2008): The effect of treatments in boron steaming medium on boron leaching and strength properties of wood. *The 39th Annual Meeting of International Research Group on Wood Protection*. Istanbul, Turkey, Document No: IRG/WP 08-40409.

Yu, L, Cao, J (2009): Leaching performance, decay and termite resistance of wood treated with boron compounds incorporated with phenol-formaldehyde resin, *the 40th Annual Meeting of International Research Group on Wood Protection*, Beijing, China. Doc. No. IRG/WP 09-30503.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 3 Wood protecting chemicals

First report on the termiticidal activity of extracts of *Annona squamosa* (Annonaceae) seeds and on its active constituent squamocins

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First report on the termiticidal activity of extracts of *Annona squamosa* (Annonaceae) seeds and on its active constituent squamocins

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ABSTRACT

Termites inflict severe damage on wood commodities and impede usage of several forest species. The biological metabolites could potentially replace synthetic termiticidal products which are becoming more restricted to use. *Annona squamosa* is well known for its edible fruits - tropical custard apple. Annonaceous tetrahydrofuran acetogenins have attracted much interest due to their broad range of biological activities, and seeds containing them are reported to show insecticidal and abortifacient properties. Under the course our exploratory investigation of non food valuable products from less known forest species (here from Benin), the fractions obtained from cake of *Annona squamosa* with solvents covering a broad range of polarity, revealed a significant termiticidal activity, when applied at 5 - 10 mg/cm² (non-polar solvent) and 1.2 - 5 mg/cm² (case of a more polar solvent). Based on the result (0: no attack) from the standard procedure EN 118, these extracts could be used for preserving wood used hazard class. A derivatization step with Kedde reagents A and B, elemental analysis C,H,O, HPLC/MS and proton NMR showed the presence of squamocin type acetogenins (MW 594 and 622 g/mole: $C_{35}H_{62}O_7$ and $C_{37}H_{66}O_7$) as main components of the most active fraction by no-choice test (FR-13 : 100% mortality within 7 days for a sample applied at 2 mg/cm²). Additional work will be undertaken to confirm the structure of most active compounds in the extracts, and to check whether some *Annona squamosa* seed extracts (non timber products of sustainable management of native forest) could show even higher activity to protect wood and be alternative active natural products to synthetic compounds.

Key words: *Annona squamosa, acetogenins, termiticidal activity, wood preservation, Benin*

1. INTRODUCTION

Termites are global problem all around the world and especially in tropical areas where relative humidity is high. The most important need of termites is cellulose in their diet. Wood, a naturally occurring polymer composite, is mainly composed of cellulose, hemicelluloses, lignin, and extractives. Due to biological nature, some wood species are susceptible to discoloration and biological deterioration, which reduce their mechanical and physical properties (Chang *et al.* 2002). Developing methods that lengthen the service life of wood have always been the interest of researchers (Chang *et al.* 2000, Klocke 1989).

Termiticidal compounds can be terpenoids, tannins, tropolones, polyphenols (Yazaki *et al.* 1982; Kinjo *et al.* 1988, Farshid 2005). Rodrigues *et al.* 2011 have demonstrated that both rubrynolide and crude ethyl acetate extract from *S. rubra* species can be used to protect woods against

termites. Sharma *et al.* 2011 have evaluated termiticidal potential of cakes derived from non-edible oil seeds (Jatropha, Karanja, Neem and Mahua) and their crude active components (phorbol esters, Karanjin, saponins and azadirachtin) *in vitro* and *in vivo* and found that Karanjin fraction was the most active.

While, as above detailed, the temiticidal activity but only few data were also recorded about the termiticidal activity of cakes (Adams *et al.* 1988; Vasant and Narasimhacharya 2008), only few data are available about the termiticidal activity of solvents extracts from defatted seed cakes. This especially the cake of *Annona squamosa* well known for its edible fruits (the tropical custard apple). Several compounds (terpenoids, alkaloids, etc.) exhibiting cytotoxic, antitumor, insecticide, antibiotic, antifeedent, immunosuppressant, etc. have been isolated from annonaceous plants, and were reviewed by Leboeuf *et al.* 1982 also cited by Araya 2004.

Annonaceous tetrahydrofuranic acetogenins have attracted much interest due to their broad range of biological activities (Alali *et al.* 1999, Morton 1973, Chopra *et al.* 1956, Araya 2004) (Fig. 1).

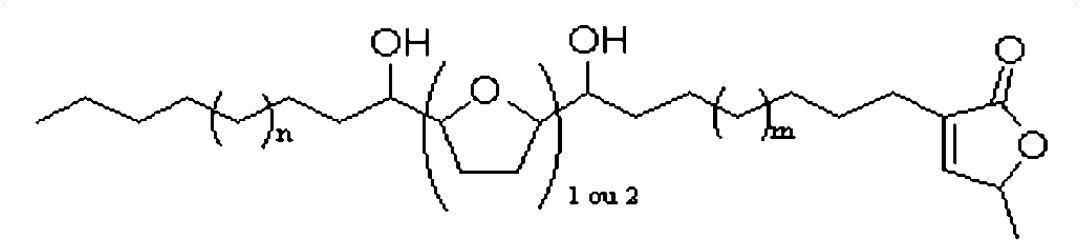


Figure 1: General structure of tetrahydrofuranic acetogenins[a]

[a]*Sahai et al. 1994, Fujimoto et al. 1994, Araya 2004*

More than 300 acetogenins, belonging to non-, mono, bis- and tri-tetrahydrofuran (THF) subgroups, have been reported (Zafra-Polo *et al.* 1998; Yazbak *et al.* 1998), and the molecular and structure of more than twenty acetogenins from the seeds of *Annona squamosa* L. were elucidated, among which squamocin and squamostatin-A, two major constituents (Fujimoto *et al.* 1988, 1990 and 1994; Sahai *et al.* 1994; Araya *et al.* 1994a and 1994b). Two bis-THF acetogenins named squamocin-O_1 and squamocin-O_2 were isolated from a methanolic extract (Araya *et al.* 2002).

The diversity of structures and the selectivity of the biological actions of acetogenins, have stimulated a continuing search for new members of this class of potent botanical products. Under the course the exploration of non food valuable products from less known forest species (Djenontin *et al.* 2009 and 2012), we investigate here by no-choice test and standard European test (EN 118 2005), on the termiticidal activity of fractions obtained from the oil seeds and defatted cake of *Annona squamosa L.* with solvents covering a broad range of polarity.

2. EXPERIMENTAL METHODS

2.1 Plant material

Fresh *Annona squamosa* fruits were collected from Cove, Zou Department, Benin (West Africa) and certified by the Service of Plants Protection at Porto-Novo (plant health certificate n°0003002/05/SPVCP/DAGRI, February 10, 2005). The seeds were separated manually, cleaned for any adhering flesh and dried at 50°C for 48 h. The dried seeds were ground in a mill type Retsch ZM 100 (GmbH and Co, Hann Germany) and screened through a mesh of 0.6 mm.

The wood used for standard test EN118 2005 is sapwwod of *Pinus sylvestris* classified as no durable species (Dirol and Deglise 2001).

2.2 Crude extracts and derived fractions

2.2.1 Solvents

All solvents (analytical or HPLC grade) were obtained from Sigma Chemicals Company Co. (St Louis, U.S.A).

2.2.2 Extraction

The cake was obtained after oil extraction in batch mode with hexane, in a stirred flask coated with aluminium foil to protect against light and under nitrogen atmosphere, during 72 h at the temperature of 20°C. Then the extracted seed powder was filtered and the solvent left in the solid was evaporated. It is also dried in the furnace at the temperature of 50°C during 2h until having a powdery aspect. 12.5 g of the powdered (0.6 mm) defatted cake of *Annona squamosa* was soaked in the dark in a glass flask in a mixture of 12.5 mL water and 50 mL ethanol under nitrogen atmosphere, during 72 h at the temperature of 20°C (Al Lawati *et al.* 2002; Laetemia *et al.* 2004). Subsequently, the solution was filtered through a filter N-EVAP^{TM} 111 (Organomation Associates Inc, USA) under nitrogen pressure. The filtered extracts were stored in the refrigerator (4°C) prior to use (Al Lawati *et al.* 2002). They were applied at 1.2; 5 and 10 mg/cm².

Table 1: *Annona squamosa* extracts from oil seeds and amounts applied for the test with termites

Name	Descritpion	Biological test	Amount applied (mg/cm^2)
Hx–As/Ex	Batch with hexane at 20°C during 72 h	No-choice	1.2 ; 5 and 10
EW–As/ Ex	Batch with EtOH/H_2O, 80/20, v/v at 20°C on almond, during 72 h	No-choice	1.2 ; 5 and 10
EW-Asdef/Ex	Batch with EtOH/H_2O, 80/20, v/v at 20°C on defatted cake during 72 h	No-choice, EN 118 (2005)	1.2 ; 5 and 10
P – MeOH	Acetogenins extract	No-choice, EN 118 (2005)	1.2 ; 5 and 10
P – Hx	Low acetogenin extract	No-choice	1.2 ; 5 and 10
FR – 13	Positive reaction with Kedde reagent A and B (squamocin fraction)	No-choice	2
FR – 19	Negative reaction with Kedde reagent A and B (no squamocin fraction)	No-choice	1

2.2.3 Isolation and fractionation

The methanolic phase of acetogenins (P-MeOH) was obtained though an adapted procedure derived from the one described by Chang *et al.* 1999. Fresh seeds of *Annona squamosa* (200 g) were extracted repeatedly with EtOAc (800 ml) at room temperature (21 ± 1°C). The solvent of the combined EtOAc extracts was evapored and partitioned to yield $CHCl_3$/H_2O (1/5, v/v) to yield chloroformic and aqueous extracts and the first phase rinsed five times with water. The layer after concentration was partitioned between n-hexane and MeOH (1/5, v/v). The MeOH

phase reactive positively to Kedde reagent, implying the presence of annonaceous acetogenins (Champy *et al.* 2004). The MeOH phase was repeatedly subjected to preparative column chromatography on silica gel 60 (Merck, 230-400 mesh) eluting successively with n-hexane, $CHCl_3$, $CHCl_3$-MeOH (99:1, 19:1, 9:1 and 4:1) and finally with MeOH. Elutions gave 30 FRs (0-29) (Table 1). Precoated silica gel plate (Merck, Kieselgel 60 F-254, 0.20 mm) were used for monitoring the column chromatography (Fig. 2).

2.3 Molecular identification

2.3.1 Detection of acetogenins with Kedde A and B reagents

This simple method was described by MPRI 1999, Naiyana 2002 and Araya 2004. Its consists a colorimetric detection of the lactone ring characteristic of the acetogenins, after derivatization by Kedde reagents A and B: 10% 3,5-dinithobenzoic acid (Panreac, ref.: 162836.1610, purity 98% to 2% w/v) in ethanol, followed by 2 N KOH in methanol), indicating the presence of unsaturated lactones as described by Champy *et al.* 2004. Two drops of Kedde reagents A and B are added successively to 0.5 mL solution of 0.2% w/v of the various fractions FRs 0-29. A persistent pink colouring purplished during 10 min is characteristic of the presence of the lactonic ring characteristics of acetogenins.

2.3.2 Analysis of extracts and structural identification

HPLC analysis were carried out with Shimadzu and JASCO systems using reverse-phase colums (Hypersil BDS-C18, 250 x 20 mm, i.d.; and Develosil ODS, 250 x 20 mm, i.d.) with UV detection at 225 nm. HPLC was performed on a Shimadzu LC-6A apparatus with a SPD-6A UV detector (220 nm).

Elemental analysis (C, H, O) was used to characterize the squamocin in purified fractions (FRs).

The identity of this compound was confirmed by NMR and mass spectrometry methods. 1H NMR (400 MHz), ^{13}C NMR (100 MHz), HETCOR, COSY, double-relayed COSY and DEPT spectra (all in $CDCl_3$) were obtained on a Varian Unity Plus 400 NMR spectrometer. FABMS and EIMS were collected on a Jeol JMS-SX/SX 102A mass spectrometer or Quattro GS/MS spectrometer having a direct inlet system. High-resolution EIMS were measured on a Jeol JMS-HX 110 mass spectrometer. Squamocins fraction was quantified by the integration of the ^1H NMR signals and then compared with the integration of the ^1H signals. The relaxation delay was set to 10 s to ensure full relaxation of the aromatic protons.

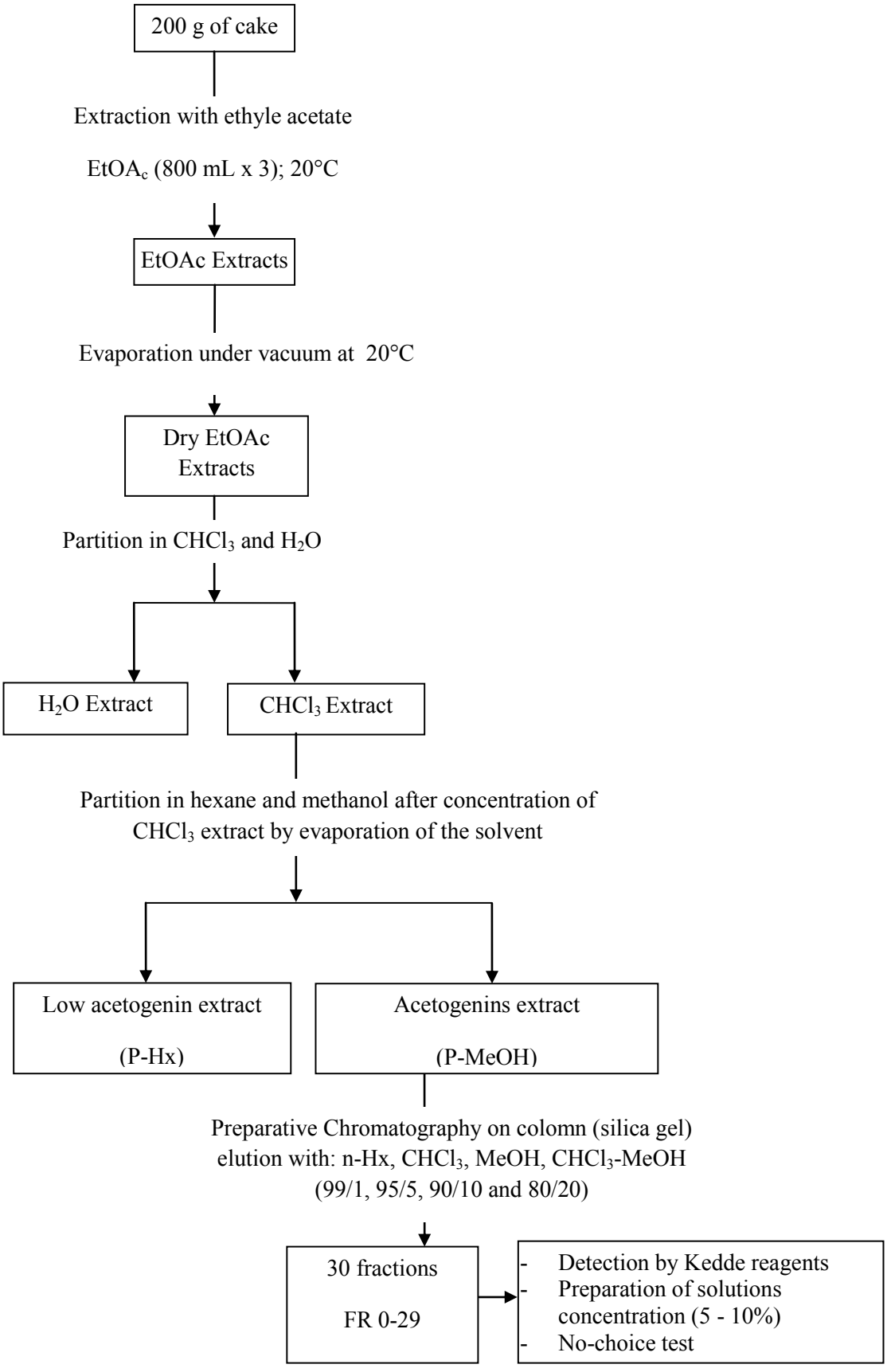


Figure 2: Obtention of acetogenin extracts and squamocin fractions[a]

[a]*Adapted from Chang et al. 1999*

2.4 Biological assays

2.4.1 Termite species

Assays were conducted with a European species of termites; belong to the family *Reticulitermes* are *Reticulitermes flavipes*. They were collected on the Oleron Island (French) and were preserved at the laboratory in vats of breeding placed in climatic chambers (27 °C – 70% RH: relative humidity). The colony is regularly supplied out of water.

2.4.2 No-choice test

The method reported by Kang *et al.* 1990 was used to evaluate the antitermitic activity of the extracts and fractions: 20µl aliquots extracts were applied to 1 cm² filter paper discs (Whatman N°42) (Table 3). After the solvent was removed from the treated papers by air drying at room temperature (65% and RH - 20°C), 50 active termites (45 workers and 5 soldiers) were placed on each filter paper impregnated with the test material housed in a Petri dish (9 cm dia. x 1.5 cm height). Wet sand of Fontainebleau (20 g) was used as substrate to maintain the humidity. The test dishes with covers were then placed in an incubator at 27°C – 70% RH. A few drops of water were periodically dripped on the bottom of each Petri dish. Filter paper samples treated with solvent alone (20 µl) were used to control the no toxicity (Solvent) and untreated paper samples were used as termite vitality controls (Diet). Also termites without paper were used as control. Each treatment was tested in triplicate and the termite mortality was scored after 14 days.

The repellent activity assays were carried out in 9 cm diameter plastic Petri dishes, containing 30g of Fontainebleau sand. A central hole, dug in the sand, contained 2 g of sawdust or a 5cm^2 filter paper square (Whatman N°42) impregnated with the extract (Table 1). 50 active termites (45 workers and 5 soldiers) were placed in a Petri dish (9 cm diam × 1.5 cm height). As a control, termites without paper and filter paper impregnated with extraction solvent were used. Repartition of termite galleries crossing concentric circles around the central sawdust was an indicator of repellent activity (Gilbert 1999, Al Lawati *et al.* 2002, Verena-Ulrike and Horst 2001).

2.4.3 Standard test EN 118 2005

Test wood blocks were cut from pine sapwood (50 × 40 × 10 mm R x T x L). One of the large surfaces of the wood samples was treated with 10 mg/cm².

Three wood samples were treated with the solvent to control the no toxicity and untreated sapwood blocks were used to control the virulence of the termites. All samples were dried in a climatic room (20°C; 65% RH) for 72 h. Surfaces of the treated wood samples and controls were exposed to 250 workers, 5 nymphs and 5 soldiers for 8 weeks in a climatic room at 27°C and 80% RH. Three replicates were conducted per assay. At the end of the exposure period, the worker mortality rate was calculated and a score related to the extent of degradation was allocated to samples according to the standard: 0 = no attacks, 1 = attempted attacks, 2 = light attacks, 3 = moderate attacks, and 4 = heavy attacks.

3. RESULTS AND DISCUSSION

3.1 Extraction

Under the course our investigation of non food valuable products from underused forest species (here from Benin), several fractions were obtained from *Annona squamosa* with solvents covering a broad range of polarity. First, oil extracted with hexane, then ethanol/water and ethyl

acetate from the resulting cake. The various extracts were subjected to no-choice and to repellent activity test. The most active fractions were then purified following literature informations from Chang *et al.* 1999 and subjected to standard test EN 118 2005. The kedde test was applied to detect the presence of acetogenins in these extracts.

3.2 Repellent activity

When expose to a "repellent substances" (Gilbert 1999, Al Lawati *et al.* 2002, Verena-Ulrike and Horst 2001), the termites organize themselves in order to limit to the maximum their contact with the active substrate (Fig. 2) (Hoffmann *et al.* 1999). They also organize themselves then in tunnels with the lower part of the sand of fontainebleau (used as model medium in the Petri dish).

The scan of the lower face of the Petri dish (2h) after the beginning of the test, have revealed the repellent activity. According to the organization of the termites observed in Fig. 3, we can note the average repellent activity of hexanic extract (Hx-As/Ex) obtained at (20°C).

3a: Hx-As/Ex 3b: Control 3c: Solvent

Figure 3: Repellent activity of oil seed (hexanic extract, Hx–As/Ex)[a]

[a]*see section 2 for description of sample and protocol)*

3.3 Validation of no-choice test and EN 118

The termiticidal activities are carried out by two tests: (i) no-choice test can evaluate the toxicity of the seed oils by allowing to compute the mortality rate; (ii) standard test EN 118 2005 can confirm the termiticidal activity revealed by screening, and evaluates the preventive activities against wood degradation. These tests on living organisms (termites) with seed oils obtained with an organic solvent are validated by the following observations:

- The termites survive during 24 days without feeding (diet). This period is quite longer than the 13 days assay required by the no-choice test,
- The rate of survival for the control (termites normally supplied with cellulose) is equal to 65 %, thus higher than the 50 % necessary for the validation,
- The effect of solvents shows only 4-10 % of mortality rate,
- The control of virulence presents the visual quotation 4 (strong wood attacks) by standard test EN 118,
- A ratio of 57 % of survivors -higher than 50 % necessary for the validation- is observed at the end of the test.

These observations do validate the assays with termites which are at the core of this work.

3.4 Termiticidal activity

3.4.1 No-choice test

Table 2 gives the results with the most interesting extracts. The activity is higher for extract obtained from cake (EW-Asdef/Ex) (100% mortality after 7 days exposure) than for the seed oil (87% mortality after 7 days exposure) at the same applied amount (10 mg/cm^2). Acetogenins fractions (P-MeOH) have shown the strongest activity (100% mortality after 3 days exposure) for the same dose (10 mg/cm²) compared with 100% mortality obtained with EW-Asdef/Ex but after 7 days exposure. The squamocins fraction (FR-13) obtained after separative chromatography on colums according to Chang *et al.* 1999, gave a pink colouring after addition of the reagents of Kedde A and B. It has been subjected to no-choice test in order to check and confirm the effectiveness of the activity due to the acetogenins in P-MeOH. The activity of the squamocins fraction (FR-13) applied at the dose of 2mg/cm^2 (100% mortality after 3 days exposure) is high although the dose is five time lower than the one of P-MeOH (10 mg/cm^2) (Table 2). This result could indicate that the termiticidal activity from *A. squamosa* extracts and fraction is caused by the acetogenins compounds as previously reported in literature by Saxena *et al.* 1993; Pardhasaradhi *et al.* 2005, Saluja 1989 and 1994; Dales 1999, Gilbert 1999 and Santos 2000 and also recorded by Hopp *et al.* 1996 about annonaceous family.

Concerning the determination of active amount, we have found a threshold situated between 5 – 10 mg/cm² for non polar solvent (Hx-As/Ex) and 1.2 – 5 mg/cm² even lower for the polar solvent (P-MeOH, EW-Asdef/Ex) (Table 2). Although feasible, the amounts to be applied remain higher than those of the synthetic products reported by Peterson 2007 (100% mortality after 7 days exposure at 69 µgg^{-1} soil). But our results are comparable with those found in literature data. For examples, Sharma *et al.* 2011 have evaluated termiticidal potential of non-edible oil seedcakes (Jatropha, Karanja, Neem and Mahua) and shown that cold water extracts of Neem cake are more active than hot water extracts of same cake and caused 100% mortality of termite in 72 h. Cheng *et al.* 2007 have also demonstrated that at 10mg/g, the heartwood and sapwood essential oils of *Calocedrus macrolepis* var. *formosana* and of *Cryptomeria japonica* and the leaf essential oil of *Chamaecyparis obtusa* var. *formosana* had 100% mortality after 5 days. Cheng *et al.* 2004 have examined the bioactivity tests against the termite *Coptotermes formosanus* and have demonstrated that the LC50 (Lethal Concentration to kill 50% of insects) value of leaf essential oil is 27.6 mg/g. Furthermore, exposure to T-muurolol caused 100% mortality at a dosage of 5 mg/g after 14 days.

Because, termiticidal compounds are being more soluble in polar solvents according to Sharma *et al.* 2011, we were interested in the binary ethanol/water (EW) solvents which have a low environmental impact contrary to acetone, chloroform and methanol. This relative termiticidal activity showed by the EW-extract could be improved by adjusting the solvent polarity and/or subsequent processing.

Table 2**:** Termiticidal activity in *Annona squamosa* seeds extracts by no-choice test

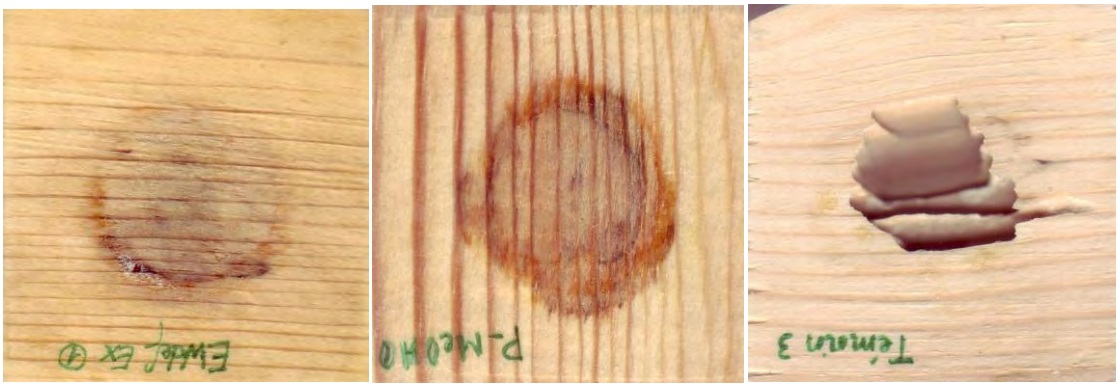
	Hx-As/Ex			EW-As/Ex			EW-Asdef/Ex : 80/20			P-MeOH		FR-13	FR-19
Applied doses (mg/cm²)	10	5	1.2	10	5	1.2	10	5	1.2	10	5	1. 2 2	1
Mortality rate (%) after 3 days exposure	70	55	50	87	82	73	100	9 5	32	100	95	33 93	31
Efficacy (mg/cm²)	5 – 10			5 - 10			1,2 - 5			1.2 - 5		< 2	

3.4.2 Preventive activity on wood – European standard test EN 118 2005

The encouraging above results obtained by no-choice test were checked by the standard test (EN 118 2005) in order to confirm this activity of more active extracts. The termites also have organized themselves in tunnels in the lower part of fontainebleau sand. We did not observed any consumption of impregnated wood.

Fig. 4 shows the level of attack on the treated wood by the ethanol/water extracts (EW-Asdef/Ex) and the acetogenins extract (P-MeOH). The rating of the attack is 0 (no attack of wood) according to the test EN 118 2005 and thus these extracts could be used for preservation. We can conclude that these extractives could be used for wood preservation according to the standard EN 599-1 (hazard class). However, P-MeOH extract has shown the highest termiticidal and repellent activities and confirm that the termiticidal activity is caused by the acetogenins and the structure confirmation will be necessary.

At this point it was useful to confirm that the termiticidal activity is caused by the acetogenins and for this purpose structure confirmation of the main compounds in extracts was necessary.



4a: 0 attack (P-MeOH) 4: 0 attack (EW-Asdef/Ex) 4c: Control

Figure 4: Attacked area of the treated wood (*Pinus sylvestris*) exposed to termites according to EN118 2005 (Dose applied: 10 mg/cm²)

3.5 Analysis of active compounds in extracts

3.5.1 Detection of acetogenins in fractions of Annona squamosa

This method is quite qualitative and moreover, is sentive of others compounds in the solution especially alkaloids. FRs-12 to 18 gave a pink colouring purplished after addition of Kedde A and B reagent,s during 10 min and then appeared a colored precipitate as described in the protocols of the MPRI 1999, and by Naiyana 2002, Araya 2004, Champy *et al.* 2004. These fractions are thus supposed to contain acetogenins and these compounds could be responsible of the termiticidal activity recorded in this study.

3.5.2 Elemental analysis (C, H, 0) of the extracts

Table 3 present the results of the elemental analysis (C, H, O). The data are compared to those computed on the basis of the molecular formula of some known acetogenins widely described in literature by Araya 2002, Sahai 1994, Chang *et al.* 1999. Elemental analysis obtained for the hexanic extract (Hx-As/Ex) (C, 76.6%; H, 11.9%; O, 12.1%) is quite different compared with squamocins (C, 71%; H, 11%; O, 18%) contrary to the P-MeOH fraction (C, 73.4%; H, 11.4%; O, 15.2%) who approach the literature value for desacetyluvaricine / squamocins-L (C, 73.3%; H, 10.9%; O, 15.8%) (Table 3) which gave a positive test with Kedde A and B reagents, and showed also the highest activity (Fig. 5).

Table 3: Elemental analysis of the extracts

Extracts	Molecular formula	% C	% H	% O
Hx-As/Ex	-	76.6	11.9	11.5
EW-As/Ex : 100/0	-	69,0	10,9	20,1
P-MeOH	**-**	**73.4**	**11.4**	**15.2**
squamocins B ou E	$C_{35}H_{62}O_7$ (594 g/mol)	70.7	10.4	18.9
desacetyluvaricine/squamocins-L	**$C_{37}H_{66}0_6$ (606 g/mol)**	**73.3**	**10.9**	**15.8**
asimicin/squamocins-H	$C_{37}H_{66}0_7$ (622 g/mol)	71.4	10.6	18.0
cis-squamostatine-D	$C_{37}H_{66}0_7$ (622 g/mol)	71.4	10.6	18.0
Squamocins-O_1 ou O_2	$C_{37}H_{66}0_8$ (630 g/mol)	70.5	10.5	19.0
12,15-cis-squamosatatine-A	$C_{37}H_{66}0_8$ (630 g/mol)	70.5	10.5	19.0

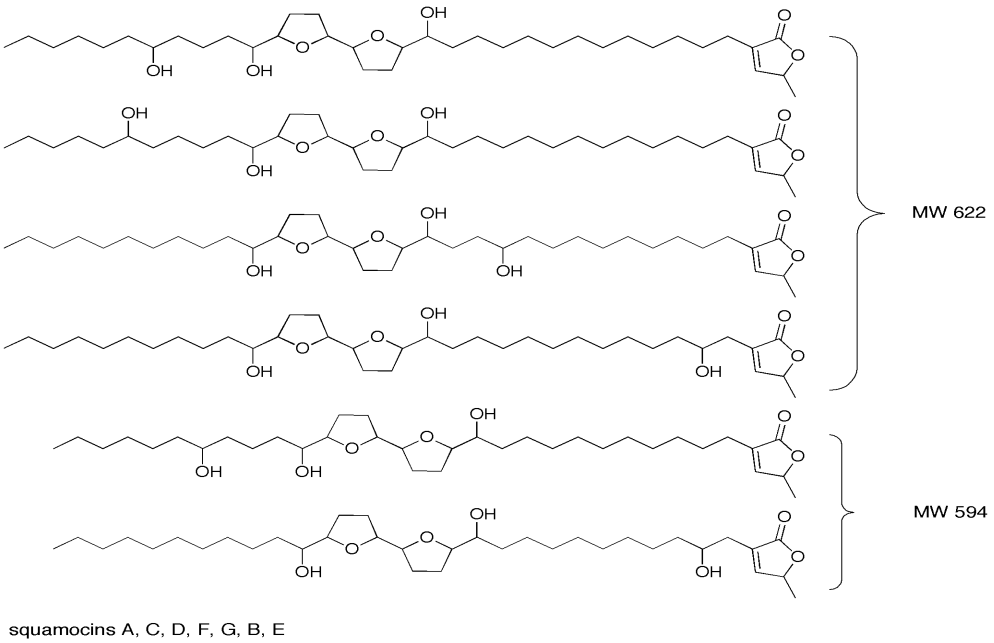


Figure 5: Structures of squamocins[a]

[a]*Sahai et al. 1994, Fujimoto et al. 1994, Araya 2004*

3.6 Potential applications

Table 4 proposes potential use of oil and others extracts from *A. squamosa* seeds as preservation agent. *A. squamosa* seed oil *(*Hx-As/Ex) is accessible *via* a simple process by pressure given the high oil content, and could be used as a repellent. Another interest of oil is that it is a hydrophobic substance which could avoid wetting by water and/or the resumption of moisture.

EW-Asdef/Ex, P-MeOH and the squamocins fraction (FR-13), could be useful for preservation of wood in class 1 uses according to the standard procedure EN 118 (2005). Additional work will be undertaken to confirm the structure of most active compounds in the extracts, and to check whether some *Annona squamosa* seed extracts (non timber products of sustainable management of native forest) could show even higher activity to protect wood under more adverse conditions and be alternative active natural products to synthetic compounds.

Table 3: Efficiency and potential uses of extracts from *Annona squamosa*

Extracts	Mortality rate (%)/days	Attack by EN118 (1990)	Efficiency (mg/cm²)	Environmental impact	Potential uses
EW-Asdef/Ex	100 /7	0	1.2 – 5	+[a]	Preservation of wood
Hx–As/Ex	68 /10	nd	5 - 10	++[b]	Repellent agent
P– MeOH	100 /3	0	1.2 – 5	+++[c]	Wood preservation
FR-13	93 /3	nd	2	+++	Wood preservation

[a]*+low* > [b] *++ relatively high* > [c]*+++ High*

4. CONCLUSIONS

Insecticidal properties in general had been widely reported in the scientific literature, but few data were available on the termiticidal activity from *Annona squamosa* seeds. We have here that the termiticidal activity depends largely on extracting solvent (polarity) and the active amount to be applied is rather high although possible. The optimization of the process could provide more active extracts not only for wood protection, but also in other fields (plant protection, human health), which still need to be investigated

The active termiticidal molecules are found preferentially in rather polar extraction phases (ethanol/water, methanol, chloroform). The chemical compounds responsible of the termiticidal activity in the cake of *A. squamosa* are supposed to be acetogenins of squamocins types. This will be confirmed after further investigation

There is still a long way before large scale applications, and additional testing would be necessary, including (i) checking of the resistance of the product (evaporation-oxidation during 4 weeks), essential to classify an extract in class 1 use, (ii) evaluating the activity on others insects and lignivores fungis.

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REFERENCES

Adams, R P, McDaniel, C A and Carter, F L (1988): Termiticidal activities in the hearthwood, Bark/Sapwood and leaves of Juniperus species from the United States. *Biochemical Systematics and Ecology*, **16**, 453-456.

Al Lawati, H T, Azam, K M, Deadman, M L (2002): Insecticidal and repellent properties of subtropical plant extracts against pulse beetle, *Callosobruchus chinensis*. *Agricultural science,* **7**, 37-45.

Alali, F Q, Liu, X.-X, McLaughlin, J L (1999): Annonaceous acetogenins: recent progress. *Journal of Natural Products*, **62**, 504–540.

Araya, H (2004): Studies on Annonaceous tetrahydrofuranic acetogenins from *Annona squamosa* L. seeds. *Bull. National Institute of Agro-Environmental Sciences*, **27**, 77-149.

Araya, H, Hara, N, Fujimoto, Y, Sahai, M (1994b): Squamostanal-A, apparently derived from tetrahydrofuranic acetogenin, from *Annona squamosa*. *Bioscience, Biotechnology,and Biochemistry*, **58**, 1146–1147.

Araya, H, Fujimoto, Y, Hirayama, K (1994c): Structural elucidation of tetrahydrofuranic acetogenins by means of precursor-ion scanning method. *Journal of Synthetic Organic Chemistry Japan,* **52**, 765–777.

Araya, H (2002): Squamocin-O_1 and squamotin-O_2 new Adjacent bis-tetrahydrofuran acetogenins from the seeds of *Annona squamosa*. *Phytochemistry,* **61**, 999-1004.

BS EN 118 (2005): Wood preservatives. Determination of preventive action against Reticulitermes species (European termites) (laboratory method).

Champy, P, Höglinger, G U, Féger, J, Gleye, C, Hocquemiller, R, Laurens, A, Guérineau, V, Laprévote, O, Medja, F, Lombès, A, Michel, P P, Lannuzel, A, Hirsch, E C and Ruberg, M (2004): Annonacin, a lipophilic inhibitor of mitochondrial complex I, induces nigral and striatal neurodegeneration in rats: possible relevance for atypical parkinsonism in Guadeloupe. *Journal of Neurochemistry,* **88**, 63–69.

Chang, F-R, Chen, J-L, Lin, C-Y, Chiu, H-F, Wu, M-J, Wu, Y-C (1999): Bioactive acetogenins from the seeds of Annona atemoya. *Phytochemistry*, **51**, 883-889.

Chang, S T, Wang, S Y, Wu, C L, Chen, P F, Kuo, Y H (2000): Comparison of the antifungal activity of cadinane skeletal sesquiterpenoids from Taiwania (*Taiwania cryptomerioides* Hayata) heartwood. *Holzforschung*, **54**, 241–245.

Chang, S T and Cheng, S S (2002): Antitermite activity of leaf essential oils and their onstituents from *Cinnamomum osmophloeum*. Journal of Agricultural and *Food Chemistry*, **50**, 1389–1392.

Cheng, S-S, Wu, C-L, Chang, H-T, KAO, Y-T, CHANG, S-T (2004): Antitermitic and antifungal activities of essential oil of *calocedrus formosana* leaf and its composition. *Journal of Chemical Ecology*, ***30, 1957-1967.***

Cheng, S.-S., Chang, H.-T., Wu, C.-L., Chang, S.-T (2007): Anti-termitic activities of essential oils from coniferous trees against *Coptotermes formosanus*. *Bioresource Technology*, **98***,* 456–459.

Chopra, R N, Nayar, S L, Chopra, I C (Eds.) (1956): Glossary of indian medicinal plants. C. S. I. R., pp. 20. New Delhi.

Dales, M J (1996): A Review of Plant Material Used for Controlling insect pests of stored products. *Bulletin of the National Research Institute*, **65**, 1-84.

Dirol, D, Deglise, X (2001): Durabilité des bois et problèmes associés. Hermes Science Europe Ltd. 415.

Djenontin, S T, Wotto, Valentin D, Lozano, P, Pioch, D and Sohounhloué, D K C (2009): Characterisation of *Blighia sapida* (Sapindaceae) seed oil and defatted cake from Benin. *Natural Product Research*, **23**, 549-560.

Djenontin, T S, Wotto, V D, Avlessi, F, Lozano, P, Sohounhloué, D C K., Pioch, D (2012): Composition of *Azadirachta indica* and *Carapa procera* (Meliaceae) seed oils and cakes obtained after oil extraction. *Industrial Crops and Products*, **38**, 39-45.

Farschid, F (2005): Etude de la durabilité d'un composé multicouche en mélange de plaçages de durabilités variables. Thèse de doctorat de l'ENGREF. *Sciences Forestières du Bois*, 246p.

Fujimoto, Y, Murasaki, C, Shimada, H, Nishioka, H, Kakinuma, K, Singh, S, Gupta, Y K, Sahai, M (1994): Annonaceous acetogenins from the seeds of *Annona squamosa*. Non-adjacent bis-tetrahydrofuranic acetogenins. *Chemical & Pharmaceutical Bulletin (Tokyo)*, **42**, 1175-1184.

Fujimoto, Y, Eguchi, T, Kakinuma, K, Ikekawa, N, Sahai, M, Gupta, Y K (1988): A new bis-tetrahydrofuran containing acetogenin from *Annona squamosa*. *Chemical & Pharmaceutical Bulletin (Tokyo)*, **36**, 4802-4806.

Fujimoto, Y, Murasaki, C, Kakinuma, K, Eguchi, T, Ikekawa, N, Furuya, M, Hirayama, K, Sahai, S, Gupta, Y K, Ray, A B (1990): Squamostatin-A: unprecedented bis-tetrahydrofuran acetogenin from *Annona squamosa*. *Tetrahedron Letters*, **31**, 535-538.

Gilbert, B (1999): Activities of medicinal, insecticidal and insect repellent Plants. *Anais da Academia Brasileira de Ciências,* **71**, 265-271.

Gutiérrez, C, Gonzalez-Coloma, A, Hoffmann, J J (1999): Antifeedant properties of natural products from *Parthenium argentatum*, *P. argentatum* x *P. tomentosum* (Asteraceae) and *Castela*

emoryi (Simaroubeaceae) against *Reticulitermes santonensis. Industrial Crops and Products*, **10**, 35-40.

Hopp, D C, Zeng, L, Gu, Z, Mc Laughin, J L (1996): Squamotacin an annonaceous acetogenin with citotoxic selectivity for the human prostate tumor cell lin (PC-3). *Journal of Natural Products,* **59**, 97-99.

Kang, H Y, Matsushima, N, Sameshima, K, Takamura, N (1990): Termite resistance tests of hardwoods of Kochi growth I. The strong termiticidal activity of kagonoki (*Litsea coreana* Léveillé). *Mokuzai Gakkaishi*, 36, 78-84.

Kenneth, G J (1997): Influence of tree extractives on foraging preference of *Reticulitermes flavipes* (Isoptera, Rhinotermidae). *Sociobiology*, **30**, 35-42.

Kinjo, K.,Doufuku, Y., Andyaga, S (1988): Termiticidal substances from thewood of *Chamaecyparis obtusa*. *Mokuzai Gakkaishi*, **34**, 451–455.

Klocke, J A (1989): Plants compounds as sources and models of insect-control agents, in economic and medicinal plant research, vol.3 Wagner H, Hikino H, Farnsworth N R Ed, Academic Press, pp. 103-144. London.

Leatemia, A, Murray, J, Isman, B (2004): Insecticidal activity of crude seed extracts of *Annona spp.*, *Lansium domesticum* and *Sandoricum koetjape* Against Lepidopteran Larvae. *Phytoparasitica*, **32**, 30-37.

Al Lawati, H T, Azam, K M, Deadman, M L (2002): Insecticidal and repellent properties of subtropical plant extracts against pulse beetle, *Callosobruchus chinensis*. *Agricultural science*, **7**, 37-45.

Morton, J (1973): Fruits of Warm Climates. Media, Inc., pp 75-80. Miami, FL.

MPRI (1999): *Medicinal Plant Research Institute, Departement of Medical Science, Ministry of Public Health. Standard of Thai herbal medicine, Andrographis paniculata (Burm. f.) Nees*, The war veterans' organization press, pp. 66. Bangkok.

Naiyana, T (2002): *Effects of Andrographis paniculata (BURM. F.) nees on performance, mortalily and coccidiosis in Broiler chichens.*, PHD of Agricultural Sciences, Institute of Animal Physiology and Animal Nutrition, pp. 114. Thailand.

Pardhasaradhi, B V V, Madhurima, R, Mubarak, A, Leela, K, Ashok, K (2005): Differential Cytotoxic effects of *Annona squamosa* seed extracts on human tumour cell lines, role of reactive oxygen species and glutathione. *Journal of Bioscience*, **30**, 237–244.

Peterson, C J (2007): Imidacloprid mobility and longevity in soil columns at a termiticidal application rate. *Pest Management Science,* **63**, 1124-1132.

Rodrigues, A M, Amusant, N, Bwaterchêne, J, Eparvier, V, Leménager, N, Baudassé, C (2011): The termiticidal activity of Sextonia rubra (Mez) van der Werff (Lauraceae) extract and its active constituent rubrynolide. *Pest Management Science*, **67**, 1420-1423.

Sahai, M, Singh, M, Gupta, Y K, Akashi, S, Yuji, R (1994): Annonaceous acetogenins from the seeds of *Annona squamosa.* adjacent bis-tetrahydrofuranic acetogenins. *Chemical & Pharmaceutical Bulletin (Tokyo)*, **42**, 1163–1174.

Saluja, A K, Santani, D D (1989): Phytochemical study of *Annona squamosa. Fitoterapia*, **64**, 359-360.

Saluja, A K, Santani, D D (1994): Pharmacological screening of an ethanol extract of defatted Seeds of *Annona squamosa*. *International Journal of Pharmacognosy*, **32**, 154-162.

Santos, D Y A C (2000): Foliar flavonoïd of annonaceae from brasil taxonomic significance. *Phytochemistry*, **55**, 567-573.

Saxena, R C, Harshan, V (1993): Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles Stephensi. Journal of the American Mosquito Control Association*, **9**, 84-87.

Sharma, S, Verma, M, Prasad, R and Yadav, D (2011): Efficacy of non-edible oil seedcakes against termite *(Odontotermes obesus)*. *Journal of Scientific and Industrial Research*,. **70**, 1037-1041.

Tsunoda, K. (1993): Termiticidal efficacy of synthetic pyrethrynoids 2. Effect of accelerated ageing on their termiticidal performance. *Wood Protection*, **2**, 67-73.

Vasant, R A and Narasimhacharya, A V R L (2008): An investigation on the termiticidal effects of certain weed plants. *'Prajna' - Journal of Pure & Applied Sciences*, **16**, 1-8

Verena-Ulrike, B and Horst, H (2001): Repellent and toxic effects of plant extracts on subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Microbiology*, **94**, 1200-1208.

Wan, J P, Wakelyn, P J (1997): Technology and Solvents for Extraction Oilseeds and Nonpetroleum Oils, Ed. Wan J. Peter, and Wakelyn P. J., AOCS Press Champaign, III., 353p. USA.

William, M A, Hron, R J (1996): In: *Baileys Industrial Oil and Fat Produ*cts, 5th edn., vol. 4, Edible Oil and Fat Products, Processing Technology, Hui, Y.H, John Wiley and Sons, Inc., 119p.

Weste, L A O, Alan, R L. and Cantrell, C L (2005): Comparison of natural and conventional Insecticides against formosan termites (isoptera: rhinotermitidae). In: *Proceedings of the fifth international conference on urban pestschow-yang lee and william h. Robinson (editors),* Malaysia.

Yazaki, Y (1982): Termiticidal Extracts from the Wood of *Ganophyllum falcatum* B1. *Holzforschung*, **36**, 249-253.

Zafra-Polo, M C, Figadère, B, Allardo, T, José, R T and Cortes, D (1998): Natural acetogenins from annonaceae, synthesis and Mechanisms of action. *Phytochemisto*, **48**, 1087-1117.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 3 **Wood protecting chemicals**

Effectiveness of CCA-C and CCB preservatives after a 30 years stake test

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Effectiveness of CCA-C and CCB preservatives after a 30 years stake test

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ABSTRACT

The objective of this experiment was to assess the durability of four pine species treated with waterborne preservatives. In order to determinate this, a stake field test, following the IUFRO (International Union of Forestry Research Organizations) recommendations, was installed at Experimental Station of Luiz Antonio (21º 32' S and 47º 42' W), State of São Paulo, Brazil.

Species under test were Slash Pine (*Pinus elliottii* var *elliottii*), Caribbean Pine (*Pinus caribaea* var *hondurensis*), Oocarpa Pine (*Pinus oocarpa*) and Benguet Pine (*Pinus kesiya*), as untreated (witness) as well treated with the preservatives CCA type C and CCB, in five retention levels (from 4.3 to 10.4 kg/m³).

These materials were inspected to evaluate the degree of attack by fungi and termites; after 8, 21 and 30 years of exposure. The results were plotted as decay index over time for all conditions (wood species and retention levels) in order to determine the mean service lives.

Differences observed among preservatives, retentions and wood species are discussed, demonstrating as main conclusions: a) the decay index presented a direct relationship to the level of retention for the stakes treated with CCA-C; b) the lowest decay index (longer durability expectation) correspond to treatments with CCA-C in retentions above 8.0 kg/m³; c) the CCB preservative showed a lower performance comparing to the CCA-C preservative.

Keywords: wood preservation; Pinus spp; CCA; CCB; field test; durability; service life.

1. INTRODUCTION

The methodology used in the evaluation of wood preservatives is a slow and costly process involving laboratory and field tests.

Preliminary laboratory screening tests of chemical compounds are normally performed in Petri dishes. When the results of these initial tests are encouraging, additional tests, such as accelerated decay (soil block) and susceptibility to insect attack, are also carried out at the laboratory. There is also the need to evaluate the permanence of the chemical substance in the wood (resistance to leaching), its corrosiveness to equipment and toxicity to humans.

A product that shows good results in all these tests do not automatically qualify for commercial release. These results simply indicate that the product has the proper credentials for being submitted to the closest possible conditions of actual use, and final approval has to be obtained in

field trials, where the treated wood is exposed for a considerable long time to the environmental conditions prevailing in real life.

Although estimative of service life could be considered speculative (Morris 2005, Brischke et al. 2011), the use of field test data is at present the most realistic and reliable approach to service life predictions (Larsson-Brelid *et al*. 2011).

This concept is more important when service life should be estimate in tropical regions, due the accelerated wood biodegradation observed in the tropics (Wong *et al*. 2004). This aggressiveness can be related to typical climate with favourable temperatures and constant rainfall (Nilsson and Edlund 1995, Wong *et al*. 2004),as well to high incidence of termites (Ling and Wong 2005).

However, a literature search on field test data is frustrating due to the scarcity of available information. In relation to CCA (Copper Chrome Arsenate), the most used wood preservative in the world, available data include no more than 10 sites with large experiments. A good example of this situation is presented by Hedley *et al*. (2000). In relation to the CCB (Copper Chrome Borate) the situation is even less favourable, and the paper of Wong and Ling (2007) is one of the few containing data of different field test.

For these reasons, the data obtained from these test plot established 30 years ago through a joint effort between the Department of Forest Sciences of ESALQ/USP and the Forest Institute of the State of São Paulo become extraordinarily important. This test field was implemented in 1980 at one SP Forest Experimental Station near the city of Luiz Antonio (21º 32' S and 47º 42' W) in the State of São Paulo, Brazil.

The objective of this experiment was to assess the durability of stakes of four pine species that had been treated with CCA (C formulation) and CCB (saline formulation); combined to five retention levels.

2. EXPERIMENTAL METHODS

Boards of *Pinus elliotti*, *Pinus caribaea* var. *hondurensis*, *Pinus oocarpa* and *Pinus Kesiya* were obtained from 14 years old plantation trees. From these boards, samples (stakes) were prepared with the following dimensions: 2.5 cm x 4.0 cm x 50.0 cm, with the grater dimension parallel to the wood grain. There were provided 10 specimens for each combination wood species x preservative retention. These stakes were treated with chromate copper arsenate (CCA type C) and with chromate copper borate (CCB, saline formulation), aiming to achieve five retention of active ingredients levels per product.

The field test was implanted at the Experimental Station of Luiz Antonio on August 1980. According Köeppen classification, the soil is type Lva and climate is Cwa. The stakes were inspected after 4, 9, 20 and 30 years, as specified by the IUFRO Method (Lepage, 1970); and evaluated according the criteria shown below in Table 1.

The local of the field test was occupied by plantation plots of *Pinus* species and natural vegetation (named "cerrado", similar to savannah vegetation). At end of 1980 decade, part of the Experimental Station of Luiz Antonio was transformed to an Area of Permanent Preservation (plantations not allowed) and regrowth of original vegetation is observed in the specific test area, as illustrated in Figure 1.

Table 1: Criteria used to evaluate sanity status of the stakes.

Stake sanity condition	Grade	Decay index
sound – no attack	0	100
light or superficial attack of fungi or termites	1	90
moderate attack of fungi or termites	2	70
intense attack of fungi or termites	3	40
break – loss of almost total stake resistance	4	00

Figure 1: Illustration of the field test at Experimental Station of Luiz Antonio.

3. RESULTS AND DISCUSSION

The effective mean retention obtained by each *Pinus* species is in Table 2, compared to the overall mean retention and to the nominal retention.

The stake Decay Index (DI) of the inspections carried out at 4, 9, 21 and 30 years were summarized in Table 3. As the DI obtained for the four species of *Pinus* have showed small variations, which probably may be attributed to the differences of effective mean retention between species and the differences between real and nominal retentions, Table 3 also shows the overall DI averages, regardless of these species.

The stakes average life, of the four species of pine without treatment (control series) was determined by Fernandes *et al*. (1990) and confirmed by Barillari (2002), as being less than one year, which shows the intensive biological activity of this site (Luis Antonio – SP). Both authors related the strong incidence of termites in the field test area.

Table 2: Mean effective retentions (kg/m³, active ingredients) by species and preservatives, compared to nominal retentions.

Product	Species				Mean retention	Nominal retention
	P. kesiya	*P. oocarpa*	*P. elliottii*	*P.c. hondurensis*		
CCA – C	5.19	5.14	4.63	5.22	5.0	5.0
	5.68	5.41	5.48	6.21	5.7	6.5
	6.86	6.74	6.29	7.12	6.8	8.0
	7.82	7.68	6.55	6.95	7.3	9.5
	8.41	8.69	7.57	8.15	8.2	11.0
CCB	4.38	4.34	4.49	4.65	4.5	5.0
	6.06	5.80	5.64	6.29	5.9	6.5
	8.61	8.19	7.31	8.10	8.1	8.0
	9.76	9.46	8.37	8.77	9.1	9.5
	10.29	10.42	9.74	10.39	10.2	11.0

Table 3: Mean values of decay index by species and preservative along exposure time, and respective effective retention.

Preservative	Species																Mean decay index				R
	P. kesiya				*P. oocarpa*				*P. elliottii*				*P.c. hondurensis*								
	4	9	21	30	4	9	21	30	4	9	21	30	4	9	21	30	4	9	21	30	
CCA (C)	100	100	87	57	100	100	81	79	98	98	85	73	100	100	80	63	100	100	83	68	5.0
	100	100	94	72	100	100	79	60	99	99	91	81	100	100	83	93	100	100	87	77	5.7
	100	99	96	87	100	100	95	92	98	98	90	82	100	100	97	75	100	99	94	84	6.8
	100	100	98	96	99	99	96	76	100	100	98	98	100	100	95	94	100	100	97	91	7.3
	100	100	97	96	100	100	97	93	100	100	92	95	100	100	98	96	100	100	96	95	8.2
CCB	99	94	88	76	93	93	91	74	96	96	81	76	97	97	90	82	96	95	87	77	4.5
	98	87	76	53	93	93	83	55	91	91	75	54	94	94	74	47	94	91	77	52	5.9
	99	98	88	68	92	92	93	73	95	95	82	59	97	97	89	51	96	96	88	63	8.1
	99	93	74	76	92	92	90	71	96	96	90	75	97	97	85	75	96	95	85	74	9.1
	99	95	92	84	97	97	94	58	96	96	89	59	97	97	91	66	97	96	92	66	10.2

4, 9, 21, 30 = years of field exposure; R = mean effective retention (kg/m³)

Based on results presented in Table 3, the decayment curves were elaborated, as can be seen in Figures 2 and 3.

Reporting the inspection done after 9 years, Fernandes et al (1990) concluded: i) the retention level did not affect the observed DI; ii) there was no effect of the species; iii) the preservative with more insecticide compound showed a better performance. The report from Barillari (2002) confirmed the previous conclusions regarding the species effect and insecticide compound, but showed a positive relationship between retention level and DI.

Barillari (2002) also estimated the average life of the worst combination between species, retention level and preservative in 30 years.

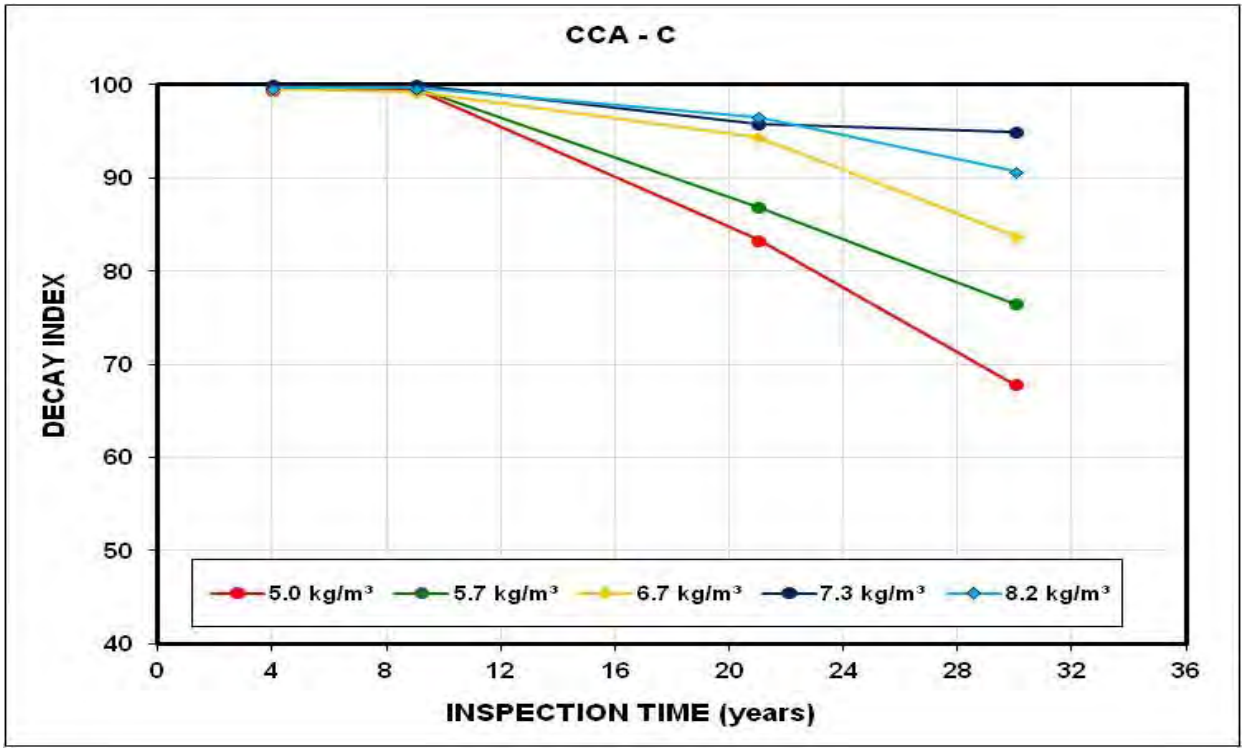

Figure 2: Decayment curve of *Pinus* stakes treated with CCA-C at five retention levels.

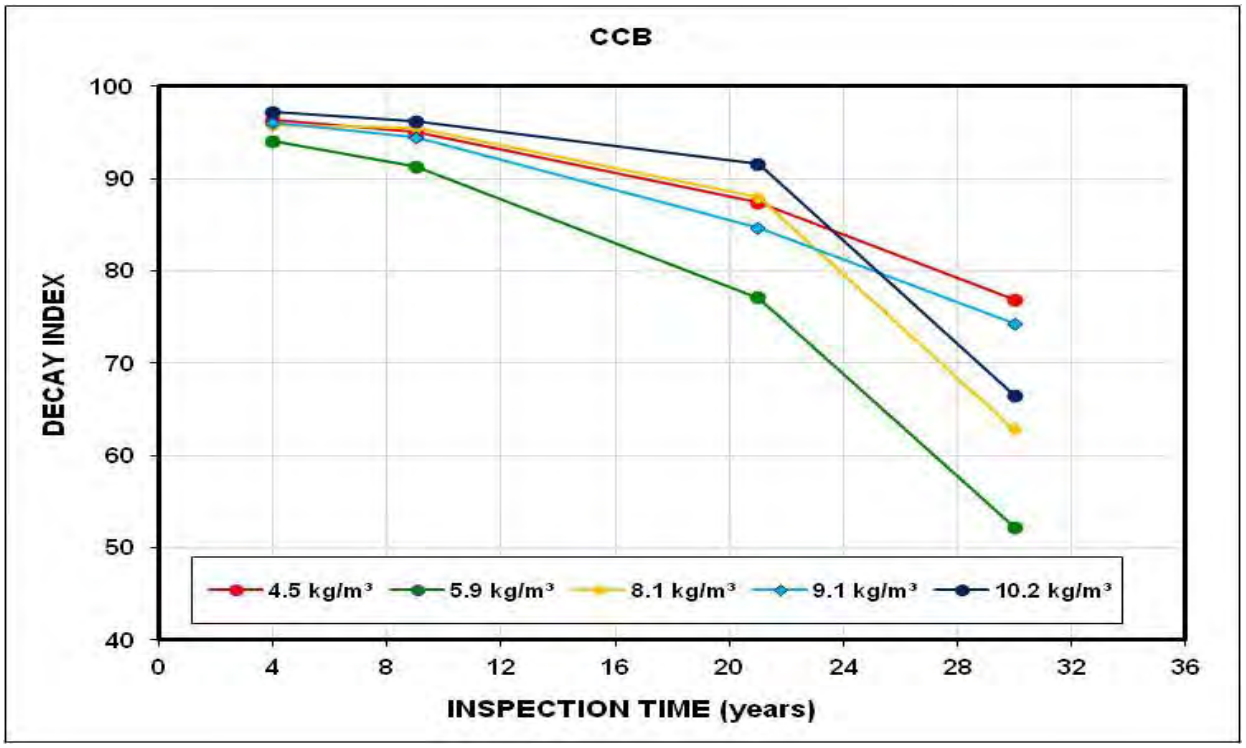

Figure 3: Decayment curve of *Pinus* stakes treated with CCB at five retention levels.

In order to estimate the average life for each product/rentention, the decayment curves were extrapolated do a DI = 50, thereby obtaining, in each case, the corresponding time. The results of the estimated lifetime of each combination can be seen on Table 4.

Table 4: Lifetime expectancy for each combination product/retention after 30 years of exposure in the field test at Luiz Antonio-SP.

CCA - C		CCB	
Retention level (kg/m³)	Expected average life (years)	Retention level (kg/m³)	Expected average life (years)
5.0	40	4.5	46
5.7	45	5.9	36
6.8	>50	8.1	34
7.3	>60	9.1	48
8.2	>60	10.2	36

After 30 years it is clear the relation between retention and decay resistance for the stakes treated with CCA-C, but the conclusion do not apply to CCB treated stakes.

The high expected average life (46 and 48 years, respectively) of CCB treated stakes correspond to retentions of 4.5 and 9.1 kg/m³ (active ingredient), while retentions of 5.9, 8.1 and 10.2 kg/m³ result in service life estimates from 34 to 36 years. One reason for this unexpected result could be the rapid Boron leaching in tropical regions (Salamah and Ani 1995) and to the possible effect of species (treatability) in Boron leaching rates (Salamah *et al*. 1992)

It is possible to conclude that for retentions of CCA-C greater than 6.8 kg/m³ the expected lifetimes are over 50 years; and the lowest decay index (longer durability expectation) correspond to treatments in retentions above 8.0 kg/m³.

The performance slight lower of CCB may have two reasons: a) the tested formulation had a salt basis and not a oxide basis; b) the site chosen for testing has a very strong incidence of termites and the content of insecticide in CCB (H_3BO_3) may not been sufficient to match the performance of CCA.

4. CONCLUSIONS

Based on results presented in this paper, the main conclusions are:

The decay index presented a direct relationship to the level of retention for the stakes treated with CCA-C;

The lowest decay index (longer durability expectation) corresponds to treatments with CCA-C in retentions above 8.0 kg/m³;

CCB preservative showed a lower performance comparing to the CCA-C preservative.

5. REFERENCES

Barillari C T. Durabilidade de Madeira do Gênero Pinus Tratada com Preservantes: Avaliação em Campo de Apodrecimento, 2002. 68p. – Thesis (Master in Forest Resources), Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, Piracicaba, 2002.

Brischke C, Welzbacher C R, Meyer L, Bornemann T, Larsson Brelid P, Pilgård A, Frühwald Hansson E, Westin M, Rapp A O, Thelandersson S, Jermer J. Service life prediction of wooden components- Part 3: Approaching a comprehensive test methodology Document IRG/WP 11-20464. International Research Group on Wood Protection, Stockholm (2011).

Fernandes, P S, Montagna, R G, Jankowsky I P, Cavalcante, A A. Estudo comparativo da durabilidade de Pinus spp tratados com preservativos de madeiras. In: *CONGRESSO FLORESTAL BRASILEIRO*, 6, 1990, Campos de Jordão. Anais... São Paulo: SBS/SBEF, 1990, v. 3, p. 689-695.

Hedley M, Page D, Patterson B. Long term performance of CCA preservatives in ground contact. Document IRG/WP 00-30223. International Research Group on Wood Protection, Stockholm (2000).

Larsson-Brelid P, Brischke C, Rapp A O, Hansson M, Westin M, Jermer J, Pilgård A. Methods of field data evaluation - time versus reliability. Document IRG/WP 11-20466. International Research Group on Wood Protection, Stockholm (2011).

Lepage E S Método padrão sugerido pela IUFRO para ensaios de campo com estacas de madeira. Preservação de Madeiras, v.1, nº 4, p. 205-216, dez. 1970.

Ling W C, Wong A H H. Tropical in-ground durability of structural Sarawak hardwoods impregnated to high retention with CCA-salts, CCA-oxide and FCAP after 20 years exposure. Document No: IRG/WP 02-30384. International Research Group on Wood Protection, Stockholm (2005).

Morris, P I. Service life prediction based on hard data. In: Proceedings of COST E 37 Workshop: Service life prediction: The contribution to Sustainability, Oslo, Norway, 19-21 June 2005.

Nilsson T, Edlund M L. Laboratory versus field tests for evaluating wood preservatives: a scientific view. Document No: IRG/WP 95-20076, International Research Group on Wood Preservation, 1995.

Salamah S, Ani S. Distribution of copper/chrome/boron preservative in dark red meranti (Shorea leprosula) before and after exposure test for 72 months. Document No: IRG/WP 95-20073. International Research Group on Wood Preservation. 1995.

Salamah S, Zaitun S, Fauzidah A. Effectiveness of copper-chrome-boron as wood preservative. Jour. Trop. For. Sci. 6, 98-115. 1992.

Wong A H H, Morsing N, Henriksen K H, Salmiah U. Above ground microbial decay test of biocide treated and untreated wood exposed to Danish and humid tropical climates. Document No: IRG/WP 04-20306. International Research Group on Wood Protection, 2004.

Wong A H H, Ling W C. Tropical In-Ground Durability of CCB-treated Keruing and other Structural Sarawak Timbers after 8 – 10 Years Exposure. Document No: IRG/WP 07-30439. International Research Group on Wood Protection, 2007.

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Section 3 **Wood protecting chemicals**

Seed oil and defatted cake proximate composition of non timber product *Annona squamosa* (Annonaceae) grown in Benin

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Seed oil and defatted cake proximate composition of non timber product *Annona squamosa* (Annonaceae) grown in Benin

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ABSTRACT

Annona squamosa L. is a small tree which grows wild in many places in the tropical regions, locally called "*xwingle"* in Benin. Its produces edible fruits, typically globular or heart-shaped which are highly appreciated and the seeds are not used. In the course of work about identifying new oil sources from a large number of oil bearing seeds grown in Benin, we report here on the chemical composition of seed oil (*ASSO*) and defatted cake (*ASDC*) of *A. squamosa*. Fatty acid composition, chemical properties of oil, unsaponifiable fraction, amino acids, lignocellulose and carbohydrates were analyzed by standard analytical procedures. Our objective is to update and to widen available data in order to check and confirm the interest of the seeds as a readily available by-product resulting of the consumption of the fruit pulp for human food. The extracted lipids (33.7%) were examined for fatty acid composition by gas chromatography. Linoleic (25.4%) and oleic (47.4%) acids were the main unsaturated fatty acids, while palmitic acid (12.6%) and stearic acid (11.6%) were the major saturated acids. The iodine value of 92 indicates that the seed oil is of non-drying type. The unsaponifiable matters (1.0wt-%) whose composition was not investigated previously especially for the sterol fraction found to contain β-sitosterol (68.7wt-%) and the tocols (143ppm) with α- and γ-tocopherol as major components (26.5 and 73.5wt-%). The defatted cake is rich in proteins (25.5g/100g), potassium (0.9%), and fibers (Van Soest; NDL 60.1%, ADF 34.7% and ADL 7.4%).

Key words: non timber product, *Annona squamosa*, seed oil, defatted cake, chemical composition, Benin.

1. INTRODUCTION

Annona squamosa L. is a small tree which grows wild in many places in the north of South America, Central America, and the Caribbean regions. It is widely cultivated and highly esteemed especially in Asia and African for its edible fruits, typically globular or heart-shaped. Therefore, the fruits are highly appreciated and the seeds are not used (Andrade *et al.* 1999).

Some reports on the chemical composition of the fruits of *A. squamosa* cultivated in Southeast Asia were previously published (Ansari *et al.* 1985, Ahmed and Naveed 1996), but were not very detailed. Ahmed and Naveed (1996) have studied the lipid fraction of *A. squamosa*. More recently, Andrade *et al*. 2001 discussed properties of the pulp, the eaten part of the fruit, and the seeds from Amazonian basin. Mariod *et al.* 2010 have reported that the oil and protein contents in the seeds were 26.8, 17.5% respectively. The major fatty acids of *A. squamosa* oil extracted by Sohxlet extraction were oleic 50.5%, linoleic 22.7%, palmitic 15.2%, and stearic 9.3%. Those authors have also reported that the tocopherol content of Soxhlet extraction oils from *A. squamosa* amounted to 155 ppm with δ-tocopherol as main component.

Benin is a large producer of conventional oilseeds such as palm, cotton seed, sesame, ground nut. Recently, our works have started to be greatly concerned about identifying new oil sources from a large number of oil bearing seeds grown in Benin (Djenontin *et al.* 2012 and 2009). Any data are available for *Annona squamosa* specimen grown in Benin. This work is part of a study aiming at adding value to underused forest biomass. Next step will deal with anti-termite properties of *A. squamosa* cake.

We report here on the chemical composition of *A. squamosa* seed oil (*ASSO*) and defatted cake (*ASDC*) to update and to widen available data. Fatty acid composition, chemical properties of oil, unsaponifiable fraction, amino acids, lignocellulose and carbohydrates were analyzed according analytical procedure published by Van Soest *et al.* 1991, Van Soest and Wine 1967 and highly discussed by Udén *et al.* 2005. The results are compared to literature data (Andrade *et al.* 2001, Ansari *et al.* 1985, Ahmed and Naveed 1996, Mariod *et al.* 2010).

2. EXPERIMENTAL METHODS

2.1 Collection and preparation of sample

Fresh *Annona squamosa* fruits were collected from Cove, Zou Department, Benin (West Africa) and certified by the Service of Plants Protection at Porto-Novo (plant health certificate n°0003002/05/SPVCP/DAGRI, February 10th, 2005). The seeds were separated manually, cleaned for any adhering flesh and dried at 50°C for 48 h. The dried seeds were ground in a mill type Retsch ZM 100 (GmbH and Co, Hann Germany) and screened through a mesh of 0.6 mm.

2.2 Chemical Properties of *Annona squamosa* seeds oil (ASSO)

The oil was extracted from powdered whole seeds in a Soxhlet extractor with hexane. The solvent-extracted meal was stored at 4°C. For determination of acid, peroxide and saponification values, unsaponifiable matters, standard IUPAC method were used. Iodine value was computed from GC results on fatty acid composition. The crude oil was analyzed as methyl esters to determine the fatty acid composition. Fatty acid methyl esters (FAMEs) were obtained through a two steps method with sodium methylate and HCl as catalysts, and then analyzed by capillary gas chromatography (GC) (Hewlett Packard HP 6890) equipped with a flame-ionization detector (FID). The unsaponifiable and sterolic fractions were obtained using standard IUPAC method. After isolation from the thin layer chromatography plate, the sterol fraction was further analyzed by GC, with a FISON GC 8000 unit equipped with a FID detector. Tocopherols in the seed oil were determined by HPLC. The apparatus was an Agilent Series 1100 liquid chromatograph coupled with a UV DAD detector set at wavelength 295nm. 10mg of the crude oil was dissolved in 10mL of hexane. An aliquot of this solution was injected onto a Luna silica Si 60 column (5µm, 25cm, 4.6mm) (Phenomenex, France) with a Rheodyne injection valve fitted with 20µL loop.

2.3 Characteristics of *Annona squamosa* defatted cake (ASDC)

Moisture, protein, lipid, ash, total carbohydrate, sugar, starch and crude fibre contents were determined following the standard AOAC methods. The minerals in defatted cake were analyzed from solutions obtained by first dry-ashing the seed flour at 550°C. Proteins in ASDC cakes were hydrolyzed by treatment with 6 N HCl in a sealed tube for 24 h at 110°C. The amino acids composition was determined in an automatic amino acid analyzer (Spectra-Physics AS3000). Lignocellulose and carbohydrates were analyzed according analytical procedure reported by Van Soest and Wine 1967; Udén *et al.* 2005.

3. RESULTS AND DISCUSSION

3.1 Chemical properties of *Annona squamosa* seeds oil (ASSO)

The oil content of ASSO (33.7wt-%) is comparable with that of the groundnut (45 – 53wt-%). The acidity which gives a good indication of the quality of the collected samples is relatively low (1.3wt-%). It is lower than the limiting values fixed for the majority of commercial oils (soya, 6% and sunflower, 4%). The oil could be consumed virgin if obtained by pressure. The peroxide value (6.9 meq/Kg) obtained is also weak compared with the limiting values (10-15 meq/Kg) because the fruit were collected on the tree, but very high compared with the data reported by Mariod *et al.* 2010. The saponification value is 161 and compatible with a preponderance of the fatty acids C18 but is lower than 184.5 found by Mariod *et al.* 2010. This value is also lower than the data concerning current vegetable oils in Benin like soya (189-195 mgKOH/g oil), groundnut (187-196 mgKOH/g oil) and cotton (189 - 198 mgKOH/g oil). The iodine value 92 shows the fairly unsaturated character of the oil and it should be relatively stable with storage with respect to oxidation. This value is lower than data recorded for soya (105 - 123) and sunflower (110 - 143), but comparable with olive and groundnut oils (85- 90; 75 - 94 respectively) and certain vegetable oil of African origin as *Coula edulis* (90 - 95), *Canarium schwenfurthii* (71 - 95) (Kapseu and Parmentier 1999). The phosphorus content is weak (14 ppm), contrary to what is awaited for oils of seeds extracted by solvent and is comparable with value recommended for a

pure vegetable oil as fuel (10-15 ppm) (Roberto *et al.* 2008). The calcium and magnesium contents could indicate a noticeable amount of hydratable phospholipids in ASSO and it would be necessary to envisage a stage of degumming in the refining process.

The fatty acids profile of ASSO grown in Benin shows the oleic type (47.4wt-%) (Table 1). It is not different from the majority of oils of great consumption in Benin and elsewhere like groundnut, cotton, olive, sunflower (Grosso *et al.* 2000). The monounsaturated fatty acids are in a majority (72.8wt-%) and the saturated fatty acids primarily consist of palmitic (12.6 wt-%) and stearic (11.6 wt-%) acids. Fatty acids composition of ASSO is comparable with data reported by Andrade *et al.* 2001 and Mariod *et al.* 2010 for the sample grown in Amazonian (Southern America) and Sudan (Eastern Africa), respectively (Table 1). This result is quite different compared with the Asian sample reported by Ansari *et al.* 1985. Therefore, further analyses have to be undertaken to study the natural fluctuations of component concentrations in *A. squamosa* fruits depending on location conditions. For example, ASSO occurring in India is characterized by a significant content of isoricinoleic acid (9.8wt-%) according to Ansari *et al.* 1985. But careful GC-MS studies of the fatty acid methyl esters revealed by Andrade *et al.* 2001 did not mentioned the above oxygenated fatty acid in ASSO from Amazonian specimen.

Unsaponifiable fraction (1.0wt-%) for *ASSO*, have a low contents sterols and tocopherols (1360 and 147 ppm respectively) (Table 1). The content of sterol is comparable with groundnut oil (147-171 mg/100g) with β-sitosterol as main component (68.7wt-%) (Grosso *et al.* 2000, Kamal-Eldin and Andersson 1997). Comparable data for the sterols fraction for *Annona squamosa* specimen were not reported in literature to our knowledge. The tocopherol fraction primarily consists of α and γ-tocopherols, with γ-tocopherol (73.5wt-%) as main component. The profile of tocopherol is quite different with the data reported by Mariod *et al.* 2010 who found that the main tocopherol of the *A. squamosa* oils grown in Sudan was δ-tocopherol (mre than 70%) of the total tocopherols (155 ppm). The tocols content is weak compared with soya oil (980 ppm), germ of corn oil (816 ppm) and palm oil (718 - 818 ppm) but comparable with olive oil (110 - 183 ppm).

Table 1: Chemical Properties of *Annona squamosa* seeds oil (ASSO)

General Properties of ASSO		This Work	Sudan specimen (Eastern Africa)[a]	Asian specimen[b]	Amazonian specimen (southern America)[c]
Oil content (wt-%)		33.7	27.5 ± 0.1	-	-
Oleic acidity (wt-%)		1.3	0.77 ± 0.10	-	-
Saponification value ($mgKOHg^{-1}$)		161	184.5 ± 0.11	-	-
Peroxide value (meq 0_2 kg^{-1})		6.9	0.9 ± 0.1	-	-
Iodine value		92	-	-	-
Unsaponifiable (wt-%)		1.0	-	-	-
Total phospholipids (wt-%)		0.35	-	-	-
Total sterols (mg/100g)		136 ± 8	-	-	-
Total tocopherols (ppm)		143 ± 4	155 ± 3	-	-
Palmitic acid	C16:0	12.6 ± 0.4	15.2 ± 0.5	25.1	14.11
Stearic acid	C18:0	11.6 ± 0.2	9.3 ± 0.4	9.3	14.43
Oleic acid	C18:1	47.4 ± 1.0	50.5 ± 1.2	37.0	45.15
Linoleic acid	C18:2	25.4 ± 0.7	22.7 ± 0.6	10.9	22.84
Linolenic acid	C18:3	0.9 ± 0.2	-	-	0.71
Isoricinoleic acid		-	-	9.8	-
Campesterol		17.8 ± 1.8	-	-	-
Stigmasterol		12.5 ± 0.5	-	-	-
β-Sitosterol		68.7 ± 1.9	-	-	-
α-tocopherol		26.5 ± 0.4	28.4	-	-
γ-tocopherol		73.5 ± 0.3	0.0	-	-
δ-tocopherol		-	71.0	-	-

[a]Mariod et al. 2010, [b]Ansari et al. 1985, [c]Andrade et al. 2001

3.2 Characteristics of the defatted cake (ASDC)

More than 90wt-% of the biomass was analyzed with moisture of 6.7wt-% lower than 10wt-% favorable for a good storage. ASDC contains a noticeable amount of crude fibre (28.3wt-%) and lignin (7.2wt-%). N, P, K, contents are relatively high for ASDC (4.7; 0.3; and 0.5wt-% respectively) and could support its probable use in formulations of manure (Table 2). These data confirm the average nutritional value of seeds of *A. squamosa* if the insecticidal compounds provide essentially from acetogenin family are isolated (Yang and Zheng 2000, Mst Shahnaj *et al.* 2003). The proteins are the main component of the defatted cake (25.5wt-%) and is highest than the data reported by Mariod *et al.* 2010 who found only (17.5 g/100g). This value is therefore comparable with groundnut (26 - 42wt-%) and cotton (28 - 50wt-%) and much lower compared with soya (36 - 53wt-%). It's also in the same order with the data recorded for colza and sunflower (20 - 25wt-%) (Greenfield and Southgate 2003). It is necessary to notice the relatively high content in lysin (1.2wt-%) and tryptophan was not found in the amino acids (Table 3).

Table 2: Characteristics of *Annona squamosa* defatted cake (ASDC)

Characteristics of ASDC	This Work	Sudan specimen (Eastern Africa)[a]
Moisture	7.02 ± 0.33	6.7 ± 0.2
Ash	3.14 ± 0.32	2.2 ± 0.1
Fat	7.85 ± 0.25	-
Proteins	25.5	17.5 ± 0.2
Starch	1.61	-
Total sugars	5.9	-
Cellulose (Wende)	26.98 ± 1.43	-
Lignocellulose (ADF)	34.66 ± 1.23	-
Lignin (ADL)	7.41 ± 0.52	-
Hemicellulose (NDF-ADF)	25.45 ± 3.53	-
N	4.7	-
K	0.9	-
P	0.5	-
Ca	0.3	-
Mg	0.3	-

[a]*Mariod et al. 2010*

Table 3: Amino acids composition of *Annona squamosa* defatted cake (ASDC)

Amino acids composition of ASDC (g/100g)	This Work	Sudan specimen (Eastern Africa)[a]
Arginine	3.1	0.7 ± 0.4
Histidine	0.4	0.1 ± 0.1
Isoleucine	1.0	0.5 ± 0.2
Leucine	2.3	0.8 ± 0.3
Lysine	1.2	0.4 ± 0.2
Methionine + cysteine	0,4	0.1 ± 0.1
Phenylalanine + tyrosine	2.0	0.7 ± 0.2
Threonine	0.9	0.3 ± 0.1
Valine	1,2	0.6 ± 0.2
Alanine	1,6	0.6 ± 0.2
Aspartic acid	2,5	0.7 ± 0.3
Glutamic acid	4,5	1.0 ± 0.5
Glycine	1,7	0.4 ± 0.2
Serine	1,2	0.3 ± 0.1
Tyrosine	0,9	-
Proline	1,7	-
Unknown	1.8	-

[a]Mariod *et al.* 2010

4. CONCLUSION

African diversity is not well known while Benin imports oils. This study provide more recent detailed analytical data about the specialty seed oils and defatted cake of underused species *Annona squamosa* grown in Benin. Fatty acids, amino acids, sterols and tocopherols fraction were quite different compared with samples from Asian, American and African areas. Especially this oil could be extracted by simple pressure, without solvent, which has an advantage for an

artisanal exploitation in terms of simplicity of implementation but also of safety and quality of the product and environmental impact. This study also has showed the average nutritional value of seeds of *A. squamosa* if the insecticidal compounds provide essentially from acetogenin family are isolated. Further investigations are needed to be undertaken the chemical fluctuations of main component. After having shown the peculiar composition of oil, a wider range of samples should now be investigated in term of chemical composition and insecticidal activity in the following works to assess variability. The different fractions could be used either for food, soap, biofuel but also for more specific targets as crop and wood preservation.

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REFERENCES
Andrade, E H A, Maia, J G S, Streich R, Marx F (1999): Seed composition of amazonian Lecythidaea species: Part 3 in the series "Studies of edible amazonian plants". *Journal of Food Composition and Analysis,* **12**, 37-51.
Ansari, M H, Afaque, S, Ahmad, M (1985): Isoricinoleic acid in *Annona squamosa* seed oil. *Journal of the American Oil Chemists' Society*, **62**(10):1514.
Ahmed, M, Naveed, M (1996): Lipid studies of *Annona squamosa*. *Pakistan Journal of Science* and *Industrial Research*, **39**,186-188.
Andrade, A H E, Zoghbi M, das Graças, B, Guilherme, S M (2001): Chemical characterization of the fruit of *Annona squamosa L* Occuring in the Amazon. *Journal of Food Composition and Analysis*, **14**, 227-232.
Mariod, A A, Elkheir, S, Ahmed, Y M, Matthäus, B (2010): *Annona squamosa* and *Catunaregam nilotica* seeds, the effect of the extraction method on *the oil compos*ition. *Journal of the American Oil Chemists' Society*, **87**, 763–769.
Djenontin, T S, Wotto, D V, Avlessi, F, Lozano, P, Sohounhloué, K C D, Pioch, D (2012): Composition of *Azadirachta indica* and *Carapa procera* (Meliaceae) seed oils and cakes obtained after oil extraction. *Industrial Crops and Products*, **38**, 39-45.
Djenontin, S T, Wotto, V D, Lozano, P, Pioch, D, Sohounhloué, D K C (2009): Characterization of *Blighia sapida* (Sapindaceae) seed oil and defatted cake f*r*om Benin. *Natural Product Research*, **23**, 549-560.
Van Soest, P J, Robertson, J B, Lewis, B A (1991): Methods for dietary fib*r*e, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci*, **74**, 3583-3597.
Van Soest, P J, Wine, R H (1967): Use of detergents in the analysis of fibrous feeds IV Determination of plant cell-wall constituents. *Assoc Off Agr Chem J.*, **50**, 50-55.
Udén, P, Robinson, P H, Wiseman, J (2005): Use of detergent system terminology and criteria for submission of manuscripts on new, or revised, analytical methods as well as descriptive information on feed analysis and/or variability. Animal Feed Science and Technology, **118**,181-186.
Kapseu, C, Parmentier, M (1999) : Composition en acides gras de quelques huiles végétales du Cameroun. *Sciences des aliments*, **17**, 325-331.
Roberto, M M, Ambrozin, A R P, Liao, L M, Ferreira, A G (2008): Critical review on analytical methods for biodiesel characterization. *Talanta*, **77**, 593-605.
Grosso, R N, Nepote, V, Guzman, C A (2000) Chemical composition of some wild Peanut Species *Arachis* L. *Journal of Agricultural and Food Chemistry,* 48, 806-809.
Kamal-Eldin, A, Andersson, R (1997): A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. *Journal of the American Oil Chemists' Society*, 74, 375-376.
Yang, R Z, Zheng, X C (2000): Studies on the chemical constituents of *Annona squamosa*. *Acta Botanica Yunnanica*, 22, 71-74.
Mst Shahnaj, P, Md Ekramul, I, Md Motiur, R, Md Ekramul, H (2003): Pesticidal activity of pure compound annotemoyin-1 isolated from chloroform extract of the plant *Annona squamosa Linn* against *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *Pakistan Journal of Biological Sciences*, **6**, 1088-1091.
Greenfield, H, Southgate, D A T (2003): Food composition data. In: Production, management and use, 2nd ed, FAO, ch 9, pp. 163-170. Roma.

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Section 3 Wood Protecting Chemicals

Treating *Bambusa vulgaris* with neem seed oil against basidiomycetic biodegradation

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Treating *Bambusa vulgaris* with neem seed oil against basidiomycetic biodegradation

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ABSTRACT

Realising maximum benefits from bamboo stems/culms in Nigeria are presently constrained by their almost non-acceptance for applications in most structural and construction purposes, except in comparatively low quality and some temporary applications, such as scaffolding, owing to their susceptibility to easy destruction by agents of biodegradation as a result of their poor inherent natural durability. Therefore, there is the need for value-addition to this lignocellulosic material using low cost preservatives, particularly the environmentally benign ones, in order to encourage sustainable utilisation for higher valued products and contribute to poverty alleviation in this part of the world. This report is an outcome of an investigation on the efficacy of neem (*Azadirachta indica* A. Juss) seed oil-treatment for bamboo (*Bambusa vulgaris* Schrad. ex J.C. Wendl.) against a fungus, also a basidiomycete, known as *Pycnoporus sanguineus* (L. ex Fr.) Murr. Oven-dried split-bamboo samples conditioned to 11.76% mean moisture content were converted to test specimens for percentage weight loss (PWL) and treated with mechanically extracted neem seed oil (NSO) at two different treatment temperature regimes by completely soaking a set in NSO at ambient room temperature of 25 ± 2^{o}C for 24 hours and soaking the other in NSO at 60^{o}C for 4 hours with untreated samples serving as control. The oil-treated and control samples were initially weighed and subsequently inoculated with cultured *P. sanguineus* and monitored in an incubating room maintained at ambient temperature of 25 ± 2^{o}C and 65 ± 5% relative humidity for 12 weeks (84 days). After the 84 days incubation period, the test specimens were then reweighed and the PWL determined. Results showed that mean values for PWL was highest for those obtained for control samples (18.21%), comparatively lower for samples soaked in NSO at 25 ± 2^{o}C for 24 hours (5.88%) and lowest for samples soaked in NSO at 60^{o}C for 4 hours (2.21%). Implications of these values were discussed while conclusions and recommendations were based on the outcome of the study.

Keywords: Bamboo; neem; oil-treatment; basidiomycetes; environment; weight loss

1. INTRODUCTION

The importance of renewable natural resources (RNR) in contributing to the efforts at achieving sustainable development in most, if not all, developing countries, particularly those in Africa, Asia, Central, and South America, cannot be overemphasised. Large proportion of the population of the inhabitants of these parts of the world is predominantly dependent on these RNR for sustenance and livelihood, making these resources to be central to their survival. Forests and its products have been noted to be a major component of these RNR with non-timber forest products (NTFPs), a valuable constituent of forests, known to contribute to household economy and income generation particularly in the rural areas of these countries.

For instance, bamboos are NTFPs that have long been identified for various traditional uses in many countries (Sattar 1996, Sekar *et al*. 1998). However, bamboo stems, a valuable source of lignocellulosic materials are prone to easy biological degradation as a result of poor inherent natural durability (Liese 1998). There is therefore the need to protect them from these agents of biodegradation in order to improve on their durability and service life (Kumar *et al*. 1994, Liese and Kumar 2003, Liese 2004) and this may be done through drying below fibre saturation point and/or chemical treatment of these and other lignocellulosic products.

However, preventing biodegradation of non-durable lignocellulosic materials using synthetic chemicals is increasingly being discouraged worldwide because of their negative impacts on the environment and life forms. There are also concerns about cost implications of these preservatives particularly in many of the developing countries where developmental challenges are still presently encountered. These have led to recent global increase in the recommendation of more environmental benign, safer and cheaper means of realising this goal, a very good example of which is the utilisation of natural extracts from plants for this purpose (Evans 2003, Stirling *et al*. 2007, Yang 2009, Erakhrumen 2010, 2012).

In line with the earlier highlighted reasons, this research was carried out in order to investigate the potentials of using the oil from neem (*Azadirachta indica* A. Juss) seed, that still have little or no economic value attached to them in most parts of Nigeria (Erakhrumen 2010, 2011, 2012), although, observed to possess some anti-microbial properties (Mishra *et al*. 1995, Puri 1999), for treating and preserving the most common bamboo (*Bambusa vulgaris* Schrad. ex J.C. Wendl.) in south-west Nigeria against a basidiomycetic fungus known as *Pycnoporus sanguineus* (L. ex Fr.) Murr. using percentage mean weight loss values as determinants. Graphical trend obtained for these values ranging from the untreated to oil-treated bamboo samples was also evaluated.

2. EXPERIMENTAL METHODS

2.1 Sourcing of bamboo culms

The bamboo culms that were converted and experimented upon in this study were sourced from wild clumps at Isale-Togun Forest, Lanlate, Ibarapa, Oyo State, Nigeria (latitude 7° 36' N and longitude 3° 27' E) in October, 2008. This area is located in between the humid and sub-humid tropical climatic zones, where mean annual rainfall ranges between 1117.10 and 1693.30mm. Only mature culms with mean circumferential length of 300mm at the second node above ground were harvested and cross cut in such a way that only the basal culm portion of 3000mm length were removed and placed in jute bags with nylon lined inner surface to avoid contamination from the soil. The harvested culms were transported to, protected and stored in the wood workshop of the Department of Forest Resources Management, University of Ibadan, Ibadan, Nigeria, for conversion to the test specimens.

2.2 Sourcing of neem seeds

The ripe neem seeds from which oil was mechanically extracted in this study were obtained from *A. indica* trees on the University of Ibadan campus located on the northern edge of the city of Ibadan, Nigeria (latitude 7° 20'N and longitude 3° 50'E). Collection of the seeds was done by placing clean nylon sheets around the stems of *A. indica* trees in such a way that it covered a substantial cross sectional area of the crown in order to directly collect the seeds as they fall. The neem seeds were secured in the months of June to early August of 2008.

The neem seeds obtained were thoroughly washed using deionised water to remove dirt and other impurities and then air dried in an open space with regular movement for aeration to ensure proper drying, a method also applied by Soetaredjo *et al*. (2008) and Erakhrumen (2010, 2012), to reduce the moisture content (MC) for proper crushing and to facilitate high oil volume recovery during mechanical extraction. The seeds were daily air dried with proper monitoring to prevent spoilage as a result of possible moisture fluctuations.

2.3 Conversion of bamboo culms to test samples

The harvested bamboo stems were carefully sawn into longitudinal strips using circular and vertical breakdown sawing. Each strip was planed on both the inner and outer surface, using a planing machine, in order to obtain mean specimen thickness of 5 ± 0.5mm for the MC determination and weight loss experiment. The strips were first conditioned in the laboratory for 14 days and then oven-dried at 103 ± 2^{o}C to constant weight. They were removed from the oven afterwards and stabilised in the laboratory for 24 hours.

The mean MC of split-bamboo samples after stabilisation was determined using test dimension 20mm (tangentially) x 20mm (longitudinally) x 5mm (radially) and calculated in accordance with ASTM D 4442 (2007) while the strips at the same MC were also converted to test specimens with dimension 20mm (tangentially) x 60mm (longitudinally) x 5mm (radially) for weight loss experiment. The nodes of the harvested and converted culms were excluded.

2.4 Extraction of neem seed oil

The neem seeds were decorticated after being completely air dried, separating the kernel from the shells and dirt, and then air-dried again to obtain constant weight. Dried kernels were carefully pulverised into smaller particles using a seed grinder ensuring no significant loss of seeds' oil. Mechanical extraction of oil was performed by cold pressing the pulverised seeds using an oil expeller at a maximum pressure of 31MPa (31Nmm^{-2} or 4500psi). Mechanical extraction was performed at this pressure until oil stopped flowing.

2.5 Treatment of split-bamboo samples with the extracted oil

The split-bamboo samples to be treated with neem seed oil (NSO) and those untreated meant to serve as control were earlier stabilised in the laboratory to a MC of 11.76%. The stabilised samples to be oil-treated were subjected to two NSO-treatment regimes through (1) soaking a set of samples in oil at ambient room temperature of 25 ± 2^{o}C for 24 hours and (2) soaking another set in hot oil at 60^{o}C for 4 hours, removed from the oil afterwards and allowed to cool in a dessicator at an ambient room temperature of 25 ± 2^{o}C.

These methods of NSO-treatments of split-bamboo specimens were also earlier adopted in the experiments reported by Erakhrumen (2009, 2010, 2012), Erakhrumen and Ogunsanwo (2009, 2010). The maximum heat treatment temperature of 60^{o}C was adopted in this research because strength and stiffness values used in practice for most lignocellulosic materials are observed to be valid for temperatures below 60°C (Homan and Jorissen 2004).

2.6 Sourcing, identification, and culturing of fungal isolates

In order to obtain a fungal species that attacks *B*. *vulgaris*, a decaying culm of this bamboo species found on the forest floor where the bamboo samples for this research were harvested was also obtained, kept in an aseptically clean container, and taken to the Pathology Laboratory of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria, where

samples of the microbes present were identified as predominantly a white rot fungus known as *Pycnoporus sanguineus* (L. EX FR.) MURR.

A nutrient medium of potato dextrose agar (PDA) in deionised water was prepared in line with the method earlier adopted by Erakhrumen (2010, 2012). The white rot fungus that was obtained from the decaying culm was first cultured and again sub-cultured, in order to obtain pure culture, in sterilised Petri dishes with the prepared PDA until the nutrient was completely covered by the mycelium of the purely cultured fungus. The prepared PDA in the culture bottles were then inoculated with the pure culture and also allowed to be completely covered by the mycelium of the pure cultured fungus.

2.7 Infection of split-bamboo samples with fungal isolates

The split-bamboo samples, i.e., both the NSO-treated (soaked and heat treated) and those not treated with the oil (control), to be infected with the pure cultured fungal species were sterilised and placed aseptically into the culture medium in chemically clean amber glass culture bottles in which there were cultures of actively growing test fungal species. The split-bamboo samples with dimensions 20mm (tangentially) x 60mm (longitudinally) x 5mm (radially) to be inoculated with the cultured *P. sanguineus* in the laboratory test were placed such that they came in contact with the aerial mycelium of the test fungus and not the culture medium itself.

The culture bottles were then properly covered with Teflon lined lids, also made of glass ware, to prevent external contamination particularly from the surrounding air. Ten test samples for each treatment (soaked and heat treated) and those not treated with oil (control) were so infected. The bottles together with their contents were left in an incubating room with ambient temperature of $25 \pm 2^{o}C$ and relative humidity of $65 \pm 5\%$ and monitored for twelve weeks (84 days) based on the method adopted by Erakhrumen (2010, 2012) in line with slight modifications to methods described by Leithoff and Peek (2001) and Luna *et al*. (2004).

2.8 Evaluation of weight loss values

The weight loss values of the oil-treated and untreated (control) samples were calculated after the twelve weeks (84 days) incubation period, in line with the procedures adopted by Luna *et al*. (2004), Arora (2006), Sarker *et al*. (2006).

2.9 Statistical analyses

The data obtained for weight loss evaluated for both the oil-treated and control split-bamboo samples exposed to fungal attack were subjected to basic descriptive statistical analyses such as mean and standard deviation (SD). Analyses of variance (ANOVA), was employed in analysing the data for statistical significant variation ($P < 0.05$) while Fishers' Least Significant Difference (LSD) was applied as a follow-up test to compare means ($P < 0.05$).

3. RESULTS AND DISCUSSION

The results obtained from the experiments to determine the percentage weight loss for both oil-treated and untreated longitudinally split-bamboo specimens exposed to *P. sanguineus* tabulated in Table 1 showed that untreated (control) split-bamboo samples had mean weight loss value of 18.21%. Similar studies such as Leithoff and Peek (2001) found that white rot fungus, *Trametes versicolor*, caused about 15% mass loss for untreated samples of bamboo species known as *Phyllostachys puberscens*. In a similar manner, another study by Cho *et al*. (2006), also obtained

a 13% mean weight loss value for untreated *P. puberscens* after 16 weeks of incubation with the fungus *Lentinus edodes*.

According to the results tabulated in Table 1, percentage mean weight loss was highest for control samples (18.21%) but comparatively lower for samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours (5.88%) while the least mean weight loss value of 2.21% was recorded for samples soaked in hot oil at 60°C for 4 hours. The likely implication of the result is that the lower values obtained for percentage mean weight loss for oil-treated bamboo samples may be as a result of prevention or retardation of the biodegradation activity of the cultured white rot fungal species (*P. sanguineus*) experimented upon in this study.

Table 1: Mean values and other statistical analytical results for percentage weight loss for the neem seed oil-treated and untreated (control) split-bamboo test samples subjected to fungal attack

Treatment	Mean weight loss [%], SD and LSD results
Control (non-treated bamboo samples)	18.21 ± 0.60^{a}
Bamboo samples soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours	5.88 ± 0.59^{b}
Bamboo samples soaked in hot oil at 60°C for 4 hours	2.21 ± 0.30^{c}

Values are means for 10 test samples per treatment
Means with different superscript are significantly different ($p < 0.05$)

In addition, the lower mean weight loss values obtained for the oil-treated split-bamboo samples when compared with the untreated ones can be depicted graphically using Fig. 1. The curve indicates the trend in mean weight loss while the straight line is the trend line. As earlier highlighted, the graph showed that percentage mean weight loss value was higher for control samples in comparison with the oil-treated samples, with the mean weight loss value lower for bamboo specimens soaked in oil at ambient room temperature of 25 ± 2°C for 24 hours and least for those soaked in hot oil at 60°C for 4 hours.

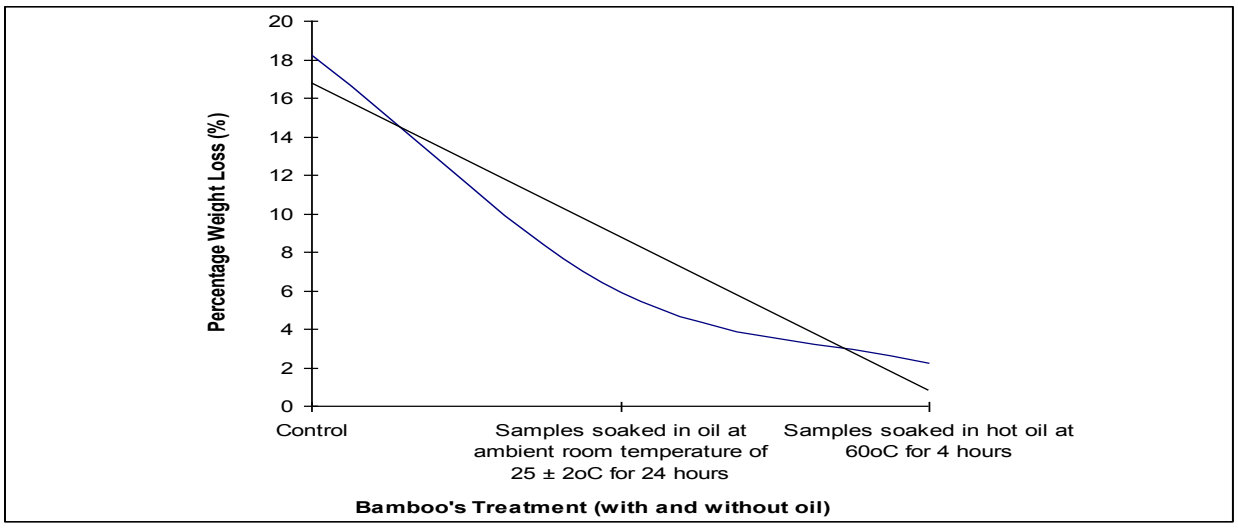

Figure 1: Graphical representation of the reduction in mean weight loss values from that for control to oil-treated bamboo samples including a linear trend line

Furthermore, the fungal species experimented upon in this study (*P. sanguineus*), one of the basidiomycetes, have been identified to be lignivorous, (lignin-degrading fungus), in many wood species (Luna *et al*. 2004), although, how it may degrade bamboo is not yet clear. However, the

lower percentage mean weight loss values obtained for the oil-treated samples, as earlier noted, are likely to be as result of anti-microbial properties of NSO in line with earlier studies that have shown that this oil have certain anti-bacterial, anti-inflamatory, antiseptic, anti-viral, anti-fungal, anti-parasitic, germicidal, pesticidal, insecticidal, anti-microbial, and other related properties (Parveen and Alam 1993, Locke 1995, Mishra *et al*. 1995, Puri 1999, Wikipedia 2007).

In addition, the lowest percentage mean weight loss value obtained for samples soaked in hot oil at 60^{o}C for 4 hours might also be as a result of some contribution from thermal modification of bamboo cell wall structure at high temperature as observed in similar studies such as Wahab *et al*. (2004) and Wahab *et al*. (2007), although, this was not investigated in this study. The ANOVA result showed that there were significant variations in the percentage weight loss experimental values obtained for the untreated and oil-treated bamboo samples. Fisher's LSD (Table 1) showed that the mean values obtained for percentage weight loss were significantly different between split-bamboo samples that were treated with NSO and the control.

4. CONCLUSIONS

The outcome of this research showed that percentage mean weight loss values were significantly lower in neem seed oil-treated split-bamboo samples (i.e. at both treatment regimes) when compared with the values obtained for the untreated (control) samples. The probable implication of this observation is that this oil might have contributed to the inhibition of fungal degradation of oil-treated samples, as weight loss in lignocellulosic materials is likely to be an indication of their deterioration due to, among other causes, activities of agents of biological degradation.

In addition, there might have also been some influence of thermal modification of the lignocellulosic matrix on the result if the mean value obtained for split-bamboo specimens soaked in hot oil at 60^{o}C for 4 hours is considered. However, obtaining comparatively lower mean weight loss value for specimens soaked in oil at ambient room temperature of 25 ± 2^{o}C for 24 hours when compared with control showed that the oil may be efficacious in this regard. Based on this outcome, the oil from neem seed may be useful as a preservative for bamboo stem.

This use is particularly imperative because neem seeds are presently with little or no economic value attached to them in most parts of Nigeria. Applying this seed's oil for this purpose is expected to contribute to series of efforts aimed at environmental benign methods of treating and preserving lignocellulosic materials and their products with organic chemicals, even as studies targeted at optimising the use of this oil for this purpose are still ongoing. This use may also be a veritable strategy that can contribute to poverty alleviation, particularly in the rural areas of the developing countries where neem trees are available with the seeds being underutilised.

5. REFERENCES

Arora, J C (2006): Method of Laboratory Testing of Wood Preservatives against Fungi and Borers (Powder Post Beetles) part 1 Determination of Threshold Values of Wood Preservatives against Fungi. Available at: http://www.bis.org accessed on the 17th of February, 2008.

ASTM D 4442 – 07 (2007): Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Base Materials. American Society for Testing and Materials.

Cho, C H, Lee, K H, Kim, J S, Kim, Y S (2006): Micromorphological Characteristics of Degradation in Bamboo Attacked by White Rot Fungus *Lentinus edodes*. IRG/WP 06-10575. A

Paper prepared for the 37th Annual Meeting for The International Research Group on Wood Protection held in Tromsø, Norway, between 18th and 22nd of June, 2006. 11pp.

Erakhrumen, A A (2009): Tensile Strength Properties of Wild Grown *Bambusa vulgaris* Treated with Neem Seed Oil in Southwest Nigeria. *Journal of Bamboo and Rattan*, **8**(1&2), 95-102.

Erakhrumen, A A (2010): Potentials of Neem (*Azadirachta indica* A. JUSS) Seed Oil as a Preservative for Bamboo (*Bambusa vulgaris* SCHRAD. EX J.C. WENDL.) against Basidiomycetes. An unpublished thesis for a Ph.D. degree of the University of Ibadan, Ibadan, Nigeria, xviii + 172pp.

Erakhrumen, A A (2011): Selected Physical and Chemical Properties of Mechanically Extracted Neem Seed Oil Sourced as a Preservative for Ligno-Cellulose in Southwestern Nigeria. *Forestry Studies in China*, **13**(4), 263-269.

Erakhrumen, A A (2012): Evaluating the Efficacy of Neem (*Azadirachta indica* A. Juss) Seed Oil-Treatment for *Bambusa vulgaris* Schrad. against *Pycnoporus sanguineus* (L. ex Fr.) Murr. using Static Bending Strength Properties. *Forest Pathology*, **42**(3), 191-198.

Erakhrumen, A A, Ogunsanwo, O Y (2009): Water Absorption, Anti-Swell Efficiency, and Dimensional Stability Properties of Neem Seed Oil-Treated Wild Grown *Bambusa vulgaris* Schrad ex J.C. Wendl. in Southwest Nigeria. *BioResources*, **4**(4), 1417-1429.

Erakhrumen, A A, Ogunsanwo, O Y (2010): Influence of Neem Seed Oil-Treatment on Static Bending Strength Properties of Wild Grown Split-Bamboo (*Bambusa vulgaris* Schrad.) in South-West Nigeria. *Silva Lusitana*, **18**(2), 167-177.

Evans, P (2003): Emerging Technologies in Wood Protection. *Forest Products Journal*, **53**(1), 14-22.

Homan, W J, Jorissen, A J M (2004): Wood Modification Developments. *HERON*, **49**(4), 361-386.

Kumar, S, Shukla, K S, Dev, I, Dobriyal, P B (1994): Bamboo Preservation Techniques: A Review. Jointly Published by International Network for Bamboo and Rattan (INBAR) and Indian Council of Forestry Research Education (ICFRE). Also available at: http://www.inbar.int/publication/txt/INBAR_Technical_Report_No03.htm accessed on the 17th of June, 2008.

Leithoff, H, Peek, R D (2001): Heat Treatment of Bamboo. IRG/WP 01-40216. Document Prepared for the 32nd Annual Meeting of the International Research Group on Wood Preservation held in Nara, Japan, between 20th and 25th of May, 2001. 11pp.

Liese, W (1998): The Anatomy of Bamboo. *INBAR Technical Report No. 18*. International Network for Bamboo and Rattan, 204pp.

Liese, W (2004): Guadua in Kolumbien. *Bambus Journal*, **16**, 4-6.

Liese, W, Kumar, S (2003): Bamboo Preservation Compendium. *INBAR Technical Report 22*. Centre for Indian Bamboo Resource and Technology, New Delhi, India.

Locke, J C (1995): Fungi. In*: The Neem Tree, Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes*, ed. H. Schmutterer. VCH, Weinheim, Germany, pp. 118-125.

Luna, M L, Murace, M A, Keil, G D, Otaño, M E (2004): Patterns of Decay caused by *Pycnoporus sanguineus* and *Ganoderma lucidum* (Aphyllophorales) in Poplar Wood. *IAWA Journal*, **25**(4), 425-433.

Mishra, A K, Singh, N, Sharma, V P (1995): Use of Neem Oil as a Mosquito Repellent in Tribal Villages of Mandla District, Madhya Pradesh. *Indian J. Malariol.*, **32**(3), 99-103.

Parveen, G, Alam, M M (1993): Bioactivity against Plant Pathogens. In: *Neem Research and Development*, eds. N.S. Randhawa, B.S. Parmar. Society of Pesticide Science, New Delhi, India, pp. 144-153.

Puri, H S (1999): Neem: The Divine Tree. *Azadirachta indica*. Harwood Academic Publications, Amsterdam. ISBN 90-5702-348-2.

Sarker, P K, Rahman, M A, Bulbul, M R, Das, T, Ilias, G N M (2006): Standard Test Methods for Wood Preservatives by Laboratory Agar-Block Test. IRG/WP06-20350. A Paper prepared for the 37th Annual Meeting of The International Research Group on Wood Protection held in Tromsø, Norway, between 18th and 22nd of June, 2006.

Sattar, M A (1996): Traditional Bamboo Housing in Asia: Present Status and Future Prospects. In: *Bamboo, People and the Environment. Vol. 3: Engineering and Utilization. INBAR Technical Report No. 8*, eds. P.M. Ganapathy, J.A. Janssen, C.B. Sastry. New Delhi, India, pp. 1-13.

Sekar, T, Balasubramanian, A, Manimekalai, V (1998): Vegetative Propagation of an Ornamental Bamboo, *Bambusa vulgaris* cv. Wamin McClure, by Branch Cuttings. *Bamboo Science and Culture*, **12**(1), 30-36.

Soetaredjo, F E, Budijanto, G M, Prasetyo, R I, Indraswati, N (2008): Effects of Pre-treatment Condition on the Yield and Quality of Neem Oil obtained by Mechanical Pressing. *ARPN Journal of Engineering and Applied Sciences*, **3**(5), 45-49.

Stirling, R, Daniels, C R, Clark, J E, Morris, P I (2007): Methods for Determining the Role of Extracts in the Natural Durability of Western Red Cedar Heartwood. Document No. IRG/WP 07-20356. The International Research Group on Wood Protection, Sweden.

Wahab, R, Samsi, H W, Sudin, M, Moktar, J (2004): Strength and Durability of Bamboo Treated Through an Oil-curing Process. *Journal of Biological Sciences*, **4**(5), 658-663.

Wahab, R, Samsi, H W, Sulaiman, O (2007): Properties of Oil-Cured Cultivated *Bambusa vulgaris*. *International Journal of Agricultural Research*, **2**(9), 820-825.

Wikipedia, the Free Online Encyclopedia, (2007): Neem Oil. Available at: http://en.wikipedia.org/wiki/Neem_oil

Yang, D Q (2009): Potential Utilization of Plant and Fungal Extracts for Wood Protection. *Forest Products Journal*, **59**(4), 97-103.

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Section 4 **Processes and properties**

Microwave Treatment of Frozen Wood Packaging Material

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ABSTRACT

As part of the *wood packaging material* (WPM) regulation in international trade, the *dielectric heating* (DH) is soon to be included in ISPM15 (IPPC 2009) as an approved phytosanitary treatment. It has been considered that when using dielectric radiation (i.e. microwaves or radio-frequencies), reaching 60°C or more throughout the entire profile of the wood during at least 60s (i.e. 60°C/60s) ensures the eradication of any noxious organism present in the wood. Regarding the treatment itself, the main requirements mentioned in the draft of ISPM15 Annex 1 (IPPC 2011) concern the way to achieve "uniformity of heating" as well as the treatment duration, which must not exceed 30min. Concerning wood characteristics, the only restriction to the DH concerns the thickness of the wood pieces, which must not exceed 20cm; no other restriction exists regarding wood moisture content, density or initial temperature. As reported in the draft of ISPM15 Annex 1, it is generally considered that "*when using microwaves as a heating source, the coldest part of the wood is the surface*". This statement implies that achieving 60°C (or more) during 60s at the surface of the wood should guarantee compliance with IPPC requirements (i.e. 60°C throughout the profile of the wood).
However, since ice and liquid water exhibit very different properties towards microwaves, initially frozen wood (9cmX9cm and 17cmX17cm in cross-section) was irradiated in a 28.8 kW microwave oven (2.45GHz) in order to assess whether achieving 60°C/60s at the surface of less than 20cm-thick planks ensures higher core temperatures. The temperature pattern observed after the treatment was compared with the one observed after the treatment of initially thawed wood pieces. It was observed that, in some conditions, initially frozen pieces exhibit inside temperature (much) lower than 60°C, despite complying with 60°C/60s all over the surface of the wood. These results show that when wood is heated with microwaves, its coldest part may not be the surface. Our results also strongly suggest that the impact of wood properties on post-treatment temperature pattern should be further investigated in order to better identify the limits of the DH (at least on frozen wood).

Keywords : wood packaging material; dielectric treatment; microwaves; ISPM15; frozen wood.

INTRODUCTION

To prevent biological invasions in forest ecosystems, ISPM15 (IPPC 2009) describes the phytosanitary measures that must be applied to *Wood Packaging Material* (WPM) used in international trade. In spite of some exceptions, methyl bromide is phased out and may not be available in the future, which makes heat treatment the only phytosanitary treatment that can be applied to WPM at present. Fortunately a *Microwave Treatment* (MT) of WPM has recently been developed and should be soon available for the Industry. The MT is part of the *dielectric heating* (DH), which consists in heating the wood through irradiation with electromagnetic waves such as microwaves or radio-frequencies.

Several studies dedicated to the dielectric heating of wood resulted in higher core than surface temperatures (Miura et al. 2004; Diaferia 2008; Bisceglia *et al*. 2009; Hoover *et al*. 2008a and 2008b). It is important to keep in mind that this particular temperature pattern is valid within the limits of those experiments or models: Extrapolation beyond these limits may require further experiments or analysis. Nevertheless, the draft of ISPM15 Annex 1 (IPPC 2011) which was submitted to the IPPC signatory countries from June to September 2011 reported the general statement that "*When using microwaves as a heating source, the coldest part of the wood is the surface*". Based on this, it was even initially proposed (before official release of the draft of ISPM15 Annex 1) – and it could still be supposed – that achieving 60°C for 60s at the surface of the wood warrants, in the specific context of the MT, higher inside temperature and therefore constitutes an efficient sanitary treatment (R. Burgess 2011, pers. com.).

It was thus decided that, in the frame of the DH, a minimum temperature of 60°C must be maintained for at least one minute throughout the profile of the wood (ISPM15 Annex 1, IPPC 2011). These conditions have been considered by the expert panels working with the FAO, which are the Technical Panel on Phytosanitary Treatments (TPPT) and the Technical Panel on Forest Quarantine (TPFQ), to eradicate all the target organisms, such as insects, fungi, and worms, among others (IPPC 2010).

In this context, the only limitation regarding the wood's characteristics is that the pieces must have a maximum thickness of 20cm: So far there is no other limitation, neither regarding their moisture content (MC) or initial temperature. The new time-temperature schedule was approved by the consultative FAO expert panels (TPPT and TPFQ), it was welcomed by the FAO Standards Committee (R. Burgess 2011, com. pers.), and the method is on its way to be included in ISPM15 (cf. ISPM15 Annex 1 draft consultation by IPPC signatory countries in June-September 2011). Finally, it is worth to mention that the draft of ISPM15 Annex 1 (IPPC 2011) proposed a limitation regarding the time needed to reach the lethal temperature (60°C): "*Heating to the prescribed temperature must occur within 30 minutes*".

However every reader has probably experienced the following situation: After taking a piece of meat out of the freezer and putting it for some minutes in the microwave oven, the outer layers of the meat may be thawed – or even cooked – whereas the core is still frozen. This undesirable situation results from the strong absorption of microwaves by free water on or close to the surface (Taher and Farid 2001). Liquid water is indeed highly absorptive material regarding microwaves, whereas ice is nearly transparent to these radiations (Purcell 1973; Fu 2004; Rattanadecho 2004). In this context a process aimed at preventing such heterogeneous heating was patented by Barbini and Jolion (1978). It consists in soaking or spraying the frozen body in/with a cryogenic substance (liquid nitrogen or carbon dioxide, for example), before or during the microwave irradiation. This practice induces considerable cooling of the outer layers of the block, which impedes the presence of free water near the surface. Microwaves entering the block

thus pass unabsorbed through the surface layer. Tiny patches of free water in the core of the block absorb the energy, which enables homogeneous and rapid thawing. Regarding wood, let us remember Lundgren *et al*. (2006), who state that there is "*a considerable change in dielectric properties when the wood changes from frozen to nonfrozen condition*".

Although the dielectric properties of wood are substantially different from those of meat, those observations prompted us to carry out some experiments in order to compare the heating pattern of frozen and thawed wood when irradiated with microwaves. In particular, if it is admitted that a surface temperature of 60°C warrants higher core temperature of 20cm-thick (or thinner) initially-thawed wood pieces, we assessed if this assumption is valid when the wood is initially frozen. This analysis may have important consequences on the treatment itself, as well as on its monitoring.

EXPERIMENTAL METHODS

The trials were performed with an industrial 2.45GHz oven provided with sixteen 1.8kW magnetrons working independently; maximum power delivery is 28.8kW. The microwaves produced by each magnetron spread through a waveguide before entering the 4m-long irradiation tunnel, in order to homogenise the energy distribution in the latter. A 45cm-wide conveyor belt enables the permanent supply of the oven. This oven had already been used in previous studies (e.g. Henin *et al*. 2008) and is structurally very similar to the one described in Diaferia (2008).

All the 60cm-long beech (*Fagus sylvatica*) pieces were irradiated individually in the oven and the conveyor speed was 1m/min. The characteristics of the samples are presented hereunder (Table 1).

Table 1. Number of samples treated, according to their thickness and initial temperature.

Cross-section [cm]	**Initial temperature** [°C]	**Samples**
9X9	-20	3
	-10	3
	-5	3
	+15	3
17X17	-20	4
	+10	4

The number of replicates may look weak. However, it should be noted that in the frame of the present experiments, evidencing only one problematic situation (e.g. the concomitance of >60°C surface temperatures with <60°C core temperatures) is enough by itself. The nature of the phenomenon that is studied (i.e. the fact it deals with Physics) and of our objective make unnecessary high number of replicates and complex statistical analysis.

ISPM15 Annex 1 states: "*the treatment temperatures are monitored at a location likely to be the coldest*". Since the coldest part of the wood is supposed to be the surface when microwaves are the heating source, temperature was measured on the surface of the wood after the treatment. Temperature measurements were performed with a VarioCAM® infrared camera (JENOPTIK). Its technical characteristics are the following: A. Sensors dimensions: 640*480 pixels; B. Accuracy: 1.5°C; C. Measuring range : -40°C to 1200 °C.

When the temperature was not higher than 60°C on the whole surface of a sample, the latter underwent one more run in the oven. For each of these additional run, the coldest face was above, in order to homogenise the heating of the wood.

The irradiation was considered successful when the temperature exceeded 60°C on the whole surface of the sample (i.e. on its four faces) one minute after it left the tunnel, that is at t_{1min} (time when samples leave the tunnel=t_{0min}). At that moment, a thermal picture of the 4 faces was taken. Immediately after recording the surface temperatures at t_{1min}, temperatures were also recorded on the sample cross-section with the infrared camera after cutting the pieces with a radial saw, at 20cm from the end. The microwave penetration from the sample ends was shown to be less than 10-15cm (Gilbert and Turcotte 1994; Gilbert and Cooper 2001): Beyond the 15cm-extremities, cross-section temperature patterns are thus free of end effects (which is shown in Antti and Perré 1999 or in Henin *et al*. 2008). It has been measured that this cutting generates, at the most, a 2° or 3°C temperature increase on the cutting plans. Moreover, since the cutting generates an increase in the wood temperature, this action can only make the MT look efficient, when in fact it is not.

The data were stored in the IRBIS®3 thermography software. For each face or cross-section analysed through thermal imagery, the mean, minimum and maximum temperatures were recorded. The surface temperature was measured on the four faces of the samples: the mean temperature corresponds to the overall mean (4 values of 4 faces), whereas the minimum and maximum temperatures are, respectively, the lowest and highest temperatures measured on any of the faces.

Analysing the temperature pattern of the samples' cross-sections through thermal imaging provides very meaningful information, notably enabling the accurate knowledge of the cross-section temperature pattern, as well as the precise identification of the coldest zone. This is an advantage compared to temperature probe measurements (for example, thermocouples or fibre optic probes), which only give partial information.

Obviously, after a microwave treatment, the temperature homogenise throughout the cross-sections of the samples with time. In some internal wood layers, the temperature may increase after the sample leaves the oven (Hoover *et al*. 2008b; pers. obs.) because of conductive heat transfer from the hottest areas. Hence, three additional cuttings were performed on the samples exhibiting less than 60°C temperature on their cross-section, in order to verify that those unsatisfactorily treated areas did not met ISPM15 Annex 1 requirement after some time. These cuts were done 3, 5 and 7 minutes after t_{0min}, every 5cm towards the centre of the sample, starting from the first transversal cut performed at t_{1min}. After the samples were cut, some pieces were oven dried in order to determine moisture content.

RESULTS AND DISCUSSION

One minute after they left the oven (t_{1min}), all the samples mentioned in Table 1 exhibited a temperature above 60°C on their surface.

Table 1. Characteristics of the samples, of the treatment, and temperature patterns recorded one minute after the treatment (t_{1min}).

Cross-section	Initial temperature [°C]	Sample*	MC (after treatment)	Number of runs	Surface temperatures [°C]		Inside temperatures [°C]	
					Mean	min-max	Mean	min-max
9X9 cm	-20	9A-20	68%	2	78	**59** - 92	80	61 - 88
		9B-20	40%	3	79	**58** - 103	95	62 - 107
		9C-20	47%	5	75	**58** - 90	80	61 - 89
	-10	9D-10	36%	2	71	**59** - 97	83	60 - 99
		9E-10	42%	2	63	**53** - 77	72	60 - 77
		9F-10	48%	5	75	**58** - 107	79	61 - 90
	-5	9G-5	42%	3	83	**58** - 102	78	60 - 92
		9H-5	51%	3	76	60 - 92	81	60 - 104
		9I-5	47%	5	70	**56** - 82	81	60 - 87
	+15	9J+15	43%	2	77	**58** - 115	83	63 - 89
		9K+15	30%	2	81	61 - 97	80	60 - 93
		9L+15	33%	2	79	66 - 87	80	62 - 86
17X17 cm	-20	17M-20	54%	8	79	61 - 126	64	**4** - 92
		17N-20	68%	13	75	60 - 96	67	**3** - 87
		17O-20	81%	10	78	61 - 150	62	**2** - 82
		17P-20	54%	9	78	60 - 102	66	**3** - 96
	+10	17Q+10	25%	6	73	**51** - 90	84	62 - 107
		17R+10	17%	7	73	60 - 87	78	61 - 88
		17S+10	46%	4	78	60 - 106	76	60 - 106
		17T+10	30%	3	74	**51** - 95	83	63 - 89

* The first number corresponds to the thickness; the letter corresponds to the identity of the sample; the last number is the initial temperature.

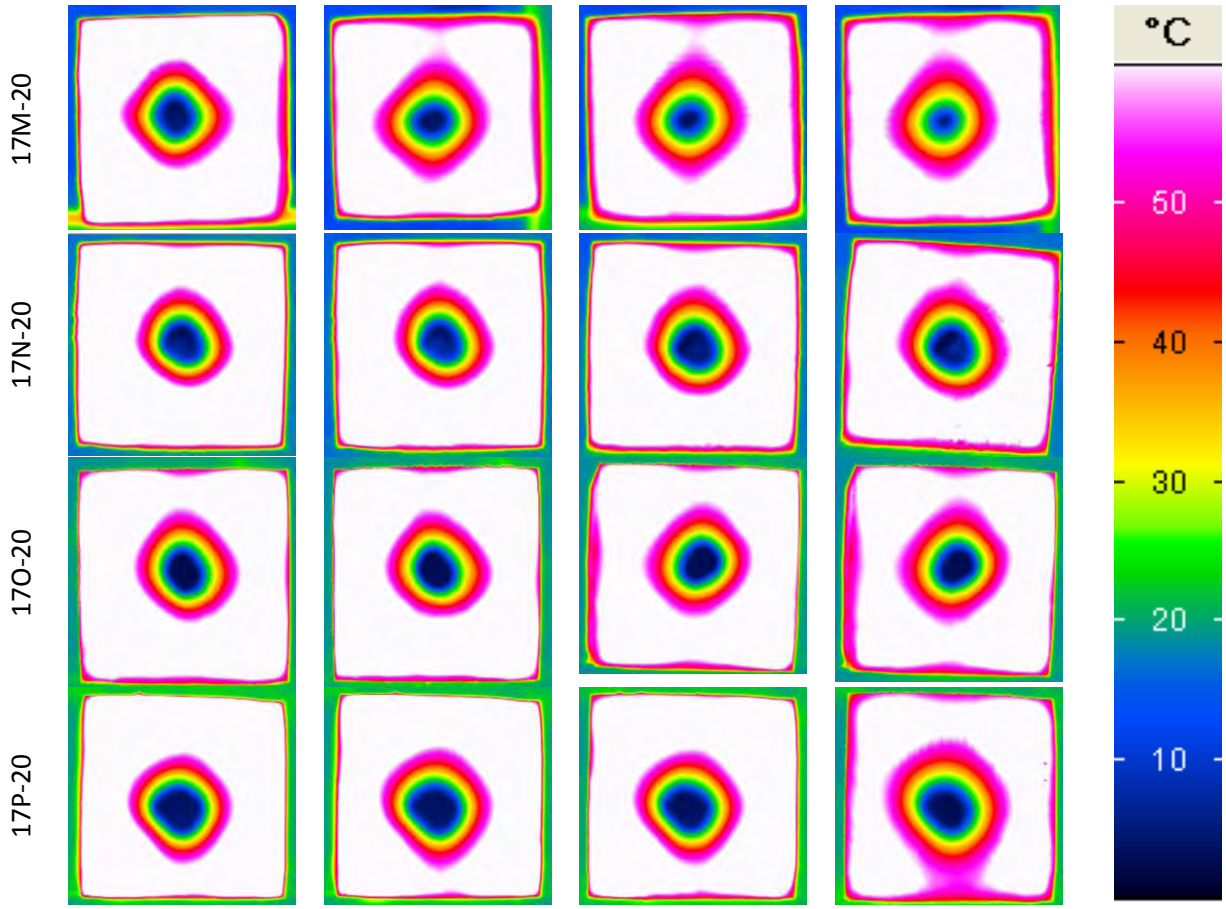
Whatever their initial temperature, it appears that all 9cm thick samples were successfully treated: they present a temperature higher than 60°C on their whole cross-section. The surface of most samples however presented tiny spots with a temperature between 53°C and 60°C (minimum values in bold character), but the latter did not impede the achievement of more than 60°C inside the wood. Obviously, achieving 60°C on 100% of the surface of the samples would have led to still higher cross-section temperatures. Whether frozen or not, achieving 60°C during 60s on the surface of the 9cm-thick samples thus warrants higher than 60°C inside temperatures and compliance with IPPC requirements. On wood with such characteristics, the monitoring of the treatment can thus be performed through the measurement of the surface temperature of the wood.

Regarding the 17cm thick samples, the success of their irradiation is strongly influenced by their initial temperature. The samples starting from 10°C clearly exhibit a satisfying temperature pattern on their cross-section and comply with ISPM15 Annex 1 requirements (despite tiny cold spots of 51°C observed on the surface of 17Q+10 and 17T+10). Conversely, the temperature patterns observed on the cross-section of the initially frozen samples do not match efficient treatment requirements. Although the temperature reached 60°C on their whole surface, the core of these pieces appears hardly thawed (minimum temperatures in bold underlined). On the one hand, it means it could be difficult to heat up to 60°C the core of frozen wood with such a thickness, and if a higher power if applied, the risks of charring in the outer wood layers must be evaluated. On the other hand, the monitoring of the treatment of these samples clearly cannot follow the same rule as for the 9cm-thick pieces: Achieving 60°C on their entire surface do not warrant higher core temperatures.

The relation between the mean temperatures is particularly interesting. On thawed samples, an average (on the 4 samples) surface temperature of 74°C corresponds to an average inside temperature of 80°C; on frozen samples, these temperatures are respectively 77°C and 65°C. Although exhibiting a surface temperature slightly higher than thawed samples, frozen pieces present a mean cross-section temperature 15°C lower. Furthermore, the difference between the maximum and minimum temperatures recorded on the cross-section of frozen 17cm-thick samples is 86°C in average (min=80°C, sample 17O-20), whereas it is only 37°C in average (max=46°C, sample 17S+10) on the corresponding thawed samples. Finally, minimum inside temperatures is in average 61°C and 3°C, for thawed and frozen samples respectively. All these facts indicate that the pattern of microwaves absorption (and consequently wood heating) significantly differs between frozen and thawed wood.

Noteworthy, frozen 17cm-thick pieces underwent 8 to 13 irradiations cycles in the oven. Since the speed of the conveyor was 1m/min and the tunnel is 4m long, more than 30 minutes (and even 52min for 17N-20) were needed to achieve 60°C on the whole surface of the pieces. Using an oven like ours could thus not enable to treat the wood within the time prescribed in ISPM15 Annex 1.

The sequences of thermal photographs presented in Fig. 1 show that, up to seven minutes after the treatment, the temperature homogenisation due to heat transfer did not result in achieving 60°C all over the cross-sections of the four unsatisfactorily treated samples (frozen 17cm-thick samples). Depending on the latter, ca 15-20% of the cross-section area do not match ISPM15 Annex 1 requirements (i.e. exhibit a temperature lower than 60°C).



bFig. 1. Cross-section temperature patterns observed (from the left to the right) 1, 3, 5 and 7 minutes after the samples left the tunnel (t_{0min}). The areas in white comply with ISPM15 Annex 1 (temperature higher than 60°C). The first picture column (patterns at t_{1min}) corresponds to the data presented in Table 1.

In the frame of previous experiments (unpublished results), a considerable drying of some samples was recorded. The amount of water lost because of the treatment was consistent with the water fluxes ranging from 0.05 to 0.3g/s (average around 0.25g/s) calculated from previous microwave (Antti 1992; Oloyede and Groombridge 2000; Fleming et al. 2004) or high frequency drying trials (Rémond and Perré 2008). Since the frozen 17cm-thick samples had to be treated during more than 30minutes before reaching 60°C on their whole surface, the considerable drying of the outer layers (some samples had an initial MC around 100%) likely contributes to enhance their heating. This phenomenon may reinforce the difference observed between core and surface temperatures.

Our results contradict the statement that the core of microwaved wood is always hotter than its outer surface, if the latter is heated up to 60°C. Conversely, these results are consistent with previous studies reporting preferential surface heating (versus core heating) of frozen wood (Gilbert and Turcotte 1994; Gilbert and Cooper 2001) or of other bodies (Fu 2004). Hence, the statement in Koubaa *et al*. (2008), who report that "*the fact that frozen material has roughly the same properties as the thawed material suggests that heating of the wood from a frozen state to 58°C can be done in the same applicator* [as for thawed wood]", must be qualified. In fact, the results presented in that study concern 5mm-thick samples: It seems hazardous to extrapolate them beyond a given threshold of thickness. Moreover, this threshold should vary with wood MC, as the latter strongly influences the microwaves' penetration depth in the wood (e.g. Torgovnikov 1993; Resch 2009).

Ideally, in order to confirm that some pests could survive after the treatment of frozen thick samples (e.g. 17cm), despite achieving 60°C/60s on their surface, it would have been opportune to perform experiments with living material. Because of their tiny size as well as of their tolerance to high and low temperatures (see Tomminen *et al*. 1991; A. Roques 2011, pers. com.), experiments with the pine wood nematode *Bursaphelenchus xylophilus* would be particularly suitable. The lethal temperature of *B*. *xylophilus*, which was demonstrated to be around 62°C (Fleming *et al*. 2005; Hoover *et al*. 2010), leads us to think that individuals could survive if present in the core of slightly frozen wood pieces (despite the thermal shock they would undergo). In order to be in more "realistic" conditions, future experiments with live quarantine organisms will be more opportune if performed on slightly frozen humid wood (-5°C to -1°C and MC>30%). The question if living individuals remain or not in such samples has however nothing to do with the fact that these samples do not comply with ISPM15 Annex 1.

CONCLUSIONS

The MT has been proved to be successful in a very wide range of situations and, when properly applied, to be as efficient as the conventional heat treatment for eradicating the target organisms. As such, there is no reason to cast doubts on its interest. Besides, taking into account the thickness of the elements they are made of, frozen pallets can be treated without particular attention, and the monitoring of their treatment can be performed as for thawed pallets: 60°C at the surface of the wood warrants higher inside temperatures. Conversely, the treatment of frozen 17cm-thick elements (e.g. dunnage) seems more problematic: in the conditions of our experiments, temperatures much higher than 60°C are needed at the surface of the wood to warrant 60°C at its core. Moreover, since 60°C at the surface of such pieces do not warrant higher core temperatures, their treatment cannot be monitored the same way as for thinner and/or thawed pieces. Additional experiments should thus aim at identifying more accurately the characteristics of the wood (thickness, moisture content and initial temperature) which alter the

"classical" cross-section temperature pattern (i.e. higher core temperatures) and makes 60°C at the surface of the wood an unsuitable benchmark for the treatment monitoring.

In its present form (draft for IPPC signatory countries consultation), Annex 1 of ISPM15 (IPPC 2011) proposes technical guidelines to ensure uniform heating of the wood. These guidelines however are somewhat hazy, and it seems advisable to better characterise the homogeneity of the energy distribution that is required to achieve uniform heating. More concrete guidelines could be based on IEC 61000-4-21 (IEC 2011) which, in another context, accurately describes how to quantify the energy heterogeneity in reverberation chambers and provides thresholds that should not be exceeded. From a broader point of view, this acknowledgement indicates that maybe some gap should be filled between the specialists of electromagnetic energy (physicists) and the technical panels dedicated to the assessment of phytosanitary treatments. When required, it would probably be relevant to associate *International Electrotechnical Commission* members or electromagnetic radiation specialists to the debates around phytosanitary treatments involving such specific and complex matters.

Finally, in the specific context of WPM dielectric heating, analysing the opportunity to use lower than 2.45GHz frequencies, within microwaves and radiofrequencies (RF), should be encouraged. Besides, some patented devices and/or processes aiming at sterilizing organic materials thanks to electromagnetic waves work at 900-1000 MHz (Novak and Burch 2002) or at lower frequencies (Diprose and Evans 1986). Indeed, wavelength influences the energy absorption pattern and therefore the temperature increase throughout the irradiated body (Rattanadecho 2006; Torgovnikov and Vinden 2010). In particular, RF offer several advantages over microwaves, whether regarding the heating of wood (Resch 2009; Watanabe *et al*. 2011; R. Taylor 2010, pers. com.) or their efficacy against pests and pathogens (Wang *et al*. 2003; Hoover *et al*. 2008a). Being nearly operational after decades of research (Thomas and White 1959; Bletchly 1965), the dielectric sanitary treatment of wood still requires, in our opinion, ultimate experiments in order to be fully mastered and properly implemented.

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REFERENCES

Antti A.L. (1992). Microwave drying of hardwood: simultaneous measurements of pressure, temperature, and weight reduction. *Forest Products Journal*, **42**(6), 49-54.

Antti A.L., Perré P. (1999). A microwave applicator for on line wood drying : temperature and moisture distribution in wood. *Wood Science and Technology*, **33**, 123-138.

Barbini S., Jolion M. (1978). Method and device for defreezing frozen projects in which the action of high-frequency radiation is combined with the action of electrostatic spraying of cryogenic liquid. Patent GB 1 534 845. 5 pp. http://v3.espacenet.com/publicationDetails/originalDocument?CC=GB&NR=1534845A&KC=A&FT=D&date=19781206&DB=EPODOC&locale=en_EP. Accessed July 6, 2011.

Bisceglia B., De Leo R., Diaferia N. (2009). MW pallets disinfestations. *Journal of Microwave Power & Electromagnetic Energy* ONLINE, **43**(4), 4-16.

Bletchly J. D. (1965). Very High Frequency Radio Waves and Wood-Boring Insect Control. *Holzforschung*, **19**(2), 47–52.

Diaferia N. (2008). Device for disinfesting and drying wooden packaging material. European Patent Office. European Patent EP 1 600 172 B1. 11 pp. http://www.freepatentsonline.com/EP1600172.pdf. Accessed July 6, 2011.

Diprose M.F., Evans G.H. (1986). Sterilisation of horticultural material. Patent GB 2 166 633. 8 pp. http://v3.espacenet.com/publicationDetails/originalDocument?CC=GB&NR=2166633A&KC=A&FT=D&date=19860514&DB=EPODOC&locale=. Accessed July 6, 2011.

Fleming M.R., Janowiak J.J., Kearns J., Shield J.E., Roy R., Agrawal D.K., Bauer L.S., Miller D.L., Hoover K. (2004). Parameters for scale-up of lethal microwave treatment to eradicate cerambycid larvae infesting solid wood packing materials. *Forest Products Journal*, **54**(7/8), 80-84.

Fleming, M.R., Janowiak, J.J., Kimmel, J.D., Halbrendt, J.M., Bauer, L.S., Miller, D.L., and Hoover, K. (2005). Efficacy of commercial microwave equipment for eradication of pine wood nematodes and cerambycid larvae infesting red pine. *Forest Products Journal*, **55**(12), 226-232.

Fu, Y.-C. (2004). Fundamentals and industrial applications of microwave and radio frequency in food processing. *In*: Smith J.S. & Hui Y.H.: Food processing: Principles and applications. Blackwell Publishing, Ames, Iowa, USA. pp. 79-100. http://club.quomodo.com/tsito21/uploads/128/Food%20Processing%20Principles%20and%20Applications.pdf#page=89 . Accessed July 18, 2011.

Gilbert A.F., Cooper D.F. (2001). A model for thawing frozen wood using microwave energy. *Journal of Pulp and Paper Science*, **27**(1), 34-40.

Gilbert A.F., Turcotte A. (1994). Microwave assisted barking of frozen wood. *Canadian Journal of Chemical Engineering*, **72**, 920-925.

Henin J.-M., Charron S., Luypaert P.J., Jourez B., Hébert J. (2008). Strategy to control the effectiveness of microwave treatment of wood in the framework of the implementation of ISPM 15. *Forest Products Journal*, **58**(12), 75-81.

Hoover K., Nehme M., Janowiak J. (2008a). Status of microwaves and radiofrequency as alternative treatments for solid wood packing materials. *In* MacManus K. & Gottschalk K.W.: Proceedings of the 19th U.S. Department of Agriculture Interagency Research Forum on Invasive Species, January 8-11th, 2008, Annapolis, Maryland, USA. U.S.D.A. General Technical Report NRS-P-36. 100 p. http://nrs.fs.fed.us/pubs/gtr/gtr_nrs-p-36.pdf#page=45. Accessed July 6, 2011.

Hoover K., Nehme M., Janowiak J. (2008b). Microwave treatment of insect-infested wood. *In*: Alien invasive species and international trade. 2nd meeting of IUFRO Unit 7.03.12, 2008 May 26-30th, Shepherdstown, West Virginia, USA. http://www.forestresearch.gov.uk/pdf/IUFRO_Shepherdstown_Hoover_Microwaving_wood.pdf/$FILE/IUFRO_Shepherdstown_Hoover_Microwaving_wood.pdf. Accessed July 6, 2011.

Hoover K., Uzunovic A., Gething B., Dale A., Leung K., Ostiguy N., Janowiak J.J. (2010). Lethal temperature for pinewood nematode, *Bursaphelenchus xylophilus,* in infested wood using microwave energy. *Journal of Nematology*, **42**, 101-110.

IEC – International Electrotechnical Commission (2011)**.** Electromagnetic compatibility (EMC) 61000-4-21: Testing and measurement techniques - Reverberation chamber test methods. International Electrotechnical Commission, Geneva : 224 pp.

IPPC – International Plant Protection Convention (2009). International Standards for Phytosanitary Measures: Revision of ISPM No. 15. Regulation of wood packaging material in international trade. Food and Agriculture Organisation of the United Nations (FAO), Rome (IT). 18 pp.

IPPC – International Plant Protection Convention (2010). Report of the meeting of the Technical Panel on Phytosanitary Treatments, 26-30 July 2010, Kyoto, Japan. 45 pp. https://www.ippc.int/file_uploaded/1303371852_Report_TPPT_2010Kyoto_2011-04-21.pdf. Accessed July 8, 2011.

IPPC – International Plant Protection Convention (2011). Draft revision to Annex 1 of ISPM 15:2009. Approved treatments associated with wood packaging material. 6pp. https://www.ippc.int/file_uploaded/1308672634_2006-011_DraftISPM_ISPM15Annex1_.doc Accessed August 26, 2011.

Koubaa A., Perré P., Hutcheon R., Lessard J. (2008). Complex Dielectric Properties of the Sapwoods of Aspen, White Birch, Yellow Birch and Sugar Maple. *Drying Technology*, **26**(5), 568-578.

Lundgren N., Hansson L., Hagman O., Antti L. (2006). FEM Simulation of interactions between microwaves and wood during thawing. AIP 2nd Conference on Mathematical Modeling of Wave Phenomena, August 14-19, 2005, Vaxjo, Sweden, AIP Conf. Proc. 834 , 260-267.

Miura M., Kaga H., Sakurai A., Kakuchi T., Takahashi K. (2004). Rapid pyrolysis of wood block by microwave heating. *Journal of Analytical and Applied Pyrolisis*, **71**, 187-199.

Novak J.F., Burch M.R. (2002). Method and apparatus for microwave utilization. WIPO, Patent WO 02/14764. 39 pp. http://v3.espacenet.com/publicationDetails/originalDocument?CC=AU&NR=2001286498B2&KC=B2&FT=D&date=20050407&DB=EPODOC&locale=. Accessed July 6, 2011.

Oloyede A., Groombridge P. (2000). The influence of microwave heating on the mechanical properties of wood. *Journal of Materials Processing Technology*, **100**, 67-73.

Purcell, E.M. (1973). Electricité et magnétisme. Berkeley: cours de physique, volume 2. Librairie Armand Colin, Paris. 460 pp.

Rattanadecho P. (2004). Theoretical and experimental investigation of microwave thawing of frozen layer using a microwave oven (effects of layered configurations and layer thickness). *International Journal of Heat and Mass Transfer*, **47**, 937-945.

Rattanadecho P. (2006). The simulation of microwave heating of wood using a rectangular wave guide: Influence of frequency and sample size. *Chemical Engineering Science*, **61**, 4798-4811.

Rémond R., Perré P. (2008). High-frequency heating controlled by convective hot air: toward a solution for on-line drying of softwoods. *Drying Technology*, **26**, 530-536.

Resch H. (2009). Drying wood with high frequency electric current. University of Natural Resources and Applied Life Sciences, Vienna, Austria. 84 pp.

Taher B.J., Farid M.M. (2001). Cyclic microwave thawing of frozen meat: experimental and theoretical investigation. *Chemical Engineering and Processing*, **40**(4), 379-389.

Thomas A., White M. (1959). The sterilization of insect-infested wood by high-frequency heating. *Wood*, **24**, 407-410.

Tomminen J., Halik S., Bergdahl D.R. (1991). Incubation temperature and time effects on life stages of *Bursaphelenchus xylophilus* in wood chips. *Journal of Nematology*, **23**(4), 477-484.

Torgovnikov G.I. (1993). Dielectric properties of wood and wood-based materials. Springer-Verlag, Berlin. 196 pp.

Torgovnikov G., Vinden P. (2010). Microwave wood modification technology and its application. *Forest Products Journal*, **60**(2), 173-182.

Wang S., Tang J., Johnson J.A., Mitcham E., Hansen J.D., Hallman G., Drake S.R., Wang Y. (2003). Dielectric properties of fruit and insect pests as related to radio frequency and microwave treatments. *Biosystems Engineering*, **85**(2), 201-212.

Watanabe K., Abubakari A., Lazarescu C., Avramidis S. (2011). Softwood heating in radio frequency fields. *Holz als Roh und Werkstoff*, **69**(2), 295-301.

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Section 4 **Processes and properties**

Wood Aging
Characteristics of aged Hinoki wood from Japanese historical buildings

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Wood Aging
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ABSTRACT

Wood has always played a major role in Japanese traditional culture. More than 90% of buildings listed as a National property or a nationally important cultural property of Japan are constructed with wood. In the ancient capitals Kyoto and Nara, many traditional wooden buildings were inscribed as World Cultural Heritage of the UNESCO. The most famous and the world's oldest wooden construction still standing is Horyu-ji temple from the latter half of the seventh century.

Wood is present in many cultural heritage objects thanks to its capacity to resist over long period of time. However, the evolution of its properties in regular use remains insufficiently known. The present study on the effect of wood aging takes advantage of the Japanese context where building traditions have been maintained for centuries.

One major difficulty for the research on "aging of wood" is the gathering of suitable samples, with well-defined origin, certified dating and permission of publication by conservation administration. The Japanese context, where traditional uses of wood have been maintained for more than 1600 years, offers a unique opportunity to address the question of wood aging. Since 2004, the wood samples from various temples and other historical buildings were being gathered by the Research institute for Sustainable Humanosphere, Kyoto University, Japan.

The matching of specimens from different origins is another typical obstacle. Wood is a variable material due to genetic variations and dependency on growing conditions of the trees. To discuss property changes due to aging, a recent reference is required. However, it is difficult and sometimes impossible to obtain recent wood that closely matches a given old wood sample. To overcome the difficulty, thermally treated wood as an accelerated aging can be used to produce corrections that will allow comparing data from slightly mismatched samples. Thermally treatment ware performed at 90, 120, 150, and 180℃ for various periods on new hinoki wood from Kiso area.

This paper deals with mechanical characteristics of aged hinoki (*Chamaecyparis obtuse* Endl.) wood from Japanese historical buildings and thermally treated hinoki wood, especially their Young's modulus, rapture energy and hygroscopisity. It is not only for the basic science study on aging of wood by using unique and indigenous Japanese hinoki wood, but also for the commonality and universality of worldwide wooden cultural assets. This research will have a positive role on preservation and conservation of wooden cultural properties in the world.

Keywords: wood aging, mechanical properties, Japanese historical buildings, hinoki (*Chamaecyparis obtusa* Endl)

1. INTRODUCTION

At the east end of the Silk Road, the islands called “The land of the rising Sun” makeup Japan. Many historical buildings over 1200-years-old still exist in Japan. The ancient capitals Kyoto and Nara have many traditional wooden buildings, some of which are also listed on the UNESCO World Cultural Heritage register. It is believed that the traditional architecture in Japan, for example, Buddhist temples, Shinto shrines and castles, is exclusive to the Japanese archipelago. Japanese architecture has many similar features to Chinese or Korean architecture, although the methodology of restoration of historic buildings is very different.

The Japanese government has been restoring and conserving historic buildings for110 years. Principally the restoration practice for historic buildings in Japan is characterized by its adherence to original techniques and materials, and also for its minimum replacement of members by new ones. As a result, it is represented by a selection of methods, which preserve the buildings for the future, without altering their structures and designs.

However it is not sufficient to apply recent scientific methods to understand these traditional techniques and materials. Since 2004, a collection of wood samples from various temples and other historical building has been gathered by at the Research Institute for Sustainable Humanosphere, Kyoto University, Japan (RISH). The aim of the project is to stimulate mutual cooperation between wood scientists and architectural conservators and to improve relations between them.

Wood is a material designed by nature to last, provided it is not attacked by biological agents. It can support trees for centuries, and as a technological material it can again sustain loads for considerable periods. It is, as a consequence, a major component of the cultural heritage of many civilizations and the assessment of wood properties from ancient objects and structures is a question of fundamental and practical interest [D. Fengel, 1991].

One major difficulty for such research is the gathering of suitable samples, with well-defined origin, certified dating and permission of publication by conservation administration. The Japanese context, where traditional uses of wood have been maintained for more than 1600 years, offers a unique opportunity to address the question of wood aging. Wood has always played a major role in Japanese culture. More than 90% or the nationally important properties of Japan are constructed with wood. The most famous and the world’s oldest wooden construction still standing is Horyu-ji temple from the latter half of the seventh century. Since 2004, a collection of wood samples from various temples and other historical building has been gathered by the Research Institute for Sustainable Humanosphere of the Kyoto University (Japan), expanding a collection gathered in the 1950s by Jiro Kohara [1958].

The matching of specimens from different origins is another typical obstacle. Wood is a variable material due to genetic variations and dependency on growing conditions of the trees. To discuss property changes due to aging, a recent reference is required. However, it is difficult and sometimes impossible to obtain recent wood that closely matches a given old wood sample. To overcome this difficulty, well-established parameters relating to the structure and properties of wood can be used to produce corrections, thus allowing the comparison of data from slightly mismatched samples.

Kohara [1958] reported that the bending strength and rigidity of aged Hinoki wood, used in temple structures for over 1300 years, initially increased for a few hundred years and then subsequently decreased with time. This paper presents new results obtained on similar materials. Representative samples of wood that were free from biological attack, weathering and visible damage were selected. Thus the properties measured reflect the intrinsic aging of the material, resulting from the long-term action of moderate mechanical stress, temperature and humidity fluctuation, and air oxidation [D. Fengel. 1991]. The results of mechanical testing of specimens of increasing age will be presented and discussed in relation to the possibility to predict the consequence of natural aging on wood properties.

2. EXPERIMENTAL METHODS

Sample origins

The aged samples were Hinoki (*Chamaecyparis obtusa*) wood from Japanese historical buildings, mostly Horyu-ji temple in Nara. The specimens used in this study were cut from aged wooden members provided from the restoration sites, which were not reused. The modern wood used for comparison was taken from a 360 year old tree from Kiso region, where the highest quality Hinoki has been grown for the last 3 centuries, and selected according of craftsman viewpoint. It was cut in 1988 and had been subjected to slow drying for 19 years before testing in 2006. Sample labelling, origin, and basic structural information are given in Table 1. To avoid the effect of UV degradation and insects, the outer layers and nails were removed, and the specimens for mechanical testing were taken from the central portion of the samples. No sapwood occurrence was detected, so that all the studied material consisted of heartwood.

Age determination

To evaluate wood age, radioactive carbon dating ^{14}C and dendrochronology were used. For each sample the wood was processed as a board containing more than 60 tree rings. Tree ring dating was performed by comparing these ring patterns with a standard pattern available for Hinoki as far back as 912 BC [Mitsutani,T.,1990]. Distinct ring patterns of Hinoki enabled dating with yearly precision. For precision dating, ^{14}C wiggle-matching method was applied [Imamura, M., et al. 2007]. As shown in Table 1, the agreement between both methods was good: the difference between ^{14}C and dendro-date ranged from -40 to 29 years. These methods can only provide information about the wood age, defined as the time elapsed since wood formation in the tree. The analysis of colour variations in the same samples suggested that most of the aging occurred after wood processing [Yokoyama, M., 2009], so that the period of time separating wood formation and tree felling should be subtracted from the wood age for the analysis of aging processes. However, in most cases this information is not available, and the time elapsed since tree felling (t_T) cannot be calculated. For the subsequent analysis, an upper bound of t_T (time elapsed since tree felling) will be considered, based on the newest visible ring on the sample. For the most recent historical sample H and the reference I, this gives a direct estimate as the bark was included in the sample. For the older samples the relative error is likely to be small. In the following, this estimate of t_T will be designated as the “age” of the sample.

Table 1: Origin and dating of the samples [5)]

	Collection	Origin	Block dimensions (R x T x L, cm)	RW (mm)	Dendro chronology* (AD)	^{14}C interval dating* (AD)	t_W (yrs)	t_T (yrs)
A	KYOw2701, RISH	HYJ	11 x 3.4 x 10	0.8	343 / 434	367 / 458	1618	1583
B	KYOw2738, RISH	HYJ	7.0 x 4.2 x 10	0.5	458 / 612	418 / 572	1467	1405
C	private	HYJ (leg)	6.7x 11.5 x 47	0.9	400 /502	418 / 520	1548	1515
D	private	HYJ (leg)	7.5 x 11.5 x 55	0.8	431 /537	421 / 527	1530	1480
E	private	HYJ (leg)	9.5 x 13 x 42	0.7	584 / 792	587 / 795	1319	1225
F	private	HYJ (leg)	5 x 7.8 x 52	1.0	1029 / 1086	1000 /1059	899	931
G	private	HYJ (leg)	2.5 x 14 x 58	0.8	1106 / 1270	1098 /1262	822	747
H	(temple donation)	SJJ	1100 (∅) x 30 (L)	0.8	1069 / 1438	1071 / 1438	753	569
I	(workshop)	Kiso forest		1.0	1622 / 1988	1631 / 1973	200	19

HYJ = Horyuji temple, Nara ; (leg) = legendly ; SJJ = Senjyuji temple, Mie ; RW =average width of annual rings ; * dates (A.D.) of first/last measured growth ring ; t_W = mean time elapsed since wood formation in the measured portion ; t_T = time elapsed since tree felling (estimated upper bound for samples A to G) ; t_W and t_T are estimated from dendro-dating.

Bending test

Wood is a highly anisotropic material, much more rigid and strong along fibres (longitudinal direction, L) than across fibres (radial direction, R, or tangential, T). Although the loading of beams is dominantly applied in L direction, in the connections parts a complex stress state occurs and the response to transverse loading may become critical. For that reason, 3 points bending tests were performed not only in L, but also in R direction whenever enough material was available. Matched specimens of dimensions 60 mm (L) × 10 mm (R) × 2 mm (T) were cut for L tests, 60 mm (R)×10 mm (T)× 2 mm (L) for R tests. The samples were initially dried at room temperature for 3 weeks in a desiccator with silica gel, then conditioned at 20°C and 60% relative humidity (R.H.). They were weighed before and after the tests performed in the air-dry condition, then oven dried at 60°C, 24 hours at atmospheric pressure and 24 hours in vacuum in presence of P_2O_5, and weighted again to calculate the moisture content during the test, as well as the oven-dry density and the air-dry density. The tests in L and R directions were performed on 5 to 10 specimens per sample and loading direction, with span length 50 mm and crosshead speed 5 mm/min.

3. RESULTS AND DISCUSSION

Stress-strain relationship

Fig. 1 shows typical stress-strain curves in L and R bending for each aged sample. In the following, the index L and R will be used to distinguish the value for each loading direction.

Our range of air dry density, 0.33~0.49 g/cm³ or of oven dry density, 0.32~0.46 g/cm³, almost covers that of modern hinoki [*Mokuzai Kogyou Handbook*. 2004, Imamura, H., et al. 1983]. As a general trend, aged wood appeared stiffer (higher E) and stronger (higher σ^m), at least in L direction, than the modern wood tested. As will be discussed below, this can be partly explained by differences in density and moisture content. The post-linear behaviour of aged wood, on the other hand, was clearly more brittle than in modern wood: this increase in brittleness, apparent from the curves of Fig. 1. All aged R specimens exhibited a fragile response, so that the elastic limit ε^e could only be estimated for the recent wood (sample I).

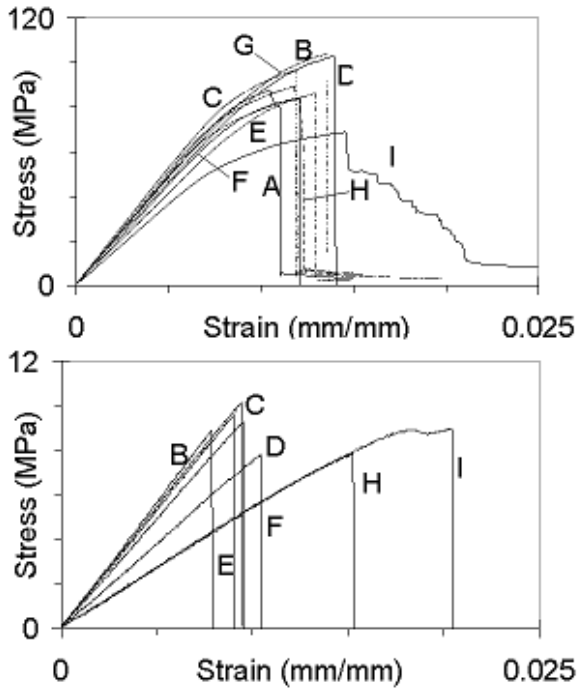


Fig.1 Typical stress-strain curves[5)]
Upper: L direction / Lower: R direction

Strain parameters

Fig.2 shows the effect on strain parameters, for which no correction was tried: the elastic limit (ε^e) in L direction and the breaking strain (ε^m) in L and R directions. A slight increase of ε^e_L, a slight decrease of ε^m_L, and a drastic decrease of ε^m_R were observed.

The difference of behaviour between L and R directions can be explained by the structural organization of wood fibres.

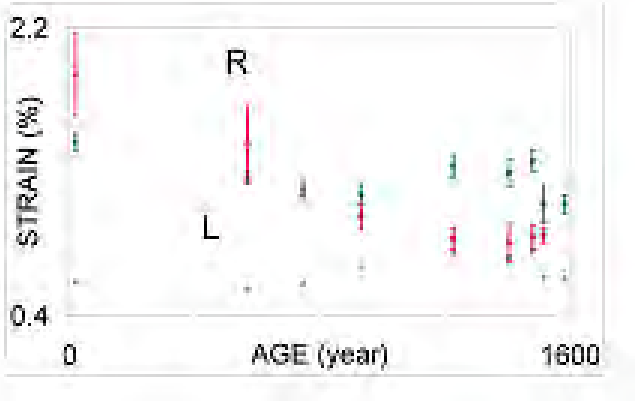
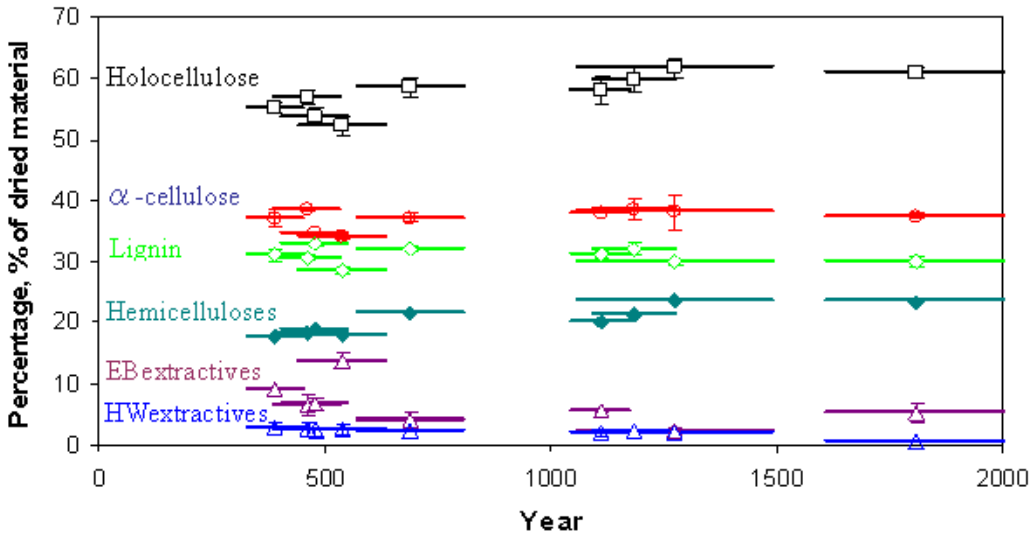


Fig.2 Relationships between strain and age. [5)]

The effect of aging on mechanical properties

Chemical analysis and thermo-mechanical testing performed in parallel to the present study. Fig.3 evidenced a decrease of hemi-cellulose content [Ragil, W., et al, 2007], as well as an increase of lignin cross-linking [Yokoyama, M., et al., 2007]. Similar observations were previously made on other aged wood by using FTIR [Takei, T., et al., 1997]. Hemicelluloses ensure the transverse cohesion between cellulosic microfibrils and lignin matrix, so that their degradation, especially in S_2, is also more detrimental to R than to L direction. The increase of cross-linking would increase the brittleness of lignified parts, especially in the middle lamella, thus accounting for the drastic decrease of R toughness without inducing any drop in rigidity [Yokoyama, M., et al, 2009].



Application for the restoration of wooden Buddhist sculpture

Fig.4 An application for the restoration of a Japanese Buddhist sculpture.
(a) "Yakushinyorai" of Okubodera temple built in the 8th century. This Buddhist sculpture was restored by Mr. Kenichiro Yano.
(b) Wooden curled hair after accelerated aging treatment, prepared to replace a loss.
©Misao Yokoyama

Hinoki (*Chamaecyparis obtusa*) specimens were subjected to the heat treatment 180℃, 150℃, 120℃ and 90℃ respectively under various treatment time by normal oven method. An accelerated aging test was performed by heat treatment to obtain different levels of accelerated aging wood samples. A comparison will be made between mechanical and chemical properties of naturally aged wood and that of heat-treated wood [Matsuo, M., et al, pending]. These results can be used as the standards in comparison with the actual naturally aged wood materials for application for the restoration of wooden artefacts.

Fig.4 is an example of an application of this basic research for the restoration of a Japanese Buddhist sculpture made from hinoki (*Chamaecyparis obtusa* Endl). These Rahotsu, curled hair on the Buddhist

head, was missing before restoration [Fig.4 (a)] and some new wooden curled hairs were made by the restorer, Mr. Kenichiro Yano. Before using the wood for restoration, the wooden curled hair was subjected to heat treatment to adjust to the condition suitable for the restoration of the Buddhist sculpture. Fig.4 (b) shows the wooden curled hairs in an antique-like finish following heat accelerated aging.

4. CONCLUSIONS

As a practical consequence, the results obtained suggest that ancient wood can be considered safe as long as it is not subject to unusual action perpendicular to the grain. The existence of large wooden structures dating back more than 1200 years is the clearest confirmation of that statement.

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6.REFERENCES

Fengel, D.. 1991. Aging and fossilisation of wood and its components. *Wood Science Technol*ogy. 25:153-177

Kohara J. 1958. Study on the old timber. *Research Report of the faculty of engineering*, Chiba University 9 (15) 1-55 (in Japanese).

1990. *National research Institute for Cultural properties, Dendrochronology in Japan, Nara. Bulletin* 48, Dohosya press (in Japanese).

Imamura, M., et al. 2007. Radiocarbon wiggle － matching of Japanese historical materials with a possible systematic age offset. *Radiocarbon*. Vol.49, No.2, 331-337.

Yokoyama, M., Gril, J., Matsuo, M., Yano, H., Sugiyama, J., Clair, B., Kubodera, S., Mitsutani, T., Sakamoto, M., Ozaki, H., Imamura, M., Kawai, S. 2009. Mechanical characteristics of aged Hinoki wood from Japanese historical buildings. *Computes Rendus Physique*. 10, pp 601-611.

2004. Forestry and Forest Products Research Institute. *Mokuzai Kogyou Handbook.* Maruzen Press (in Japanese)

Imamura, H., et al. 1983. *Mokuzai riyou-no kagaku*. Kyoritsu Press, p 38 (in Japanese)

Ragil, W., Yokoyama, M., Kubodera, S., Mitsutani, T., Sakamoto, M., Ozaki, H., Imamura, M., Kawai, S. 2007. Evaluation of aged wood from historical Japanese buildings – Changes in Chemical component. *Proceedings of the 57th Annual meeting of the Japanese Wood Research Society*

Yokoyama, M., et al. 2007. Evaluation of aged wood from historical Japanese buildings Ⅰ - Changes in Mechanical properties of Hinoki wood. *Proceedings of the 57th Annual meeting of the Japanese Wood Research Society*

Takei, T., et al. 1997. Fourier Transform Infrared Spectroscopic Analysis of the Degradation of Structural Lumber in Horyu-ji Temple. *Mokuzai Gakkaishi* 43(3), 285-294(in Japanese)

Matsuo, M., Yokoyama, M.., Umemura, K., Gril, J., Yano, K., Kawai, S. (pending). Color changes in wood during heating: kinetic analysis by applying a time-temperature superposition method. *Applied Physics* A.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 4 **Properties of treated wood**

Development of a quality control assessment method to predict properties of heat treated wood

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ABSTRACT

Heat treatment has been used to improve properties of non durable European species. Chemical modification of some of the wood components provides improved dimensional stability and biological performance against decay fungi while mechanical properties such as modulus of rupture are reduced. Quality control of commercially made thermally treated wood is one of the major challenges to allow its industrial development. The variability inherent within wood specie and between wood species, density and chemical compositions variation combined with the heat treatment parameters such as temperature duration and levels contribute to the production of heterogeneous heat treated wood. The development of a heat treatment process by conduction which monitors the weight changes during the process will facilitate and help in controlling, in understanding and predicting the properties of heat treated wood. Data collected from a pilot study of heat treated wood using conditions similar to commercial process show that hardwood species were more susceptible to thermal degradation as compared to softwood. It was also established that wood chemical composition is directly connected to the percent of weight loss due to thermodegradation, allowing the use of chemical composition to predict fungi durability. Carbon and oxygen contents and/or oxygen to carbon ratio of heat treated wood can be therefore used as valuable markers to develop quality control assessment of heat treated wood.

Keywords: heat treatment, thermodegradation, mass loss, elemental composition, durability, treatment intensity, quality assessment, wood.

1. INTRODUCTION

The use of wood as building material is subjected to increasing interest due to its intrinsic properties and its ability to fix carbon dioxide. Wood heat treatment by mild pyrolysis has been reported to improve some of its properties such as its biological durability and its dimensional stability. This process avoids the use of wood preservatives constituting an attractive "non biocidal" alternative to classical preservation treatments. Indeed, even if heat treatment generated some volatile organic compounds and waste water having strong acidic and corrosive effect, it remains globally relatively safe for the environment. The influence of treatment conditions on chemical composition and conferred properties of heat-treated wood have therefore been intensively studied. Wood heat treatment induces chemical modifications of the main wood cell wall components. The lignin network is modified (Alen *et al*. 2002, Tjeerdsma and Militz 2005, Nguila *et al*. 2006, Nguila *et al*. 2007a, Esteves *et al*. 2008), the ratio between amorphous and crystalline cellulose is also changed (Fengel and Wegener 1989, Sivonen *et al.* 2002, Yildiz *et al.* 2006), hemicelluloses are strongly degraded,(Sivonen *et al.* 2002, Nuopponen et al. 2004) leading to formation of carbonaceous material within the wood (Nguila *et al*. 2007b). These chemical modifications confer to the material new properties such as improved dimensional stability (Mouras *et al*. 2002, Esteves *et al*. 2007), improved fungal resistance (Kamdem *et al*.

2002, Hakkou *et al*. 2006, Shi *et al.* 2007), lower affinity for water due to increase of wood surface hydrophobicity (Kamdem *et al.* 2002, Pétrissans *et al.* 2003, Hakkou *et al.* 2005, Gérardin *et al.* 2007, Kocaefe *et al.* 2008). Different heat treatment conditions have been described in the literature (Militz 2002, Patzelt *et al.* 2002). Among these, the wood torrefaction corresponds to heat treatment carried out at temperatures inferior to 300 °C under inert gaseous atmospheres. Chemical reactions involved during torrefaction as well as final properties of the material depend strongly on the treatment temperature and of its duration. During torrefaction, wood is thermally decomposed at a slow rate (Degroot *et al.* 1988). Recently, it was reported that the anhydrous mass loss during the heat treatment could be a reliable and accurate marker to predict decay resistance of heat-treated wood (Hakkou *et al.* 2006, Welzbacher *et al.* 2007). Hence, the control of the mass of the material during the heat treatment process will allow predicting its final durability. Due to the importance of dehydration reactions occurring during the heat treatment, the behaviour of carbon and oxygen contents have been evaluated through determination of wood's elemental composition and reactivity of free hydroxyl groups with acetic anhydride (Nguila *et al*. 2007a). Indeed, previous studies have shown that heat treatment resulted in numerous dehydration reactions due to degradation of amorphous polysaccharides (Fengel and Wegener 1989, Sivonen *et al.* 2002, Yildiz *et al.* 2006) jointly with the formation of carbonaceous materials within the wood structure leading to a strong decrease of wood's O/C ratio ((Nguila *et al*. 2006, Nguila *et al*. 2007b). Moreover, dehydration reactions occurring during heat treatment have been reported to be at the origin of the lower reactivity of hydroxyl groups of wood cell wall polymers during acetylation (Tjeerdsma and Militz 2005, Nguila *et al*. 2007a). The aim of this communication is to make an overview of different papers already published concerning the utilization of wood elemental composition to develop a quality control assessment method to predict properties of heat treated wood (Nguila et al., 2009; Šušteršic et al., 2010, Chaouch et al., 2010). We will describe in a first time the correlations existing between O/C ratio obtained for different heat treatment temperatures and mass losses due to the thermo-degradation reactions before to investigate, in a second time, the correlations with durability against fungal decay. Finally, the study was extended to evaluate the correlations between the improvement of the durability of different softwood and hardwood species and the mass losses induced by the heat treatment estimated on the basis of their O/C ratio. The study leads to conclude that carbon content and O/C ratio can be used to predict wood durability conferred by heat treatment.

2. MATERIALS AND METHODS

2.1 Materials

Samples were cut from heartwood boards of different softwood and hardwood species: beech, *Fagus sylvatica L.*; poplar, *Populus nigra*; ash, *Fraxinus excelsior L.*; pine, *Pinus sylvestris* and Silver fir, *Abies pectinata*.

2.2 Heat treatment

Two experimental devices were used in this work. The first one is a Shimadzu GC-14A oven. Wood specimens with dimensions 10×20×50 mm^3 (R, T, L) were oven dried at 103°C for 48 hours and subjected to heat treatment at temperatures of 220, 240 and 250°C under nitrogen for different times to reach the desired mass losses. The oven temperature was increased by 20°C min-1 from ambient to final temperature. Temperature accuracy was estimated to ± 1% of the set temperature (absolute temperature). Mass loss due to chemical degradation during heat treatment was calculated according to the formula:

$$ML\ (\%) = 100 \times (m_0 - m_1) / m_0 \qquad (1)$$

where m_0 is the initial oven dried mass of the sample before heat treatment and m_1 the oven dried mass of the same sample after heat treatment. Two series of experiments (experiment 1 and 2) were done at different times to check repeatability of the measurements.

In the second device heat treatment was performed under nitrogen on the different species by conduction between two metallic heating plates placed on a precision balance allowing recording of dynamic mass loss as a function of time and temperature (Fig. 1). Dimension of the boards used for heat treatment were 2.5×11×25 cm^3 (R, T, L). Wood specimens were oven dried at 103°C during 48 h prior to heat treatment. Weight of the sample was recorded automatically all along the experiment and mass loss due thermo-degradation calculated using Eq. 1

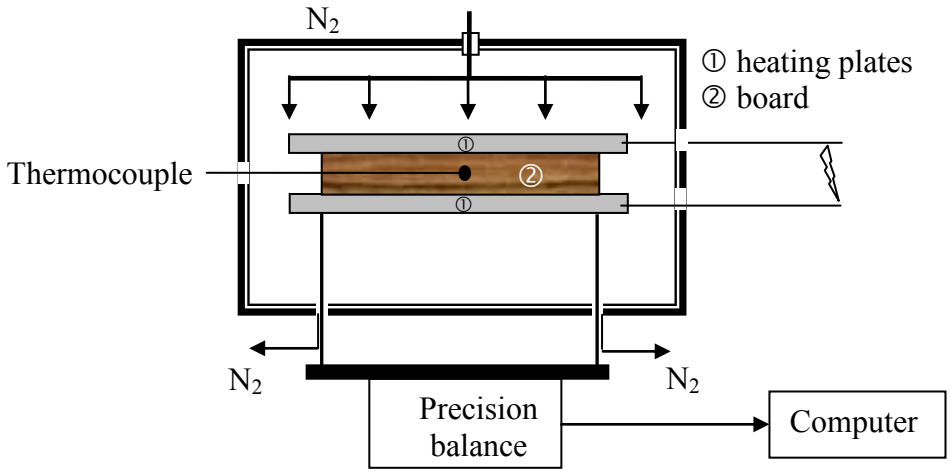

Figure 1: Schematic equipment used for heat treatment

2.3 Microanalysis

Wood was grounded to fine sawdust and passed through different sieves to obtain a powder of granulometry comprised between 0.2 and 0.5 mm. Sawdust was conditioned at 103°C for 24 h. and stored in closed bottle before analysis. Elemental analyses were performed on a Thermofinnigam Flash EA1112 micro-analyser.

2.4 Fungal durability

Wood blocks of 5×10×30 mm^3 were cut from heat treated boards for fungal durability evaluations. Petri dishes (9 cm diameter) were filled with sterile medium (20 mL) prepared from malt (40 g) and agar (20 g) in distilled water (1 L) and inoculated with a piece of mycelium of a freshly grown *Poria placenta* culture. Petri dishes were incubated at 22°C and 70% HR until full colonization of the surface's medium by the mycelium. Two heat treated specimens and one untreated sample were placed in each Petri dishes and exposed to the brown rot fungus for 16 weeks. Each experiment was duplicated. At the end of test period, mycelia were removed and the blocks were dried at 103°C and weighed (m_2) to determine the weight loss caused by the fungal decay:

$$WL\ (\%) = 100 \times (m_{0\ or\ 1} - m_2) / m_{0\ or\ 1} \qquad (2)$$

where $m_{0\ or\ 1}$ are respectively the initial oven dried mass of untreated or heat treated wood blocks before fungal exposure and m_2 is the oven dried mass after fungal attack.

3. RESULTS AND DISCUSSIONS

3.1 Influence of the heat treatment temperature

Influence of the heat treatment temperature on wood elemental composition and on its decay durability was investigated in two previous papers (Nguila et al., 2009; Šušteršic et al., 2010).
Heat treatment was performed on Scots pine sapwood (*Pinus sylvestris L.)* in the first device (Shimadzu GC-14A oven). Evolution of mass losses as a function of treatment time for different temperatures is graphically presented in Fig. 2. The results showed that mass loss is directly related to treatment severity, which depends on the treatment duration and the temperature. An interesting point to note is that a same mass loss can be obtained at shorter treatment duration with higher temperature or by using longer treatment time with lower temperature.

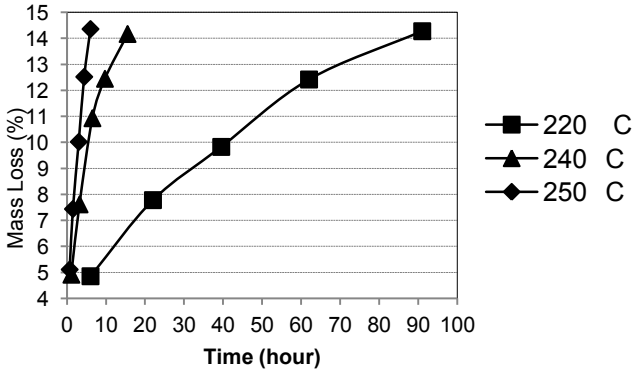

Figure 2: Evolution of mass losses according to treatment temperature

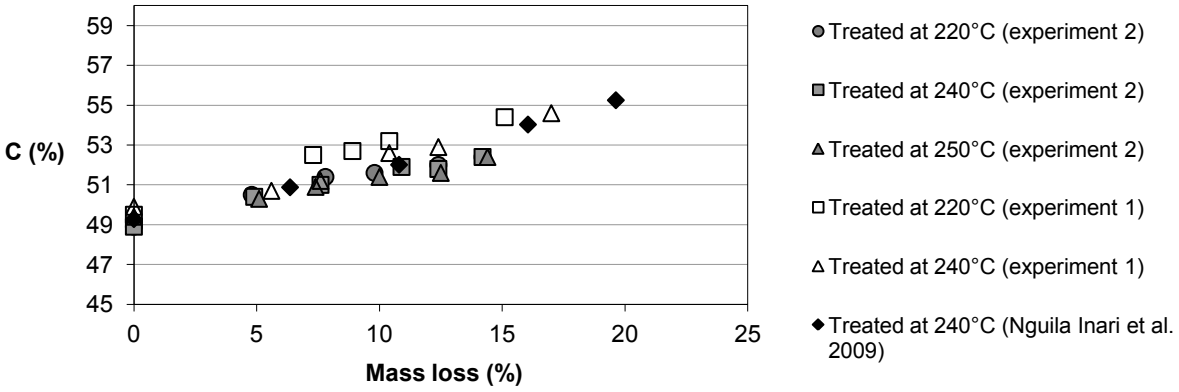

Figure 3: Correlation between mass loss resulting from different heat treatment conditions and C%

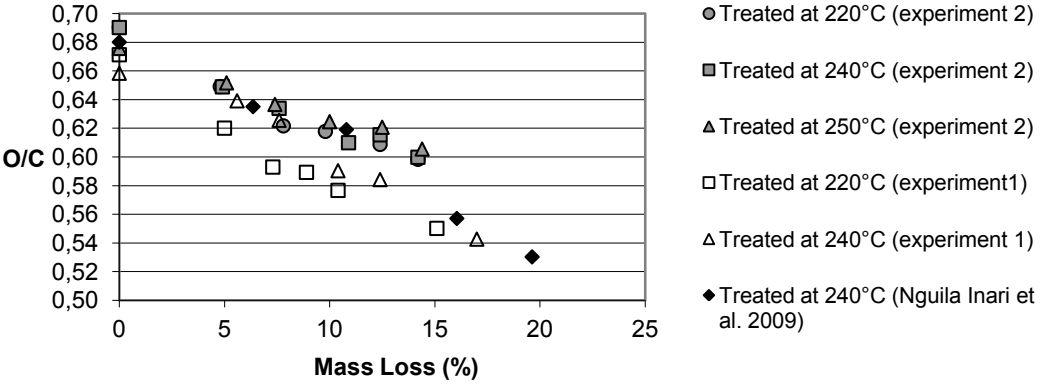

Fig. 4: Correlation between mass loss resulting from different heat treatment conditions and O/C ratio

Table 1: Elemental composition of wood according to different heat treatment temperatures

Experiment	Mass Loss (%) [a]	C (%)	H (%)	O (%)	O/C [b]
Experiment 1, 220°C	0	49.5	6.2	44.3	0.67
Experiment 1, 220°C	5.0	51. 4	6.0	42.6	0.62
Experiment 1, 220°C	7.3	52.5	6.0	41.5	0.59
Experiment 1, 220°C	8.9	52.7	5.9	41.4	0.59
Experiment 1, 220°C	10.4	53.2	5.9	40.9	0.58
Experiment 1, 220°C	15.1	54.4	5.7	39.9	0.55
Experiment 1, 240°C	0	49.9	6.3	43.8	0.66
Experiment 1, 240°C	5.6	50.7	6.1	43.2	0.64
Experiment 1, 240°C	7.6	51.2	6.1	42.7	0.63
Experiment 1, 240°C	10.4	52.6	6.0	41.4	0.59
Experiment 1, 240°C	12.4	52.9	5.9	41.2	0.58
Experiment 1, 240°C	17.0	54.6	5.9	39.5	0.55
Experiment 2, 220°C	0	48.9	6.1	45.0	0.69
Experiment 2, 220°C	4.8	50.5	5.8	43.7	0.65
Experiment 2, 220°C	7.8	51.4	6.0	42.6	0.62
Experiment 2, 220°C	9.8	51.6	5.9	42.5	0.62
Experiment 2, 220°C	12.4	52.0	5.8	42.2	0.61
Experiment 2, 220°C	14.2	52.4	5.8	41.8	0.60
Experiment 2, 240°C	0	48.9	6.1	45.0	0.69
Experiment 2, 240°C	4.9	50.4	6.0	43.6	0,65
Experiment 2, 240°C	7.6	51.0	5.9	43,1	0,63
Experiment 2, 240°C	10.9	51.9	5.8	42.2	0,61
Experiment 2, 240°C	12.4	51.8	5.7	42.5	0,61
Experiment 2, 240°C	14,1	52.4	5.7	41.9	0,60
Experiment 2, 250°C	0	49.4	6.1	44.5	0.68
Experiment 2, 250°C	5.1	50.3	6.0	43.7	0.65
Experiment 2, 250°C	7.4	50.9	5.9	43.2	0.64
Experiment 2, 250°C	10.0	51.4	5.8	42.8	0.62
Experiment 2, 250°C	12.5	51.6	5.7	42.7	0.62
Experiment 2, 250°C	14.4	52.4	5.7	42.3	0.61

[a] mass loss due to thermal treatment
[b] atomic ratio

Elemental composition of Scots pine sapwood blocks treated under different time and temperature conditions for more or less similar mass losses are reported in Table 1 and Fig. 3. For comparison purposes, previously published data were also added (Nguila *et al.* 2009). The results revealed that carbon content increases significantly with heat treatment intensity, while oxygen and hydrogen contents decrease. Independently of the treatment conditions, evolution of carbon content is strongly correlated with wood mass losses. Moreover, the elemental compositions recorded for a given mass loss obtained after more or less prolonged heat treatment time at a selected temperature, are quite similar. These results clearly indicated that no significant differences can be detected between the different treatment conditions. Even if the

kinetic of the reactions involved in thermo-degradation processes may slightly differ according to treatment temperatures influencing activation energies, observed mass losses remain quite similar in the range of the tested temperatures. The relation between mass loss and O/C ratio is illustrated in Fig. 4. For each heat treatment conditions, O/C ratio decrease linearly with the increase of the mass loss indicating that O/C ratio could be a valuable marker of treatment intensity. This decrease can be mainly attributed to thermal degradation of wood polysaccharides. Indeed, it is reported that hemicelluloses are easily deacetylated to form acetic acid catalysing dehydration and depolymerization reactions leading to anhydromonosaccharides like furfural or levoglucosan (Boonstra and Tjeerdsma 2006, Nguila *et al.* 2006, Nguila *et al.* 2007b). As a results of these degradations reactions, wood O/C ratio decrease significantly (Nguila *et al.* 2006, Nguila *et al.* 2009). Even if the behaviours recorded for each treatment condition were not completely similar, the tendencies observed for the different calibrations curves remained quite similar. The small differences between experiments 1 and 2, can be attributed to the natural variability of wood elemental composition rather than to real differences of reactions occurring during thermo-degradation. Indeed, initial O/C ratio vary between 0.69 and 0.66 according to the origin of the tested pine wood, which is the key factor for explaining the differences observed between the different heat treated samples. Even if a same elemental composition is not sufficient to assume that chemical composition of the different pine samples are similar, the strong correlation observed with the evolution of mass losses due to thermal degradations allow to envisage the use of O/C ratio as a valuable marker of the heat treatment intensity.

The results of decay resistance of heat treated samples to the brown rot fungus *Poria placenta* and evolution of weight loss due to fungal attack as a function of mass loss due to thermal degradation are reported in Fig. 5.

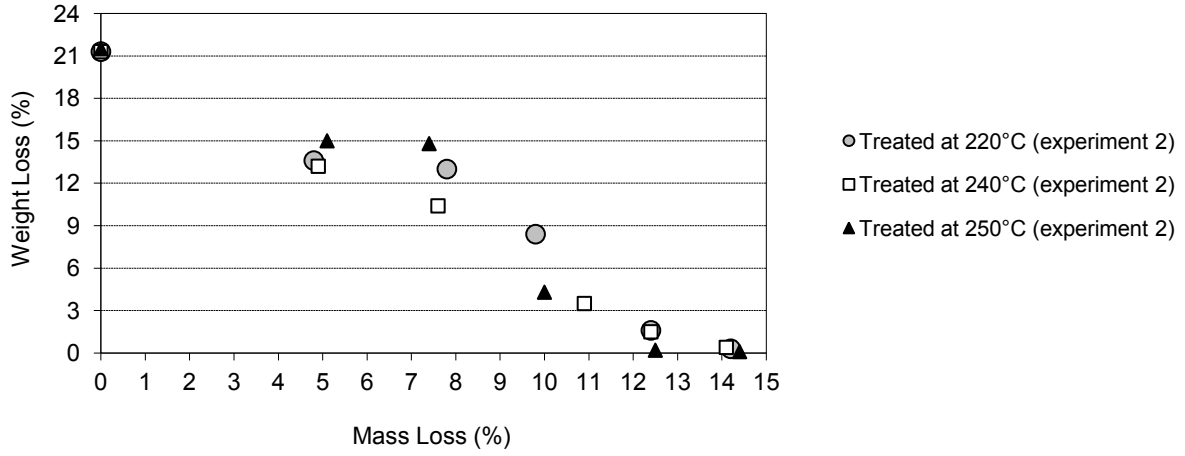

Figure 5: Correlation between mass loss resulting from different heat treatment conditions and weight loss due to fungal degradation

After 12 weeks of fungal exposure, the recorded weight losses are well correlated with the intensity of heat treatment. For a given temperature, increase of the curing time improves gradually pine wood durability against fungal decay, which becomes totally resistant for mass loss of about 12%. The same behaviour was observed for the different treatment temperatures allowing full protection of treated blocks at more or less similar treatment intensities. Similarly to our previous report, results indicated that decay durability is strongly correlated to mass losses due to thermal degradations (Hakkou *et al*. 2006). Results are also in good agreement with recently published data obtained by Welzbacher et al. 2007. In this study, the authors investigated the effect of heat treatment conditions on wood decay durability at different temperatures (180, 200, 210, 220 and 240°C). They observed that decay resistance for a given

treatment intensity was better at higher temperatures. However, if only temperatures of 220 and 240°C were considered similarly to the temperatures used in our study, results becomes quite similar showing no significant differences between the two curing conditions. For the range of temperatures used, wood chemical composition appeared therefore to be a valuable marker of wood durability due to the strong correlations existing between treatment intensity and decay resistance. Even if mass losses due to thermo-degradation reactions are not always easily available on heat treated timbers resulting from industrial processes, it seems possible to establish easily calibration curves for given treatment conditions allowing further correlations between the elemental composition and the treatment intensity estimated or wood decay durability.

3.2 Heat treatment of softwood and hardwood species.

The study on the five given below wood species is performed in the second experimental device. Mass losses of the different wood species as a function of time are presented in Fig. 6 for similar curing conditions. Mass loss depends of the density but also of the nature of the wood species. Until 160°C, only very small mass losses were observed corresponding to vaporization of volatile extractives and of bound water absorbed on the wood fibres. Thermo-degradations begin at higher temperatures and are effective at 230°C. Species of lower density present better stability to thermo-degradation than species of higher density.

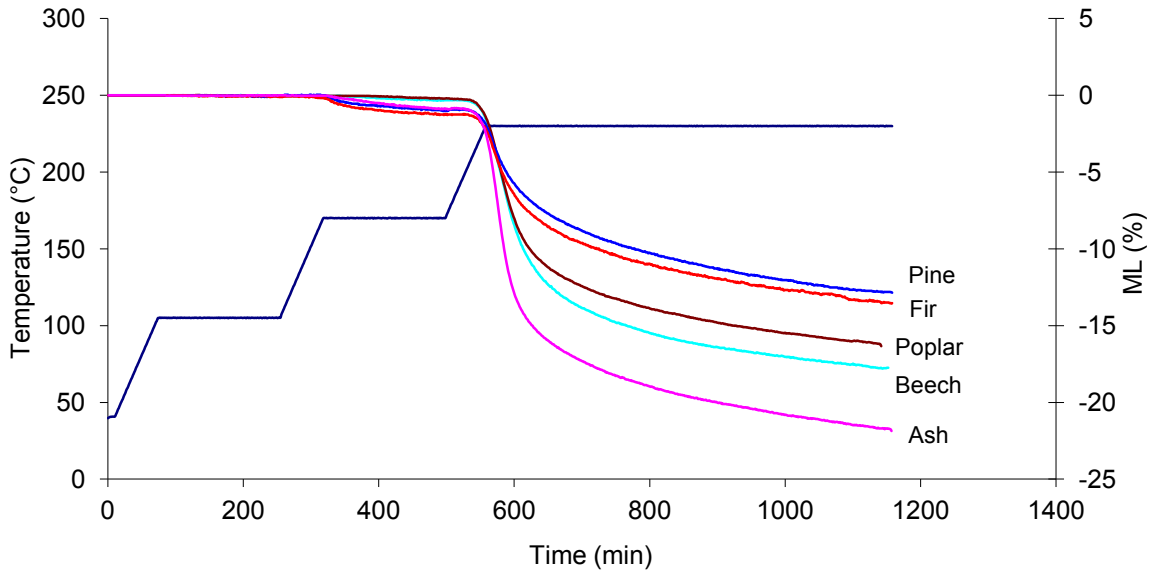

Figure 6: Evolution of mass during heat treatment of different wood species

Some characteristics should be involved to explain the differences observed between the two softwoods species and poplar. Softwoods and hardwoods differ in the percentage and the composition of hemicelluloses. Softwoods contain galactoglucomannan and arabinoglucuronoxylan, while hardwoods contain mainly glucuronoxylan and low amount of glucomannan. Moreover, xylan units of hardwood glucuronoxylan are strongly acetylated, comparatively to softwood hemicelluloses for which acetyl groups are attached to the glucomannan backbone (Fengel, and Wegener 1989, Sjöström 1981). Deacetylation of hemicelluloses causes liberation of acetic acid, which catalyses depolymerisation of the less ordered carbohydrates like hemicelluloses and amorphous cellulose. Higher content of acetyl groups present in hardwoods may be at the origin of the higher kinetic of thermo-degradation observed for poplar comparatively to softwoods species. Similarly, differences in chemical composition should be involved to explain differences of thermo-degradation patterns observed for beech and ash.

Starting from thermo-degradation curves, it was possible to estimate curing times to reach a given mass loss. Each wood species was heat treated during different times to reach mass losses of approximately 5, 10 and 15%. Elemental composition and durability against the brown rot fungus *Poria placenta* were then measured. Correlations between mass losses and carbon content, O/C ratio as well as fungal durability are presented in Figs. 7, 8 and 9.

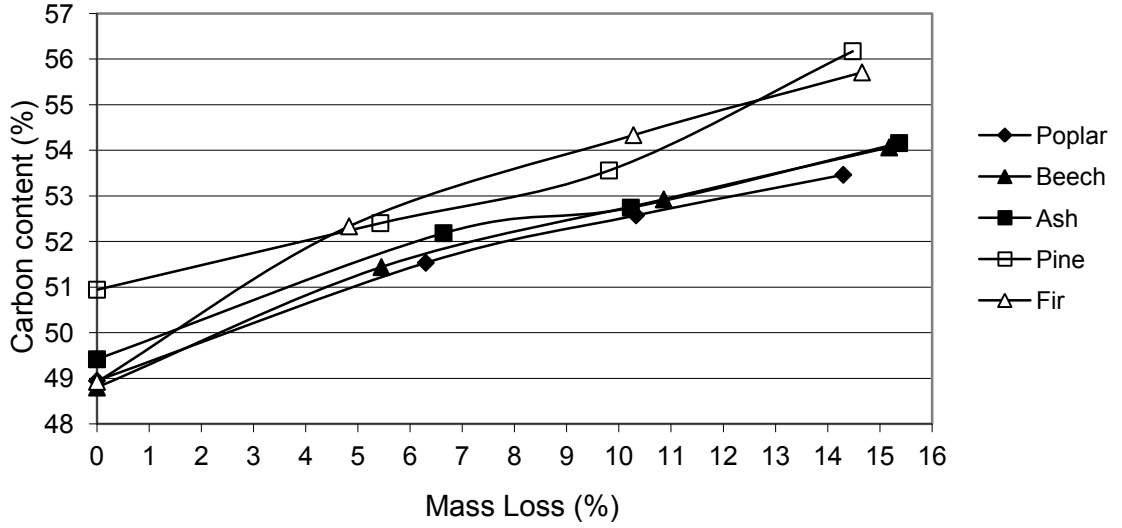

Figure 7: Correlation between mass loss resulting from heat treatment at 230°C and carbon content for different wood species

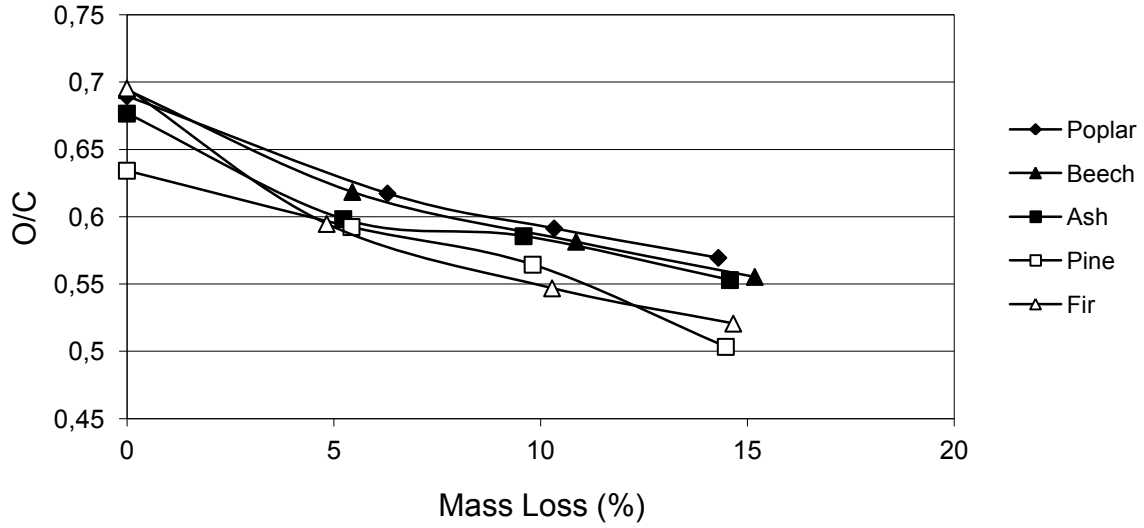

Figure 8: Correlation between mass loss resulting from heat treatment at 230°C and O/C ratio for different wood species

As expected, wood carbon content increases as the mass loss increases, while oxygen content decreases (Nguila *et al.* 2009, Šušteršic *et al.* 2010). For a similar mass loss, softwoods present higher carbon content than hardwoods. In all cases, evolution of carbon content increases linearly as the mass loss increases showing a good correlation between mass loss and carbon content. Even if the kinetics of thermo-degradation are different, their effects on wood chemical composition for a same level of degradation are relatively close. This is not surprising considering that the main modifications occurring during wood heat treatment are due to degradation of hemicelluloses through depolymerisation reactions to C5 and C6

monosaccharides leading after dehydration to furfural, hydroxymethylfurfural or levoglucosenone. These products may either be loss by evaporation or subject to further degradation leading to furan, formaldehyde, formic acid, levulinic acid and other by-products involved in thermo-condensation or thermo-reticulation with lignin (Alen *et al.* 2002, Nguila *et al.* 2007b, Rowell *et al.* 2009, Shen and Gu 2009).

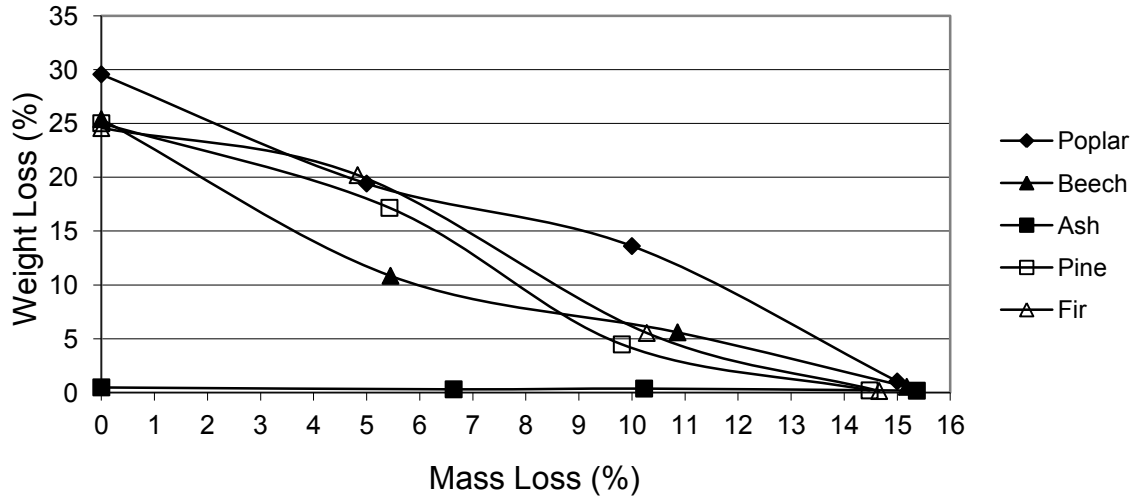

Figure 9: Correlation between mass loss resulting from heat treatment at 230°C and weight loss due to fungal degradation for different wood species

From a chemical point of view, it seems reasonable to think that quite similar products were formed for a similar mass loss making abstraction of the initial chemical differences existing between softwoods and hardwoods. The main difference concerns the kinetic of thermo-degradation, which differs significantly between softwoods and hardwoods. This difference may be explain by the higher acetyl content present in hardwoods leading to the formation of higher quantities of acetic acid involved in acidic catalysis of hydrolysis and dehydration reactions, but also by the higher susceptibility to hydrolysis of pentoses present in hardwoods hemicelluloses (Xu *et al.* 2009). Correlations between mass loss and atomic O/C ratio are shown in Fig. 8. O/C ratio decreases linearly with the increase of the mass loss indicating that O/C ratio could be a valuable marker of heat treatment intensity. This decrease can be mainly attributed to thermal degradation of wood mentioned above and subsequent dehydration reactions explaining the decrease of oxygen content. Even if each wood species presents different behaviour, the tendencies observed remained the same indicating formation of degradation products with relatively similar structures. Except for untreated fir, higher atomic O/C ratios were obtained with hardwoods. This observation can be explained by the higher syringyl content of hardwoods lignins compared to softwoods, which contain mainly guaiacyl units. Similarly to our previous results obtained with pine, the strong correlations observed between mass losses due to thermal degradations and O/C ratios allow envisaging O/C ratio or carbon content as valuable markers of the heat treatment intensity.

Correlation between mass loss due to heat treatment and weight loss due to fungal degradation are shown in Fig 9. In a way similar to what is practised on industrial scale, we have chosen to perform heat treatment on the heartwood of the different wood species studied. Indeed, all these species are characterized by a relatively low natural durability and are therefore particularly suitable for heat treatment. Except in the case of ash, which was totally resistant to biodegradation in the tested conditions, all wood species present weight losses in direct connection with their degree of thermo-degradation. Similarly to our previous results obtained with pine, conferred durability increase with treatment intensity. Independently of the wood

species used, wood samples become totally resistant to decay for mass losses around 15%. This value can be associated to a carbon content of approximately 56% for softwoods and 54% for hardwoods, confirming the possibility to use wood elemental composition as a marker to predict decay durability of heat treated wood.

4. CONCLUSION

Treatment intensity estimated from mass losses due to thermo-degradation depends on treatment duration and temperature. In the range of studied temperatures ranging from 220 to 250°C, treatment intensity determined for a similar mass losses is strongly correlated to wood elemental composition and do not depend on treatment temperature. The results of this study have shown that even if wood species of lower densities seems to be more resistant to thermodegradation, other parameters should be also involved to explain differences in thermodegradation patterns observed between softwood and hardwood species of similar densities or hardwoods species of similar densities. The kinetic of the mass loss during the heat treatment is strongly dependent to the wood composition and especially to its acetyl groups content. The study has also shown a good correlation between the mass loss, the elemental composition and the fungal durability, confirming the possibility to predict the decay durability of heat treated woods on the basis of their carbon content or O/C ratio. Further studies are in progress to validate this method on industrially heat treated samples in order to develop a quality control method aimed to certify the product quality to the customers.

5. REFERENCES

Alen R, Kotilainen R, Zaman A. (2002) Thermochemical behavior of Norway spruce (Picea abies) at 180–225°C. *Wood Science and Technology*, 36, 163-171

Boonstra MJ, Tjeerdsma B. (2006) Chemical analysis of heat treated softwoods. *Holz als Roh - und Werkst*; 64: 204-211.

Chaouch M., Pétrissans M., Pétrissans A., Gérardin P. (2010). Use of wood elemental composition to predict heat treatment intensity and decay resistance of different softwood and hardwood species. *Polymer Degradation and Stability*, 95, 2255-2259.

Degroot WF, Pan WP, Rahman D, Richards GN. (1988) First chemical events in pyrolysis of wood. *J. Anal. Appl. Pyrol.*, 13, 221-31.

Esteves B, Domingos I, Pereira H. (2007) Improvement of technological quality of eucalypt wood by heat treatment in air at 170-200°C. *For. Prod. J.*, 57 (1/2), 47-52.

Esteves B, Graça J, Pereira H. (2008) Extractive composition and summative chemical analysis of thermally treated eucalypt wood. *Holzforshung*, 62, 344-351.

Fengel, D., Wegener, G. (1989) *Wood Chemistry Ultrastructure Reactions*. Walter de Gruyter.

Gérardin P, Petrič M, Pétrissans M, Erhrardt JJ, Lambert J. (2007) Evolution of wood surface free energy after heat treatment. *Polymer Degradation and Stability*, 92, 653-57.

Hakkou M, Pétrissans M, Gérardin P, Zoulalian A. (2005) Investigation of wood wettability changes during heat treatment on the basis of chemical analysis. *Polymer degradation and stability*, 89, 1-5.

Hakkou M, Pétrissans M, Gérardin P, Zoulalian, A. (2006) Investigations of the reasons for fungal durability of heat-treated beech wood. *Polymer Degradation and Stability*, 91, 393-97.

Kamdem DP, Pizzi A, Jermannaud A. (2002) Durability of heat-treated wood. *Holz als Roh- und Werkstoff*, 60, 1-6.

Kocaefe D, Poncsak S, Boluk Y. (2008) Effect of thermal treatment on the chemical composition and mechanical properties of birch and aspen. *Biores*, 3(2), 517-537.

Militz H. (2002) Thermal treatment of wood: European processes and their background. *The International Research Group on Wood Preservation*, Document IRG/WP 02-40241.

Mouras S, Girard P, Rousset P, Permadi P, Dirol D, Labat G. (2002) Propriétés physiques de bois peu durables soumis à un traitement de pyrolyse ménagée. *Annals of Forest Science*, 59, 317-26.

Nguila Inari G, Pétrissans M, Lambert JL, Erhardt JJ, Gérardin P. (2006) XPS Characterization of wood chemical composition after heat treatment. *Surface & Interface Analysis*, 38, 1336-42.

Nguila Inari G, Pétrissans M, Gérardin P. (2007a) Chemical reactivity of Heat treated Wood. *Wood Science Technology*. 41, 157-68

Nguila Inari G, Mounguengui S, Dumarcay S, Pétrissans M, Gérardin P. (2007b) Evidence of char formation during wood heat treatment by mild pyrolysis. *Polymer Degradation and Stability*. 92, 997-1002

Nguila Inari G, Pétrissans M, Pétrissans A, Gérardin P. (2009) Elemental composition of wood as a potential marker to evaluate heat treatment intensity. *Polym Degrad Stab*.; 94: 365-368.

Nuopponen M, Vuorinen T, Jamsa S, Viitaniemi P. (2004) Thermal modifications in softwood studied by FT-IR and UV resonance raman spectroscopie. *Journal of Wood Chemistry and Technology*. 24 (1), 13-26.

Patzelt M, Stingl R, Teischinger A. (2002) Termische Modifikation von Holz und deren Einfluss auf ausgewählte Holzeigenschaften. In: *Modifiziertes Holz Eigenschaften und Märkte*. Lignovisionen Band 3, ISSN 1681-2808, 101-49.

Pétrissans M, Gérardin P, El Bakali I, Serraj M. (2003) Wettability of heat-treated wood. *Holzforschung.* 57(3), 301-307.

Ross, R J, DeGroot, R C, Nelson W J (1994): Technique for the non-destructive evaluation of biologically degraded wood. *Experimental Techniques*, 18(5), 29-32.

Rowell RM, Ibach RE, McSweeny J, Nilsson T. (2009) Understanding decay resistance, dimensional stability and strength changes in heat-treated and acetylated wood. *Wood Material Science and Engineering*, 4, 14 - 22.

Shen DK, Gu S. (2009) The mechanism for thermal decomposition of cellulose and its main products. *Bioresource Technology*, 100, 6496-6504

Shi JL, Kocaefe D, Amburgey T, Zhang J. (2007) A comparative study on brown rot fungus decay and subterranean termite resistance of thermally-modified and ACQ-C-treated wood. Holz als Roh- und Werkstoff. 65, 353-358.

Sivonen H, Maunu SL, Sundholm F, Jämsä S, Viitaniemi P. (2002) Magnetic resonance studies of thermally modified wood. Holzforschung, 56:648-654.

Sjöström E. (1981) Wood chemistry. Fundamentals and Applications. In: Chapter 3 *Wood Polysaccharides*. Academic Press., pp. 49-67.

Šušteršic Z., Mohareb A., Pétrissans M., Tetrîc M., Gérardin P. (2010). Prediction of the decay resistance of heat treated wood on the basis of its elemental composition. *Polymer Degradation and Stability* 95, 94-97.

Tjeerdsma B, Militz H. (2005) Chemical changes in hydrothermal treated wood: FTIR analysis of combined hydrothermal and dry heat-treated wood. *Holz Roh Werkst*., 63, 102-111.

Welzbacher C, Brischke C, Rapp A. (2007) Influence of treatment temperature and duration on selected biological, mechanical, physical and optical properties of thermally modified wood. *Wood Material Science and Engineering*. 2, 66-76.

Xu C, Pranovich A, Hemming J, Holmbom B, Albrecht S, Schols HA, Willför S. (2009) Hydrolytic stability of water-soluble spruce O-acetyl galactoglucomannans. *Holzforschung*, 63 (1), 61-68

Yildiz S, Gezer D, Yildiz U. (2006) Mechanical and chemical behaviour of spruce wood modified by heat. *Building Environ*. 41, 1762-66

IRG/WP 12-40612

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 4 **Processes and properties**

Effect of preservative treatment on mechanical performance of round and square poles made of small diameter Scots pine

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Effect of preservative treatment on mechanical performance of round and square posts made of small diameter Scots pine

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ABSTRACT

A growing proportion of harvested Scots pine (*Pinus sylvestris* L.) timber originates from the first or second commercial thinning stands. There is a need to find economically viable and high quality wood products that can be manufactured from this raw material. Small log volume means not only demands of increased efficiency for material handling in logistics and manufacturing processes, but also challenging wood properties such as high proportion of juvenile wood and sapwood. Due to these facts, products made of small diameter logs are prone to twist and check, and have poor durability against weather. These shortcomings can be improved by a number of preservation methods. In this study, we concentrated in pressure impregnation with copper based preservative, and pine oil impregnation. The objective of the study was to define the modulus of elasticity (MOE) and modulus of rupture (MOR) of non-treated, pressure impregnated and pine oil treated fencing posts in static bending.

The material originated from five stands in south-eastern Finland. We prepared two diameters of round posts (80 and 120 mm), and two square-sawn dimensions (50 x 50 mm and 100 x 100 mm). The material was divided into three treatments: 1) not impregnated, 2) pressure impregnated into AB-class with copper-based preservative in commercial process, and 3) impregnated with pine oil using the process of Ekopine Ltd. After the impregnation, the specimens were further divided into two groups. The first one headed straight to the bending tests, and the other group was subjected to weather exposure test. After the weather exposure test, also these specimens were tested in bending. The bending tests were done according to EN 408.

Unlike expected, both MOE and MOR of square sawn posts were higher than those of round posts. The AB class pressure impregnated specimens had, on average, slightly lower strength than the other treatments. This was also the case with stiffness of round posts, whereas the square-shaped AB impregnated specimens had almost equal stiffness compared to the non treated and pine oil impregnated specimens.

Keywords: pine oil impregnation, *Pinus sylvestris,* pressure impregnation, stiffness, strength

1. INTRODUCTION

The volume, annual growth and annual harvesting removal of Scots pine (*Pinus sylvestris* L.) timber in Finland are 1098, 45, and 23 million m^3, respectively (Ylitalo 2011). Sequential thinnings of forest stands at least twice during the rotation period belong to the modern forestry practice in Finland. At the time of the first thinning, the log diameter and quality do not fulfil the requirements of logs for traditional saw mill production as far as structural products and carpentry are concerned. A growing proportion of harvested timber originates from the first or second commercial thinning stands, which means that the average volume of logs gets smaller than is the case in conventional final felling stands. Utilisation of small diameter logs has been increased, *e.g.*, in small scale and garden construction (*e.g.*, Boren 1999, 2001; Ranta-Maunus 1999, Wall *et al.* 2005). However, there are many industrial sawmills using logs as small as 70 mm in top diameter. Such raw material can be processed economically only if the production efficiency is very high; for instance, the saw line speed is typically 150-180 m/min, logs are run almost without log-to-log spaces (fixed sawing pattern), and the lines are virtually unmanned. The current products made of small diameter logs include *e.g.*, moldings and lamellae for glulam boards. However, there is a global and growing need to find new economically viable and high quality wood products that can be manufactured from small sized log material, both from softwood and hardwood species (see: Spelter *et al.* 1996, Boren 1999, Kilpeläinen *et al.* 2011).

Small log volume means not only demands of increased efficiency for material handling in logistics and manufacturing processes, but also challenging wood properties such as high proportion of juvenile wood, knotty wood, and sapwood. Due to these facts, products made of small diameter logs are prone to twist and check, and have poor durability against weather. In addition, stiffness and strength of products made of small diameter timber is supposed to be lower than those of products originating from larger logs (*e.g.*, Bodig & Jayne 1982, Zobel & van Buijtenen 1989, Ranta-Maunus 1999, Boren & Barnard 2000, Heräjärvi *et al.* 2000, Boren 2001, Wall *et al.* 2005, Stöd & Kilpeläinen 2006, Stöd *et al.* 2006).

The need for an increase in the utilisation of small diameter logs in structural uses means higher demand for controlling and modifying the challenging wood properties such as high proportion of juvenile wood and sapwood. In addition to the physical challenges, there are chemical ones, as well. Products with increased water resistance and resistance against decay fungi and mould growth are needed. The most common method to preserve wood against microbial growth is pressure impregnation with chromium-copper (CC) based additives. Finland alone produces more than 200,000 m^3 of CC-impregnated sawn timber annually (Production statistics…2012). Pine oil (or "crude tall oil") impregnation, either alone or combined with some other modification such as heat treatment, has been considered as an alternative for CC-impregnation (*e.g.*, Cartwright & Findley 1958, Banks 1973, Sailer *et al.* 1998, 2000, Paajanen *et al.* 1999, Van Eckeveld 2001, Van Eckeveld *et al.* 2001, Paajanen & Ritschkoff 2002, Koski 2008). According to the experience of industry representatives, pine oil penetrates in wood as its best via sawn or peeled surfaces, whereas planed surfaces are more challenging to impregnate.The time period between surface machining and impregnation also appears to have an influence on the impregnability, which is in line with the experiences from the glue joint behaviour.

There is no information available concerning the effects of pine oil impregnation on mechanical performance of wood. We know that the equilibrium moisture content (EMC) of pine oil impregnated wood is 3-6 percent units lower than in untreated wood, which apparently causes certain indirect influences on the mechanical performance of wood, as well. During the impregnation process, however, the moisture content of wood (MC) decreases down to 1-2%. It

is unclear whether or not this causes destructive effects on the anatomical structure of cell walls or intercellular connections.

The objective of the study was to define the modulus of elasticity (MOE) and modulus of rupture (MOR) of non-treated, pressure impregnated and pine oil treated round and square shaped Scots pine (*Pinus sylvestris*) fencing posts in static bending tests.

2. EXPERIMENTAL METHODS

The study material originated from five thinning stands in south-eastern Finland, which were harvested in the beginning of year 2009. The top diameters of harvested logs varied between 80 and 155 mm on bark. All logs were butt logs.

Two diameters of round posts (80 and 120 mm) were peeled, and two square dimensions (50x50 mm and 100x100 mm) were sawn from the logs (Fig. 1). Altogether, we prepared 192 posts that were divided into four treatment groups. Treatments 1 and 2 were pine oil impregnated with pine oil types 1 and 2 using the oil drying process of Ekopine Ltd, treatment 3 was pressure impregnation into AB-class with copper-based preservative in commercial process, and treatment 4 was a reference that was not impregnated with any additives. The difference between pine oil types 1 and 2 was in composition and amount of thinner, which was used to get differences in the viscosity and absorption of the pine oil.

Figure 1: Examples of the round (80 and 120 mm) and square (50×50 mm and 100×100 mm) post specimens.

After the impregnation, mass, dimensions, deformations, checks, as well as other possible defects were measured from the specimens. Then, the material was further divided into two sub-groups the first one heading straight to the bending tests, whereas the other sub-group was subjected to a relatively mild weather exposure test in a weather chamber. The cyclic weather chamber test lasted 14 days, and consisted of seven cycles with two phases. Phase 1 lasted 36 hours and had RH of 95% and temperature of 10 degrees Celcius, in which conditions the EMC

of untreated wood should be approximately 21%. Phase 2 lasted 12 hours, and had a RH of 15% and temperature of 40 degrees Celcius. These conditions take the EMC of untreated wood down to 4.2%. The above mentioned EMC values were not likely achieved in our weather chamber tests due to the short time period in each condition.

The dimensions as well as the MC were measured from the posts again prior to the four-point bending test. In addition, the size and location of knots, checks and other defects were recorded. The position of the post in bending test was decided based on its defects. The weakest face (square posts) or side (round posts) was visually determined, and marked in the specimens using a felt-tipped pen. The stength related defects were measured or evaluated from the mid section of the post at 1/3 length. Thus, we concentrated in the section with maximum bending momentum during the four-point bending test (see: Fig. 2). The weakest side was positioned downwards in the bending test. Therefore, the bending results indicate the expected lowermost strength value for each individual post.

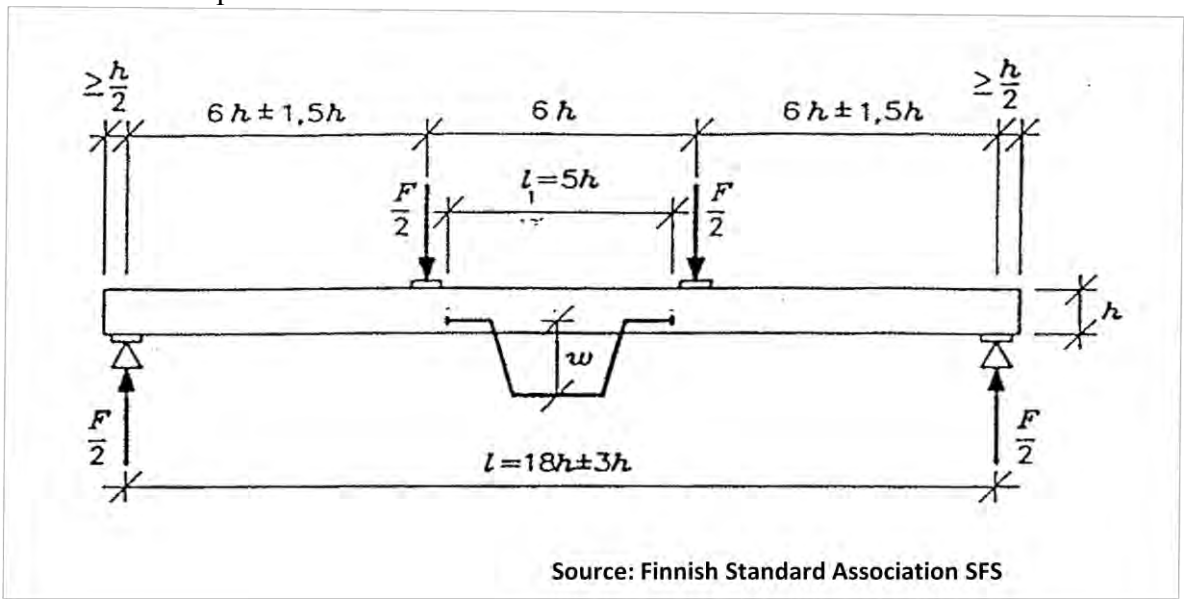

Figure 2: Experimental setup in static four-point bending test according to SFS-EN 408 (2003).

The static bending tests were made according to SFS-EN 408 (2003) standard using a TIRAtest 28100 material testing device of Savonia University of Applied Sciences, Kuopio, Finland. Global modulus of elasticity (MOE) was calculated according to equation 1:

(1)

where $E_{m,g}$ = static MOE (global) at 12% MC [GPa], l = distance between the lower supports [mm], b = specimen width [mm], h = specimen height [mm], a = distance between the load point and the closest support point [mm], $F_2 = 0.4F_{max}$ [N], $F_1 = 0.1F_{max}$, w_2 = displacement at the point F_2 [mm], w_1 = displacement at the point F_1 [mm].

The static modulus of rupture (MOR) was calculated according to equation 2:

(2)

where f_m = static MOR at 12 % MC [MPa], a = distance between the load point and the closest support point [mm], F_{max} = maximum load [N], W = section modulus, [mm^3]. The section moduli for square and round shaped specimens were calculated according to equations 3 and 4, respectively:

$$W = (bh^2)/6 \quad (3)$$

and

$$W = (\pi r^3)/32 \quad (4)$$

where W = section modulus, b = specimen width [mm], h = specimen height [mm], π = the pi factor, and r = radius of the round post.

The dimensions of the specimens were measured with a calliper prior to the bending tests. The MC's were determined with a resistance moisture meter (Gann HT85T) after the bending tests. For the calculations of the static MOE and MOR, the MC's were adjusted to correspond to 12% as follows (Boström 1994):

(5)

(6)

where E_{12} = static global MOE at 12% MC [GPa], ω = MC at the time of the bending test [%], E_ω = static global MOE at the MC of ω [GPa], $f_{m,12}$ = static MOR at 12% MC [MPa], f_ω = static MOR at the MC of ω [MPa].

Wood density was calculated after the impregnation processes using the mass, volume and moisture content of the specimens. Since the MC's varied to some extent, densities were computationally adjusted to correspond to the value at 12% MC.

The MOE and MOR values are reported graphically in this paper.

3. RESULTS AND DISCUSSION

The global MOE values varied from 6,000 to 12,000 MPa, on average. Square shaped posts had clearly higher MOE compared to the round ones. Figure 3 shows the mean values of global MOE for square and round specimens by treatment groups, dimensions and weather exposure. The weather exposure causes negative effects only in the stiffness of 50x50 mm square post, whereas in case of 120 mm diameter round posts the weather exposure has even improved the stiffness of the specimens. Since the possible statistical differences in knottiness features between the post types has not been analysed yet, we cannot state any reasonable explanation to these findings. However, it appears that the weather chamber test was too mild to cause any real damages to the structure of the posts.

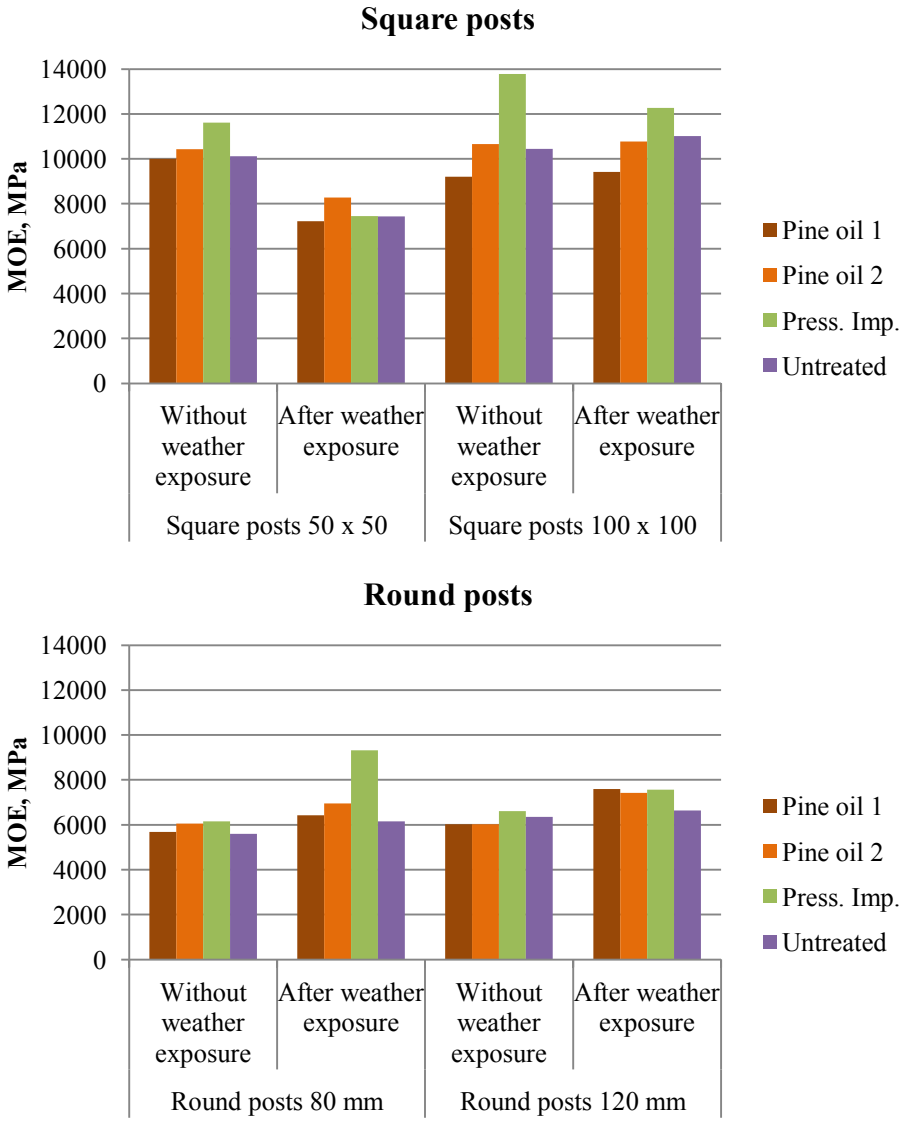

Figure 3: Global modulus of elasticity (MOE) of the round and square posts by dimension, weather exposure and preservative treatment.

The average bending strength values for round and square shaped posts are shown in Figure 4. The MOR values between 35 to 50 MPa for pine oil impregnated round posts are clearly lower than for untreated (approximately 55 MPa) and CC-impregnated (60-80 MPa) posts. Also in case of square shaped posts, the CC-impregnated specimens are clearly stronger (average MOR 45-80 MPa) than untreated (50-60 MPa) and pine oil impregnated (40-50 MPa) ones. The weather chamber test did not have any clear effects on the strength of pine oil impregnated or untrated specimens, whereas the CC-impregnated specimens systematically lost some of their strength as a result of the weathering test.

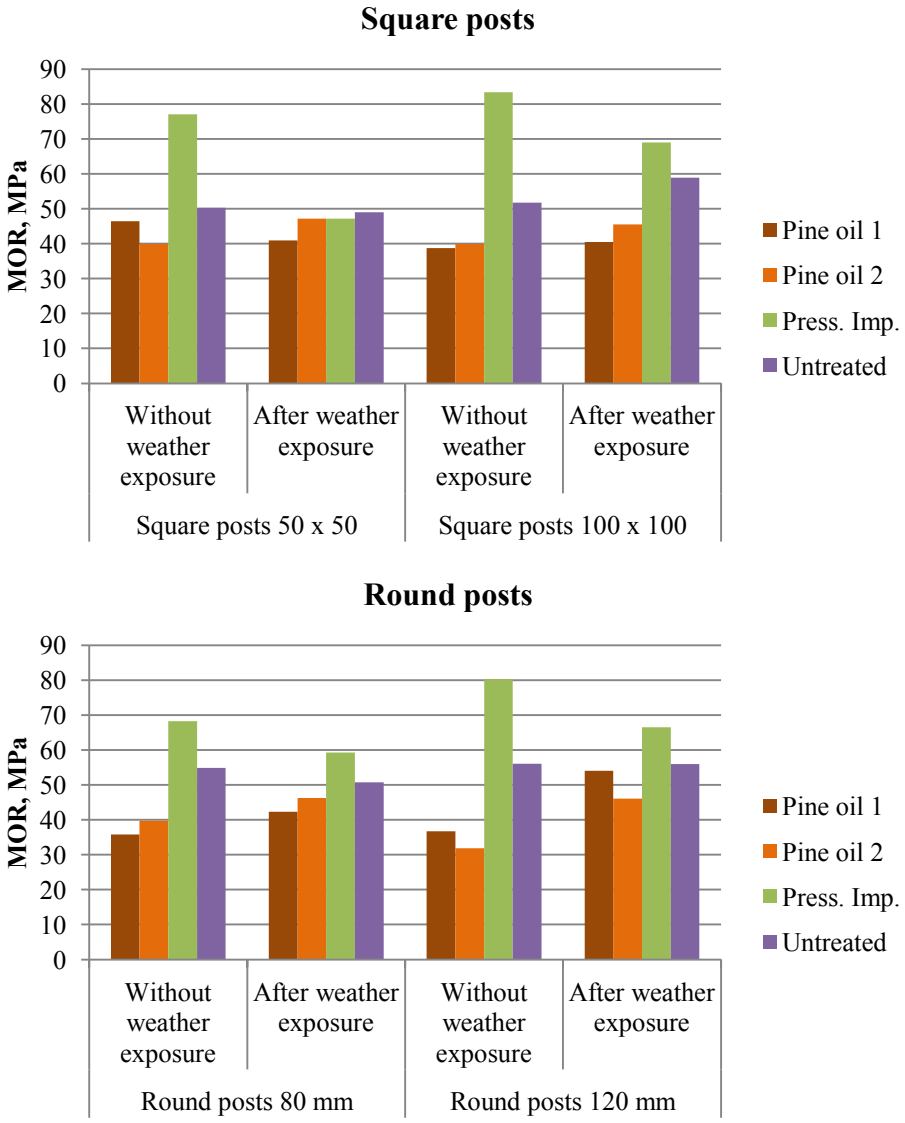

Figure 4: Bending strength (MOR) of the round and square posts by dimension, weather exposure and preservative treatment.

Knots (*i.e.*, large knot / smaller knot being the only defect in the vicinity of the failure / knot group) were the predominant origin of failure in the bending tests in most cases (Figures 5 and 6). In some cases, larger cracks as well as cross-grain were assessed to be the main reason. However, if knots are excluded, no realistic conclusions can be made concerning the reasons of rupture, since the number of observations is too small.

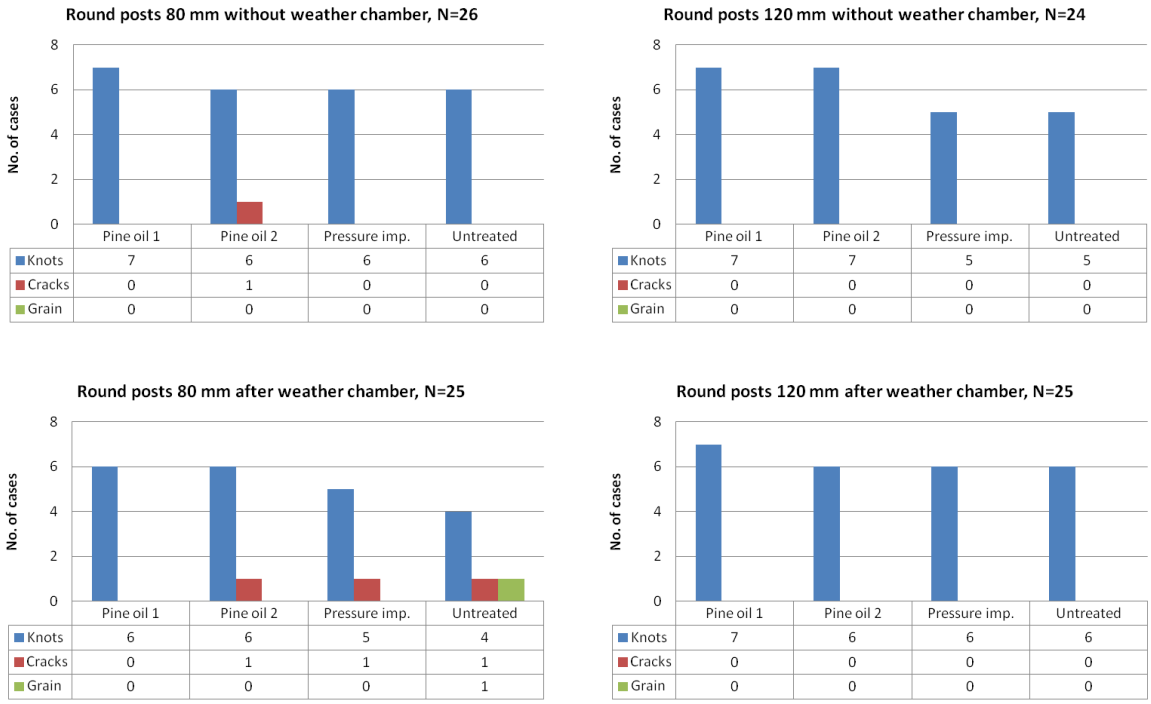

Figure 5: Reason of rupture in static 4-point bending test of round posts.

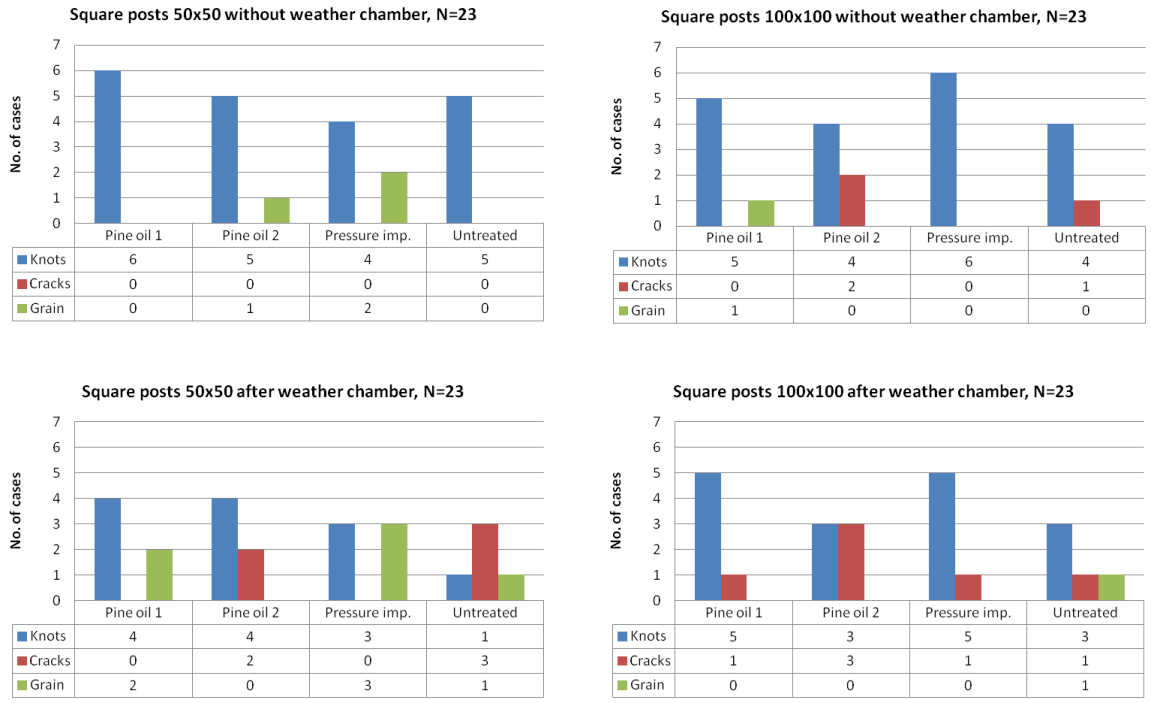

Figure 6: Reason of rupture in static 4-point bending test of square shaped posts.

4. CONCLUSIONS

In this study, we were interested in stiffness, strength and durability of pine oil impregnated, CC-impregnated and untreated round and square shaped fencing posts made of Scots pine. Pine oil impregnation appears to be a promising method to protect wood from cracking and other weather related challenges. In order to be able to characterise the changes in anatomical structure (cell walls or middle lamellae) of pine oil impregnated wood, we still have to carry out microscopic analyses. Knots can be considered to be practically the only reason for origin of breakage in the fencing posts. Therefore, strength grading should be carried out based on knot characteristics.

REFERENCES

Banks, W.B. (1973): Water uptake by Scots pine sapwood, and its restriction by the use of water repellents. *Wood Science and Technology* **7**, 271-284.

Bodig, J. & Jayne, B.A. (1982): Mechanics of wood and wood composites. Van Nostrand Reinhold, New York. 712 p.

Boren, H. (1999): [Increasing the utilisation of small sized timber in wood product industries. Final report of a preliminary survey]. *Finnish Forest Research Institute, Research Papers 761.* 58 p. (In Finnish)

Boren, H. (2001): Factors affecting the knottiness, twisting and mechanical properties of pith enclosed round and sawn timber of Scots pine (Pinus sylvestris) and Norway spruce (Picea abies) from thinnings in Southern Finland. *Finnish Forest Research Institute, Research Papers 807.* 154 p + App.

Boren, H. & Barnard, G. (2000): Analysis of the strength and stiffness properties for small diameter round Scots pine timber tested in bending and compression parallel to the grain. *Paperi ja Puu – Paper and Timber* **82**(2), 48-56.

Boström, L. (1994): Machine strength grading, comparison of four different systems. *Swedish National Testing and Research Institute, Building Technology, SP Report, 49.*

Cartwright, K.St.G., & Findley, W.P.K. (1958): Decay of timber and its prevention. 2nd edition. H.M. Stationery Office, London. 332 p.

SFS-EN 408 (2003): Timber structures. Structural timber and glued laminated timber. Determination of some physical and mechanical properties. 31 p.

Heräjärvi, H., Boren, H., Pietilä, J. & Stöd, R. (2000): Properties and potential of Scots pine and Norway spruce harvested in first commercial thinnings for manufacture of round timber and saw milling. In: *Proceedings of the Third Workshop on Measuring of Wood Properties, Grades and Qualities in the Conversion Chain and Global Wood Chain Optimisation*, 19th-21st June, 2000, Dipoli, Espoo, Finland. Eds. Usenius, A. & Kari, P. Cost Action E10 Wood Properties for Industrial Use. VTT Building Technology, Espoo. p. 103-122.

Kilpeläinen, H., Lindblad, J., Heräjärvi, H. & Verkasalo, E. (2011): Saw log recovery and stem quality of birch from thinnings in southern Finland. *Silva Fennica* **45**(2), 267-282.

Koski, A. (2008): Applicability of crude tall oil for wood protection. *Acta Universitas Ouluensis 293.* 99 p. + App.

Paajanen, L., Koskela, K. & Viitaniemi, P. (1999): Treatment of wood with a mixture of tall oil and maleic anhydride. Espoo, Technical Research Centre of Finland. *VTT Publications 836.* 75 p. (In Finnish)

Paajanen, L. & Ritschkoff, A.-C. (2002): Effect of crude tall oil, linseed oil and rapeseed oil on the growth of decay fungi. *The International Research Group on Wood Preservation, IRG/WP 02-30229.* 6 p.

Production statistics of pressure impregnated wood. (2012): [http://www.kestopuu.fi/tuotanto_ja_tilastot.html]

Ranta-Maunus, A. (ed.) (1999): Round small-diameter timber for construction. Final report of project FAIR CT 95–0091. *VTT Publications 383.* 191 p. + appendix 19 p.

Sailer, M., Rapp, A.O. & Peek, R.-D. (1998): Biological resistance of wood treated with waterbased resins and drying oils in a mini-block test. *The International Research Group on Wood Preservation, IRG/WP 98-40107.*

Sailer, M., Rapp, A.O. & Leithoff, H. (2000): Improved resistance of Scots pine and spruce by application of an oil-heat treatment. *The International Research Group on Wood Preservation, IRG/WP 00-40162.* 17 p.

Spelter, H., Wang, R., & Ince, P. (1996): Economic feasibility of products from inland West small-diameter timber. *General Technical Report FPL-GTR-92.* Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 17 p.

Stöd, R. & Kilpeläinen, H. (2006): Knot properties of Scots pine saw timber trees from thinning stands. In: *Proceedings of the 5th IUFRO symposium Wood Structure and Properties '06 held on September 3-6, 2006 in Sliac - Sielnica, Slovakia.* Eds. Kurjatko, S., Kúdela, J. & Lagana, R. Arbora Publishers, Zvolen. p. 149-154.

Stöd, R., Kilpeläinen, H. & Wall, T. (2006): Yield and technical quality of Scots pine saw timber from thinnings on drained peatlands and mineral soils in Finland. *Baltic Forestry* **12**(2), 170-183.

Van Eckeveld, A. (2001). Natural oils as water repellents for Scots pine. *Wageningen University, Thesis AV 2001-15.* 30 p.

Van Eckeveld, A., Homan, W.J. & Miliz, H. (2001): Increasing the water repellency of Scots pine sapwood by impregnation with indiluted linseed oil, wood oil, coccos oil and tall oil. *Holzforschung und Holzerwertung 6.*

Wall, T., Fröblom, J., Kilpeläinen, H., Lindblad, J., Heikkilä, A., Song, T., Stöd, R. & Verkasalo, E. (2005): [Development of procurement and sawing of Scots pine from thinnings, final report of a research concortium in Wood Wisdom research programme]. *Finnish Forest Research Institute, Research Papers 943.* 129 p. + App. 13 p. (In Finnish)

Ylitalo, E. (2011): Finnish Statistical Yearbook of Forestry. Official Statistics of Finland, Agriculture, forestry and fishery. Finnish Forest Research Institute. 472 p.

Zobel, B.J. & van Buijtenen, J.P. (1989): Wood variation – its causes and control. Springer Verlag, Berlin. 363 p.

IRG/WP 12-40613

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 4 **Processes and properties**

The effect on moisture content of water trapped in wood joints

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The effect on moisture content of water trapped in wood joints

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ABSTRACT

To predict the service life of a structure, a model where the *exposure* of a structure is compared to its *resistance* can be used. Which exposure and resistance parameters that are relevant depend on which materials the structure consist of. This approach, with an exposure and a resistance parameter, is similar to the one used in structural engineering where a load (exposure) is compared to the bearing capacity (resistance) of a structural element.

For wood outdoors the relevant exposure parameter is a combination of wood moisture content and temperature and the resistance parameter is the ability to withstand decay by rot fungi. This study concerns the exposure parameter of wood outdoors above ground. To predict moisture and temperature conditions in the wood from climate data, the macro climate (precipitation, temperature, RH etc.) needs to be transformed into a micro climate, i.e., the climate at the wood surface. The moisture and temperature conditions in the wood can then be calculated using heat and mass transfer models with the micro climate as boundary condition.

The micro climate is influenced by the design of a structure. If water is trapped in joints and stays on wood surfaces, the time during which water is absorbed by the wood increases as well as the risk for decay. The aim of this work is to provide information about the relationship between micro climate and wood moisture content. The study concerns structures exposed to liquid water where high moisture contents are reached. Three different types of joints were exposed to artificial rain in the laboratory. Three different gap sizes between the boards were tested for each joint type to create different micro climates at the wood surfaces. Both the micro climate (the duration of water on surfaces and in gaps) and moisture content profiles were monitored during wetting and drying. The measurements were performed using small glued resistance electrodes.

Keywords: durability, service life, duration, surface moisture, moisture content measurements, joints, wood, exposure

1. **INTRODUCTION**

The service life of wood structures outdoors is mainly governed by the resistance to decay. Since decay only occurs when the wood moisture content is high, it is important that the critical moisture content level is not exceeded or at least that the duration of high moisture contents is limited. To predict the service life of a structure, a model where the *exposure* of a structure is compared to its *resistance* can be used (see e.g. Isaksson et al. 2010). This approach, with an exposure and a resistance parameter, is similar to the one used in structural engineering where a load (exposure) is compared to the bearing capacity (resistance) of a structural element.

Which exposure and resistance parameters that are relevant in service life prediction depend on which material the structure consists of. For concrete, where chloride ingress is one mechanism that causes reinforcement corrosion, one relevant exposure parameter is the chloride concentration at the surface. Corresponding resistance parameter is the permeability of the concrete and the thickness of the concrete layer that covers the reinforcement (see e.g. Luping et al. (2012)). For wood outdoors, the relevant exposure parameter is a combination of wood moisture content and temperature since these parameters influence the decay rate. The resistance parameter is the wood's ability to withstand decay by rot fungi and is therefore dependant on wood species, heartwood or sapwood, treatment etc. However, the relevant exposure and resistance parameters are not only material specific but can also depend on the application. For instance, when wood is used in the building envelope, the relevant exposure parameter is a combination of relative humidity and temperature and the resistance parameter is the critical level for mould growth.

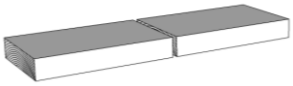
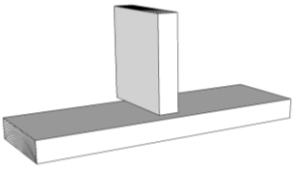
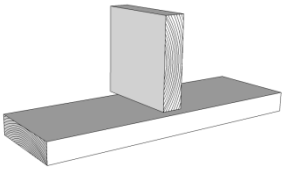
This study concerns the exposure parameter of wood outdoors above ground, i.e. wood exposed to liquid water where high moisture contents are reached. Examples of such structures are wooden decking, claddings, balconies and bridges. To predict moisture and temperature conditions in the wood from climate data, the macro climate (precipitation, temperature, RH etc.) needs to be transformed into a micro climate, i.e., the climate at the wood surface. The moisture and temperature conditions in the wood can then be calculated using heat and mass transfer models with the micro climate as boundary condition. The micro climate is influenced by the design of a structure; if water is trapped in joints and stays on wood surfaces, the time during which water is absorbed by the wood increases. Such "water traps" therefore give high local moisture contents (Gaby and Duff 1978) and joint type therefore has a considerable influence on the decay rate (De Groot and Highley).

This paper presents the first results from moisture content and micro climate measurements in three different joint types of exposed to artificial rain in laboratory. Three different micro climates were created by changing the gap size between the two boards in the joints. Both the micro climate (the duration of water on surfaces and in gaps) and moisture content profiles were monitored during wetting and drying. The experiments were performed for both sapwood and heartwood of Norway spruce (*Picea abies* L. Karst.) of two provenances.

2. METHODS

Three different joint types were included in the experiments: joint type A with two end grain surfaces facing each other, joint type B with an end grain surface facing a non-end grain surface and joint type C with two non-end grain surfaces facing each other (Table 1). Wood of two provenances, from northern (provenance 1) and southern (provenance 2) Sweden, was used and heartwood and sapwood was separated. Table 1 shows which materials that was used for each joint type, sapwood of provenance 1 (northern Sweden) was used for all three joint types.

Table 1. The three joint types and the different types of wood used for each joint type in the experiments.

	Joint type A end grain surface facing end grain surface			Joint type B end grain surface facing non-end grain surface	Joint type C non-end grain surface facing non-end grain surface
					
wood type:	sapwood	heartwood	sapwood	sapwood	sapwood
provenance:	1	1	2	1	1

2.1 Specimens

2.1.1 Joint type A

The design of the joint type A specimen is shown in Fig. 1. Two pieces of wood (295×95×22 mm^3, longitudinal×radial×tangential) was mounted in an poly(methyl methacrylate) (PMMA) box so that only two sides of each wood board, the upper surface and the end grain surface facing the gap, were exposed to water. A rubber sealing was fastened between the PMMA and the wood to ensure that no water could enter. The two parts of the wood/PMMA boxes were held together by two aluminium bars. The gap between the two end grain surfaces could be regulated between 0 and 10 mm. The wood specimens were fastened in the PMMA box by screws, but no screws were inserted in the wood within a distance of 100 mm from the gap. The wood within this area was free from visual defects.

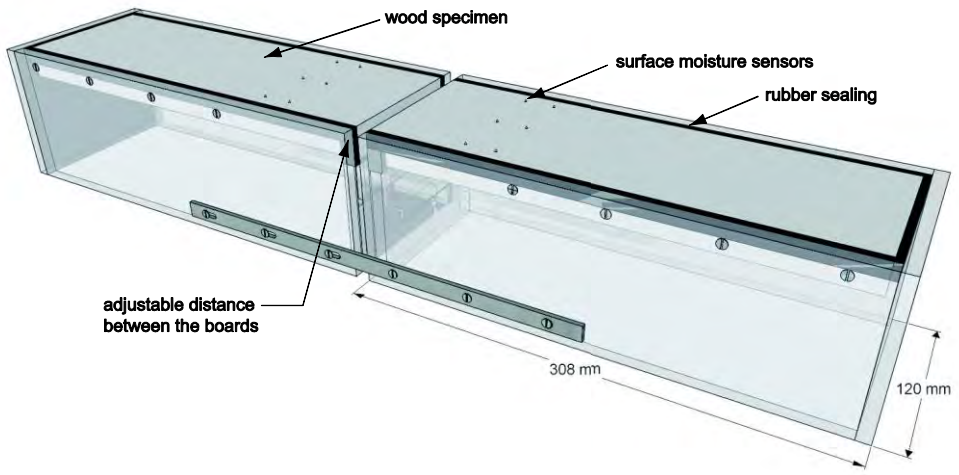


Figure 1. The design of the joint type A specimen. Two wood boards were mounted in a PMMA box. A rubber sealing was placed between the PMMA box and the wood to ensure that no water could enter. The two parts of the PMMA box were held together by two aluminium bars.

Moisture contents sensors (Fig. 3a) consisting of electrically conductive adhesive (EPO-TEK E4110, Epoxy Technology Inc., Billerica MA, USA), capillary tubing (PEEK tubing outer diameter: 1.6 mm, inner diameter: 1 mm) and copper wire (diameter 0.5 mm) (Fredriksson 2010) was inserted at three depths and at four distances from the end grain surface (Fig. 2). The sensors were inserted from the back of the specimen, i.e. the side of the specimen that was not exposed to water, to ensure that no water could enter along the sensors and cause high local moisture contents. In addition, the duration of surface moisture and the presence of water in the gap were monitored by sensors described by Fredriksson (2010). The design of the gap sensors was similar to the moisture content sensors but these sensors were mounted at the specimen edge facing the gap (Fig.3b) (Fredriksson 2010) . Both the duration of surface moisture and water in the gap was monitored by pairs of three sensors connected in parallel.

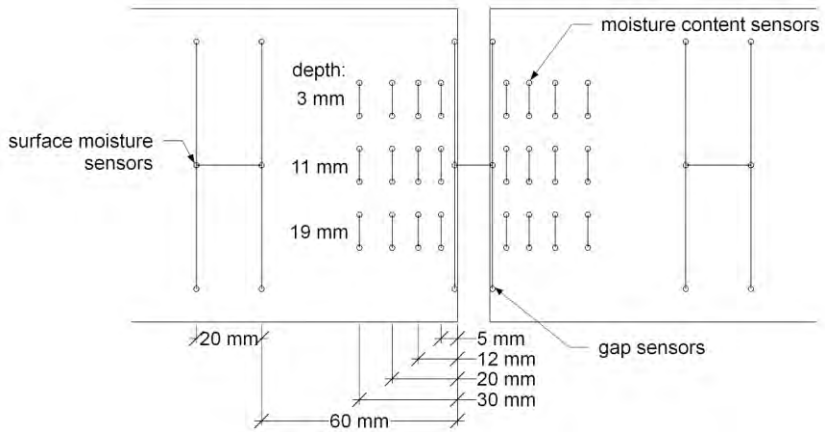
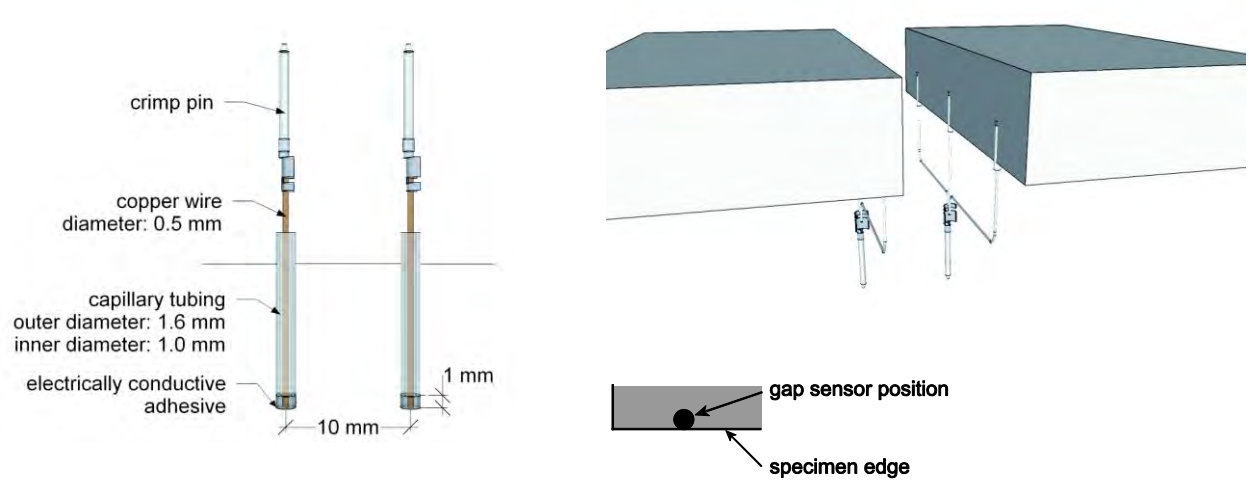


Figure 2.The positions of the moisture content sensors, surface moisture sensors and the sensors that monitor presence of water in the gap in the joint type A specimen.



a. *b.*

Figure 3. The design of the moisture content sensors (*a*) and the gap sensors mounted in a joint type A specimen (*b*).

2.1.2 Joint type B

The design of the joint type B specimen is shown in Fig.4. Two pieces of wood, 590×95×22 mm^3 and 200×95×22 mm^3 (longitudinal×radial×tangential), was mounted in two PMMA boxes so that only the upper surface of the horizontal board and two sides of the vertical board was exposed to water. All other surfaces were kept dry by the sides of acrylic plastic and rubber sealing as for joint type A. Also here the two parts of the wood/PMMA boxes were held together by two aluminium bars and the gap could be regulated between 0 and 10 mm. The wood specimens were fastened in the PMMA box by screws. In the vertical wood board, no screws were inserted within a distance of 100 mm from the lower end grain surface. In the horizontal board, no screws were inserted within a distance of ±100 mm from the middle of the board. The wood within in these areas was free from visual defects.

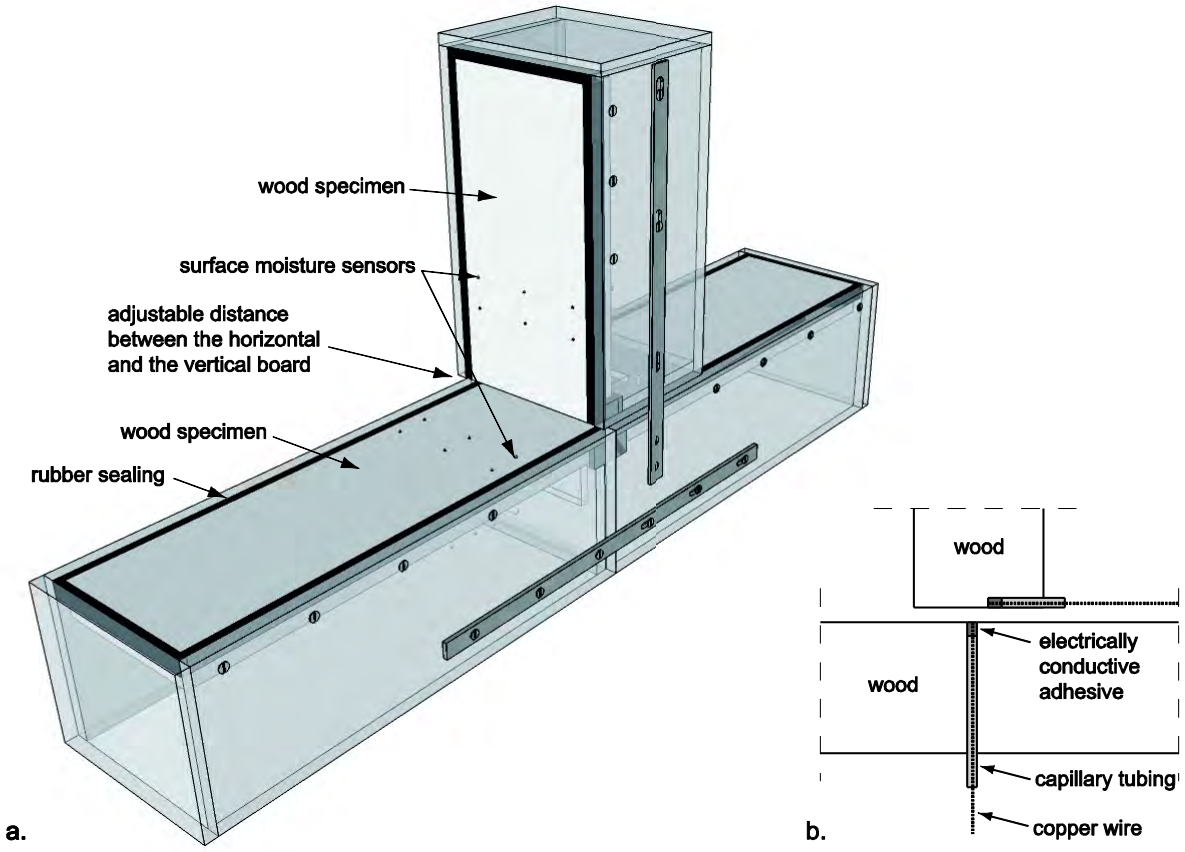


Figure 4. The design of the joint type B specimen (*a*) where two boards, one vertical and one horizontal, were mounted in a PMMA box. A detail of the sensors that monitor presence of water in the gap is shown in *b*.

Moisture content sensors were mounted from the back of the wood boards at the positions shown in Fig.5. Surface moisture sensors were mounted on both the horizontal and vertical surfaces. Also the presence of water in the gap was monitored but the position of the gap sensors was slightly different than described by Fredriksson (2010). Here, the presence of water in gap was monitored using two sensors connected in parallel on each side of the gap. The sensors in the horizontal board were mounted by drilling holes through the horizontal board opposite to the positions of the gap sensors in the vertical board (Fig. 4b). Capillary tubing was inserted from the back until the upper end of the tubing was about 1-2 mm below the surface. Electrically conductive adhesive was then inserted through the tubing until the space above the tubing (below the wood surface) was filled with glue. Finally, a copper wire was inserted from the back of the

specimen through the glue until it was slightly below the wood surface. The gap sensors in the vertical board were mounted as for joint type A.

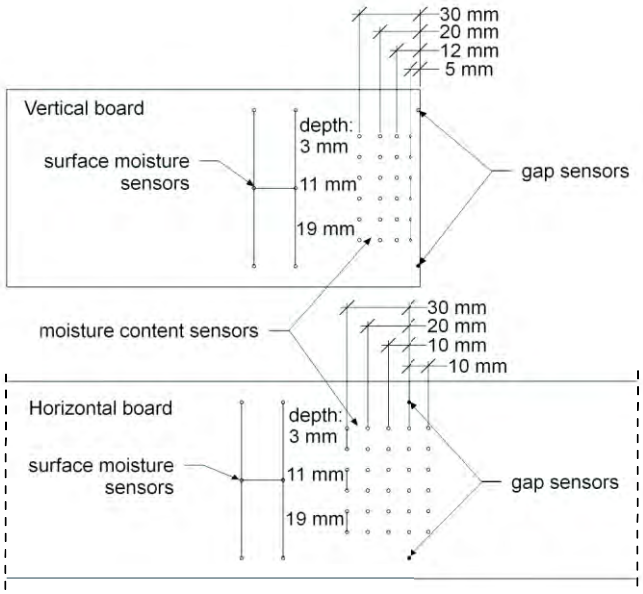


Figure 5. The positions of the moisture content sensors, surface moisture sensors and the sensors that monitor presence of water in the gap in the joint type B specimens.

2.1.3 Joint type C

The design of the joint type C specimen was similar to joint type B, except that the non-end grain surface of the vertical board was facing the gap. Moisture content sensors, surface moisture sensors and gap sensors were mounted as for joint type B. However, for joint type C, no moisture content measurements were made in the vertical board.

2.2 Experimental set-up

Three specimens with three different gap sizes (0 mm, 2 mm and 5 mm) were used in each batch. The gap sizes 2 and 5 mm were chosen since pre-experiments showed that water tended to stay in gaps smaller than 4 mm. Therefore, these two gap sizes would give one case when water stayed in the gap after the water spraying ceased and one case where no water stayed in the gap. One water spray gun (Hozelock Multi-spray gun 2676) was mounted above each specimen and the duration of the water spraying was regulated using a water timer (Gardena Water Computer C1060 plus).

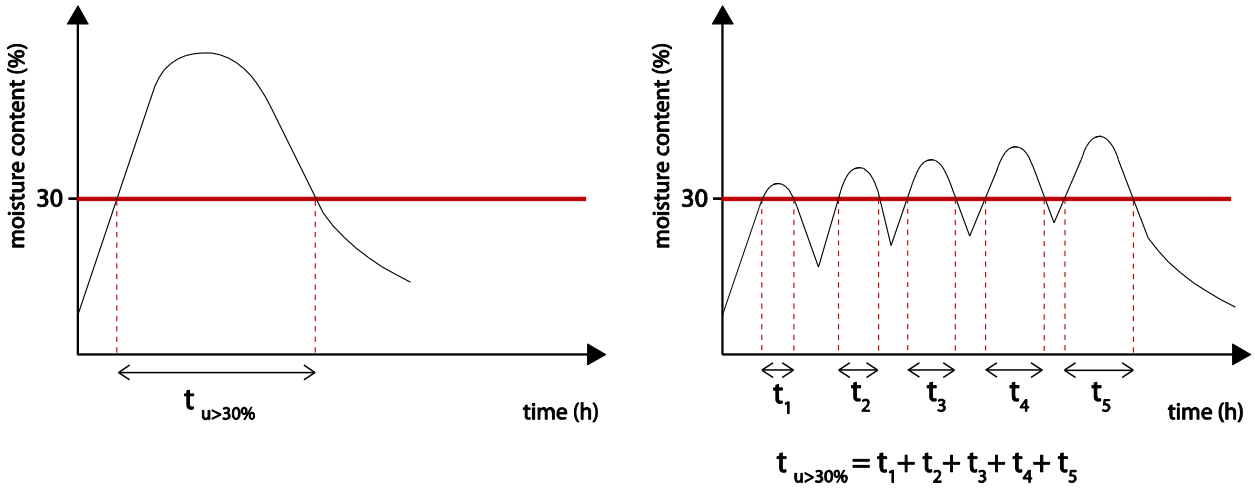
Two different watering schedules were used in the experiments. The specimens were first sprayed with water during 6.5 hours and this wetting period was followed by drying in 65% RH and 20 °C. When the specimens had dried a cyclic watering schedule was started; they were then sprayed with water during 1 hour each day during five days. Also here the climate was 65% RH and 20 °C between the watering periods.

During and after the wetting periods the moisture content, duration of surface moisture and presence of water in the gaps were logged every five minutes. The logger was custom built and the measurement range was 0.0001-200 µS. The conductance reading was performed as follows; a voltage of 2 V was applied and after 0.5 s the first reading was made during 0.5 s. The polarity was then switched and after 0.5 s another reading was made during 0.5 s. The registered conductance reading was the average between these two measurements.

2.3 Evaluation

2.3.1 Time above 30% moisture content, $t_{u>30\%}$

The measured conductance from the moisture content sensors was transformed into moisture content by the calibration curve given by Fredriksson (2010). Since rot fungi need moisture contents above the fibre saturation point and since the duration of moisture contents above this critical limit influences the decay rate (Carll and Highley 1999), the time above the fibre saturation point, $t_{u>30\%}$, was evaluated in all points of measurement. During the first watering schedule, with one wetting period and one drying period, $t_{u>30\%}$ was determined as time above 30% moisture content as shown in Fig. 6a. During the second watering schedule, with five short wetting periods, this parameter was determined as the sum of all periods of time when 30% moisture content was exceeded (Fig. 6b).



a. *b.*

Figure 6. The parameter $t_{u>30\%}$ was defined as time above 30% moisture content. During the first watering schedule with one wetting period and one drying period the parameter $t_{u>30\%}$ was the time during which 30% moisture content was exceeded as shown in *a*. During the second watering schedule with five short wetting periods, the parameter $t_{u>30\%}$ was the sum of all periods of time with a moisture content above 30% (*b*).

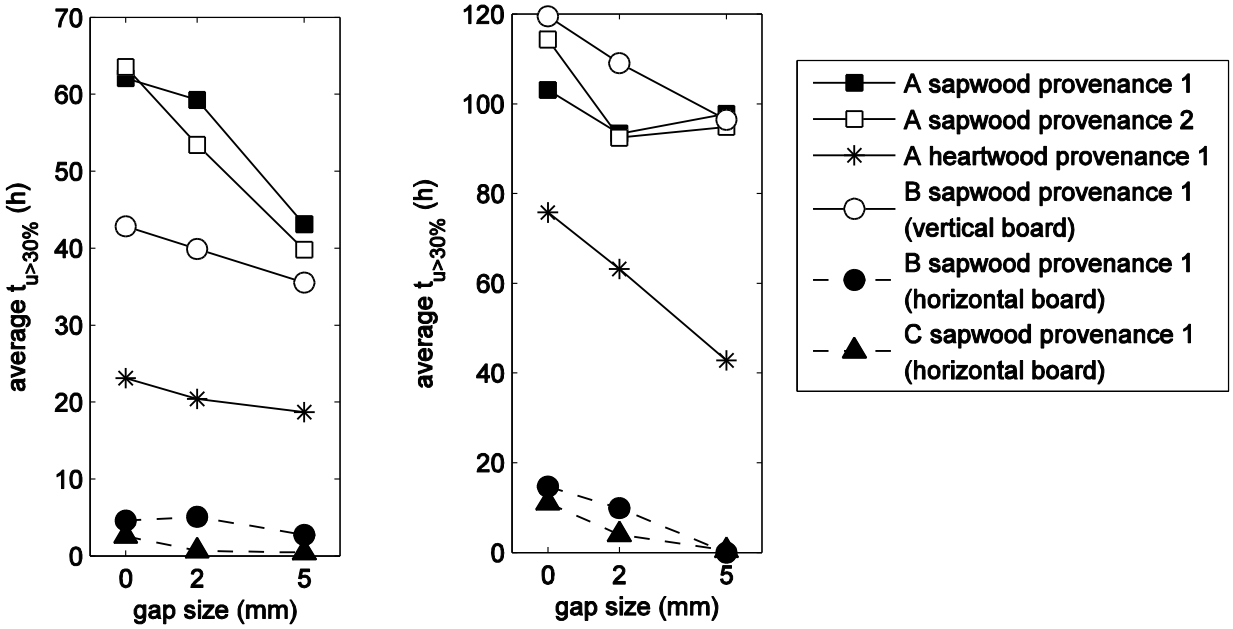
2.3.2 Duration of water in the gap

To evaluate the duration of water in the gap the reading before the measurement started was used as a threshold value. Before the measurement started the gap sensors gave low readings (between 0 and 0.0009 µS) even though there was no water in the gap. This small value was slightly different between specimens and an individual threshold value for each specimen was therefore used. The maximum value one hour before the watering started was used as a threshold value for each specimen. The time above this value was then calculated. The evaluation of presence of water in the gap when the gap size was 0 mm was slightly different since the gap sensors had contact through the wet wood surfaces. Here, the maximum conductance from the moisture content sensors closest to the surface was used as a threshold value; when the reading from the gap sensors was lower than the maximum reading from the moisture content sensors close to the surface the gap was considered empty. In both cases, the watering time was subtracted from the duration of water in the gap; i.e. if no water was held in the gap after the water was turned off the duration of water in the gap was zero.

3. RESULTS AND DISCUSSION

3.1 Influence of joint type on the average moisture content

In Fig. 7 the average time above 30% moisture content in all points of measurements in each specimen is shown. For watering schedule 1, the longest durations of high moisture contents were found for the sapwood specimens in joint type A where two end grain surfaces were exposed to water. The average time above 30% moisture content was however less than half if heartwood was used in this joint type instead. For the second watering schedule, the duration of high moisture contents were longer for all joint types. However, the longest durations of high moisture contents were here found in the vertical board of joint type B. The horizontal boards in joint type B and C, where no end grain surface were exposed to water, had the shortest durations of moisture contents above 30% and therefore also the lowest risk for decay by rot fungi.



a. *b.*

Figure 7. The average $t_{u>30\%}$ for all points of measurements in each specimen.
a. watering schedule 1: wetting during 6.5 hours.
b. watering schedule 2: wetting during 1 hour each day during five days.

3.2 Influence of gap size on $t_{u>30\%}$

The greatest influence of gap size was seen for joint type B (Figs. 8 and 9) during cyclic wetting when the specimens were sprayed with water during one hour each day for five days. In the horizontal board, the moisture content never exceeded 30% when the gap between the boards was 5 mm (Fig. 8). Also in the vertical board the influence of a gap between the boards was significant at 20 and 30 mm from the end grain surface (Fig. 9).

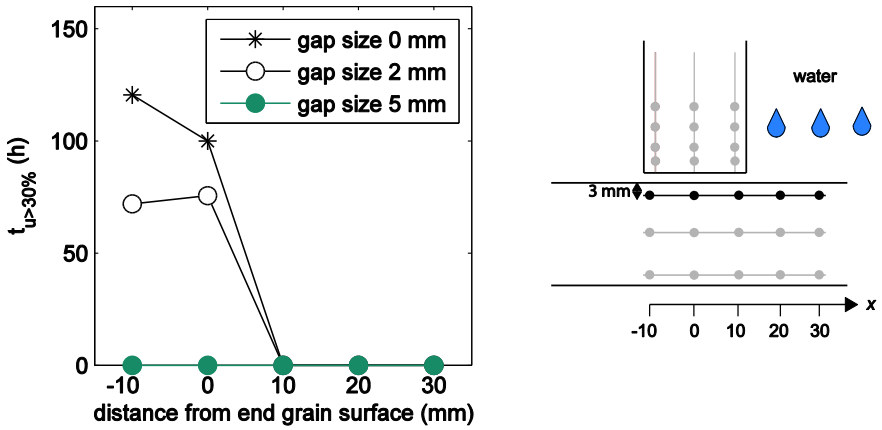


Figure 8. The time above 30% moisture content, $t_{u>30\%}$, for the horizontal board in joint type B (left). The results are for the second watering schedule where the specimens were sprayed with water during one hour each day for five days. The measurements were made 3 mm below the water exposed surface in five positions. These positions are indicated by black filled circles in the figure to the right.

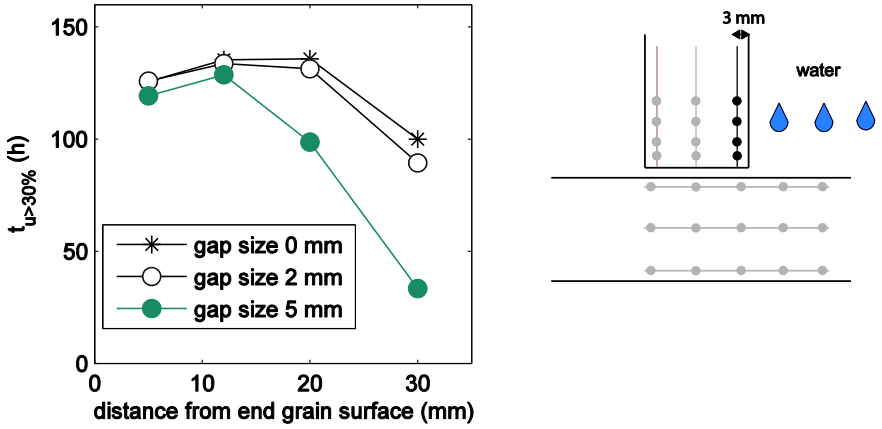


Figure 9. The time above 30% moisture content, $t_{u>30\%}$, for the vertical board in joint type B (left). The results are for the second watering schedule where the specimens were sprayed with water during one hour each day for five days. The measurements were made 3 mm below the water exposed surface at four distances from the end grain surface facing the gap. These points of measurement are indicated by black filled circles in the figure to the right.

3.3 Influence of heartwood/sapwood and provenance on $t_{u>30\%}$

Large differences were seen between heartwood and sapwood specimens (joint type A) of the same provenance; the duration of high moisture contents were lower for the heartwood specimens, especially 20 and 30 mm from the end grain surface (Fig. 10a). The influence of gap size was larger for the sapwood specimens. However, no significant difference in duration of high moisture contents was seen comparing sapwood from northern and southern Sweden (Fig. 10b and 10c).

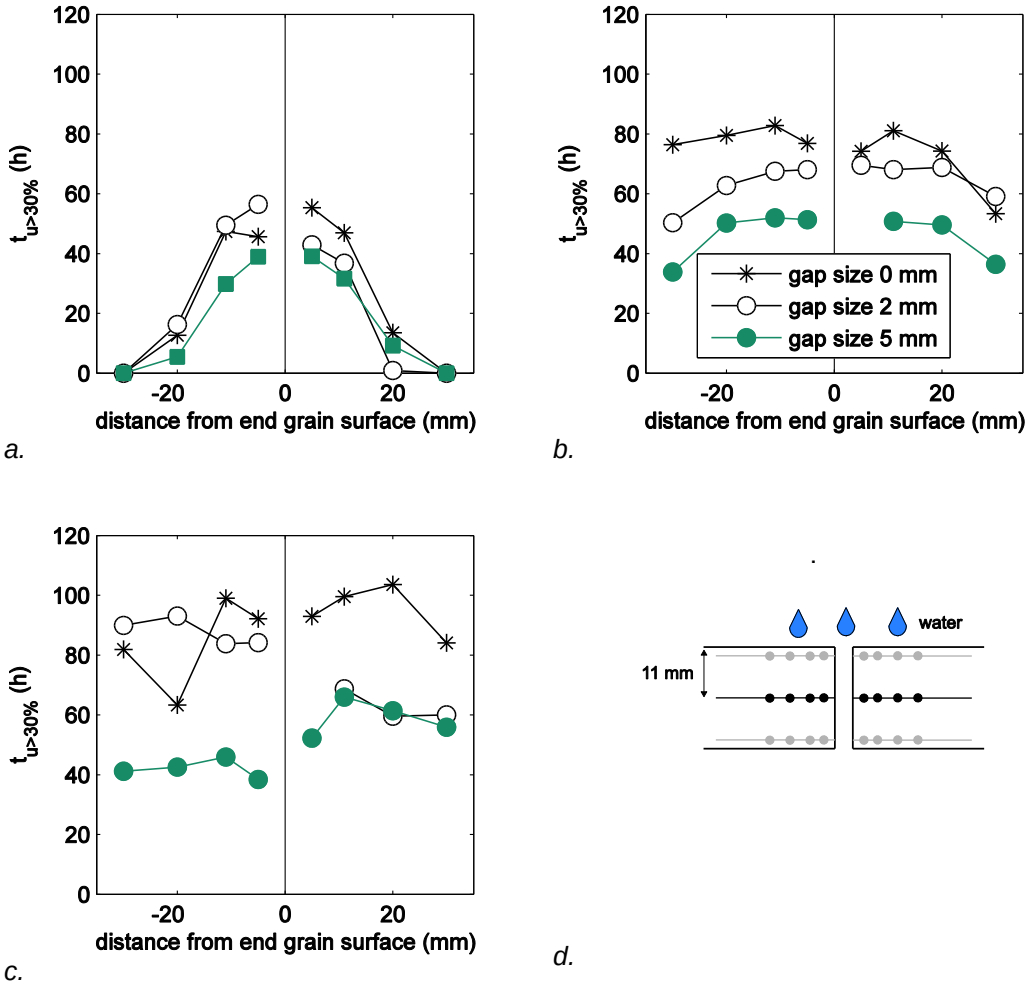


Figure 10. The duration of moisture contents above 30%, $t_{u>30\%}$, for heartwood from northern Sweden (*a*), sapwood from southern Sweden (*b*) and sapwood from northern Sweden (*c*). The measurements were made 11 mm below the water exposed surface, the positions are indicated by black filled circles in *d*. The results are for the first watering schedule where the specimens were wetted during 6.5 hours and dried in 65% RH, 20 °C.

3.4 Wetting in cycles versus one wetting period

Two different watering schedules were used in the experiments:

1. Water during 6.5 hours then drying in 65% RH 20 °C
2. Water during 1 hour every day during five days, i.e. 1 hour of watering and 23 hours of drying in 65% RH 20 °C repeated five times.

In Fig. 11 the time above 30% moisture content for these two watering schedules are compared for the vertical board in joint type B. Even though the total time of wetting is shorter for the second watering schedule (5 hours instead of 6.5 hours) the time above 30% moisture content is considerably higher for the second watering schedule. This is true for all joint types used in this study.

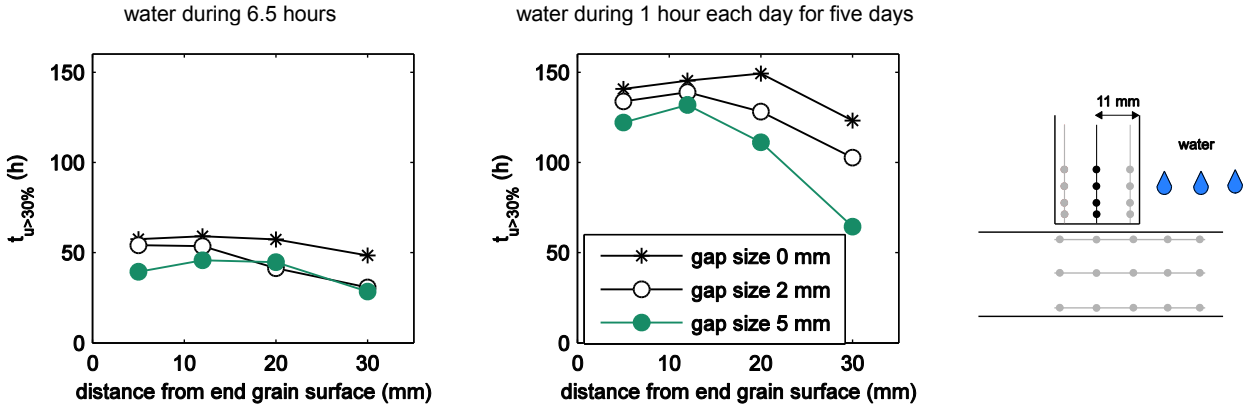


Figure 11. The duration of moisture contents above 30%, $t_{u>30\%}$, for the vertical board in joint type B for the first watering schedule when the specimens were sprayed with water during 6.5 hours (*a*) and the second watering schedule where the specimens were sprayed with water during 1 hour each day for five days (*b*). The measurements were made 11 mm below the water exposed surface, i.e. in the middle of the vertical board. The positions of the moisture content sensors are indicated in *c* by black filled circles. The measurement were made in Norway spruce sapwood of provenance 1.

3.5 Duration of water in the gap

The results from the measurements of duration of water in the gap for all joint types are shown in Fig. 12. When the gap between the boards was 5 mm, none of the joints trapped water after the wetting period ended.

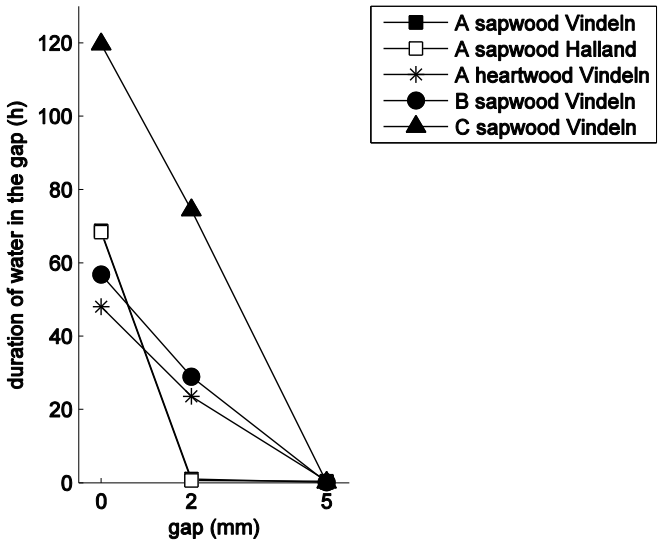


Figure 12. The duration of water in the gap after a wetting period of 6.5 hours.

4. CONCLUSIONS

Changes in micro climate, i.e. duration of water trapped in gaps, influences the duration of moisture contents above the fibre saturation point. The duration of high moisture contents was in most cases lowered by a larger gap between the boards. Joint types with end grain surfaces have higher average time above the fibre saturation point but the time above the critical level is less than halved if heartwood is used instead of sapwood.

ACKNOWLEDGEMENTS

Thomas Ulvcrona, Swedish University of Agricultural Sciences, is gratefully acknowledged for providing the wood material used in this study.

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REFERENCES

Carll, C. G. and T. L. Highley (1999). Decay of wood and wood-based products above ground in buildings. *Journal of Testing and Evaluation* 27(2): 150-158.

De Groot, R. C. and T. L. Highley Forest Products Laboratory methodology for monitoring decay in wood exposed above ground. *Proceedings of IRG Annual Meeting*. IRG/WP 95-20074.

Fredriksson, M. (2010). *Methods for determination of moisture conditions in wood exposed to high moisture levels.* Division of Building Materials, Lund University. Lund. TVBM-3157.

Gaby, L. and J. Duff (1978). *Moisture content changes in wood deck and rail components.* USDA Forest service research paper. SE-190.

Isaksson, T., S. Thelandersson, A. Ekstrand-Tobin and P. Johansson (2010). Critical conditions for onset of mould growth under varying climate conditions. *Building and Environment* 45(7): 1712-1721.

Luping, T., L.-O. Nilsson and P. M. Basheer (2012). *Resistance of concrete to chloride ingress*. Taylor and Francis, London.

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Section 4 **Processes and Properties**

Protective nanoparticle coating reducing water absorption of wood species

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Protective nanoparticle coating reducing water absorption of wood species

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ABSTRACT

Nanoparticle coatings were deposited onto different tropical wood samples for controlling the surface hydrophobicity. The styrene(maleimide) nanoparticles were synthesized in aqueous dispersions under pure conditions or in combination with 70 wt.-% palm oil. A first evaluation of the non-coated wood surfaces indicates a high dependence between the water contact angles, the average surface roughness and wood density: dewetting phenomena occur on high-density wood and penetration phenomena happen on low-density wood. These interactions further determine the behaviour of the nanoparticle dispersions in contact with the different wood surfaces. The nanoparticle coatings are consequently more homogeneous on low-density wood compared to high-density wood. The water contact angles of pure nanoparticle coatings increase for all wood types, although instabilities are observed due to the porous coating structure. The hybrid nanoparticle coatings including palm oil provide highest hydrophobicity, as the vegetable oil acts as a natural binder component and provides more continuous coatings. The coatings are further evaluated with optical roughness measurements, indicating that the surface structure and coating performance is mainly determined by the original wood density.

Keywords: surfaces, hydrophobicity, nanoparticles, roughness

1. INTRODUCTION

The protection of wood against environmental factors of humidity, moisture and water is a primary requirement for improving dimensional stability, preservation, and for reducing stress-fracture by cyclic swelling and shrinkage. Common chemical surface treatments improving the hydrophobicity and water repellence include, e.g., heat treatment (Gérardin *et al.* 2007), fluorination (Hsieh *et al.* 2007), covalent grafting of silicone polymers (Sèbe and Brook 2001), or silylation treatments (Mohammed-Ziegler *et al.* 2005). However, severe chemical surface modifications often mask the natural image of wood and more sustainable alternatives should be developed. The wettability of wood surfaces can be controlled by using waterborne coatings (Petric *et al.* 2007). One technique to reduce water absorption is inspired by the Lotus-leaf effect, where the hydrophobic moieties are located on the surface in combination with micro- to nanoscale surface structures. Recently, the durability of nanoparticle wood coatings (Vlad-Cristea *et al.* 2012) and introduction of vegetable oils (Mahendran *et al.* 2012, Philipp *et al.* 2012) received attention. In previous research, we successfully applied waterborne nanoparticle coatings on papers (Samyn *et al.* 2010, Samyn *et al.* 2011). In present research, we further explore possibilities of those coatings for compatibility and modification of wood surfaces.

2. EXPERIMENTAL METHODS

2.1 Wood Species

Different types of tropical wood species were used in this study, as summarized in Table 1. Their selection was based on different density and availability. Further characterisation of the wood samples was made elsewhere (Paredes *et al.* 2011). For ease of further interpretation and discussion of the following test results, the wood samples will be mainly grouped as high-density samples (HD-wood) and low-density samples (LD-wood). The wood samples were sawn into poles with free tangential and radial surfaces, and air-dried before further use. The tangential surfaces of the wood samples were used for further surface modification and analysis. The radial surfaces were not coated nor characterised.

Table 1: Selected wood species that were used as substrates.

Wood sample	Specimen	Density (g/cm^3)
1	Senna sp.	0.88 ± 0.07
2	Acacia glomerosa	0.89 ± 0.07
3	Machaerium capote	0.93 ± 0.05
4	Pseudobombax maximum	0.35 ± 0.11
5	Schizolobium parahybum	0.32 ± 0.07
6	Gyrocarpus americanus	0.35 ± 0.05

2.2 Coating Materials

The coating materials contain organic nanoparticles that were synthesized in the laboratories of Topchim N.V., Belgium. Therefore, high-molecular weight poly(styrene maleic anhydride) or SMA was imidized according to a protocol that was reported before (Samyn *et al.* 2012), and that is schematically represented in Fig. 1. The polymer precursor has an average molecular weight of 80.000 g/mol and contains 26 mol-% maleic anhydride.

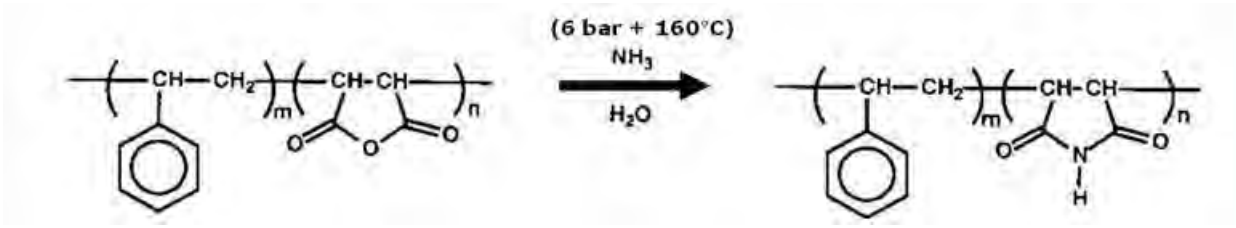

Figure 1: Synthesis of poly(styrene maleimide) nanoparticles.

For synthesis of pure poly(styrene maleimide) nanoparticles, or SMI, about 140 g of SMA was charged into a 1 liter autoclave reactor, together with an 1:1.01 molar ratio of ammonium hydroxide relatively to the maleic anhydride (MA) moieties and 700 ml water. After adding the ammonium hydroxide, the temperature was progressively raised to a maximum of 160°C at a maximum reaction pressure of 6 bar under continuous stirring at 1 rpm. As the reaction mixture attained 90 to 120°C, the viscosity increased significantly through gel formation. While further heating at 150 to 160°C, a sudden drop in viscosity was characteristic for the initiation of nanoparticle formation. After a total reaction time of approximately 6 hours, the reaction mixture was cooled down to room temperature. Finally, an aqueous dispersion of SMI nanoparticles have white to yellow colour and are stable without further addition of surfactants or stabilizing agents. The aqueous nanoparticle dispersions were characterized as before, with a solid content of 35 %, and Zetapotential ζ = -60 mV.

For synthesis of hybrid poly(styrene maleimide), or SMI/PO, 70 wt.-% of palm oil was added to the reaction. The aqueous nanoparticle dispersions were characterized as before, with a solid content of 65 %, and Zetapotential ζ = -45 mV.

2.3 Coating application

The coating dispersions were applied onto the wood surfaces by means of bar coating, using a laboratory-scale K303 Multi-coater (RK Print Coat Instruments Ltd, UK). With a metering bar number 2 (close wound wire diameter 0.15 mm) operating under controlled pressure and a bar moving speed of 6 mm/s, a wet coating thickness of approximately 12 µm was obtained. Generally, the wet coating thickness is determined by the wire diameter of the bar (higher diameter equals thicker wet coating deposit) and speed (higher speed equals thinner wet coating deposit) for a dispersion with given solid content. The coated wood samples were placed into a circulating hot-air oven at 120°C for 2 min, allowing to dry the coating.

2.4 Surface characterisation

The wood surfaces were first characterized by water contact angle measurements. Using a Digidrop equipment (GBX, France), a water droplet of about 6 µL was placed onto the surface over a time span of 60 seconds. The contact angle upon initial contact θ_i was characterised as a measure for the surface hydrophobicity, and the decay of the water contact angle over time $\Delta\theta$ is a measure for the stability of the droplets in contact with the surface. The contact angle results were averaged from five measurements with an average standard variation of ± 1.5°.

The surface morphology was observed by optical microscopy, using an Olympus BX51M microscope with objective magnification of 5.

The surface topography was characterized by optical profilometry, using a WYKO NT-2000 (VEECO) profilometer. Using vertical scanning interferometry, sampling sizes of L x L = 1000 x 1000 µm^2 were scanned by using a stitching function. An objective magnification of 50 x and field of view magnification of 0.5 x was used, with a sampling resolution of 314 nm. The pixel size was 1.26 µm for all images and the light intensity of one pixel represents an average surface height within this pixel. As such, the sampling interval τ remains constant as more data points N are recorded at increasing sampling sizes. A rectangular stitching function was used to preserve the lateral resolution over the entire sampling area L x L, with an overlap of 20 % between the individual field-of-view areas. The vertical scan length for a single field-of-view picture is 50 µm with a backscan length of 15 µm, which allows focussing the system at a medium height without losing surface information above the focussing point. The scan length and backscan define the total scan length of a single measurement, and are uniform for each unit of the multipart scan. The images were processed with Wyko Vision 32 software (Version 2.21), allowing to calculated the average surface roughness Sa. Test results were averaged from three independent image captures per sampling area.

3. RESULTS AND DISCUSSION

3.1 Surface characterisation of non-coated wood samples.

The measurements of contact angles on non-coated wood samples over time, are shown in Fig. 2. There is significant difference between the different wood types in the initial contact angle θ_i upon first contact. In general, we observe that the LD-wood species have a high initial contact

angle θ_i and a relatively sharp decrease in contact angle over contact time resulting in a high value for $\Delta\theta$. On the other hand, the HD-wood species have a lower initial contact angle θ_i and a relatively rapid stabilisation in contact angle value, resulting in lower values for $\Delta\theta$. The differences in intrinsic wettability of wood species mainly depend on the density of LD- and HD-wood samples, and the resulting surface properties upon initial contact: we may conclude that different interaction mechanisms of water droplets include mainly *penetration* effects on LD-wood and *spreading* effects on HD-wood samples. The different behaviour of water in contact with the respective wood species is important to control the future application of the nanoparticle coatings from aqueous dispersions. Indeed, the differences in wetting behaviour of the nanoparticle dispersion over the wood surface will in particularly influence either the penetration or spreading of the coating pigments over the surface of the wood species, depending on the wood origin.

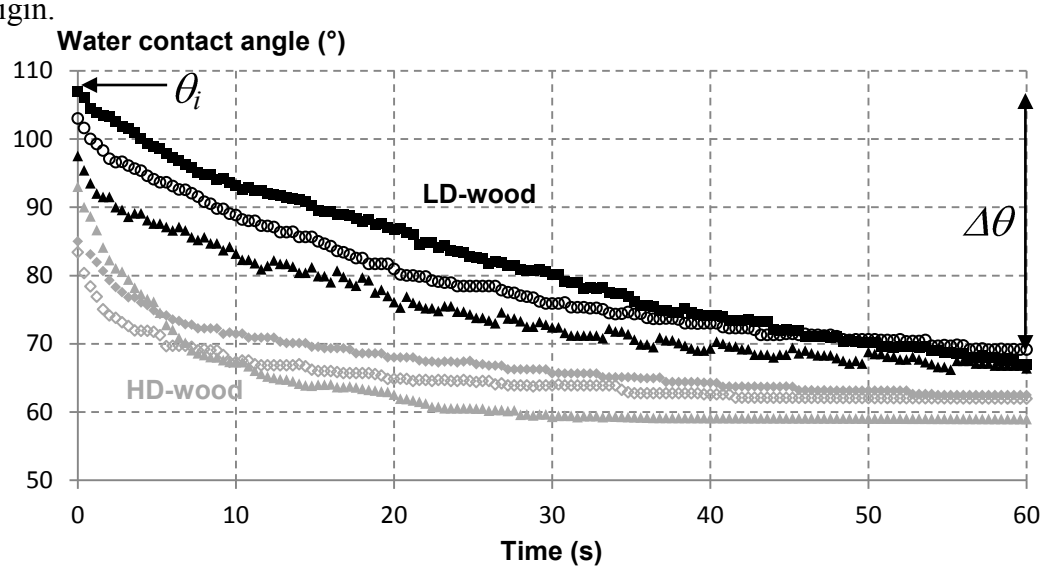

Figure 2: Time-dependent evolution of water contact angles deposited onto non-coated wood samples.

The surface profiles of HD- and LD-wood species are illustrated in the Fig. 3, with an overview of the surface roughness data. We observe that the surfaces of HD-wood species correspond to a dense structure, while the surfaces of the LD-wood species correspond to a more open and porous structure. These observations are reflected in differences in average surface roughness Sa, which is indeed higher for the LD-wood compared to the HD-wood species.

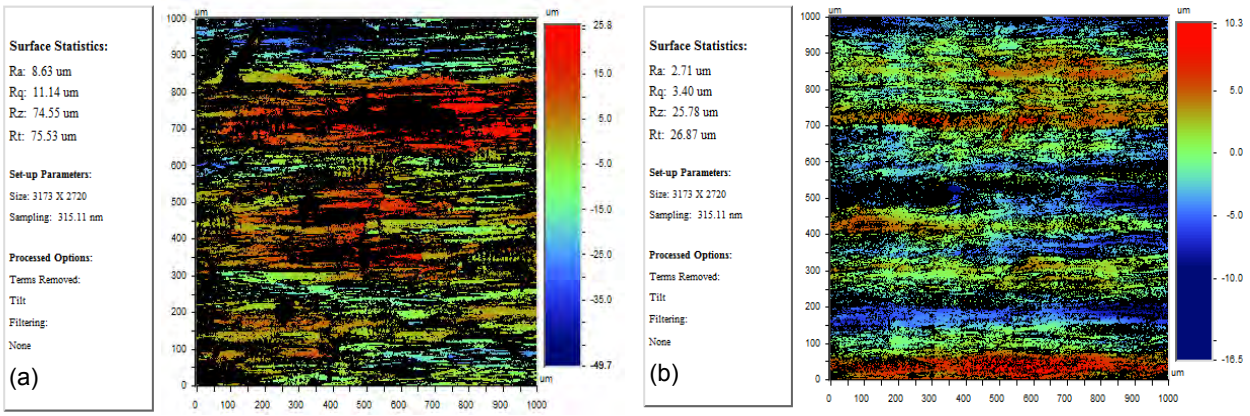

Figure 3: Surface profiles and roughness data obtained by optical profilometry for (a) LD- and (b) HD-wood species.

By comparing the surface roughness data and the contact angles for the non-coated wood samples in Fig. 4, a direct relationship between the average surface roughness Sa and the initial contact angle θ_i can be drawn, indicating that wood samples with a higher value of Sa also present a higher initial water contact angle. Also the reduction of contact angle $\Delta\theta$ over time relates to the average surface roughness Sa, as the wood samples with a low surface roughness present lower values for $\Delta\theta$ compared to wood samples with a high surface roughness presenting higher values for $\Delta\theta$. As such, we conclude that the initial wettability and time-dependent water interactions of different wood species depend on the average surface roughness Sa as an important parameter.

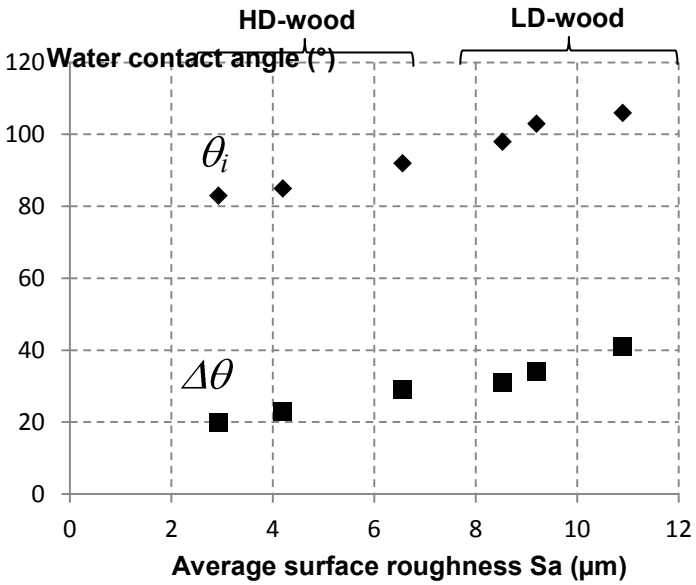

Figure 4: Relation between the average surface roughness Sa, and water contact angle data θ_i, $\Delta\theta_i$ for non-coated wood species.

3.2 Surface characterisation of wood samples with nanoparticle coatings.

3.2.1 Surface hydrophobicity of nanoparticle coated wood samples

For all wood types, the pure SMI nanoparticle coated samples and hybrid SMI/PO nanoparticle coated samples were evaluated by measuring water contact angles over a contact time of 60 sec. As characteristic values for the water contact angles, the initial contact angle θ_i and the difference $\Delta\theta$ are summarized in Fig. 5, and compared to the values for non-coated wood samples.

The initial contact angles θ_i for non-coated wood samples, wood samples with a pure SMI nanoparticle coating, or wood samples with a hybrid SMI/PO nanoparticle coating are plotted in Fig. 5a. For all of the wood samples, we observe a higher initial contact angle for nanoparticle coated samples compared to the non-coated samples. The contact angle for coatings of pure SMI nanoparticles is higher than the non-coated woods, and the contact angle for coatings of SMI/PO nanoparticles is higher than the pure SMI nanoparticle coatings. For pure SMI nanoparticle coatings, the increase in contact angle is not directly related to the contact angles of the individual non-coated wood species, but the surface hydrophobicity differentiates between both categories of wood samples, with a coating on HD-wood samples providing relatively lower contact angles compared with a coating on LD-wood samples.

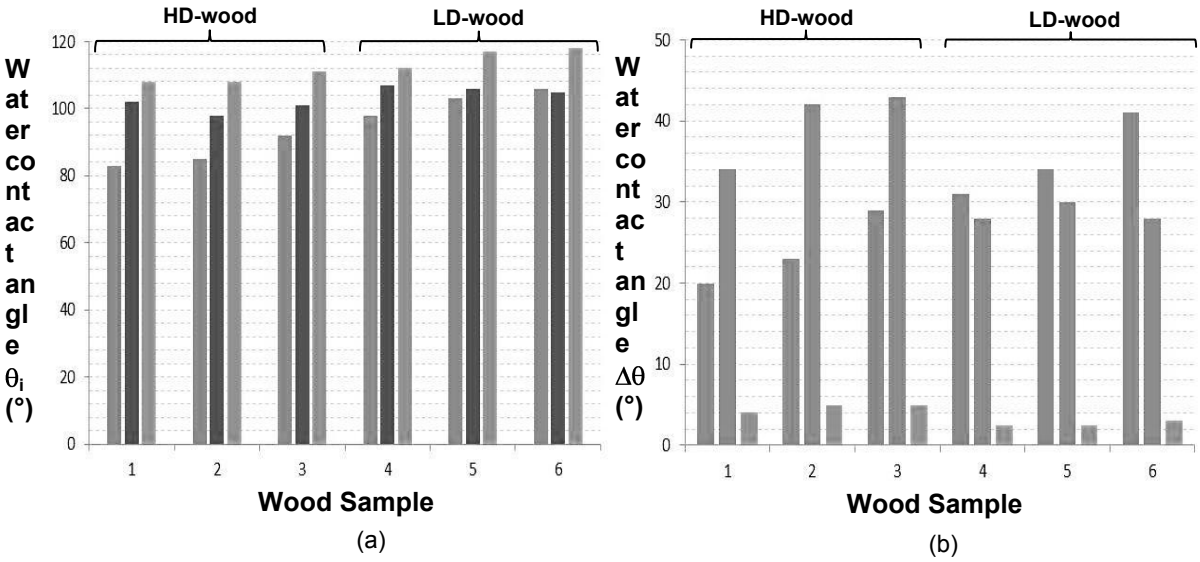

Figure 5: Water contact angle data for nanoparticle coated wood samples, (a) initial contact angle θ_i, (b) contact angle decrease $\Delta\theta$: non-coated wood (first column), pure SMI nanoparticle coating (second column), and hybrid SMI/PO nanoparticle coating (third column).

The contact angle for wood species coated with pure SMI nanoparticles seems mainly determined by the properties of the nanoparticle coating itself: e.g., for LD-wood species the contact angles of the non-coated wood samples vary between 98 and 106° while the contact angles of SMI-coated wood samples vary between 105 to 107°. For hybrid SMI/PO nanoparticle coatings, the presence of vegetable oils stimulates the hydrophobicity of the wood surfaces and provides highest contact angles. There is again a trend that the hydrophobicity on coated LD-woods is higher than on coated HD-wood, in parallel with the pure SMI nanoparticle coatings. The latter trend even is somewhat more pronounced for the hybrid SMI/PO nanoparticle coatings than for the pure SMI nanoparticle coatings.

The values for decrease in contact angles $\Delta\theta$ over 60 sec contact time for non-coated wood samples, wood samples with a SMI nanoparticle coating, or wood samples with a SMI/PO nanoparticle coating are plotted in Fig. 5b. For pure SMI nanoparticle coated samples, the decrease in contact angles depends on the original wood specimens, in parallel with the values for initial contact angle: (i) for HD-wood samples, a higher decrease of contact angles $\Delta\theta$ than the non-coated samples indicates some instabilities, (ii) for LD-wood samples, there is a slight improvement as the contact angle decrease $\Delta\theta$ is somewhat lower than for non-coated samples and HD-wood samples, but the values still remain high. For hybrid SMI/PO nanoparticle coated samples, the decreases in contact angle $\Delta\theta$ are much lower, as an indication for better stability. However, the values for $\Delta\theta$ on HD-wood samples are somewhat higher than the values for $\Delta\theta$ on LD-wood samples, with a maximum of 6° (HD-wood) and a minimum of 2° (LD-wood). In this respect, the lowest values for $\Delta\theta$ on LD-wood agree with the high initial contact angles θ_i and high surface hydrophobicity of LD-wood. The best hydrophobicity overall is obtained for the SMI/PO nanoparticle coatings for all wood species. Based on initial contact angle values and decrease in contact angle over time, it is illustrated that the presence of vegetable oils significantly contributes to the hydrophobic behaviour. The sensitive trend in increasing the contact angles indicates that the oil is effectively located at the wood surface as a hydrophobic agent, and the oil does not preferentially penetrate into the bulk of the wood sample.

3.2.2 Surface morphology of nanoparticle coated wood samples

Optical microscopy of the coated wood samples provides a first idea of the coating deposition and its homogeneity. The pictures in Fig. 6 were taken at relatively low magnification in order to image the coating morphology over a relatively broad area, both on HD- and LD-wood samples. Only the coating on one of each wood sample type is shown, which is representative for similar HD- or LD-wood types. The pure SMI nanoparticle coatings form an 'island-like' morphology with coverage depending on the density of the original wood sample: (i) the HD-wood samples are covered inhomogeneously, while (ii) the LD-wood samples are more homogeneously covered with an even distribution of the coating having the same morphology over the entire wood sample. These two morphologies confirm different interaction mechanisms of the coating with the wood samples, depending on the sample density: (i) the coating obviously dewets over the HD-wood samples, while (ii) the coating penetrates into the surface pores over the LD-wood samples. These observations explain the differences observed in contact angles as before. More detailed studies, however, show that all pure SMI nanoparticle coatings are highly porous and form macro-scale domains. These phenomena can be related to the high glass transition temperature T_g of the SMI nanoparticles (T_g = 180°C) and lack of inter-diffusion of the coating pigments under drying. The resulting coating porosity explains the instabilities in contact angle measurements and overall high decrease in contact angle $\Delta\theta$.

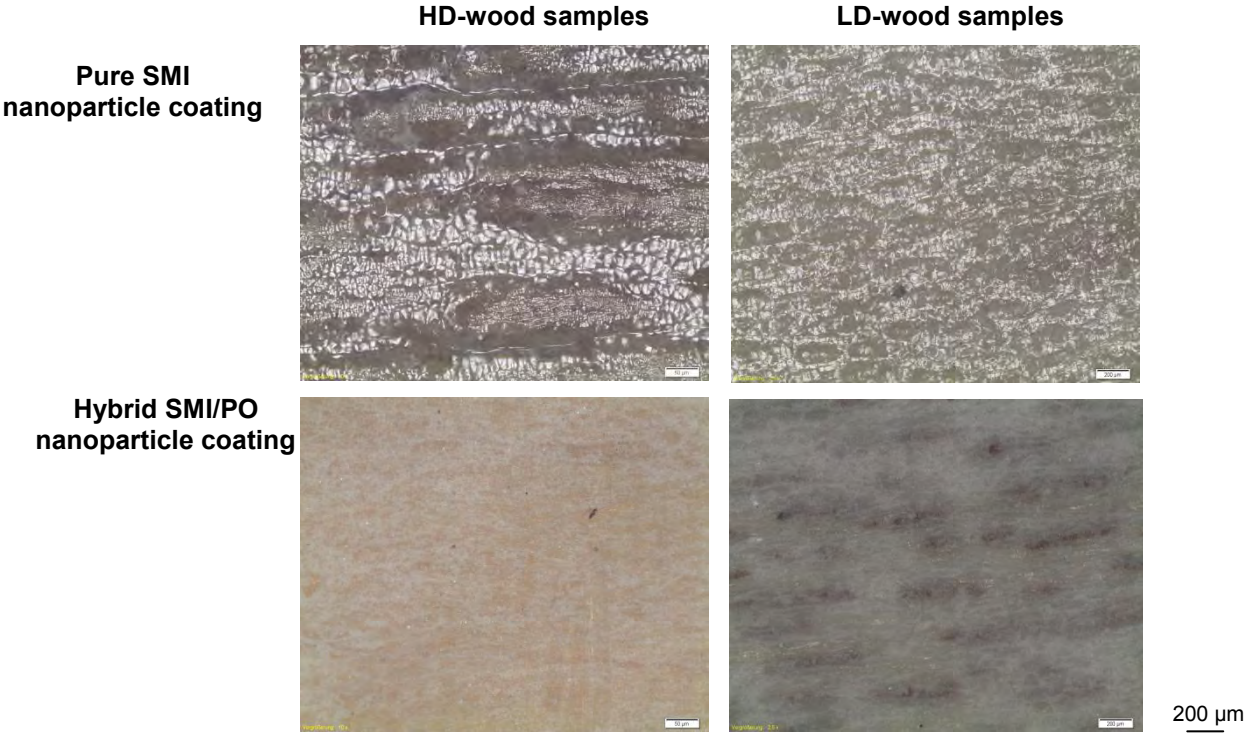

Figure 6: Optical microscopy of HD- and LD-wood samples with pure SMI nanoparticle coating, or hybrid SMI/PO nanoparticle coating.

The hybrid SMI/PO nanoparticle coatings cover both the HD- and LD-wood species more homogeneously in comparison with the pure SMI nanoparticle coatings. The formation of the macro-scale domains and consequent cracking of the coating does not appear in presence of vegetable oils. The vegetable oils prevent dewetting of the nanoparticle dispersions over the wood surface, leading to more stable contact angles and good protection against water penetration as a function of time. The vegetable oils encapsulated in the hybrid SMI/PO

nanoparticles consequently act as a natural binder for the nanoparticle coatings, and are also compatible with the different wood surfaces.

3.2.3 Surface profilometry of nanoparticle coated wood samples

An example of surface profiles of pure SMI nanoparticle coatings and hybrid SMI/PO nanoparticle coatings is illustrated in Fig. 7. The characteristic features as discussed before, are clearly revealed by optical profilometry, i.e. the formation of either macro-domains of pure SMI nanoparticles, or continuously covering coatings of hybrid SMI/PO nanoparticles.

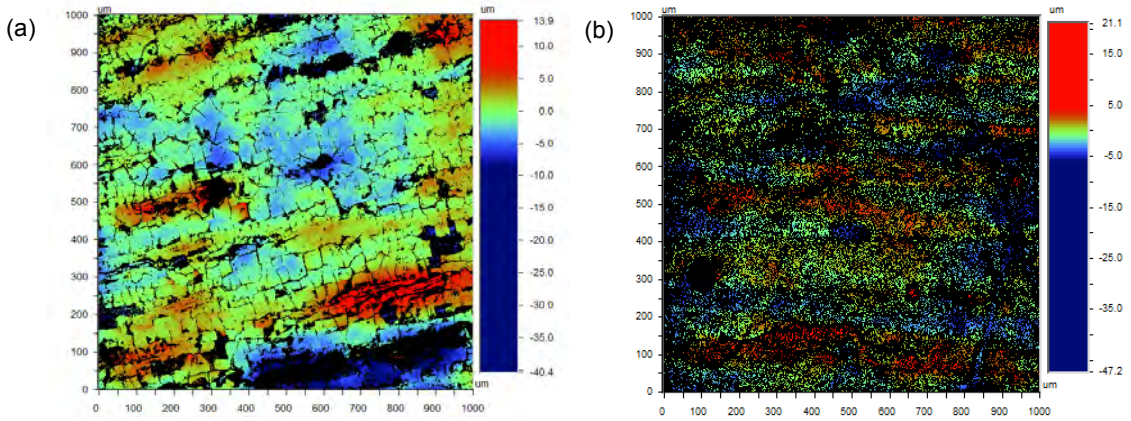

Figure 7: Illustration of surface profiles for (a) pure SMI nanoparticle coating, (b) hybrid SMI/PO nanoparticle coating deposited on LD-wood.

The values for average surface roughness Sa, are summarized in Fig. 8 for the different non-coated and coated wood types. These values were obtained from the originally measured surface profiles, without any further flattening procedure performed by the software. As such, they give data from the non-coated and coated wood surfaces, including macro- and microscale defects.

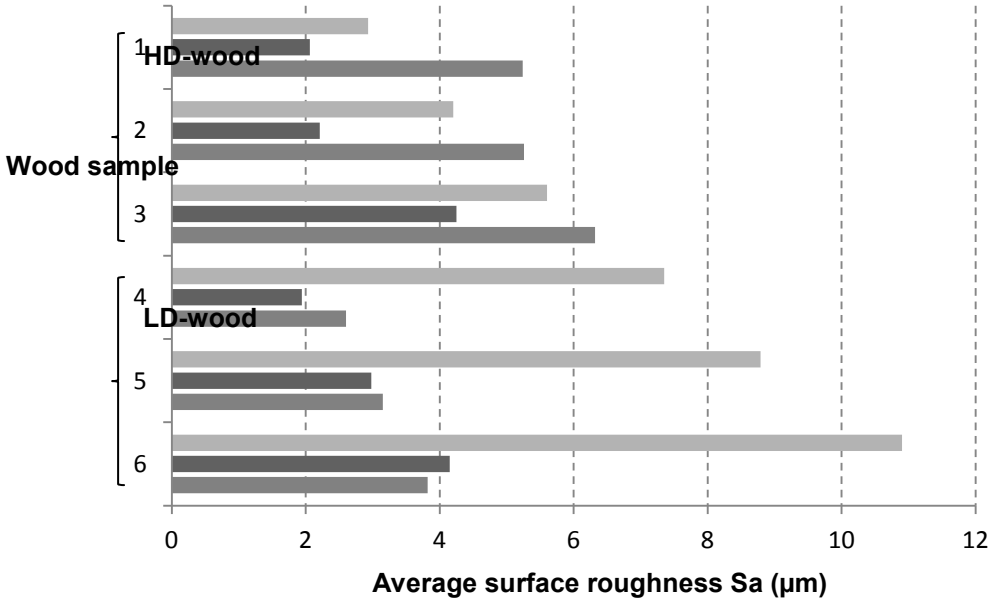

Figure 8: Average surface roughness Sa for nanoparticle coated wood samples: non-coated wood (1st bar), pure SMI nanoparticle coating (2nd bar), hybrid SMI/PO nanoparticle coating (3rd bar).

After coating with pure SMI nanoparticles, the original roughness of the wood samples has decreased for both the HD- and LD-wood types, compared with the non-coated wood. As such, the average roughness values indicate that the original pores and porosities of the original wood surfaces are covered, and the final roughness is mostly related to the condition of the coating surface layer. In order to provide a homogeneous coverage, a coating layer thickness of approximately 7 µm (measured by profilometry) is needed. In general, an overall relationship between the original roughness of the non-coated wood surface and the roughness of the coated wood samples does not held by comparing all the different wood types. There is no direct relation that a higher surface roughness of the original non-coated wood sample provides a higher surface roughness to the coated wood samples. However, we observe that influences may apply if the HD- and LD-wood samples are evaluated separately. Indeed, there is a strong correlation for the LD-wood samples, while the trend is not so strong for the HD-wood samples because two of the coated wood samples (1, 2) obtain nearly similar roughness while their original wood surface roughness was clearly different. These measurements suggest also that the interaction mechanisms between the coating and the wood samples are different for both wood types, including rather *dewetting* phenomena on HD-wood samples and *penetration* phenomena on LD-wood samples. Finally, the surface roughness of coated samples is fully determined by the presence of the macro-domain structures in the coating layer, and eventual surface defects. From the latter, we can estimate that the adhesion of the pure SMI nanoparticle coating to HD-wood is inferior compared to LD-wood.

After coating with hybrid SMI/PO nanoparticles, the roughness on HD-wood samples has characteristically increased compared to the non-coated wood samples. The roughness values are likely determined by a combination of both the coating layer and the wood structure, with wood fibers that remain contributing to the roughness through the coating. This observation interestingly points to an increase in roughness that may favourably contribute to the increase in surface hydrophobicity. The roughness on LD-wood samples, however, has decreased in respect to the original wood surfaces providing a more even surface. The present observations, comparing hybrid SMI/PO nanoparticle coatings onto HD- and LD-wood samples may at first sight contradict the general rule describing the wetting of nanoscale structured surfaces. According to wetting models of structured surfaces (Wenzel *et al.* 1947), the hydrophobicity on rough surfaces is enhanced in comparison to a smooth surface with the same chemical composition. Here, we mainly observe that the hydrophobicity of the hybrid SMI/PO nanoparticle coatings onto LD-wood surfaces is higher than the same hybrid SMI/PO nanoparticle coatings onto HD-wood surfaces, although the coating roughness on LD-wood samples is lower. We have to consider that the measured roughness values provide an overall value for the coated wood substrates and are measured at the macroscale level. Therefore, the presented roughness values highly depend on the coating homogeneity and rather indicate different mechanisms of interaction of the nanoparticle dispersion with LD-wood and HD-wood samples, respectively. Indeed, the *dewetting* phenomena observed on HD-wood samples highly increase the surface roughness while this structure is not favourable for good and stable hydrophobicity. In this case, the heterogeneity of the wood substrate is enhanced. On the other hand, the *penetration* phenomena on LD-wood are clearly better in providing continuous and smooth coatings that are favourable for higher and more stable hydrophobicity. In this case, the macroscopic defects in the wood surfaces are covered into the formation of an even film at the surface. We may predict that the roughness profiles of coated LD-wood samples are around an optimum between the different tested samples for enhancing hydrophobicity and consequently providing high water repellence. This behaviour is likely enhanced by including a multi-scale surface roughness profile with contributions of the original wood fibers and the decorations with nanoparticles at the surface. The creation of multi-scale surface roughness profiles with SMI nanoparticle coatings may favourably enhance the surface hydrophobicity and advancing contact

angles, depending on the base substrate. After previous experiences of such nanostructured coatings onto porous paper substrates, the present study let us project good potentials to apply these coatings also to various wood types with good homogeneity, adhesion and water repellence. The local interactions with cellulose and lignin moieties are presently further investigated to better understand the coating performance in relation to different wood substrates.

4. CONCLUSIONS

A hydrophobic coating was formed by deposition of organic nanoparticles from a stable aqueous dispersion, onto tropical wood types with low densities (LD-wood) and high densities (HD-wood). The overall formation of a water-protecting nanoparticle coating depends on the balance between substrate porosity/roughness and wood density.

The interaction of uncoated HD-wood (low surface roughness) and LD-wood (high surface roughness) with water is determined by predominant *dewetting* or *penetration* phenomena, respectively related to the surface roughness. The pure SMI nanoparticle coatings increase the surface hydrophobicity (98 to 107°) with a small tendency for better hydrophobicity on LD-wood substrates. However, instabilities occur due to the formation of a porous structure with micro-scale domains and consequently low adhesion. The latter inhomogeneities were most prominent on HD-wood samples, and somewhat better homogeneity was observed on LD-wood samples. Hybrid nanoparticle coatings provide better surface hydrophobicity, with highest contact angles measured on coated LD-wood samples, most likely through the formation of a more continuous film and change in surface roughness. The vegetable oils provide more stable contact angles, as the oil serves as a natural binder and provides more homogeneous coatings. To understand the interactions between coating substances and wood surfaces or for interpretation of hydrophobicity in terms of surface roughness, the LD- and HD-wood samples should be considered separately.

A future development of present nanoparticle coatings with up to 70 wt.-% vegetable oils seems promising for the replacement of more severe chemical wood surface treatments.

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5. REFERENCES

Gérardin, P, Petric, M, Petrissans, M, Lambert, J, Ehrhardt, J J (2007): Evolution of wood surface free energy after heat treatment. *Polymer Degradation and Stability*, **92**, 653-657.

Hsieh C T, Chang, B S, Lin, J Y (2011): Improvement of water and oil repellency on wood substrates by using fluorinated silica nanocoating. *Applied Surface Science*, **257**, 7997-8002.

Mahendran, A R, Wuzella, G, Aust, N, Kandelbauer, A, Müller, U (2012): Photocrosslinkable modified vegetable oil based resin for wood surface coating application. *Progress in Organic Coatings*, **74**(4), 697-704.

Mohammed-Ziegler, I, Hórvölgyi, Z, Tóth, A, Forsling, W, Holmgren, A (2006): Wettability and spectroscopic characterization of silylated wood samples. *Polymers for Advanced Technologies*, **17**, 932-939.

Paredes, A, Nutto, L, Becker, G (2011): Columbian lesser known hardwood species: is there a potential for sustainable forest management? *International scientific conference on hardwood processing, Blacksburg, USA,* 17th October 2011.

Petric, M, Knehtl, B, Krause, A, Militz, H, Pavlic, M, Petrissans, M, Rapp, A, Tomazic, M, Welzbacher, C, Gerardin, P (2007): Wettability of waterborne coatings on chemically and thermally modified pine wood. *Journal of Coatings Technology*, **4**(2), 203-206.

Philipp, C, Eschig, S (2012): Waterborne polyutethane wood coatings based on rapeseed fatty acid methyl esters. *Progress in Organic Coatings*, **74**(4), 705-711.

Samyn, P, Deconinck, M, Schoukens, G, Stanssens, D, Vonck, L, Van den Abbeele, H (2010): Modification of paper and paperboard surfaces with a nanostructured polymer coating, *Progress in Organic Coatings*, **69**(4), 442-454.

Samyn, P, Schoukens, G, Vonck, L, Stanssens, D, Van den Abbeele, H (2011): How thermal curing of an organic paper coating changes topography, chemistry and wettability. *Langmuir*, **27**(13), 8509-8521.

Samyn, P, Deconinck, M, Schoukens, G, Stanssens, D, Vonck, L, Van den Abbeele, H (2012): Synthesis and characterization of imidized poly(styrene-maleic anhydride) organic nanoparticles in stable aqueous dispersion. *Polymers for Advanced Technologies*, **23**(3), 311-325.

Sèbe, G, Brook, M A (2001): Hydrophobization of wood surfaces: covalent grafting of silicone polymers. *Wood Science and Technology*, **35**(3), 269-282.

Vlad-Cristea, M, Riedl, B, Blanchet, P, Jiminez-Pique, E (2012): Nanocharacterization techniques for investigating the durability of wood coatings. *European Polymer Journal*, **48**, 441-453.

Wenzel, T N (1949): Surface roughness and contact angle. *Journal of Physics and Colloidal Chemistry*, **53**, 1466-1467.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 4 Processes and properties

Wood-leather panels – A biological, fire retardant building material

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Wood-leather panels – A biological, fire retardant building material

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ABSTRACT

The poor flame retardant properties of wood-based products are among the severest obstacles, hindering its use in the commercial building sector. Recently, some attempts to improve the fire properties, relying on inflammable salts or reactive halogen compounds, have been presented, although they either cause problems with machining or embody harmful compounds (halogen derivates).
In this paper, the fire retardant properties of a novel material, wood-leather panels, are determined by the use of flame tests in a furnace according to ÖNORM EN ISO 1363:2011. The specimens were evaluated according to integrity and surface temperature.
For the test specimens, wet white (WW) and wet blue (WB) leather shavings, with varying contents were used.
The main finding is that both, panels containing WW and WB leather shavings, show properties superior to current flame-retardant medium density fibre boards, MDF B1,s2-d0. An optimum was found here at a leather content of 50%.
In order to describe this behaviour towards fire in further detail, the calorific value of the material as well as the thermal conductivity were determined. As the leather panels produce a foam-like structure during the fire treatment, it is assumed, that this is caused by the exhaust of gases, leading to decreased temperature flow through the specimen, resulting in the observed properties.
It can be concluded that the panels show superior fire retardant properties, compared to commonly available flame retardant material. Therefore further research in this field is proposed, with the aim to produce a certified product.

Keywords: wood, leather particles, wet white, wet blue, wood-based panel, fire protection, flame retardant, MDF

1. INTRODUCTION

Facing increasing problems with raw materials supply for wood based panels, recent developments in this field aim to diversify the material supply. Many attempts have been made to incorporate ligno-cellulosic materials such as straw (Han et al. 2001), rice husk (Leiva et al. 2007), bamboo or bagasse (Lee et al. 2006). The presented project focuses on a new way using waste residues from leather production for the development of a biogenous building material with improved material characteristics e.g. fire resistance.

Regarding the performance of wood -based panels, fire resistance is clearly among the largest issues. For instance, an untreated MDF panel shows a performance of D-s2,d0 according to the standard EN 13986 (M. Kaindl Holzindustrie,o.J.), which means that the material is burning normally, shows smoking but creates no burning droplets. Comparatively new materials like fire-

retardant MDF show a performance of B-s2,d0 (Finsa 2006) which means that the material is fire retardant, shows smoking but creates no burning droplets. This performance is usually carried out by impregnation with inflammable salts such as borax or phosphate compound or reactive compounds, mostly halogen derived (Dunky and Niemz 2002, Wang et al. 2010). Deducing this principle of inflammable salts, also tanning agents could serve as flame retardants as long as they are inflammable.
Beside the price of such a material these materials always represent a mixture of natural and artificial resources which means merging a biosphere material with a technosphere material according to the Cradle to Cradle principle defined by Braungart and MacDonough (McDonough und Braungart 2002).

For the development of the wood-leather panels the leather material used is originated from the trimming of tanned wet blue and wet white hides. Annually a remarkable amount of 0,2mio tons of these residues is generated in Europe. At current this material can only be disposed in landfills as it is non-burning. Till current, only few suitable attempts to use this material have been presented. Sundar (2011) proposes applications such as lubricants or protein glues, Ramaraj (2006) and Ambrósio (2011) describe in their papers the use of WB shavings as fillers in a thermoplastic matrix.
With regard to the leather particle panels only few studies have been published. The underlying patent was presented by Lackinger (2009) claiming the combination of wood fibres and leather particles. Grünewald et al (2012) presented some work on the distribution of fibres and particle within the panel and how to determine the structure by means of Raman spectroscopy.
Generally it can be concluded that leather shavings are an interesting new source of raw materials, leading to panels with interesting properties.
The aim of this study is to investigate the fire retardant properties of wood-leather panels, find correlations between different panel parameters such as leather type, -content, density and resin and to describe the behaviour of the different constituents qualitatively.

2. EXPERIMENTAL METHODS

2.1 Materials

The leather material for the tests was specified and provided by “Mag. Gerald Lackinger Consulting”, Salzburg, Austria. They are derived from cattle hide and are produced during the shaving to thickness phase in the leather preparation. The wet blue material comes from hides with chromium tanning whereas the wet white material is originated from hides with chromium-free tanning. The material was dried to a final moisture content < 10% in a drying kiln and the material was sieved to a grid size below 4 mm, prior to use. The wood fibers were provided by MDF Hallein, Hallein, Austria. The provided fibers were generated with a defibrator power consumption of 135 kWh/t (atro) and were dried to a moisture content of 10.0 – 10.5 %. They are exclusively derived from spruce (Picea abies). All samples were prepared using an Urea-Formaldehyde glue “Kaurit K-350” by BASF, Ludwigshafen, Germany with a solid content of 66%. The formaldehyde-free Soy-protein adhesive“Hercules PTV-D-41063”was kindly provided by Ashland Hercules Water Technologies, Wilmington, USA.

2.1.1. Panel production

The aim of this study was to investigate on the influence of density, leather content and –type and resin on the flame retardant properties of the panels.
Therefore boards of the dimension 700 x 700 x 12 mm were prepared. The densities of the produced panels are shown in Table 2 Specimens used for the fire test. The resin content was 10%

for all specimens. The specimens were prepared using a Hoefer HLOP 280 automated hot-press at 180°C and a pressing factor of 0.5 min/mm. The resin was sprayed with a Schlick two-substance nozzle with a pressure of 2.5 bars and the resin fed gravimetrically with a cup. In order to break fiber lumps after resin application, the fiber material was soaked with a conventional vacuum cleaner "Bosch GAS 50-M". The turbulent air stream ensures even glue spread because it creates conditions which resembles the conditions in a blow-line for MDF production. After the sample preparation they were stored in a standard climate (20°C / 65 % r.H).

2.2 Methods

2.2.1.Pre-tests

Preliminary tests were conducted as described by ÖNORM EN ISO 11925-2:2011 for small flame tests. It features a small flame, treating the edges of the specimen for 30 seconds, as shown in Figure 1. After removing the flame, the following factors were evaluated:

- Whether the Afterflame-time lasted shorter than 3 seconds –
- Whether the flame spread was below 150 mm and the respective time to reach 150 mm
- Droplet occurrence
- Occurrence of flaming droplets

Table 1 shows the specimens used for the Pre-tests. For all mixtures three specimens were tested. The flame exposition lasted 30 seconds.

Table 1: Specimen used for Pre-tests

Specimen	Wood content [%]	Leather content [%]	Density [kg/m^3]	Resin
Pre-1 0	100	0	700*	UF
Pre-2 25	75	25	700*	UF
Pre-3 50	50	50	700*	UF
Pre-4 75	25	75	700*	UF
Pre-5 100	0	100	700*	UF

*Density estimated from production parameters, not directly determined.

Figure 1: Procedure of small flame test

2.2.2.Fire tests

The fire resistance tests were carried out in vertical furnace. The proceeding was oriented on ÖNORM EN ISO 1363:2011. The aim of this test was to determine the fire resistance of the specimen and the temperature-time curve. Therefore five temperature sensors were used per specimen. In accordance to the standard, a temperature change of 140°C for the average and a temperature change of 180°C for a single sensor were monitored. The criteria of failure were defined as loss of integrity (fire resistance) with regard to the standard.
With respect to maximum specimen size of lab-made panels, the test chamber with an opening of 1400 mm was divided into 4 squares of 575 x 575 mm. A metal frame, cladded with promat panels for protection, was used to hold the specimens. The specimens were set into a bed of heat-resistant silicon "Hilti CP 601 S" and fixed with strips of Promat panels. The detailed setup can be seen in Figure 2.

Figure 2: Fire test preparation (left: flame exposed, right: flame averted)

The temperature was controlled according to the standard temperature curve, as given by (1)

(1)

, with T being the average furnace temperature (°C) and t the time (min).

The used specimens and their composition are given Table 2. They were produced with dimensions of 575 x 575 x 12 mm.

Table 2 Specimens used for the fire test

Specimen	Wood [%]	WW [%]	WB [%]	Density [kg/m³]	Resin type
1 MDF commercial	100	0	0	714	unknown
2 MDF B-1fire retardant	100	0	0	827	unknown
3 MDF lab-made	100	0	0	814	UF
4 25 WW	75	25	0	806	UF
5 50 WW	50	50	0	806	UF
6 75 WW	25	75	0	800*	UF
7 100 WW	0	100	0	800*	UF
8 25 WB	75	0	25	840	UF
9 50 WB	50	0	50	796	UF
10 75 WB	25	0	75	816	UF
11 100 WB	0	0	100	843	UF
12 2-layer WW	50	50	0	866	UF
13 3-layer WW	33	66	0	809	UF
14 12.5 WW 37.5 WB	50	12.5	37.5	798	UF
15 25 WW 25 WB	50	25	25	902	UF
16 37.5 WW 12.5 WB	50	37.5	12.5	841	UF
17 50 WW soy	50	50	0	817	soy

*Density estimated from production parameters, not directly determined.

2.2.3. Calorific Value

The gross calorific value of the different constituents wood (MDF fibers, derived from spruce), WW and WB was determined using a mass calorimeter "IKA C2000 Basic". The tests were carried out in accordance to DIN EN ISO 1716:2010. Prior to the tests, all specimens were stored in climate of 20°C and 65% r.H. to achieve an equilibrium moisture content within the material.

2.2.4. Thermal Conductivity

The thermal conductivity of three specimens (100% wood, 100% WW and 100% WB) was tested with a Thermal conductivity testing device "λ-Meter EP 500e, Lambda-Messtechnik, Dresden". The temperature difference was set to 15 K, with a specimen temperature of 23 °C.

3. RESULTS AND DISCUSSION

3.1. Pre tests

The pre-tests showed the general performance of the material under the exposition to a small flame. Not only were the results according to criteria of the standard, shown in Table 3 interesting but foremost the general behaviour of the material. The results show, that there is an influence of the leather content on the performance, as an increasing leather content decreased afterflame-time and the flame spread.

Table 3: Results of the Pre-tests

Specimen	Afterflame-time < 3 sec?	Flame spread > 150 mm, time?	droplets?	flaming droplets?
Pre-1 0	no, 4	yes, 28	no	no
Pre-2 25	yes	yes, 30	no	no
Pre-3 50	yes	no	no	no
Pre-4 75	yes	no	no	no
Pre-5 100	yes	no	no	no

Even more interesting were the observations possible during the test. It was apparent, that increasing leather content influences the fire properties. It decreased the afterflame-time substantially, at level of 75% leather and above, there was no apparent afterflame. Also the incineration during flame exposition changed. The flame stability seemed to decrease massively with increasing leather content. For low leather contents up to 25%, a clear-shape was visible, being clearly nourished by the panel material. For higher leather contents, the flame length decreased and finally seemed limited to a small area adjacent to the burner flame.

3.2. Fire tests

The results of the fire tests are shown in Table 4. Regarding the influence of leather content on the integrity of the material, Figure 4 shows the influence of WW and WB. It can be seen, that both have an optimum at 50% leather content, although the integrity is maintained for a longer time in the case of WB. Nevertheless both WW and WB outperform the MDF B1 with regard to integrity.

Table 4: Results of the fire tests

Specimen No.	Integrity [min]	Specimen No.	Integrity [min]
1 MDF commercial	12	10 75 WB	24
2 MDF B-1fire retardant	18	11 100 WB	14
3 MDF lab-made	14	12 2-layer WW	16
4 25 WW	17	13 3-layer WW	13
5 50 WW	19	14 12.5 WW 37.5 WB	21
6 75 WW	17	15 25 WW 25 WB	20
7 100 WW	17	16 37.5 WW 12.5 WB	18
8 25 WB	20	17 50 WW soy	16
9 50 WB	25		

As already stated during the discussion of the Pre-tests, the fire tests featured some behaviour which couldn't be measured quantitatively but still need mentioning. As visible in Figure 3, the integrity failed in the case of low leather contents (0 – 50%) randomly within the panel due to burn-through of the material. For higher leather contents (75 – 100%) the integrity failed due to deformation of the panel, leading to slits, offering the fire a way to pass out of the furnace.

Figure 3: Differences in integrity fail for 50 WB (left) 100 WB (right)

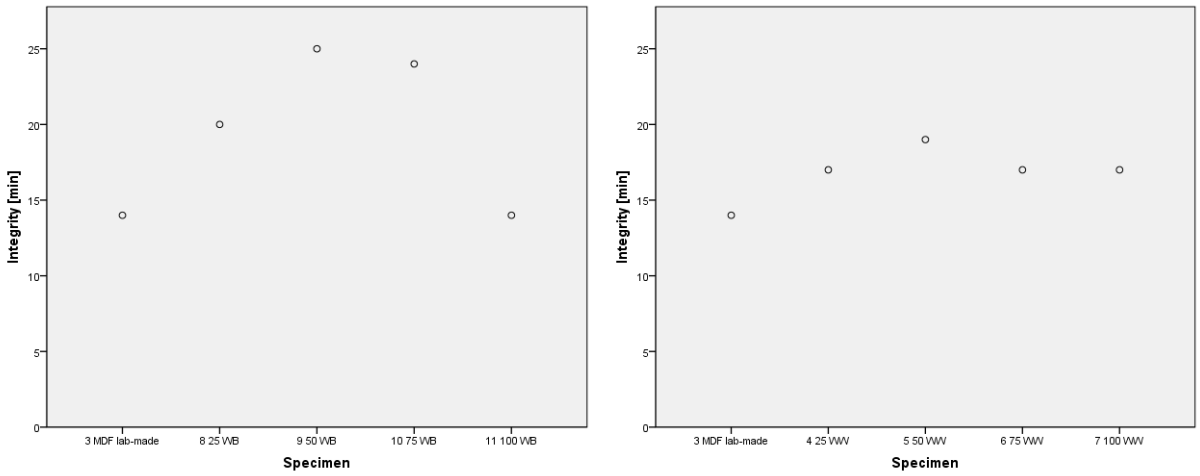

Figure 4: Integrity as a function of leather content

Another interesting effect of the leather content is visible in the averaged surface temperature of the panels. In Figure 5 and Figure 6 it can be seen, that there is a decrease in surface temperature

with increasing leather content. Especially in the case of WW, a dramatic decrease can be seen, compared to MDF of similar density.

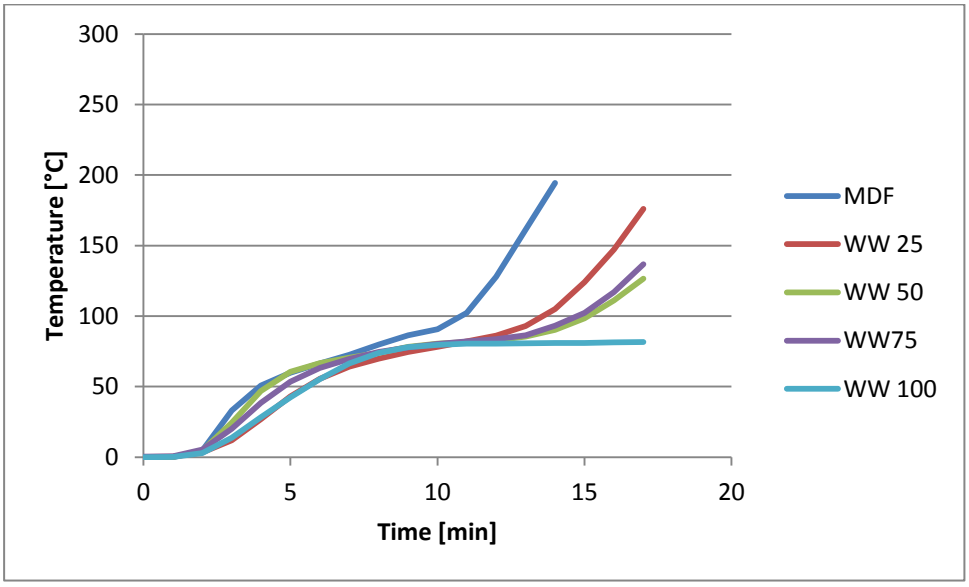

Figure 5: Wet white panel Surface temperature as a function of time

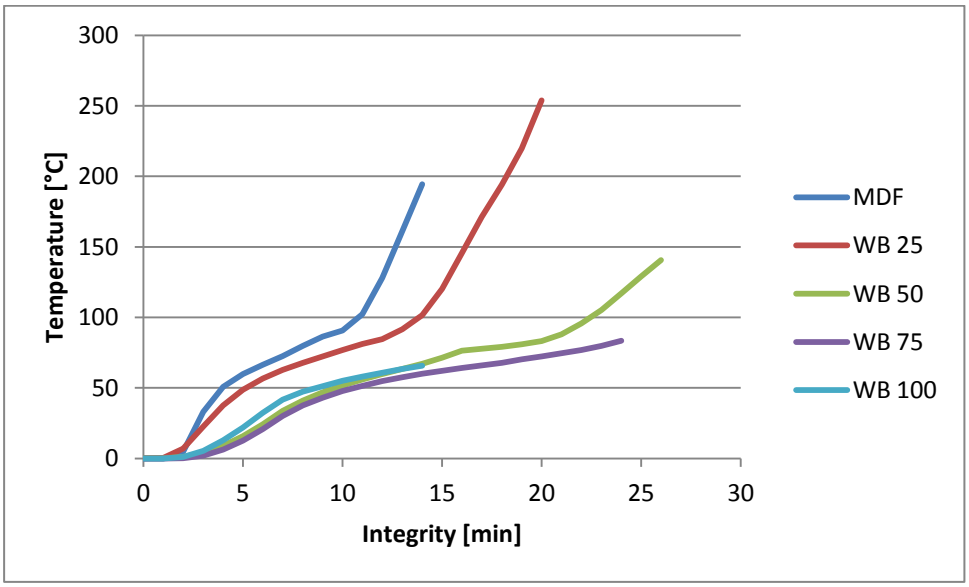

Figure 6: Wet blue panel Surface temperature as a function of time

Corresponding to this observation the panels showed a tendency to foam, even increased by higher leather contents. This tendency was more pronounced in the case of WW than in the case of WB. Figure 7 shows the comparison between the foaming of WB and WW.

Figure 7: Foaming of 50 WB panel (left) and foam thickness of 100 WW (right)

As a conclusion of this foaming, the decrease of surface temperature for increasing leather content and the deformation of 75 and 100 WW/WB panels, a 2- and 3-layered material composition was also evaluated. The idea was to use the positive effect of the leather and combine it with a wood fibre core for higher dimensional stability. But these panels showed either a strong tendency to warp (2-layer) or poor performance with early burn-through. After the experiment it was visible, that the foam layer didn't adhere to the wood core. Therefore is seems that either a stronger layer of leather or a different type of adhesive could support the positive effects observed. The same thought of combining the positive effect of the higher integrity values of the WB leather particles with the apparently better foaming behaviour of the WW leather particles was applied for the mixtures of WW and WB (specimen 14-16). But, as visible from the data, these materials didn't perform substantially better than pure WB panels.
The specimen with soy adhesive (specimen 17) showed a lower performance compared to the UF specimen. Is seems that the use of the UF adhesive has a positive effect on the integrity of the specimen if compared to the specimen where the soy adhesive was used. When looking at the Internal Bond values of these two panels as shown in Table 5, this effect might be due to the higher internal bond value of the 50 % WW UF panel resulting in a higher dimensional stability.

Table 5: Internal Bond values of 50 % WW UF and 50% WW Soy wood leather panels

Specimen	Wood [%]	WW [%]	WB [%]	Internal Bond [N/mm²]	Resin type
5 50 WW	50	50	0	0.91*	UF
17 50 WW soy	50	50	0	0.52	Soy

3.3. Calorific Value

The gross calorific values can be seen in Table 6.

Table 6: Gross calorific values

Specimen	gross calorific value [MJ/kg]
Wood	17.381
WB	15.572
WW	18.428

It is apparent, that the values do not differ substantially, which could be at least expected from the differences in the flame retardant properties. Therefore these properties do not seem to be determined by overall energy release but rather by the rate of pyrolysis of the material.

3.4. Thermal conductivity

The results of the thermal conductivity tests are shown inTable 7.

Table 7: Thermal conductivity

Specimen	Density [kg/m³]	conductivity [mW/m*K]
C-1 Wood	856	128.38
C-2 WB	910	128.99
C-3 WW	903	118.31

The results show, that there is not much difference, although the WW panels seem to show a somehow decreased conductivity. With regard to the differences in the surface temperature, these cannot be attributed to differences in thermal conductivity.

4. CONCLUSIONS

The conducted tests unveil some clear differences in the fire properties of the tested materials. With regard to different parameters, optimum mixtures could be found.
However not all the observed phenomenon such as foaming or lowered surface temperature could be beneficially used in materials compositions. The reasons could be manifold as pointed out before but the authors are still convinced that further research in this field will lead to a synergetic combination of effects.
An aspect, not yet fully understand, is the reason for the improved fire properties of the material. The experimental values of integrity show this at least most impressively. As the gross calorific values do not differ, it is not a matter of energy release.
An observation made during the experiments is the decreasing surface temperature with increasing leather content as well as the foaming of the leather material. In combination with the results of the thermal conductivity, also not differing substantially, this leads to the conclusion that there are two possible ways how the leather influences the fire properties. The pyrolysis of leather could lead to the release of gases, inhibiting the fire. As leather is containing a substantial stake of nitrogen, this could be a possible explanation.
The other possibility is the decrease of the pyrolysis rate. This could be attributed to the decreased temperatures on the surface for increasing leather contents. In this case, the foam layer hinders the transport of pyrolysis gas and decreases the temperature in the pyrolysis zone.

In order to clarify this point, further research is proposed. In a first step, an investigation of the pyrolysis gases by means of gas chromatography could be beneficial. To determine the chemical composition of the panels, techniques such as elemental analysis or X-ray fluorescence spectroscopy could be beneficial.
With regard to the current material and its fire properties, all carried-out tests indicate, that a rating in the class B1 is possible. Therefore tests with larger scale material would be necessary to characterize the material and in a second step, constructions could be tested according to their fire retardant properties.
All in all, the presented material is in its way of uniting biological constituents and fire retardance outstanding. Although the characteristics have to be defined in further depth, wood-leather fibre panels offer an interesting new scope on the topic of fire-retardant material and provide a sustainable alternative among the conventional materials.

5. REFERENCES

Ambrósio, J.D.; Lucas, A.A.; Otaguro, H.; Costa, L.C. (2011): Preparation and characterization of poly(vinyl butyral)-leather fiber composites. In: *Polymer Composites* **32**(5), 776-785.

Dunky, M.; Niemz, P. (2002): Holzwerkstoffe und Leime: Technologie und Einflussfaktoren. Springer, Heidelberg.

Finsa (2006): Datasheet MDF fire retardant.

Grünewald, T.;Ostrowski, S.; Petutschnigg, A.; Musso, M.; Wieland, S. (2012): Structural analysis of wood-leather panels by Raman Spectroscopy. In: *BioResources* **7**(2), 1431-1439.

Han, G.; Umemura, K.; Zhang, M.; Honda, T. (2001): Development of high-performance UF-bonded reed and wheat straw medium-density fiberboard. In: *Journal of Wood Science* **47**(5), 350–355.

Lackinger, G. (2009). "Composite body", Applied by Vogl, W.,17.06.2009. Applicationnr.: EP 09007907.0. Publicationnr.: EP2135892.A2.

Lee, S.; Shupe, T.F; Hse, C.Y (2006): Mechanical and physical properties of agro-based fiberboard. In: *HolzalsRoh- undWerkstoff***64**(1), 74–79.

Leiva, P; Ciannamea, E; Ruseckaite, R. A; Stefani, P. M (2007): Medium-density particleboards from rice husks and soybean protein concentrate. In: *Journal of Applied Polymer Science* **106**(2), 1301–1306.

M. Kaindl Holzindustrie: Datasheet Kaindl MDF E1.

McDonough, W.; Braungart, M. (2002): Cradle to cradle. Remaking the way we make things. 1. edition. New York: North Point Press.

Ramaraj, B. (2006): Mechanical and thermal properties of ABS and leather waste composites. In: *Journal of Applied Polymer Science* **101**(5), 3062-3066

Sundar, J. V.; Gnanamani, A.; Muralidharan, C.; Chandrababu, N. K.; Mandal, A.B. (2011): Recovery and utilization of proteinous wastes of leather making: a review. In: *Review in Environmental Science and Biotechnology* **10**, 151-163.

Wang, F.; Wang, Q.; Wang, X. (2010): Progess in Research on Fire Retardant-Treated Wood and Wood-Based Composites: A Chinese Perspective. *Forest Products Journal***60**(7/8), 668-678.

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THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 4 Processes and properties

Water absorption and desorption of non treated, pressure impregnated, and pine oil treated glulam made of small diameter Scots pine and Norway spruce

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ABSTRACT

A growing proportion of harvested timber originates from the first or second commercial thinning stands in Finland, which means smaller average log volumes in comparison to final felling stands. Smaller log volume means challenging wood properties such as higher proportion of juvenile wood and sapwood. Due to these facts, products made of small diameter logs are prone to twist and check, and have reduced durability against weather. The objective of the study was to define the water absorption and desorption velocity of non treated, pressure impregnated and pine oil treated glulam.

The 6 inner lamellae of the glulam beams originated from small-sized logs, whereas the surface lamellae were made of larger logs. Beams with 44 x 200 mm cross cut dimensions were glued using MUF resin and divided into three treatment groups. Treatment 1 was not impregnated, treatment 2 was impregnated into AB class with copper-based preservative in commercial pressure process, and treatment 3 was impregnated with pine oil using the process of Ekopine Ltd. After the treatments, 20 pieces of 200 mm-long specimens were sawn from each treatment group. The cross cut surfaces of the specimens were sealed using waterproof varnish to ensure that the water movement took place via the side surfaces of the specimens. The air-dry specimens (MC 7.7–12.6%) were immersed into water for 6 weeks. After that, they were brought to a standard climate (65% RH, 20 °C temperature). Again, their mass was recorded until it did not change anymore.

Pine oil impregnated glulam resisted the water absorption more than non treated and pressure impregnated glulam. Due to the low initial MC after the absorption period, pine oil impregnated glulam dried rapidly below 20% MC, while the drying of non treated and pressure impregnated glulam to the same level took 3-4 weeks. In conclusion, pine oil impregnation of timber from small-sized logs can be considered an effective and ecological preservation method for timber used in outdoor constructions. For load carrying structures, pine oil impregnation appears to be an efficient way to maintain the MC of wood below the level that enables mould growth.

Keywords: absorption, desorption, pine oil impregnation, Pinus sylvestris, pressure impregnation.

1. INTRODUCTION

Sequential thinning of forest stands at least twice during the rotation period are part of the modern forestry practices in Finland. The properties of wood raw material originating from these commercial thinning stands varies in a great extent, due to the great variation in the diameter of the harvested logs. Obviously, the share of small diameter logs with diameter less than 15 cm will increase as a result from increment of early thinnings. At the time of the first thinning, the log diameter and quality do not fulfil the requirements of logs for traditional sawmill production as far as structural products and carpentry are concerned. Therefore, the utilisation of small

diameter logs has been increased mostly in small scale and garden construction (*e.g.*, Boren 1999, 2001; Wall *et al.* 2005).

The need for an increase in the utilisation of small diameter logs in structural uses means higher demand for controlling and modifying the challenging wood properties such as high proportion of juvenile wood and sapwood. In addition to the physical challenges, there are chemical ones, as well. Products with increased water resistance and resistance against decay fungi and mould growth are needed. The most popular method to preserve wood against microbial growth is pressure impregnation with chromium-copper (CC) based additives. Finland alone produces more than 200,000 m^3 of CC-impregnated sawn timber annually (Production statistics…2012). Pine oil (or "crude tall oil") impregnation has been considered as an alternative method for wood protection (*e.g.*, Koski 2008). Pine oil penetrates in wood as its best via sawn or peeled surfaces, whereas planed surfaces are more challenging to impregnate.

Finland produced 330 000 m^3 of glulam in 2011. The raw material for glulam beams is mainly Norway spruce (*Picea abies*) but some Scots pine (*Pinus sylvestris*) is used, as well (Glulam Handbook 2009). There is a growing interest to use small diameter logs in glulam production. The main driver for this interest is the price of the logs: small diameter logs typically cost only 50-60 per cent of the price of conventional logs.

The objective of this study was to define the water absorption and desorption velocity of non treated, pressure impregnated and pine oil („tall oil') treated glulam.

2. EXPERIMENTAL METHODS

The inner lamellae of the glulam beams were sawn from small-sized logs originating from south-eastern Finland. Inner lamellae had dimensions of 25x44 mm. The surface lamellae with dimensions of 25x44 mm were commercial lumber originating from larger logs. Each beam had six inner lamellae.

Beams with 44x200 mm cross cut dimensions were manufactured in an industrial glulam production line. Melamine-urea-formaldehyde (MUF) resin was used. A part of the Scots pine made beams were then taken to CC impregnation, another part to pine oil impregnation that was made by company Ekopine Ltd. The reference beams made of Norway spruce were stored indoors. After the impregnation treatments, a sample from all three treatment groups was taken to a mild weather chamber test to study their dimensional stability and glue joint performance. The cyclic weather chamber test lasted 14 days, and consisted of seven cycles with two phases. Phase 1 lasted 36 hours and had a relative humidity of 95% and temperature of 10 degrees Celcius, in which conditions the equilibrium moisture content of wood should be approximately 21%. Phase 2 lasted 12 hours, and had RH of 15% and temperature of 40 degrees Celcius. These conditions get the equilibrium moisture content of wood down to 4.2%.

After the weather chamber tests, a total of 20 pieces of 200 mm-long water absorption specimens were sawn from the beams in each three treatment groups, thus obtaining a total of 60 specimens with 44x200x200 mm dimensions for the water absorption tests (Fig 1).

The cross cut surfaces of the specimens were sealed using several layers of waterproof varnish. Air-dry specimens (MC 7.7–12.6%) were immersed into water in 11th of April, 2011 for six weeks. During that time their mass was regularly recorded (Fig. 2). After each weighing, the specimens were immediately returned back in the water. After the 6-week immersion period, the specimens were removed from the water for the last time, weighed, and brought to a standard

climate (RH: 65%, T: 20 °C). Again, their mass was repeatedly recorded until it did not change anymore. The specimens were weighed the last time in 2nd of November 2011, almost seven months after initiating the test.

In this paper, we analyse the results by graphical illustration.

Figure 1: Examples of tested 44x200x200 mm glulam specimens. From the left: untreated, CC-impregnated, and pine oil impregnated. The material from small sized timber was mainly sapwood which can be seen as high penetration of preservative liquid in the inner lamellae of the CC-impregnated specimen, whereas virtually no preservative has penetrated in the surface lamellae that were made of larger logs with higher proportion of heartwood. (Photo: Erkki Oksanen, Metla)

3. RESULTS AND DISCUSSION

The pine oil impregnated glulam was more resistant against water absorption than the other treatment groups. During the six week water immersion, the MC increased up to 71.0, 58.4 and 22.7% for CC-impregnated, non treated and pine oil impregnated specimens, respectively (Fig. 2). Due to the low initial MC after the water absorption period, pine oil impregnated specimens also dried below 20% MC very fast, *i.e.*, in 3 days, while the drying of non treated and pressure impregnated specimens down to 20% MC took 3-4 weeks.

The initial moisture content of wood before the experiment started was the highest in the pressure impregnated specimens. The initial MC did not differ markedly between the pine oil impregnated and reference specimens. Consequently, the treatment with CC-preservative increased the MC of specimens compared with the untreated or pine oil impregnated specimens.

The CC-impregnated specimens absorbed water most rapidly during the immersion period. The difference in the MC between CC-impregnated and untreated specimens increased from 4.9% to 12.6%. The MC of the pine oil impregnated specimens increased clearly slower than that of CC-impregnated and untreated specimens. The difference in MC at the end of the immersion period between pine oil impregnated and untreated specimens was 35.7%. The increase of the MC had decelerated at the end of the immersion period in each treatment group.

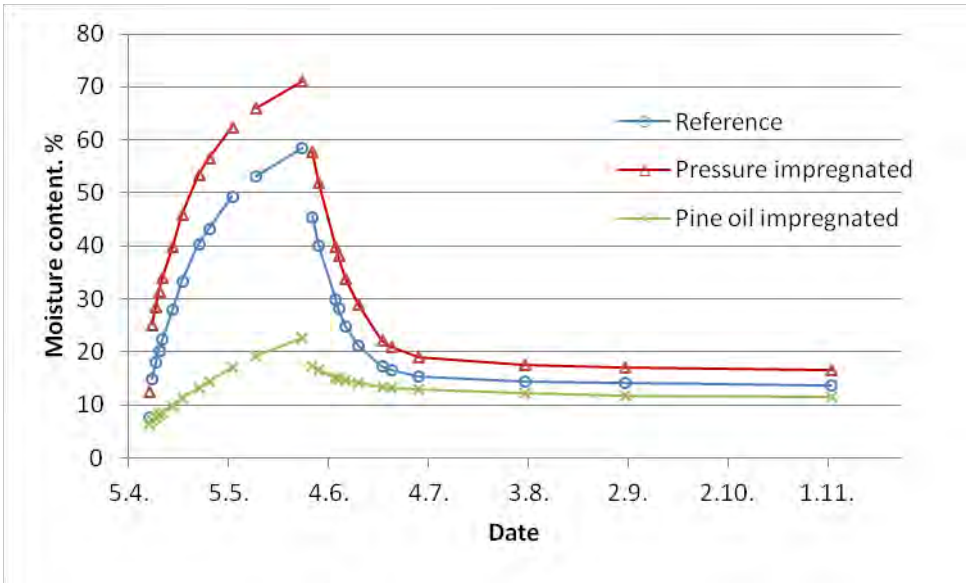

Figure 2: Change in average MC (%) of pine oil impregnated, pressure impregnated and reference samples during the absorption (water immersion) and desorption (T, 20°C; RH, 65%) periods

During the immersion period, the moisture content of CC-impregnated and untreated specimens increased to a level that corresponds to the moisture content of green wood. Independently of the treatment, all specimens desorbed moisture faster than they absorbed it. Due to the relatively low moisture content of the specimens before water immersion period, the final moisture content did not reach the initial moisture content in any treatment group.

4. CONCLUSIONS

In conclusion, pine oil impregnation of timber from small-sized logs can be considered to be an effective and ecological preservation method for timber used in structures exposed to high moisture variations, such as garden structures, load carrying structures in humid conditions, etc. It appears that pine oil impregnation is an efficient way to maintain the moisture content of wood below the level that enables the growth of mould and decaying fungi. The increase of moisture content above the fibre saturation point, as observed during the immersion test for CC-impregnated and untreated specimens, markedly decreases the stiffness and strength of wood along with increasing the susceptibility for microbial growth. These problems are not equally obvious for pine oil treated specimens, since their moisture content remained clearly under the fibre saturation point of wood even during the two-month soaking period

5. REFERENCES

Boren, H. 1999. [Increasing the utilisation of small sized timber in wood product industries. Final report of a preliminary survey]. Finnish Forest Research Institute, Research Papers 761. 58 p. (In Finnish)

Boren, H. (2001). Factors affecting the knottiness, twisting and mechanical properties of pith enclosed round and sawn timber of Scots pine (Pinus sylvestris) and Norway spruce (Picea abies) from thinnings in Southern Finland. Finnish Forest Research Institute, Research Papers 807. 154 p + App.

Glulam Handbook. (2009). Finnish Glulam Association. [http://www.glulam.fi/liimapuu/?__EVIA_WYSIWYG_FILE=28190&name=file]

Koski, A. (2008). Applicability of crude tall oil for wood protection. Acta Universitas Ouluensis 293. 99 p. + App.

Production statistics of pressure impregnated wood. (2012). [http://www.kestopuu.fi/tuotanto_ja_tilastot.html]

Ranta-Maunus, A. (ed.) (1999). Round small-diameter timber for construction. Final report of project FAIR CT 95–0091. VTT Publications 383. 191 p. + appendix 19 p.

Stöd, R., Kilpeläinen, H. & Wall, T. (2006). Yield and technical quality of Scots pine saw timber from thinnings on drained peatlands and mineral soils in Finland. Baltic Forestry, 12(2), 170-183.

Wall, T., Fröblom, J., Kilpeläinen, H., Lindblad, J., Heikkilä, A., Song, T., Stöd, R. & Verkasalo, E. (2005). [Development of procurement and sawing of Scots pine from thinnings, Final report of a research concortium in Wood Wisdom research programme]. 129 p. + App. (In Finnish)

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Section 4 Processes and properties

Differences between heat treated *Pinus pinaster* heartwood and sapwood

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Differences between heat treated *Pinus pinaster* heartwood and sapwood

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ABSTRACT

Heat treatment is a well known process to improve the durability and dimensional stability of less noble woods. The treatment can be applied for heartwood unlike the traditional treatments based on impregnation due to the difficulty of impregnating heartwood.

Pure sapwood and pure heartwood samples were treated in an oven at 190ºC and 200ºC for 2h, 4h and 6h. Dimensional stability, measured as Anti Shrinking Efficiency (ASE) between 0% and 65% relative humidity, durability, mechanical resistance (MOE and MOR) and density were determined for both treated and untreated sapwood and heartwood.

One of the main differences between treated sapwood and heartwood was the presence of resin on the surface of the heartwood treated wood samples. The results showed that for the same treatment conditions the dimensional stability improved more for sapwood than for heartwood. However when the comparison is made at the same mass loss, the differences were not significant. ASEradial reached 80% for sapwood and 50% for heartwood while ASEtangential reached 50% and 40% respectively. In relation to mechanical properties, MOE increased slightly at the beginning of treatment decreasing afterwards. No significant differences were found between sapwood and heartwood. MOR decreased for both heartwood and sapwood reaching almost a 50% and 30% decrease for sapwood and heartwood respectively. Once again if the comparison is made at the same mass loss, no significant differences were found between sapwood and heartwood. Durability against *Postia placenta* was evaluated, by the mini-block method, for sapwood and heartwood samples treated at 190ºC and 200ºC for 2h and 4h. A significant increase in durability was found for both heartwood and sapwood at the higher temperature and for heartwood only at 190º for 4h.

The results showed that the heat treatment is equally efficient for sapwood and heartwood when comparing at the same mass loss. Since temperature and treatment time influenced differently on heartwood and sapwood i.e. on mass loss, the extent of improvements varied between sapwood and heartwood with the same treatment conditions i.e. the improvement was higher for sapwood.

Keywords: dimensional stability; durability; heartwood; heat treatment; mechanical properties; sapwood.

1. INTRODUCTION

Even though there are several papers about the properties of heat treated wood their aim is always sapwood and no treatment has been made with heartwood samples. An advantage of this wood modification in relation to other methods like chemical or impregnation modification is that it can be applied on both heartwood and sapwood and to any species of wood. One exception is the work of Metsä-Kortelainen et al. (2006) who studied the water absorption of sapwood and heartwood of Scots pine and Norway spruce heat-treated at 170°C, 190°C, 210°C and 230°C. They concluded that only the treatment at 230ºC lead to a decrease in water absorption (based on a floating test) for pine sapwood, while it decreased for heartwood even at the lowest temperature. In relation to spruce the treatment decreased water absorption similarly for sapwood and heartwood. In a study about wettability of treated sapwood and heartwood of Scots pine and Norway Spruce (Metsä-Kortelainen and Viitanen, 2012) referred that in general, wettability of pine sapwood was higher than that of pine heartwood and that water repellence of pine sapwood was only increased for wood treated at 230ºC while for pine heartwood wettability decreased for samples treated at temperatures higher than 170ºC. No significant difference between spruce sapwood and heartwood was found, and wettability decreased with the increase in treatment severity. The decay resistance of sapwood and heartwood of untreated and thermally modified Scots pine and spruce was reported by (Boonstra, et al., 2007). According to these authors, Scots pine heartwood showed a higher resistance against brown rot, *C. puteana* and *P. placenta*; and also against white rot fungus *C. versicolor*. (Metsä-Kortelainen and Viitanen, 2009) also reported the heat treated heartwood durability of Scots pine and Norway spruce, and concluded that the treatment increased the durability of all the wood materials. This paper intends to enlighten the differences between heat treated *Pinus pinaster* sapwood and heartwood and to discuss the feasibility and the difficulties in treating heartwood samples.

2. EXPERIMENTAL METHODS

2.1 Treatment

The samples were cut from two centre boards of pine (*Pinus pinaster*) stems. Three types of samples were prepared. The samples for dimensional stability were cubic with approximately 40 mm edge and with clear radial, tangential and transversal faces. The dimensions of the samples for static bending were 340 x 20 x 20 mm^3 (axial x radial x tangential) and for biodegradation were 40x10x10 mm^3 (axial x radial x tangential). All the samples were kept in a conditioned oven at 65% relative humidity and 20 ºC before the treatment. The heat treatment was made at atmospheric pressure inside an oven for 2 to 6 hours and at 190 ºC and 200 ºC. The warm up period was about one hour to reach the treatment temperature, which was kept approximately constant at a range of ±5ºC. At the end of each treatment the samples were cooled in a dry environment and weighted. Mass loss was determined in relation to dry wood.

2.2 Equilibrium moisture and dimensional stability

After the treatment, treated and untreated samples were kept in an oven at 100ºC overnight, cooled, weighed and measured in radial, tangential and longitudinal directions. Then the samples were put in an oven at 20ºC and 65 % relative humidity for at least 5 weeks. After stabilization, the samples were again weighted and measured in radial, tangential and longitudinal directions. Equilibrium moisture content was determined for 65% RH as the mass difference between 65% and dry state and dimensional stability was determined by the Anti Shrinking Efficiency (ASE) method used by (Stamm, et al., 1946) and described in (Esteves, et al., 2006, 2007, 2008) This

method gives the shrinking difference between treated and untreated samples calculated in percent, and determined between 65% RH and dry state.

2.3 Bending strength

Bending strength and modulus of elasticity were determined by a three point bending device. Measurements were made using a constant velocity of 40 kgf/min MOE and bending strength were determined according to:

$$\text{MOE(MPa)} = \frac{\Delta F * L^3}{\Delta x * 4 * b * h^3}$$

$$\text{Bending strength (MPa)} = \frac{3F_{máx} * L}{2 * b * h^{10/6}}$$

Where $F_{máx}$ is the load on rupture in N, $\frac{\Delta F}{\Delta x}$ is the slope of the elastic zone in N/mm, L is the arm length, h the height and b the width all expressed in mm.

2.4 Durability

A brown rot fungi, *Postia placenta* (Fr.) Lars et Lomb. (FPRL 280) was used to evaluate the resistance to decay by the method generally described in CEN/TS 15083-1 (2005) though using mini-blocks (30x10x10mm). The number of test specimens was adapted to the availability of material (4 to 6 replicates per variable tested) and the exposure period was 8 weeks.

3. RESULTS AND DISCUSSION

The possibility to treat both sapwood and heartwood is an advantage of the heat treatments over other wood modification processes. Nevertheless, some difficulties may arise when heat treating heartwood. One of the main difficulties is the presence of high contents of resin on the surface of the heartwood samples, giving wood a less aesthetic look (Figure 1). Even though there is also some resin on the surface of sapwood samples the surface is much smoother (Figure 1).

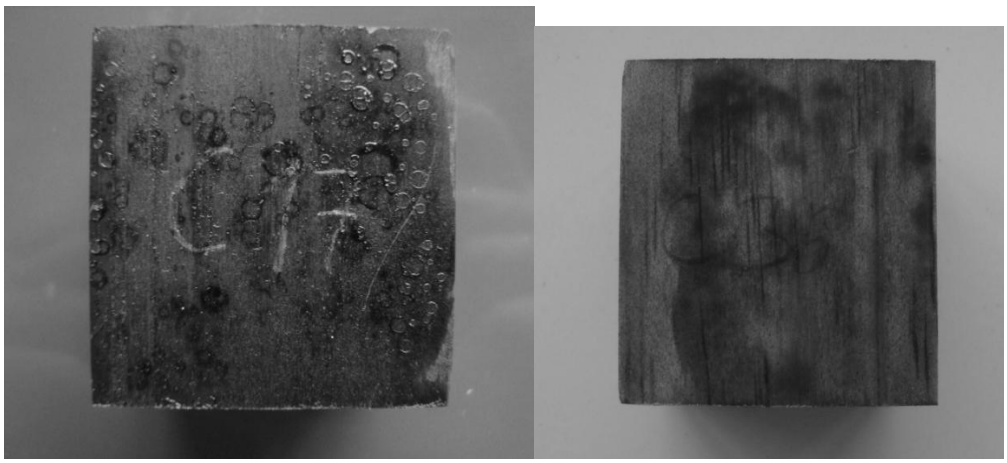

Figure 1: Heat treated heartwood (left) and sapwood (right)

Equilibrium moisture content (EMC) was determined for the untreated and heat treated wood at 65% relative humidity. EMC was about 12% for both sapwood and heartwood, decreasing sharply with the treatment until about 3% mass loss, and then decreasing much more slowly (Figure 2). The minimum EMC was 7.0% for sapwood and 7.4% for heartwood corresponding to 42% and 39% improvement in relation to untreated wood. In spite of the slightly higher decrease for sapwood, there are no significant difference between sapwood and heartwood EMC. Similar results were reported by Metsä-Kortelainen and Viitanen (2009). According to these authors equilibrium moisture content for spruce decreased from 10.6% to 5.9% and from 10.3% to 5.3%, and for Scots pine from 10.1% to 5.4% and 9.8% to 4.7% for sapwood and heartwood respectively. When the comparison is made a the same treatment conditions there is also a higher decrease for sapwood, on average the decrease is 7% higher than for heartwood. Nevertheless there isn't a significant difference between sapwood and heartwood.

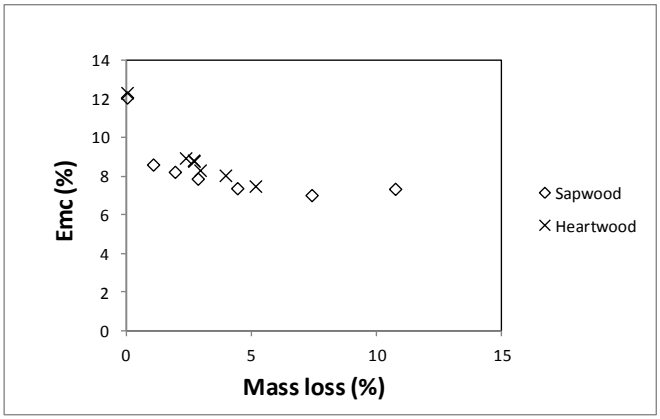

Figure 2: Equilibrium moisture content (EMC) vs mass loss due to treatment

Radial ASE reached 52% for sapwood and 50% for heartwood while tangential ASE reached 50% and 40% respectively. The results showed that for the same treatment conditions the dimensional stability had a similar improvement for softer treatments but for wood treated at 200ºC for 6 h the improvement was higher for sapwood than for heartwood. However when the comparison is made at the same mass loss (Figure 3), the differences were not significant. In the tangential direction ASE was higher for sapwood.

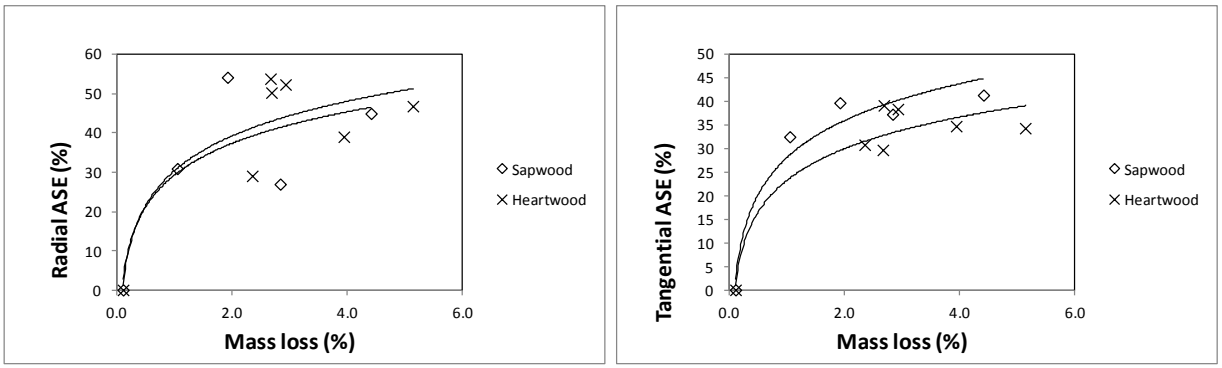

Figure 3: Radial and tangential ASE vs mass loss due to treatment

The fungal durability results obtained showed a significant decrease of mass loss with the temperature used and with the time of treatment (Figure 4). The pattern of decrease was similar for sapwood and heartwood but the highest level of protection against *P. placenta* was achieved

for sapwood treated at 200ºC for 4 hours. Nevertheless, for the lower treatments, mass loss was smaller for heartwood samples.

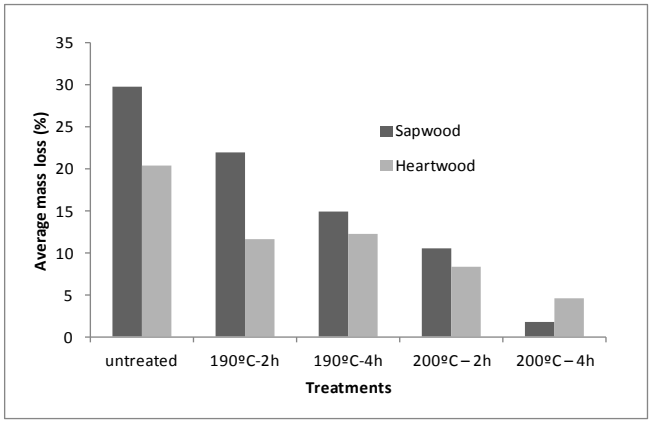

Figure 4: Fungal durability of untreated and treated wood

In relation to mechanical properties, MOE increased slightly at the beginning of treatment decreasing afterwards for both sapwood and heartwood. This is in agreement with earlier findings by Esteves et al. (2006). Even though there are no heartwood results for mass loss over 4%, until this mass loss there are no significant differences between sapwood and heartwood (Figure 5). MOR decreased for both heartwood and sapwood reaching almost a 50% and 30% decrease (in relation to initial MOR) for sapwood and heartwood respectively. The decrease for sapwood was almost linear (R^2=0.99) while for heartwood the decrease was inconsistent. Once again if the comparison is made at the same mass loss, no significant differences were found between sapwood and heartwood.

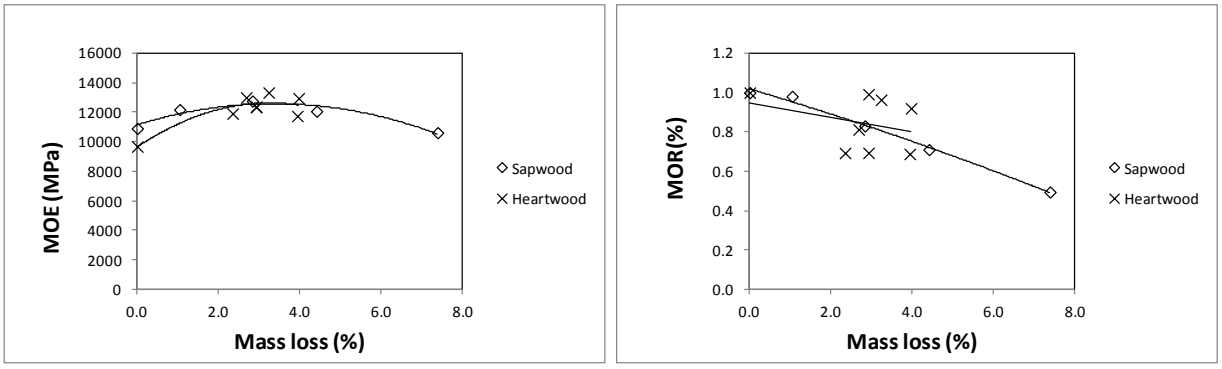

Figure 5: MOE and MOR vs mass loss due to treatment

4. CONCLUSIONS

The results showed that the heat treatment is equally efficient for sapwood and heartwood when comparing at the same mass loss. Since temperature and treatment time influenced differently on heartwood and sapwood i.e. on mass loss, the extent of improvements varied between sapwood and heartwood with the same treatment conditions (time and temperature) i.e. the improvement was higher for sapwood. The presence of resin in the surface of heartwood samples may lead to some aesthetic problems.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

Boonstra, M., Van Acker, J., Kegel, E., and Stevens, M. (2007): "Optimisation of a two-stage heat treatment process: durability aspects." Wood Science and Technology, 41(1), 31-57.

CEN/TS 18083-1 (2005) Durability of wood and wood-based products - Determination of the natural durability of solid wood against wood-destroying fungi, test methods - Part 1: Basidiomycetes. European Committee for Standardization, Brussels.

Esteves, B., Marques, A. V., Domingos, I., and Pereira, H. (2006): "Influence of steam heating on the properties of pine (*Pinus pinaster*) and eucalypt (*Eucalyptus globulus*) wood." Wood Science and Technology, 41, 193-207.

Esteves, B., Domingos, I., and Pereira, H. (2007): "Improvement of technological quality of eucalypt wood by heat treatment in air at 170-200 degrees C." For. Prod. J, 57(1-2), 47-52.

Esteves, B. M., Domingos, I. J., and Pereira, H. (2008): "Pine wood modification by heat treatment in air." BioResources, 3(1), 142-154.

Metsä-Kortelainen, S., Antikainen, T., and Viitaniemi, P. (2006): "The water absorption of sapwood and heartwood of Scots pine and Norway spruce heat-treated at 170 C, 190 C, 210 C and 230 C." European Journal of Wood and Wood Products, 64(3), 192-197.

Metsä-Kortelainen, S., and Viitanen, H. (2009): "Decay resistance of sapwood and heartwood of untreated and thermally modified Scots pine and Norway spruce compared with some other wood species." Wood Material Science & Engineering, 4(3-4), 105-114.

Metsä-Kortelainen, S., and Viitanen, H. (2012). "Wettability of sapwood and heartwood of thermally modified Norway spruce and Scots pine." European Journal of Wood and Wood Products, 70(1), 135-139.

Stamm, A. J., Burr, H. K., and Kline, A. A. (1946): "Staybwood—heat-stabilized wood." Industrial & Engineering Chemistry, 38(6), 630-634.