DURABILITY OF PINE WOOD MODIFIED WITH A SERIES OF LINEAR CHAIN CARBOXYLIC ACID ANHYDRIDES AGAINTS SOFT ROT FUNGI

Antonios N. Papadopoulos
Technological Educational Institute of Kavala, Branch of Drama, Department of Forestry and Management of Natural Environment, Laboratory of Wood and Wood Products, Drama, Greece

Holger Militz, Antje Pfeffer
Georg August University Göttingen, Burckhardt Institute
Wood Biology and Wood Products
Göttingen, Germany

ABSTRACT

The work described in this paper has demonstrated that chemically modified Corsican pine sapwood with a homologous series of linear chain carboxylic acid anhydrides afforded bio-protection against soft rot decay. By varying the reaction time, various levels of modifications were obtained. The results indicated that the breakdown caused by fungi is reduced by chemical modification at these levels of modification. There was no significant reduction in feeding above 20 % WPG (weight percent gain due to modification) suggesting that modification to WPG's greater this certain level did not cause additional protection. The type of anhydride employed had little influence on breakdown behaviour.

KEYWORDS: Wood, modification, anhydrides, durability, decay, soft rot.

INTRODUCTION

The microbiological degradation of lignocellulosic materials is one of the most important processes in nature. Fungal activities in wood limit its use by reducing its density, strength and aesthetic properties. Preservation of wood by conventional methods has long been established to prevent, or eradicate wood-inhabiting fungi. Conventional wood impregnation methods (water

or oil type preservatives) are based primarily on the use of biocides. Environmental concerns, particularly with regard to disposal of treated wood at the end of product life, are now causing restrictions to be imposed upon the utilization of conventional chemical treatments. An alternative method of enhancing the durability of wood without the use of conventional biocides is chemical modification. It is well established that the chemical modification of wood is able to provide protection against fungal attack (Rowell 1983, Hon 1996, Militz 1991). A chemical modification reaction involves the formation of a chemical bond between the reagent and the cell wall polymers of wood. In almost all cases, this reaction occurs between some of the hydroxyl groups of these polymers and the reagent molecules. Such a reaction leads to a change in the chemical and physical properties of the substrate. The effectiveness of linear chain carboxylic anhydrides used in this study at conferring decay resistance against the brown rot fungus *Coniophora puteana* in a laboratory test has already been demonstrated (Papadopoulos and Hill 2002) where it was found that a weight gain (WPG) of 18 % following reaction conferred complete protection. Additionally, it was reported that decay protection is independent of the degree of substitution of the cell wall hydroxyl groups, but correlates with the degree of bulking of the cell wall.
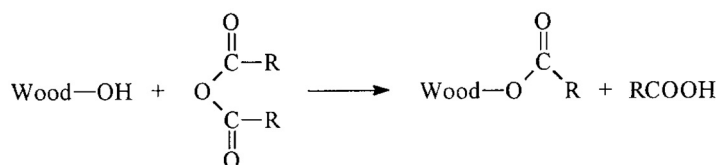

Fig. 1: Anhydride modification scheme, where $R=CH_3$ (acetic anhydride), $R= C_2H_5$ (propionic anhydride), $R=C_3H_7$ (butyric anhydride), $R= C_4H_9$ (valeric anhydride), $R=C_5H_{11}$ (hexanoic anhydride) (Rowell 1983).

In soil contact the type of fungi differs. Due to the wet circumstances the conditions in the soil are optimised for the development of mainly soft rot fungi and bacteria. Therefore the effectiveness of the treatment against soft rot fungi is to be investigated. In this paper we report on the protection afforded to Corsican pine sapwood, against soft rot fungi, modified to a range of WPG's with various linear chain anhydrides, namely acetic anhydride (AA), propionic anhydride (PA), butyric anhydride (BA), valeric anhydride (VA) and hexanoic anhydride (HA). The reaction between wood and linear chain anhydrides is a single site reaction, as depicted in Fig. 1. All the anhydrides yield the corresponding carboxylic acid as a by-product of their reaction with wood.

MATERIAL AND METHODS

Chemical modification of wood samples

Corsican pine (*Pinus nigra* Schneid) sapwood samples were chosen because of their good permeability, essential in wood modification reactions. In order to minimise any within species variability, the samples were prepared from a single board, from knot free sapwood. Also, samples with the same number of growth rings were preferentially chosen. Samples were screened for defects, such as small knots, sloping or curvy grain, and splits or cracks. The specimen geometry was chosen in order to minimise the longitudinal dimension ensuring rapid reagent penetration into the cell lumen (average softwood fibre length is 3 - 4 mm). Hence, samples 20 x 20 x 5 mm (radial x tangential x longitudinal) were cut from kiln dried wood and carefully selected so that

growth rings were parallel to the tangential face. Samples were carefully sanded to remove loosely adhering fibres, then placed in a Soxhlet extractor for solvent extraction using toluene/methanol/acetone (4:1:1 by volume) for eight hours to remove the extractives of the wood samples. Samples were dried in an oven for eight hours at 105°C. Prior to weighing (four figure balance), samples were transferred to a vacuum desiccator and allowed to cool to ambient temperature over silica gel. Weighed samples (W_1), were vacuum impregnated with pyridine for one hour then transferred to a flask containing pyridine set in an oil bath at 100°C. Pyridine swells the wood and acts as a catalyst for the modification reaction. Pyridine was chosen for the following reasons: (i) each of the modifying chemicals used in this study was found to be highly soluble or miscible with pyridine, (ii) the longer chain anhydrides used in this study do not react with wood otherwise (Hill and Hillier 1999) and (iii) pyridine is an organic tertiary amine, considered by Rowell (1983) to be ideal for the catalysis of wood modification of pine. The action mechanism of pyridine in the anhydride reactions has been commented in detail previously (Hill and Papadopoulos 2002). Samples were allowed to equilibrate in the hot pyridine for one hour. Sets of hot samples were added to a flask containing a one molar solution of the anhydride in pyridine set in an oil bath at 100°C for a specific length of time to achieve the desired level of modification. The reaction time varied (Tab.1) in order to achieve equivalent levels of modification (Papadopoulos 2001). At the end of the reaction period, the flask was removed from the oil bath, the hot reagent decanted off and ice cold acetone added to quench the reaction. Samples were allowed to sit in the acetone for one hour before being transferred to the Soxhlet apparatus for solvent extraction as detailed previously. Samples were re-weighed (W_2) after oven drying as detailed previously. Samples were free of reagent, by-product and pyridine after this procedure.

Tab. 1: *Weight percent gain (WPG) of oven dried Corsican pine samples after reaction with a homologous series of linear chain carboxylic acid anhydrides. (Standard deviation in parentheses).*

Anhydride	Reaction time (min)	WPG[a] (%)
Acetic	45	7.5 (0.3)
Acetic	120	15 (0.2)
Acetic	240	19.5 (0.4)
Acetic	300	22.3(0.3)
Propionic	90	10.2 (0.1)
Propionic	120	14.8 (0.2)
Propionic	270	20.5 (0.2)
Propionic	300	25.1 (0.2)
Butyric	330	23.3 (0.3)
Valeric	360	26.1 (0.2)
Hexanoic	360	25.1 (0.2)

[a] Each value represents the mean of four replicates

The extent of reaction was calculated as weight percent gain determined by the differences in oven dry weight of the sample before modification (W_1) and after modification (W_2) according to equation WPG = (W_2 - W_1) / W_1 x 100 (%).

The number of hydroxyl groups per gram of wood that are substituted when reacted with the

corresponding anhydride can be calculated as follows:

$$\text{OH}_{\text{substd.}} = \frac{W_{\text{mod.}} - W_{\text{unmod.}}}{W_{\text{unmod.}} \times M_w} \times 1000 \quad (\text{mmoles.g}^{-1})$$

Where:
$W_{\text{mod.}}$ is the dry weight of modified wood
$W_{\text{unmod.}}$ is the dry weight of unmodified wood
M_w is the molecular weight of the adduct -C(O)-R

Infra-red (IR) analysis

For Infra-red (IR) analysis, the treated samples were grounded up by using a microdismembrator (20.000 rpm for 6 min). The fibre flour was then mixed with oven dry potassium bromide (KBr) powder (the fibre flour/KBr ratio was 1:100) and placed in a vibratory ball mill capsule. The mixture was grounded for about 2 min. The grounded mixture was then transferred to a press and the bolts of press screwed down. The bolts were tightened with a spanner to press the disk. After a few minutes, the bolts were loosen and removed. The press was placed directly into a sample beam of a Mattson FTIR spectrometer, Nicolet 750, series II.

Unsterile soil bed test

The durability of treated and untreated samples of Corsican pine sapwood was tested in soil contact under unsterile conditions according to the European standard ENV 807. The sample size was differed from the standard. Treated and untreated samples (four replicates for each treatment, i.e. 48 samples) with a sample size of 20 mm x 20 mm x 5mm (as described above) were used. Natural soil, composted and prepared in Göttingen, was applied. It was mixed with sand to provide a water holding capacity (WHC) of 48 %. During the test, a soil moisture of 95 % of the WHC was set and weekly controlled by weighing the test container and adding water by spraying if required. After 32 weeks the samples were carefully cleaned from adherent soil and oven dried at 103°C and weighed (m_2) to determine the mass loss. The mass loss (ML) was calculated by the differences in oven dry weight of the samples before test (m_1) and after test (m_2) according to equation: ML = (m_1-m_2) / m_1 x 100 (%).

RESULTS AND DISCUSSION

By varying the reaction time, various levels of modifications (WPG) of pine wood with linear chain carboxylic acid anhydrides were obtained (Tab. 1). Esterification of wood was established by infra-red spectroscopy (Fig. 2) and by increase in weight (Tab. 1). Infra-red spectra confirmed the occurrence of wood-anhydride reaction. The strong vibration obtained in the region of 1 736 and 1 730 cm^{-1} (C=O) was a distinct pattern present in modified samples, which indicates ester bond formation. As expected, absorption was not present in unmodified wood.

The biological behaviour of pine wood modified with linear chain carboxylic acid anhydrides against soft rot fungi is presented in Fig. 3. Fig. 3 depicts the mass loss (%) of modified wood samples caused by soft rot decay. It can be observed that the highest mass loss was recorded on the untreated control samples (37.9 %) and on samples modified with acetic anhydride (9.5 %) at the lowest modification level (7.5 %) employed in this study. Wood modified with valeric anhydride at 26.1 % WPG showed the greatest resistance (0.70 % mass loss). Wood samples modified at higher WPG's have shown light attack. The only exception seems to be the samples modified

with hexanoic anhydride at 25.1 % weight gain, where a mass loss of 5.6 % is observed. However, this discrepancy can be explained on the basis of prolonged reaction time (360 min) which caused further sample degradation and therefore affected the durability against soft rot decay. There was no significant reduction in mass loss above approximately 20 % WPG suggesting that modification to WPG's greater than this did not offer additional protection at least during the research period of 32 weeks. Overall, chemical modification with a homologous series of linear chain carboxylic acid anhydrides afforded substantial bio-protection of Corsican pine against soft rot decay.
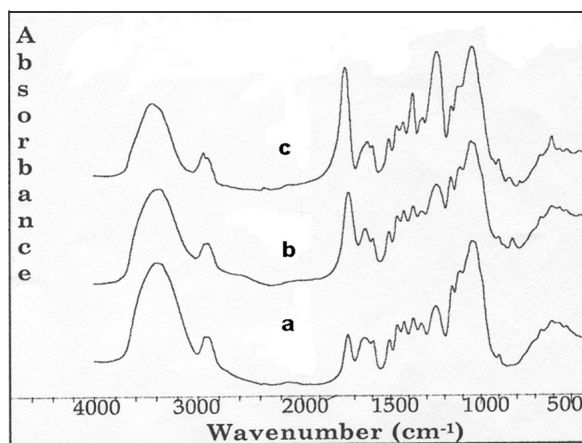

Fig. 2: FTIR spectra of esterified and control wood: a) control, b) modified with acetic anhydride and c) modified with hexanoic anhydride
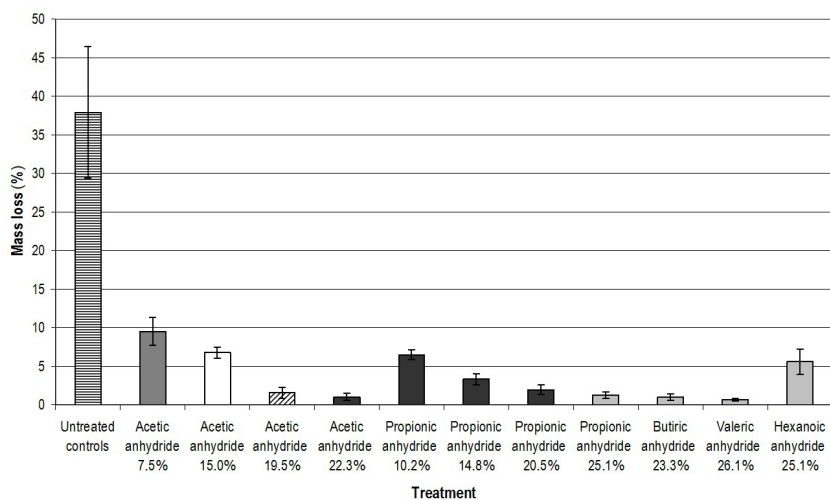

Fig. 3: Mean values (± 1 SE) of mass loss (%) caused by soft rot decay of control and chemically modified wood. Each value is a mean of four replicates

Our results are in line with those reported in the literature. Many studies have been performed investigating the biological degradation resistance of acetylated wood to fungi, and other organisms. It is generally reported that a weight percent gain of ca. 20 % is required before full protection is achieved. Goldstein et al. (1961) acetylated *Ponderosa pine* using acetic anhydride in xylene. The modified wood was tested against six basidiomycete fungi, five brown rot and one white rot, with a WPG of 18 % reported to be sufficient to provide decay resistance. Peterson and Thomas (1978), acetylated loblolly pine, green ash and yellow poplar using acetic anhydride in xylene. The modified samples were tested against the brown rot fungus *Gloeophyllum trabeum* and the white rot fungus *Coriolus versicolor*. It was found that the white rot was generally easier to control than the brown rot, with levels of acetylation as low as 7 % being able to provide protection. However, ash was still degraded by white rot, even at WPG levels of 20 %. It was stated that 'blocking of action of fungal catalysts appears to be the primary protection mode of the acetylation technique'. Levels of acetylation of 17-20 % WPG were found to provide decay protection (with the exception of ash). The effect of the acetylation level on the decay resistance of Japanese red pine, red beech and albizzia was studied by Takahashi et al. (1989). Modified samples were exposed to the brown rot fungi *Tyromyces palustris, Serpula lacrymans*, the white rot fungus *Coriolus versicolor* and to soft rot in unsterile soil. Protection against *Tyromyces palustris* was observed in all wood species at a WPG of 20 %. With *Coriolus versicolor*, a WPG of 6 % was sufficient to protect softwood, but hardwoods required a WPG of 16 %. Beckers et al. (1994), determined the decay protection threshold levels for acetylated Scots pine to a variety of wood decay fungi. It was found that WPG levels of 18 % were required against *Coniophora puteana* and *Gloeophyllum trabeum*, over 20 % against *Poria placenta* and 11 % was required in an unsterile soft rot test. In ground contact stake tests of acetylated pine samples, it was found that an acetyl content of 20 % prevented attack by brown, white and soft rot fungi (Larsson Brelid et al. 2000). Laboratory unsterile soil tests on acetylated mini-stakes showed that an acetyl content of 18.5 % was able to provide significant protection against fungal attack. Vapour phase acetylated makamba (*Betula maximowiczii*) were exposed to a brown (*Tyromyces palustris*) and white-rot (*Coriolus versicolor*) fungus (Ohkoshi et al. 1999). Mass loss due to decay with the brown rot fungus was zero at 20 % WPG, and with the white rot fungus at 12 % WPG. Protection against soft rot has been reported for a WPG of 10.7 % for pine, 14.4 % for poplar and 12.8 % for beech (Beckers et al. 1995). Suttie et al. (1999), modified Scots pine with acetic, propionic, butyric, or hexanoic anhydrides and determined decay resistance against the brown rot fungi *Coniophora puteana, Gloeophyllum trabeum, Poria placenta* and a white rot fungus (*Coriolus versicolor*) using European Standard method EN 113 and a vermiculite overlay method. It was found that a threshold of ca. 23 % was required to ensure protection, regardless of the anhydride used. Papadopoulos and Hill (2002), reported that a WPG of 18 % following reaction with a series of anhydrides conferred complete protection against the brown rot fungus *Coniophora puteana* in a laboratory test.

The type of anhydride employed in this study has little influence on the break down, since similar numbers of mass loss were recorded. This is in line with the observation made earlier as far as the effect of the anhydride type upon the decay resistance of modified softwood against brown and white rot fungi (Forster 1998, Papadopoulos and Hill 2002), to attack by the marine wood borer *Limnoria quadripunctata* Holthius (Papadopoulos et al. 2008a) and to attack by the subterranean termites *Reticulitermes flavipes* (Papadopoulos et al. 2008b).

Tab. 2: Molecular weight of the adduct –C(O)-R due to reaction with an homologous series of linear chain carboxylic acid anhydrides

Anhydride	Molecular weight of the adduct	WPG (%)	OH Groups substd
Acetic	43	15	3.48
Propionic	57	15	2.62
Butyric	71	15	2.11
Valeric	85	15	1.76
Hexanoic	99	15	1.51

The results from this study indicate that the degree of cell wall bulking caused by the adduct, rather than the extent of hydroxyl substitution is the primary factor controlling resistance against soft rot decay, since similar mass losses were found for wood modified with five different anhydrides at equivalent levels of modification (acetic 22.3 %, propionic 25.1 %, butyric 23.3 %, valeric 26.1 % and hexanoic 25.1 % WPG). This may be illustrated more clearly in Tab. 2. For example, it can be seen, that at hypothetical 15 % weight gain, 3.48 mmoles of OH groups per gram of wood are substituted when reacted with acetic anhydride, but only 1.51 moles of OH groups per gram when reacted with hexanoic anhydride (Papadopoulos 2001). Despite the large difference in OH substitution level, reaction with different anhydrides results in the same level of protection against decay, marine borers and termites. These observations suggest that the mechanism of protection is not chemical/biochemical in origin, but relates to the bulking of the cell wall by the reacted adduct. Stamm and Baechler (1960), considered a number of mechanisms by which chemical modification of wood imparted decay resistance. In a study where low levels of formaldehyde were reacted with wood, yet good decay protection was obtained nonetheless, they proposed a mechanism whereby cell wall microcapillary blocking prevented access by fungal enzymes. Although it is now known that fungal enzymes are too large to enter the cell wall, the theory still applies in the case of the low molecular weight degradative agents. In a comprehensive study of this topic, Forster (1998) also came to the conclusion that cell wall capillary blocking was the mechanism for decay protection.

CONCLUSIONS

Results from this study have shown that chemically modified Corsican pine sapwood with a homologous series of linear chain carboxylic acid anhydrides afforded bio-protection against soft rot decay. There was no significant reduction in break down caused by soft rot fungi above 20 % WPG suggesting that modification to WPG's greater than this did not confer additional protection. The type of employmyed andride has had little influence on break down, since the protection imparted by chemical modification was independent of the degree of substitution of the cell wall hydroxyl groups, but correlates with the degree of bulking of the cell wall.

REFERENCES

1. Beckers, E.P.J., Militz, H., Stevens, M., 1994: Resistance of acetylated wood to basidiomycetes, soft rot and blue stain. International Research Group on Wood Preservation. Document No: IRG/WP/94-40021.

2. Beckers, E.P.J., Militz, H., Stevens, M., 1995: Acetylated solid wood. Laboratory durability test (part II) and field tests. International Research Group on Wood Preservation. Document No: IRG/WP/95-40048.

3. Forster, S., 1998: Decay resistance of modified softwood. Ph.D thesis. University of Wales, Bangor.

4. Goldstein, I.S., Jeroski, E.B., Lund, A.E., Nielson, J.F., Weaver, J.W., 1961: Acetylation of wood in lumber thickness. For. Prod. J. 11(8): 363-370.

5. Hill, C.A.S., Hillier, J.G., 1999: Studies of the reaction of carboxylic acid anhydrides with wood. Experimental determination and modelling of kinetic profiles. Physical Chemistry Chemical Physics 1(7): 1569-1576.

6. Hill, C.A.S., Papadopoulos, A.N., 2002: The pyridine catalysed acylation of sapwood and phenolic model compounds with carboxylic acid anhydrides. Determination of activation energies and entropy of activation. Holzforschung 56(2): 150-156.

7. Hon, D.N.S., 1996: Chemical modification of lignocellulosics. Marcel Dekker, New York.

8. Larsson Brelid, P., Simonson, R., Bergman, O., Nilsson, T., 2000: Resistance of acetylated wood to biological degradation. Holz als Roh- und Werkstoff 58(5): 331-337.

9. Militz, H., 1991: Die Verbesserung des Schwind- und Quellverhaltens und der Dauerhaftigkeit von Holz mittels Behandlung mit unkatalysiertem Essigsäureanhydrid. Holz als Roh- und Werkstoff 49(4): 147-152.

10. Ohkoshi, M., Kato, A., Suzuki, K., Hayashi, N., Ishihara, M., 1999: Characterization of acetylated wood decayed by brown-rot and white-rot fungi. J. Wood Sci. 45(1): 69-75.

11. Papadopoulos, A.N., 2001: Swelling, cell wall porosity and chemical modification of wood. Ph.D Thesis, University of Wales, Bangor.

12. Papadopoulos, A.N., Hill, C.A.S., 2002: The biological effectiveness of wood modified with linear chain carboxylic acid anhydrides against *Coniophora puteana*. Holz als Roh-und Werkstoff 60(5): 329-332.

13. Papadopoulos, A.N., Duquesnoy, P., Cragg, S.M., Pitman, A.J., 2008a: The resistance of wood modified with linear chain carboxylic acid anhydrides to attack by the marine wood borer *Limnoria quadripunctata* Hothius. International Biodeterioration & Biodegradation 61(2): 199-202.

14. Papadopoulos, A.N., Avtzis, D., Avtzis N., 2008b: The biological effectiveness of wood modified with linear chain carboxylic acid anhydrides against the subterranean termites *Reticulitermes flavipes*. Holz als Roh-und Werkstoff 66(4): 249-252.

15. Peterson, M.D., Thomas, R.J., 1978: Protection of wood from decay fungi by acetylation. An ultrastructural study. Wood and Fiber 10(3): 149-163.

16. Rowell, R.M., 1983: Chemical modification of wood. For. Prod. Abstr. 6(12): 366-382.

17. Stamm, A.J., Baechler, R.H., 1960: Decay resistance and dimensional stability of five modified woods. For. Prod. J. 10(1): 22-26.

18. Suttie, E.D., Hill, C.A.S., Jones, D., Orsler, R.J., 1999: Chemically modified solid wood. I. Resistance to fungal attack. Mat. und Org. 32(3): 159-182.

19. Takahashi, M., Imamura, Y., Tanahashi, M., 1989: Effect of acetylation on decay resistance of wood against brown rot, white rot and soft rot fungi. International Research Group on Wood Preservation. Document No: IRG/WP 3540.

Antonios N. Papadopoulos
Technological Educational Institute of Kavala
Branch of Drama
Department of Forestry and Management of Natural Environment
Laboratory of Wood and Wood Products
66100 Drama
Greece
Corresponding author: antonios1974@hotmail.com.
Phone: 0030 2521060445

Holger Militz, Antje Pfeffer
Georg August University Göttingen
Burckhardt Institute
Wood Biology and Wood Products
37077 Göttingen
Germany