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The biological effectiveness of wood modified with linear chain carboxylic acid anhydrides against *Coniophora puteana*

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This paper presents an assessment of the effectiveness of linear chain carboxylic acid anhydrides namely, acetic, propionic, butyric, valeric and hexanoic anhydride, in improving the biological resistance of Corsican pine (*Pinus nigra* Schneid) sapwood. A brown rot fungus [*Coniophora puteana* (Schum.:Fr)] was selected in order to determine and compare the effectiveness (threshold value) of the linear chain anhydrides. The work described in this paper has demonstrated that chemically modified Corsican pine sapwood afforded substantial bioprotection against *Coniophora puteana.* With all anhydrides studied, a weight gain of 18% following reaction ensured complete protection. The results indicate that degree of cell wall bulking by the bonded adduct, rather than extent of hydroxyl substitution is the primary factor controlling decay resistance.

Biologische Wirksamkeit der Holzmodifikation mit Fettsäureanhydriden zum Schutz gegen Braunfäule (Coniphora puteana)

Diese Arbeit beschreibt die Wirksamkeit von unverzweigten Fettsäureanhydriden zum Verbessern der biologischen Resistenz von Kiefernsplintholz (*P. nigra Schneid.*). Geprüft wurden die Anhydride von Essig-, Propion-, Butter-, Valerian- und Capronsäure. Als Testpilz diente der Braunfäulepilz *Caniophora puteana* (Schum. : Fr.). Die Ergebnisse zeigen eine erhebliche Verbesserung der biologische Resistenz gegen diesen Pilz nach der chemischen Modifizierung des korsischen Kiefernholzes. Mit allen Anhydriden konnte mit einem Massenzusatz von 18% ein vollständiger Schutz erreicht werden. Die Ergebnisse deuten darauf hin, dass das Ausmaß der Zellwandbelegung und nicht so sehr die Substitution der Hydroxylgruppen der ausschlaggebende Faktor zum Schutz vor biologischem Abbau ist.

1
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 toxic chemicals. 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. The consumption of preservative treated wood in Western Europe is estimated at 6 million m^3 per year, with about 30,000 tonnes of toxic preservatives used. An alternative method of enhancing the durability of wood without the use of conventional biocides is chemical modification. Chemical modification is accomplished by reacting the wood with selected chemicals, which modify the cell wall wood polymers without leaving toxic residues within the wood. The use of chemical modification to stabilise wood against the activities of decay organisms has been the subject of numerous studies (Rowell 1983; Hon 1996). Of all the modification methods investigated, the chemical modification of wood with acetic anhydride has been studied the most and is nearest to commercial exploitation.

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 weight percent gain (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 *G. 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 level of acetylation on the decay resistance of Japanese red pine, red beech and albizzia was studied by Takahashi

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et al. (1989). Modified samples were exposed to the brown rot fungi *T. palustris*, *Serpula lachrymans*, the white rot fungus *C. versicolor* and to soft rot in unsterile soil. Protection against *T. palustris* was observed in all wood species at a WPG of 20%. With *C. 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 *C. puteana* and *G. trabeum*, over 20% against *P. 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 (*Coriolis 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 EN113 and a vermiculite overlay method. Resistance to soft rot attack was also determined using a modified ENV 807 stake test in unsterile soil. The effect of different levels of reaction upon decay resistance was only tested with the soft rot experiment. In this, it was found that a threshold of *ca.* 23% was required to ensure protection, regardless of the anhydride used.

It is not clear whether protection arises from a decrease in the moisture content of modified samples, because the cell wall polymer OH groups are masked, because the cell wall is bulked by adduct, or a combination of these phenomena. Brown rot fungi preferentially attack the cellulose and hemicellulose components of the cell wall, with depolymerisation of these polymers. The hemicellulose component is particularly susceptible to attack during the initial phase of degradation. Lignin attack is largely limited to demethoxylation of the aromatic residues, loss of the propyl side chain and incorporation of oxygen, with minor depolymerisation. It is known that the enzymes associated with the degradation of these components are too large to enter the cell wall of undegraded wood. For this reason, various low molecular weight diffusible agents have been proposed to initiate degradation thereby allowing enzymes to penetrate as decay proceeds (Eaton and Hale 1993; Green and Highley 1997). Any proposed mechanism by which chemical modification provides biological protection against fungal attack should therefore take account of the presence of these low molecular weight diffusible agents.

Results reported by Suttie et al. (1999) have shown that with soft rot attack, the WPG level, rather than the extent of hydroxyl substitution is the sole factor determining protection from decay. This indicates that a physical, rather than a chemical mechanism may be responsible for decay protection, at least as far as soft rot is concerned. In this paper, we report on the protection afforded to Corsican pine sapwood modified to a range of WPG's with various linear chain anhydrides (Fig. 1), against attack by the brown rot fungus *Coniophora puteana*.

2 Materials and methods

2.1 Chemical modification of wood samples

Corsican pine (*Pinus nigra* Schneid) sapwood samples of dimension 20 mm × 20 mm × 5 mm (radial × tangential × longitudinal) were cut from freshly felled kiln dried logs. 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. 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. Samples were allowed to equilibrate in the hot pyridine for one hour. Sets of hot samples (sixteen replicates) 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. 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.

The extent of reaction was calculated as weight percent gain (WPG) determined by the differences in oven dry weight of the sample before modification (W_1) and after modification (W_2) according to equation [Weight percent gain $(\mathrm{WPG}) = (W_2 - W_1)/W_1 \times 100$].

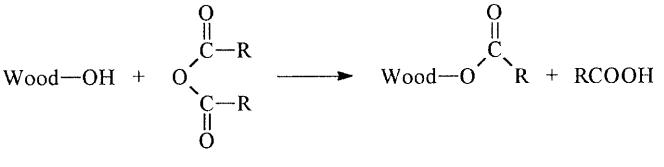

Fig. 1. Anhydride modification scheme, where R = CH_3 (acetic anhydride), R=C_2H_5 (propionic anhydride), R = C_3H_7 (butyric anhydride), R = C_4H_9 (valeric anhydride), R = C_5H_{11} (hexanoic anhydride) (Rowell 1983)

Bild 1. Modifikationsschema. R bedeutet jeweils: CH_3 (Acetonhydrid); C_2H_5 (Propionanhydrid); C_2H_9 (Valerianlhydrid); C_5H_{11} (Capron anhydrid) (nach Rowell 1983)

2.2
Decay tests

Samples were packed in an argon atmosphere and sterilised by irradiation (2.5 Mrad.) prior to decay tests. Each set of 16 modified and unmodified control samples at each level of treatment were subdivided into four groups of four replicates. Laboratory pure strains of the brown rot fungi *Coniophora puteana* (No FPRL 11E) were used, grown on malt agar. Four unmodified control or modified wood samples were planted on sterile specimen supports placed on the cultures of the test fungus actively growing on 5% malt agar in 500 ml capacity jars (EN 113 1996; the only modification was the sample size, see 2.1). An additional set of sterile control samples were used to assess operational control losses. The closed jars were incubated for 16 weeks, at 22 ± 1°C and 75 ± 5% relative humidity to evaluate the efficacy of the treatments. After incubation, the samples were removed from the jars, cleaned, dried overnight at 105°C and weighed. Weight loss was expressed as a percentage of the initial oven dried weight of the sample. Weight losses from sterile controls were subtracted from the decay results to give corrected data.

3
Results and discussion

Chemical modification with a homologous series of linear chain carboxylic acid anhydrides afforded substantial bioprotection of Corsican pine against *Coniophora puteana*. The data from all tests were plotted and the fit shown by linear regression analysis indicates a positive relationship between extent of modification and decay resistance (Fig. 2). The data shows that a WPG of approximately 18% is required following reaction to ensure complete protection, irrespective of the modifying chemical used.

According to Rowell (1982), at a WPG of 20% all of the lignin OH groups are substituted, whilst the holocellulose has a degree of substitution of 0.48 of the accessible OH groups. Investigations by Ohkoshi and co-workers using NMR methods, have shown that hemicellulose is preferentially substituted in acetylation reactions, with cellulose participating in reactions only above a WPG of 20%. Full substitution of the hemicellulose OH groups was found to occur only at a WPG of 35%, whereas free OH groups were detected on the lignin even at a WPG of 27% (Ohkoshi and Kato 1993, 1997; Ohkoshi et al. 1997). Determination of the distribution of acetyl groups in the cell wall of wood or plant fibres has shown that the S_2 layer shows the highest levels of substitution at moderate WPG's (10%), with the middle lamella showing high levels of reaction at WPG's in excess of 25% (Rowell et al. 1994; Hill et al. 1998). Evidence from the literature therefore suggests that not all accessible OH groups are reacted at the WPG threshold where decay protection occurs.

Different levels of OH substitution also occur at comparable WPG levels for each of the anhydrides studied. At 18% WPG, 4.3 mmoles of OH groups per gram of wood are substituted when reacted with acetic anhydride, but only 1.8 mmoles of OH groups per gram when reacted with hexanoic anhydride (Hill and Jones 1996). Despite the large difference in OH substitution level, reaction with different anhydrides results in the same level of protection against decay. These observations suggest that the mechanism of protection with brown rot is not chemical/biochemical in origin, but rather 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.

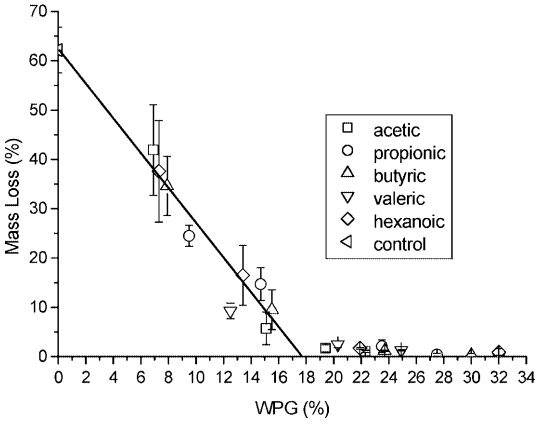

Fig. 2. Data from decay studies corrected for weight loss from sterile controls
Bild 2. Ergebnisse der Abbauversuche, korrigiert für Gewichtsverlust gegenüber Kontrollproben

4
Conclusions

Results from this study have shown that decay protection imparted by chemical modification of Corsican pine sapwood against *C. puteana* is independent of the degree of substitution of the cell wall hydroxyl groups, but correlates with the degree of bulking of the cell wall. It is proposed that the mechanism for decay protection in this case is due to blocking of the cell wall microcapillaries, preventing access by the low molecular weight degradative agents produced by the fungus.

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