The Influence of Wood Species upon the Decay Protection Mechanisms Exhibited by Anhydride Modified Woods

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Keywords: Anhydride, chemical modification, *Coniophora puteana*, decay resistance

ABSTRACT

By modifying wood with anhydride reagents of different molecular weight it is possible to determine whether property changes arise due to the extent of substitution of hydroxyl groups or bulking of the cell wall. This strategy has been adopted in studies of the decay resistance of anhydride modified wood. When Corsican pine sapwood is modified with acetic or hexanoic anhydride, it is found that the extent of decay resistance (as determined by mass loss) is a function of the weight percentage gain due to reaction rather than extent of hydroxyl substitution. This shows that decay resistance is related to bulking by bonded adduct and this has in turn been linked to a reduction in the cell wall water content due to space taken up by acyl groups in the cell wall. However, in ECWM2 it was reported that rubberwood exhibited the opposite behaviour, with decay resistance correlating with extent of OH substitution, whereas beech appeared to show anomalous behaviour. In order to shed further light on this phenomenon a range of hardwoods and softwoods have been reacted with acetic or hexanoic anhydride and the thus modified woods have been exposed to *Coniophora puteana*. Results indicate that cell wall bulking is indeed the protection mechanism, although there are differences in behaviour which are attributed to the topochemistry of the reaction with the cell wall. Finally, there is some discussion of how universal this protection mechanism may be.

INTRODUCTION

The decay properties of acetylated wood have been investigated for over sixty years in pure culture tests, unsterile tests and in the field (Hill 2006). Although many studies have been performed over the years, the scientific literature was not able to provide any conclusive evidence as to the mechanism by which acetylated wood was protected from microbiological decay. Various hypotheses were advanced which included protection due to enzyme recognition blocking, reduction in cell wall moisture content, or physical blocking of the cell wall micropores (Stamm and Baechler 1960, Hill 2006). The first study showing that the decay protection mechanism was related to cell wall bulking was that of Papadopoulos and Hill (2002). Subsequently, Hill *et al*. (2005a) demonstrated that the mechanism was related to the volume occupied by the covalently bonded acyl groups in the cell wall and that a reduction in cell wall moisture content was the most likely explanation for the decay resistance imparted. Hill *et al*. (2006) then presented the results from a study of the effect of chemical modification upon the decay resistance of Corsican pine (*Pinus nigra*) when exposed to *Coniophora puteana* where the decay resistance was confirmed to be related to WPG. Since chemically bonded acyl groups

occupy space within the cell wall, it can be hypothesised that this volume is thereby denied to water molecules and that as a consequence the fibre saturation point (FSP) of the wood cell wall is reduced. It has been shown that when the theoretical FSP at different WPGs is calculated from the volume of bonded acyl group as determined from helium pycnometry, then this correlates very well with FSP values directly determined from solute exclusion (Hill 2005a). This data has been combined with mass loss data from decay experiments to show that zero mass loss occurs at a FSP value of 20% (Hill *et al*. 2005b). From this work, it was further suggested that the protection mechanism is indeed a function of the cell wall moisture content.

Since the above mentioned studies have been solely applied to Corsican pine, a logical next step in this work is to apply the methodology to other wood species. A preliminary report was presented at the Second European Conference on Wood Modification suggesting that the situation may be more complex than previously thought (Hill *et al*. 2005b). In particular, it was reported that with rubberwood (*Hevea brasiliensis*) decay protection was a function of OH substitution rather than WPG, whereas data for European beech (*Fagus sylvatica*) was ambiguous. Further work is clearly necessary and in the work described in this paper a wider range of hardwoods and softwoods (both heartwood and sapwood) have been studied.

EXPERIMENTAL

Samples of European beech (*Fagus sylvatica*) sapwood, oriental white oak (*Quercus aliena*) (sapwood and heartwood) Korean pine (*Pinus koraiensis*) (sapwood and heartwood) and Japanese larch (*Larix kaempferi*) (sapwood and heartwood) were cut to dimensions of 20 x 20 x 5 mm^3 (tangential x radial x longitudinal). After sanding to remove loosely adhering fibres, the wood samples were extracted using a mixture of toluene/acetone/methanol (4:1:1 by volume) in a Soxhlet extractor for 12 hours, allowed to air dry in a fume hood overnight, then in an oven set at 103 ^{o}C for 12 hours. Samples were subsequently removed from the oven and placed in a desiccator over silica gel to cool to ambient temperature before weighing on a four figure balance. Wood samples were reacted with either pure acetic anhydride or with hexanoic anhydride in pyridine at 100 ^{o}C for time intervals ranging from 15 minutes to 24 hours to obtain a range of WPGs. After reaction, the samples were quenched in acetone and extracted in Soxhlet extractor, dried and weighed as detailed above. Samples were then leached according to EN84 before they were exposed to *Coniophora puteana* (FPRL 11E) for a 16 week period. Samples were exposed in squat jars with four samples placed on plastic mesh sitting on inoculated agar nutrient in each test jar.

RESULTS AND DISCUSSION

Results from the decay resistance of the hardwoods are shown in Figure 1, and the softwoods in Figure 2, for the wood species as mass loss due to decay against WPG and against degree of OH substitution. In all cases the decay resistance correlates best with the WPG of the wood rather than extent of OH substitution for the two anhydrides in this study. In Figure 1a, beech exhibits the same behaviour as that reported previously for Corsican pine and this contrasts with the behaviour reported in ECWM2 for this species. The threshold for zero mass loss is of the order of 17-20% WPG for acetic and hexanoic anhydride modified beech.

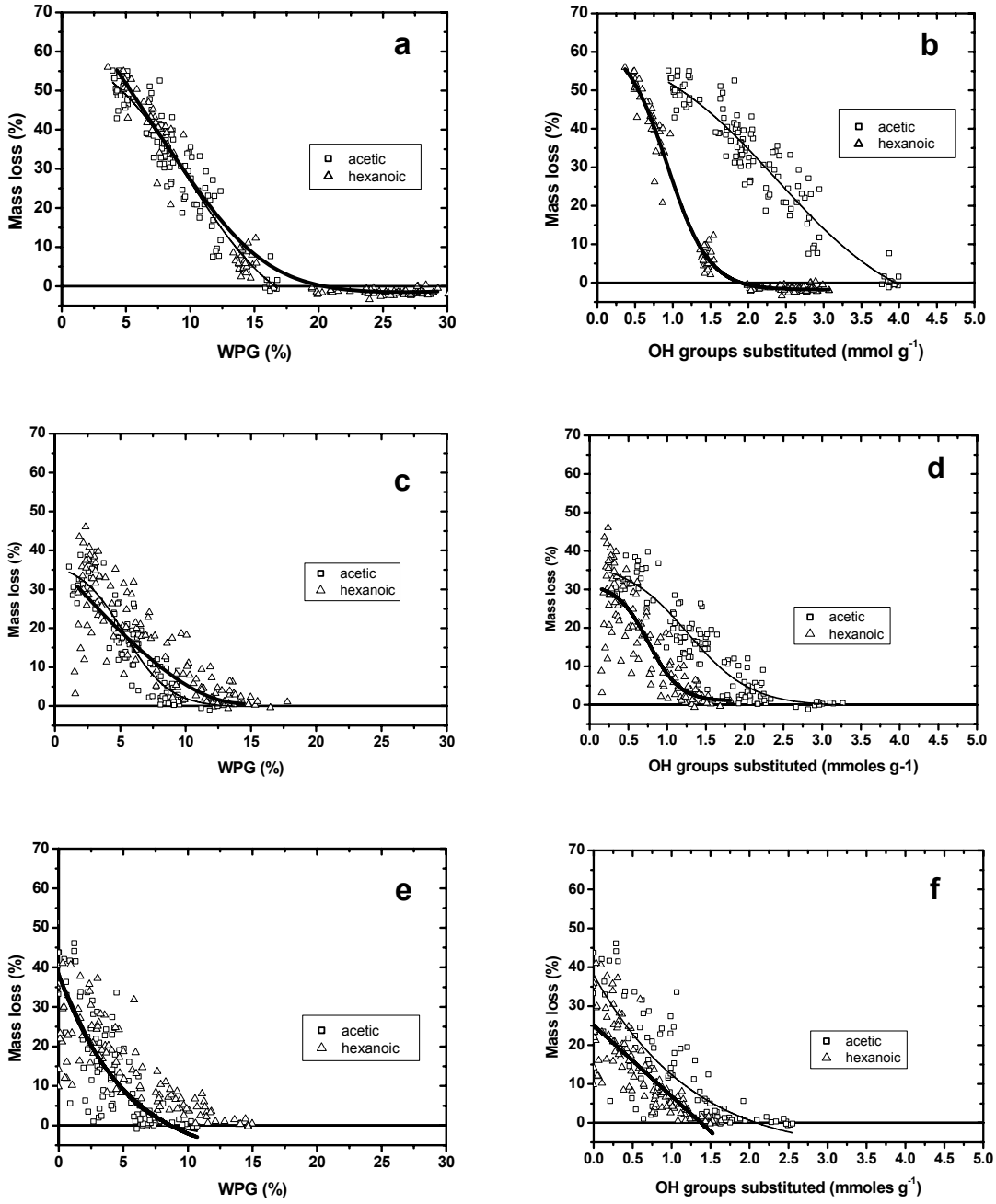

Figure 1: Plots of mass loss due to decay against WPG or OH groups substituted for European beech (a,b), oak sapwood (c,d) and oak heartwood (e,f). The lines are best fit sigmoidal curves to the data points, the thicker line is a fit to the hexanoic data points

The data for modified oak sapwood and heartwood shows considerable scatter and the best fit sigmoidal curves cannot be considered statistically reliable. Nonetheless there is no evidence suggesting that the behaviour is different from that reported previously for Corsican pine. Thresholds for oak are of the order of 14-16% WPG for zero mass loss. The above behaviour contrasts with that previously reported for rubberwood (Hill *et al*. 2005b). It is not known why this difference in behaviour was observed. In addition to the hardwood species for which the data is reported in Figure 1, Japanese larch (heartwood and sapwood) and Korean pine (heartwood and sapwood) were also modified with acetic or hexanoic anhydride and the decay resistance against *C. puteana* investigated (Figure 2).

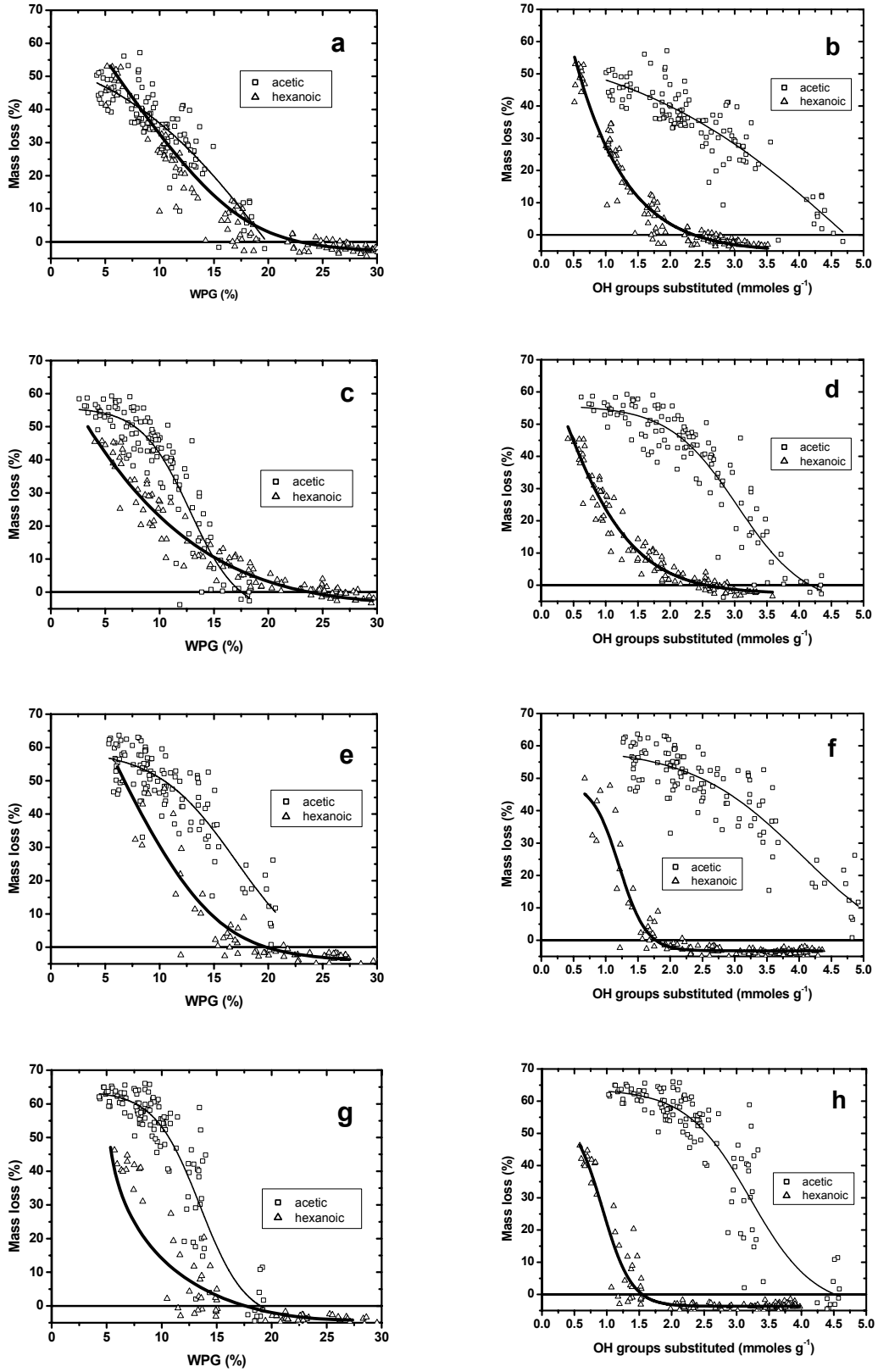

Figure 2: Plots of mass loss due to decay against WPG or OH groups substituted for Japanese larch sapwood (a, b), Japanese larch heartwood (c, d) and Korean pine sapwood (e, f) Korean pine heartwood (g, h). The lines are best fit sigmoidal curves to the data points, the thicker line is a fit to the hexanoic data points

The situation with softwoods appears to be more complex than that exhibited by hardwoods. For Japanese larch sapwood, the mass loss due to decay correlates with WPG irrespective of anhydride (Fig. 2a) rather than number of OH groups substituted; as has been previously noted for Corsican pine sapwood. However, with Japanese larch sapwood the situation is more complex (Fig. 2c). It can be seen that hexanoic anhydride modification provides greater protection up to about 15% WPG, but above this level mass loss due to decay is higher. With Korean pine sapwood or heartwood meanwhile, decay protection due to hexanoylation is superior compared with acetylation at all WPG levels. However, there is no example where the decay protection is correlated with the degree of OH substitution (Figs. 2b, 2d, 2f, 2h). Thus the behaviour reported for rubberwood in this respect appears to be anomalous. Although there is therefore no reason to invoke substrate recognition as the decay protection mechanism, there is nonetheless more complex behaviour shown with the softwoods when compared to that observed with Corsican pine.

Clearly this variation in behaviour requires interpretation, but before a discussion of the results it is necessary to add a note of caution. There has been very little work where different anhydrides have been used to modify wood in order to investigate the decay protection mechanisms and the bulk of such work has largely been confined to the laboratories at Bangor University. Most of the work has also concentrated upon *C. puteana* (as is the case here). To gain a definitive insight clearly requires a lot more results from different laboratories and using different fungi. However, whilst noting the above; thus far, the bulk of the evidence gathered indicates that cell wall bulking is related to the mechanism of protection.

The question arising from the work presented in this paper is why are there small but nonetheless significant differences in decay protection exhibited by acetylated and hexanoylated wood when plotted against WPG for Japanese larch heartwood, and Korean pine (heartwood and sapwood)? The reason for the differences can be attributed to the topochemistry of the reaction of the two anhydrides in the cell wall. Many studies of the kinetics of the reaction of acetic anhydride with wood have indicated that the reaction is diffusion limited (Hill 2003, Hill and Hillier, 1998, 1999, Hill and Papadopoulos 2002). In practice, this means that there is a reaction front that progresses into the cell wall as the reaction proceeds. It follows that at low WPGs there are relatively high levels of acetylation close to the lumen surface, but further into the cell wall the level of substitution falls off (a situation akin to envelope protection but at a cell wall level). With hexanoic anhydride there is different behaviour. During the early stages of reaction (at lower WPGs) the reaction kinetics are rate limited, resulting in a more even distribution of adduct throughout the cell wall. At higher WPGs it would be expected that there would be less difference in acyl distribution in the cell wall with the two reagents. A further complication arises in that the reaction with hexanoic anhydride requires the use of pyridine, which acts to ‘super-swell’ the cell wall as well as acting as a catalyst for the reaction. Reagent distribution will be subject to reaction conditions, such as time and temperature of reaction, the use or not of a catalyst, reagent concentration, whether the wood is pre-impregnated with reagent prior to initiation of the reaction. Substrate factors affecting reagent distribution include wood density, moisture content and cell wall accessibility, with differences expected in reactivity between heartwood and sapwood, earlywood and latewood, hardwoods and softwoods. Other confounding factors include differences in fungal species, differences in fungal strains (Lee *et al*. 2004), differences in laboratory methods, and differences due to

experimenter variability (Van Acker *et al*. 2002). When the foregoing is taken into consideration, it is not surprising that there may be such variations observed, but perhaps more remarkable that there is such close correspondence when the data is plotted in terms of WPG versus decay mass loss.

Further experimentation is clearly required with microscopic examination of the decay process and determination of the cell wall distribution of reagent molecules. Determination of cell wall moisture content using solute exclusion has shown very clearly that the cell wall water holding capacity decreases linearly as WPG increases and that this in turn correlates with the cell wall volume occupied by bonded acyl group as determined by helium pycnometry (Hill 2008, Hill *et al*. 2005a). By combining this data with mass loss data, it is possible to produce plots such as those shown in Fig. 3a for Korean pine and Fig. 3b for European beech.

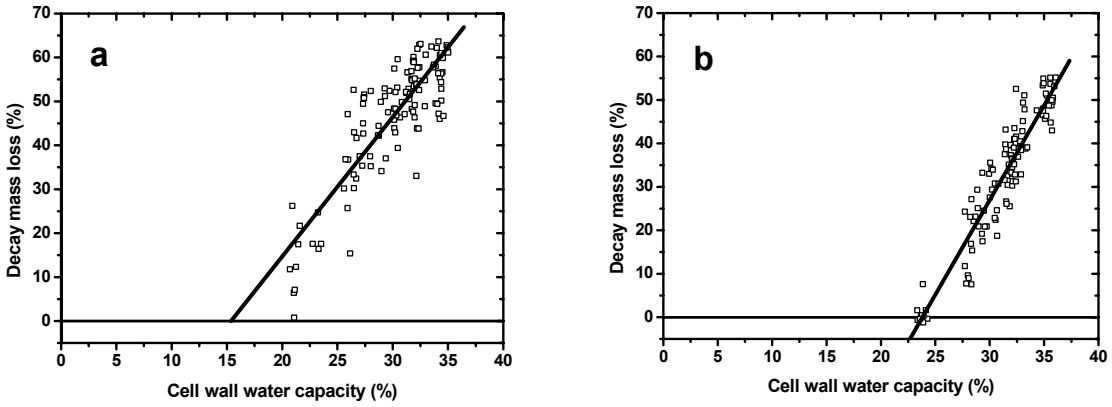

Figure 3: Plot showing cell wall water capacity (calculated) against decay mass loss for Korean pine (a) and European beech (b)

The cell wall water capacity (akin to FSP) was calculated from the molar volume of the acetyl group in Korean pine sapwood (39.22 cm^3 mol^{-1}) and European beech sapwood (41.71 cm^3 mol^{-1}) reported by Kwon *et al*. (2007). The cell wall water capacity was calculated by subtracting the volume occupied by the acetyl group in the cell wall from a nominal FSP of 40%; a value invariably reported from solute exclusion work. As such, the plots obtained rest on a chain of assumptions and should be take as no more than an indication of threshold cell wall moisture contents at zero decay mass loss. Verification using real experimental data for cell wall water content is required. Furthermore, the linear regressions used for this analysis may not be appropriate. Stamm and Baechler (1960) used similar analyses in their work but plotted instead anti-shrink efficiency against decay mass loss for a range of wood modifications. More studies of this nature are required in order to further investigate the decay protection mechanisms.

It is important to be aware that when considering all such studies of the decay resistance of modified wood that the situation is not static and that decay mass loss experiments represent a 'snap shot' in time of a dynamic process. Hill *et al*. (2006) showed this very clearly in an experiment where mass loss due to decay was plotted against time for unmodified wood and wood modified to 8 or 18% WPG. This study found that given sufficient exposure time, even wood acetylated at 18% WPG showed some decay mass loss after 24 weeks exposure. Similar results were found in a test conducted at BRE and reported in ECWM2 (Hill *et al*. 2005b).

There is clearly much to do before we are able to arrive at a definitive explanation for why modification is able to provide decay resistance. All the data reported in this study supports the hypothesis that cell wall bulking is responsible and there is no need to invoke enzyme recognition as a mechanism. The most likely explanation is that the cell wall has insufficient water to support decay (Stamm and Baechler 1960). Further support for the lack of an enzyme recognition mechanism is the observation that formaldehyde modified wood is decay resistant at low levels of WPG (Stamm and Baechler 1960), which is entirely consistent with a cross-linked cell wall being unable to expand to accept water, but hard to reconcile with an enzyme recognition blocking mechanism, due to the high levels of unsubstituted OH groups in such formaldehyde modified wood.

Finally, there is clearly a need for conducting experiments where modified wood is exposed to enzymes and indeed a number of studies of this nature have been performed already. Experiments of this type usually expose wood flour to enzymes and the assumption is that by doing so, a much large surface area is available. In numerous nitrogen sorption experiments it has been shown that the BET surface are of dry wood flour is no greater than 5 $m^2 g^{-1}$, which is hardly much greater than that of solid wood (1 $m^2 g^{-1}$).Thus although the surface area is slightly greater, this has no significance at the molecular length scales relevant to enzymatic mechanistic pathways.

CONCLUSIONS

The modification of wood with different anhydride reagents is a very useful tool for determining whether property changes arise due to cell wall bulking or due to substitution of hydroxyl groups. In all of the studies conducted on wood modified with different anhydride reagents thus far, ALL property changes have been found to be determined by cell wall bulking (WPG), with two exceptions: (i) surface contact angle of water (ii) decay mass loss of rubberwood. No evidence has been found in this latest study to suggest that the bulking mechanism originally proposed for the decay protection of acetylated Corsican pine should be modified. The rubberwood data remains an anomaly.

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