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Decay resistance of acetylated and hexanoylated hardwood and softwood species exposed to *Coniophora puteana*

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Abstract

The effect of chemical modification with acetic or hexanoic anhydride upon the decay resistance of wood was studied. Both sapwoods and heartwoods of the following trees were investigated: Japanese larch, *Larix kaempferi* (Lamb.) Carrière; Korean pine, *Pinus koraiensis* Siebold *et* Zucc. as softwoods and European beech (only sapwood), *Fagus sylvatica* L.; oriental white oak, *Quercus aliena* (Blume) as hardwoods. After chemical modification, the samples were exposed to the brown rot fungus *Coniophora puteana* (FPRL 11E). The study investigated whether weight percentage gain or degree of hydroxyl substitution were the main factors controlling decay resistance. It was found that decay resistance is associated primarily with cell wall bulking rather than hydroxyl substitution. However, there are differences in behaviour between the acetylated and hexanoylated wood and the possible reasons for these differences are discussed.

Keywords: acetylation; brown rot; *Coniophora puteana*; decay resistance; hexanoylation.

Introduction

The decay properties of acetylated wood have been investigated for over 60 years (Hill 2006), but the scientific literature was not able to provide any conclusive evidence regarding 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 (Rowell 2005; Hill 2006).

The first study showing that cell wall bulking was related to the decay protection mechanism was that of Papadopoulos and Hill (2002). In their study, Corsican pine (*Pinus nigra*) was modified with a variety of linear chain anhydride reagents and it was shown that the decay resistance imparted upon exposure to the brown rot fungus *Coniophora puteana* was a function of weight percentage gain (WPG) rather than extent of hydroxyl substitution. Building upon this work, Hill et al. (2005a) were able to demonstrate that the mechanism could be correlated with 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 mechanism for imparting decay resistance. More recently, Hill et al. (2006) presented the results from a study of the effect of chemical modification upon the decay resistance of Corsican pine when exposed to *C. puteana* where the decay resistance was confirmed to be related to WPG. Thus far, all such studies of this nature reported in the peer-review literature have been confined to Corsican pine, but it is not yet known whether the behaviour is generic; although there has been some indication that other species may exhibit different properties when modified with anhydride reagents (Hill et al. 2005b).

Since chemically bonded acyl groups occupy a certain 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 of the modified wood cell wall is reduced. The theoretical fibre saturation point (FSP) at different WPGs can be calculated from the volume of bonded acyl group as determined from helium pycnometry, and it was shown that these data correlate very well with FSP values directly determined from solute exclusion (Hill et al. 2005a). These data have also been combined with mass loss data from decay experiments and it was demonstrated that zero mass loss occurs at a FSP value of 20% (Hill et al. 2005b). From this work, it was concluded that the protection mechanism is indeed a function of the cell wall moisture content.

In this context, two alternative explanations that are consistent with a bulking mechanism require consideration. One is that the cell wall micropores are blocked by the presence of bonded acyl groups and that, as a consequence, diffusible agents with low molecular weight are physically prevented from entering the cell wall (Forster 1998). Solute exclusion experiments of anhydride modified wood indicate that accessibility to the cell wall is not altered by acetylation, since the maximum cell wall micropore diameter does not change as WPG increases (Hill et al. 2005a). The other mechanism which may be suggested involves a steric ‘masking’ of OH groups by acyl groups. Whereas an acetyl group effectively masks one OH group by virtue of chemical substitution, the hexanoyl group may be able to mask several proximal OH groups because of its greater physical size. The evidence presented thus far does not support a masking mechanism.

Apart from the above-mentioned studies on Corsican pine one further report has appeared (Hill et al. 2005b) indicating the complexity of these questions. In particular, it was reported that with rubberwood (*Hevea brasiliensis*) decay protection was a function of OH substitution rather than WPG. In the present paper, a wider range of hardwoods and softwoods (both heartwood and sapwood) have been studied.

Materials and methods

The experimental procedure of acylation has been detailed a number of times (Hill et al. 1998; Hill and Hillier 1999; Papadopoulos and Hill 2002; Hill et al. 2005a). All solvents and reagents (Aldrich) were ACS grade and not purified prior to use, although pyridine was dried by storing over KOH. Sapwood (SW) and heartwood (HW) of the following trees were investigated:

- Japanese larch, *Larix kaempferi* (Lamb.) Carrière, (SW + HW)
- Korean pine, *Pinus koraiensis* Siebold *et* Zucc., (SW + HW)
- European beech, *Fagus sylvatica* L., (SW)
- Oriental white oak, *Quercus aliena* Blume, (SW + HW)

Samples were cut to dimensions of $20 \times 20 \times 5$ mm^3 (tang./rad./long.). After sanding to remove adhering fibres, the wood samples were extracted with a mixture of toluene/acetone/methanol (4:1:1 by vol.) in a Soxhlet extractor for 12 h, allowed to air dry in a fume hood overnight, then in an oven set at 103°C for 12 h. Prior to weighing on a four figure balance the samples were allowed to cool to ambient temperature in a desiccator over silica gel. Acylation was performed with undiluted acetic anhydride or with hexanoic anhydride in pyridine at 100°C for time intervals ranging from 15 min to 24 h to obtain a range of WPGs. After reaction, the samples were quenched in acetone and extracted in a Soxhlet extractor, dried and weighed as detailed above. Samples were then leached according to EN84 (CEN 1997a) and then oven dried to constant weight at 103°C according to EN113 protocols (CEN 1997b) 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 malt agar nutrient in each test jar.

Hydroxyl content substituted by the acyl groups was calculated using the following formula [Eq. (1)]:

$$[OH]\ (\text{mmol g}^{-1}) = (WPG/100)/(MW\text{-}1) \times 1000 \quad (1)$$

where MW is the molecular weight of the acyl group (43 for acetyl and 99 for hexanoyl). Note that one atomic mass unit is subtracted from these values to account for the loss of the hydrogen atom from the OH group.

Percentage mass loss (ML) due to decay by the fungus was calculated according to Eq. (2):

$$ML\ (\%) = [(M_1\text{-}M_2)/M_1] \times 100 \quad (2)$$

where M_1 is the oven dry mass of the wood sample prior to decay (after leaching according to EN84) exposure and M_2 is the oven dry mass of the same sample after exposure. The values of the oven dry mass (M_1 and M_2) were recorded as mass of sample including the mass of bonded reagent.

The data were plotted using Origin 7.5 and the sigmoidal fits to the data were performed using the non-linear least squares fitting routine in the software using a sigmoidal fitting function. The 95% confidence limits reported by the software refer to the statistical probability of the fit being within the bounds plotted.

Results and discussion

How acetylation imparts decay resistance to wood is a complex phenomenon. Furthermore, there are several experimental factors influencing the results and their interpretation. Mass loss (ML) is usually determined as a function of WPG and Figure 1a shows an idealised and simplified relation for exposure of a brown rot fungus, where the dotted line at 75% represents the point at which the wood sample is composed entirely of lignin (in reality, a 'dose response' sigmoidal curve will be observed). A much wider range of behaviours are illustrated in Figure 1b. Here, a series of lines are drawn labelled (i) through to (v) representing increasingly extended exposure periods in the fungal decay experiment. Thus, as the period of exposure increases, the 'protection threshold' shifts to higher WPG values (Hill et al. 2006). Although 75% ML has been chosen arbitrarily as the value for a sample with 25% lignin content, it should be noted that if the lignin is acetylated, then ML will be somewhat lower if the fungus cannot remove the covalently bonded acetyl groups from the lignin. The relationship between WPG and ML (Figure 1b) could equally well refer to species exhibiting different natural durabilities, where natural durability would decrease from (i) through to (v). This figure might also refer to the virulence of the test fungus increasing from (i) through to (v). These circumstances complicate interlaboratory comparisons of decay experiments (Van Acker 2003). In this context,

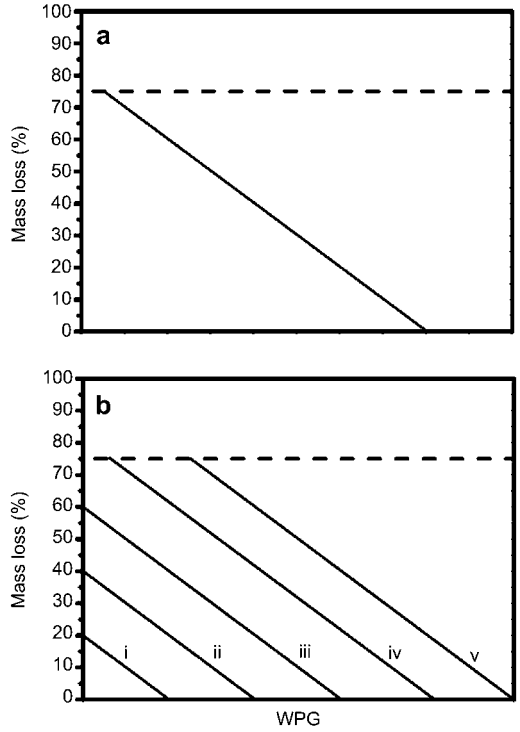

Figure 1 (a) Diagram showing the expected relationship between mass loss due to decay and weight percentage gain (WPG), where the dotted line represents the residual mass of lignin after the brown rot decay process has gone to completion. (b) Diagram showing how the apparent threshold value determined in the decay experiment can vary as a function of time of exposure, with (i) being the shortest exposure time and (v) the longest exposure time.

it should also be mentioned that the threshold referred to throughout this text is the WPG at a decay ML of 0%. This contrasts with the more commonly used criterion of a ML of less than 3% employed in the EN113 protocol (Eaton and Hale 1993). This is not to suggest that a 0% ML is a preferred criterion to the 3% ML as used in EN113. Indeed, it has been shown that thresholds in such tests for acetylated wood can be rather variable (Van Acker 2003; Hill et al. 2006) and should not be employed as the sole determinant for evaluating the in-service performance of such modified wood.

The results for different wood species modified with acetic or hexanoic anhydride to different WPGs exposed to *C. puteana* for 16 weeks are illustrated in Figures 2 (HWs) and 3 (SWs).

The result for beech sapwood (SW) is shown in Figure 2a for ML due to the brown rot decay plotted as a function of WPG or against the number of OH groups substituted by the acyl moieties (acetyl or hexanoyl) (Figure 2b). The behaviour exhibited by this species is entirely consistent with that reported previously for Corsican pine (Papadopoulos and Hill 2002; Hill et al. 2006). On the other hand, the behaviour of beech is different in this case compared to that presented in a previous study by Hill et al. (2005b). The decay protection threshold for this particular experiment is approximately 17–20% WPG for both acetylated and hexanoylated beech, corresponding to different levels of OH substitution. It is clear that at higher WPG levels with hexanoic anhydride modified wood particularly, there is a negative ML reported

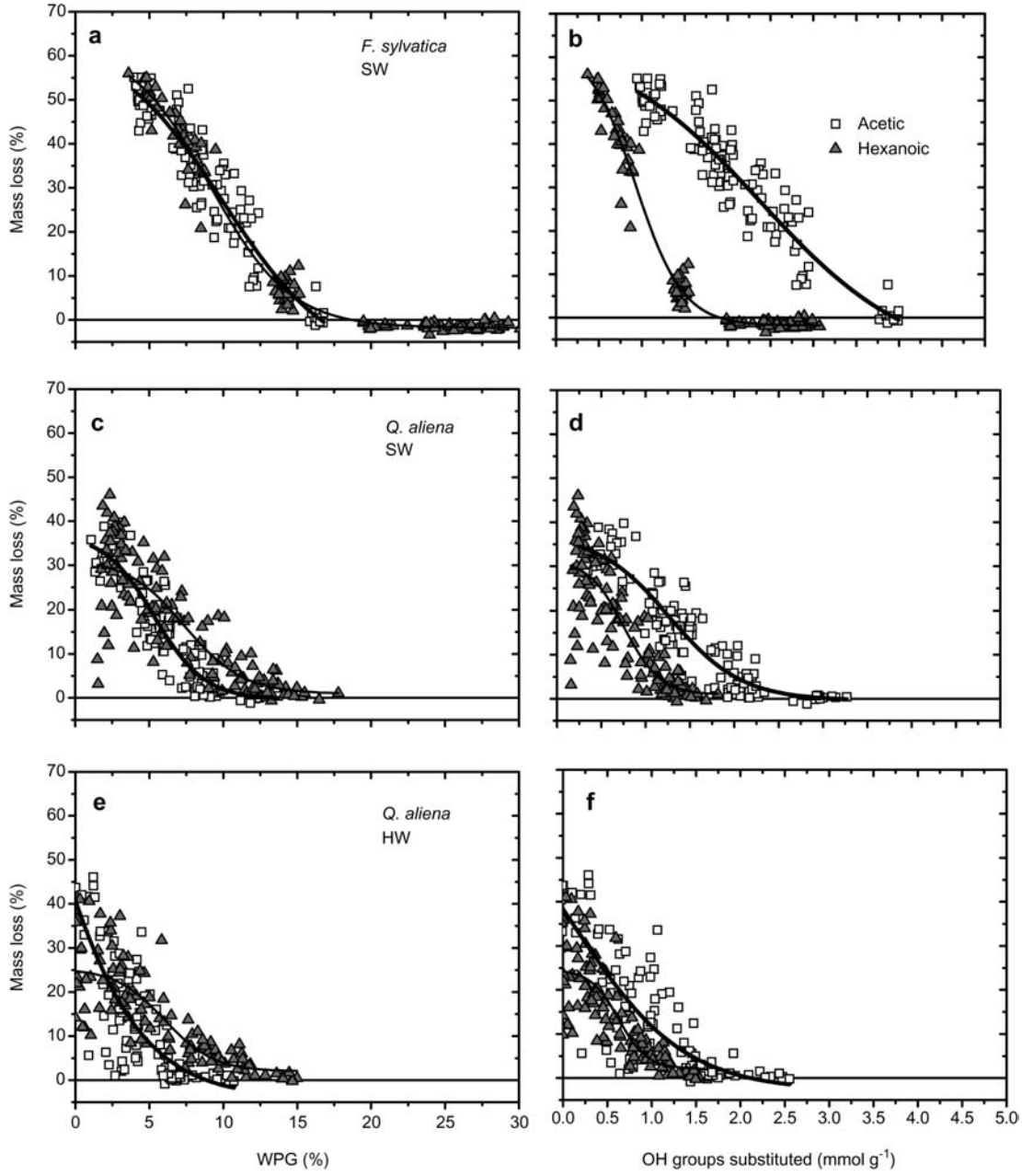

Figure 2 Results for the exposure of acylated hardwoods to *Coniophora puteana* plotted as weight percentage gain (WPG) or extent of hydroxyl substitution (in mmol g^{-1} of dry cell wall substance) against percentage mass loss due to decay. Open squares are for acetylated wood samples and filled triangles are for hexanoylated wood samples and lines are best fit sigmoidal curves. The species shown are European beech (a, b), oriental oak sapwood (c, d) and oriental oak heartwood (e, f).

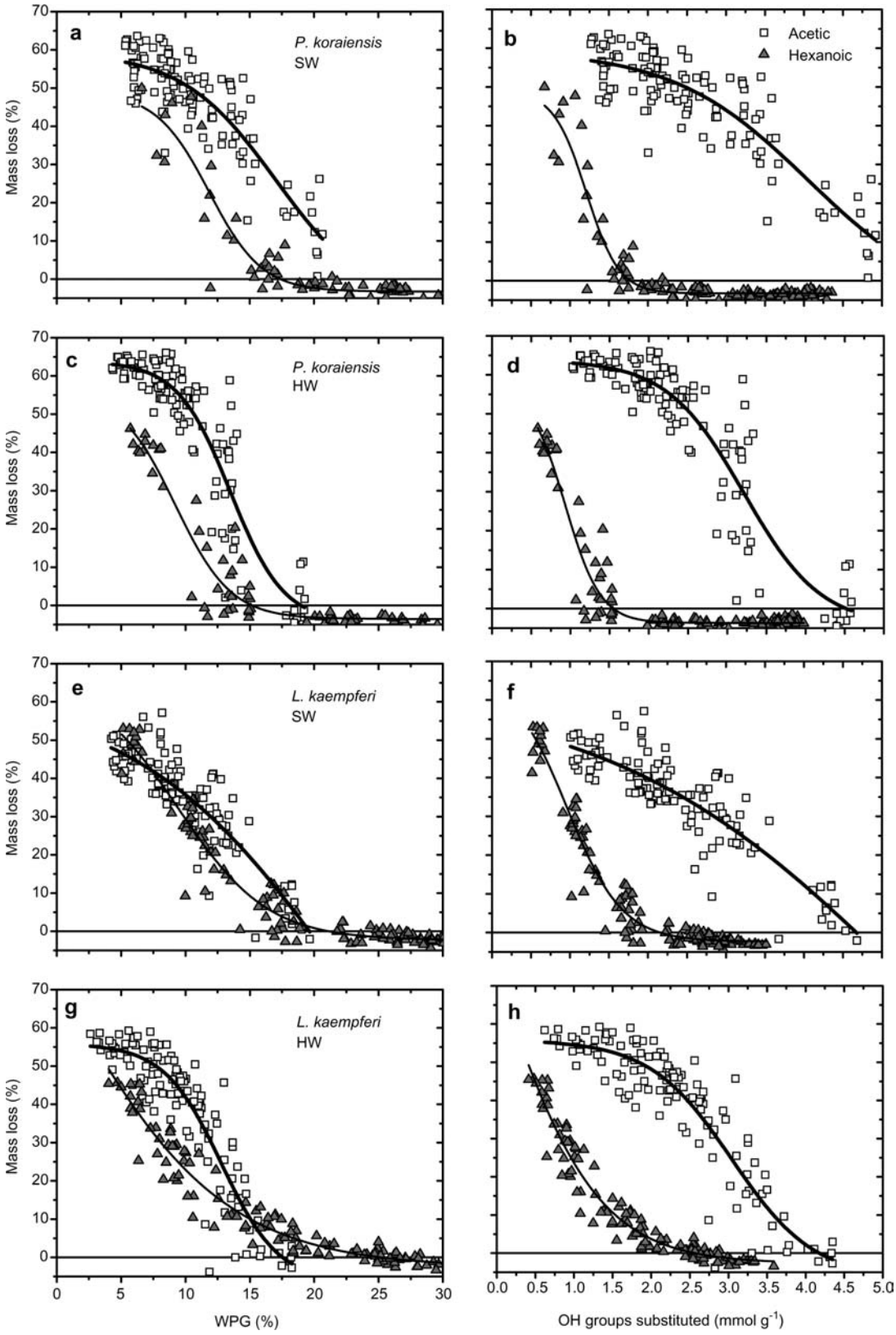

Figure 3 Results for the exposure of acylated hardwoods to *Coniophora puteana* plotted as weight percentage gain (WPG) or extent of hydroxyl substitution (in mmol g^{-1} of dry cell wall substance) against percentage mass loss due to decay. Open squares are for acetylated wood samples and filled triangles are for hexanoylated wood samples and lines are best fit sigmoidal curves. The species shown are Korean pine sapwood (a, b), Korean pine heartwood (c, d), Japanese larch sapwood (e, f) and Japanese larch heartwood (g, h).

which is explained by the presence of hyphal and other extraneous fungal debris in the macroscopic void space of the wood samples. The high level of correlation seen by visual inspection of the sigmoidal fits of ML against WPG for both the acetylated and the hexanoylated beech is further confirmed by reference to Figure 4a. In this plot, the confidence limits for both of the fits overlap, indicating the two curves can be considered as coincident.

A similar result is found for oriental white oak SW (Figure 2c, d) and HW (Figure 2e, f), although there is considerable scatter in the experimental data making it more difficult to discern differences between the acetylated and hexanoylated samples. Nonetheless, there are significantly different fits for the two sigmoidal best fit curves through the data points (Figure 4b oak SW, Figure 4c oak HW).

The decay data for the SWs are presented in Figure 3. Acetylated Korean pine SW (Figure 3a, open squares) shows a decrease in ML as WPG increases, although there is no clear threshold for 0 ML. With acetylated Korean pine HW, the decay protection threshold is around 20% WPG (Figure 3c). In both cases, hexanoy-

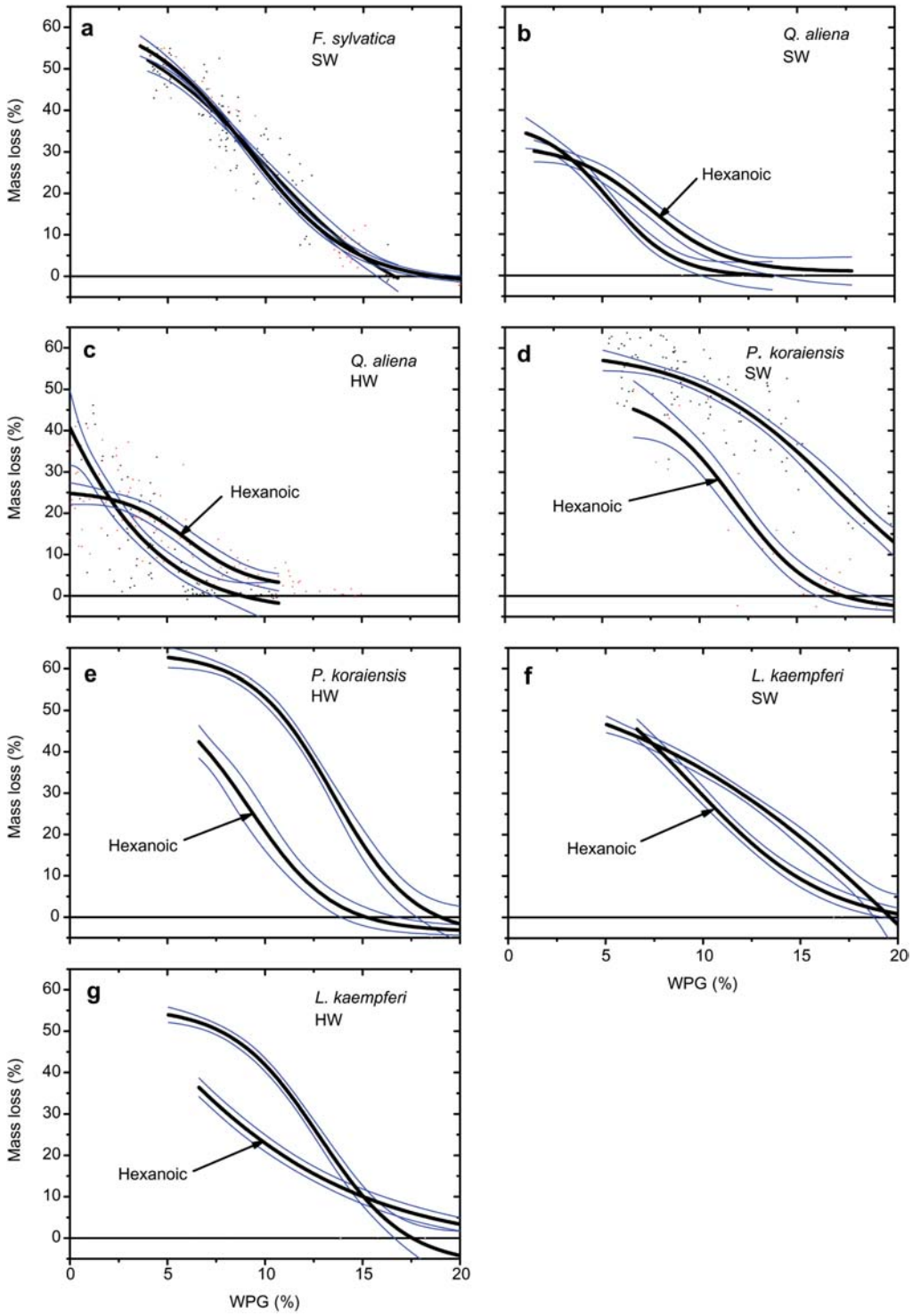

Figure 4 Sigmoidal curve fits to the data showing the 95% confidence limits (thinner lines) for the different woods in this study.

lation provides better protection against decay over the entire range of WPGs. With Japanese larch SW (Figure 3e), thresholds are around 20% WPG for both acetylated and hexanoylated samples, whereas in the case of HW acetylated samples exhibited threshold of approximately 20% WPG, but this is of the order of 25% WPG for the hexanoylated wood (Figure 3g).

All of the species in this study showed differences in decay behaviour between the acetylated and hexanoylated wood samples exposed to *C. puteana* when the ML results were plotted against degree of OH substitution (with the possible exception of oriental white oak). However, the relationship between ML and WPG was not as simple as that reported for Corsican pine. Only with European beech was the relationship between ML and WPG identical for wood modified with acetic or hexanoic annydride, with the other species there were significant differences between the behaviour of the acylated wood samples. With the softwoods, hexanoylated samples showed superior decay resistance over most of the WPG range studied, the opposite behaviour was found with oak, with acetylated samples showing marginally better protection against decay. It is not known why there are differences in behaviour when the ML data are plotted against WPG, but this could relate to variations in the distribution of reagent at the cell wall level, or may reflect changes in the decay protection mechanism.

The reason why there may be differences in the cell wall distribution of the bonded reagent arises from previous kinetic studies of the reaction of different anhydrides with wood. The reaction kinetics of acetic and hexanoic anhydride with wood has been shown to comply with different reaction kinetics (Hill and Hillier 1998, 1999; Hill et al. 1998; Hill and Papadopoulos 2002; Hill 2003). Reaction with acetic anhydride is diffusion limited, meaning that the distribution of acetyl groups within the cell wall will be characterised by a diffusion front moving into the cell wall. With hexanoic anhydride the reaction is rate limited, which would be expected to result in a more even distribution of hexanoyl groups in the cell wall at lower WPGs. Thus, assuming this to be the case, the topochemistry of the distribution of groups within the cell wall will be different, especially at WPGs below 20%, although this has not been proven experimentally. Furthermore, with hexanoylation, the use of pyridine as a cell wall swelling agent/catalyst will also affect the substrate. Indeed, with hexanoic anhydride WPGs in excess of 20% are readily achieved, which will undoubtedly involve some element of cell wall damage occurring. The reaction kinetic processes are further influenced by factors, such as temperature, reagent concentration, wood density and cell wall composition.

The other possibility is that the decay protection is not simply related to the cell wall moisture content, but there may be other phenomena involved in this process. It is very well known that the enzymes involved in wood decay processes are not able to penetrate the lignified cell wall and various low molecular weight diffusible agents have been posited as initiating the decay process by, for example, breaking open the lignin network (Hill et al. 2005a). Due to the complexity of this process, it is not possible to postulate with any degree of confidence how acetylation could prevent these degradative agents from functioning. The ability of these reagents to diffuse will certainly be compromised if the moisture content is too low, or if micropore blocking occurs. Whether there is a substrate recognition effect or steric hindrance effect in addition is open to debate. What is, however, clear from the present study is that whilst the level of OH substitution does not appear to influence the decay resistance of the acylated wood, it does not appear to be simply related to the WPG of the materials either. Further work is certainly required to understand this phenomenon.

Conclusions

This study has shown that the decay resistance due to modification with hexanoic or acetic anhydride is not a function of degree of OH substitution (in contrast to a previous study on rubberwood). However, there are variations in behaviour between different species when decay ML is plotted against WPG. The reasons for these differences are not known at this time, although two possible explanations can be posited:

(a) Differences in distribution of the bonded acyl groups at a cell wall level.
(b) There may be more than one mechanism operative, in which case a range of behaviour will be observed depending upon the relative importance of substrate recognition compared to cell wall bulking.

Further work is required in order to increase the scientific understanding of how acetylation is able to provide decay resistance against microbiological attack. The use of anhydride reagents of different molecular weight in order to determine whether cell wall bulking or OH substitution are the main determinants of decay resistance remains a relatively little studied area.

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