DOCTOR OF PHILOSOPHY

The effect of treatment with succinic anhydride and its derivatives on the decay resistance of wood

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The Effect of Treatment with Succinic Anhydride and Its Derivatives on the Decay Resistance of Wood

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The Effect of Treatment with Succinic Anhydride and Its Derivatives on the Decay Resistance of Wood

Summary

Succinic anhydride (SA), a 4-carbon cyclic unsaturated anhydride and its derivatives ASAC12, SA wiith a 12-carbon aliphatic chain containing a carbon-carbon double bond, ASAC17, a similar compound with a 17-carbon chain and ASAC20, a similar compound with a 20-carbon chain were used to chemically modify Scots pine sapwood. The reactions were carried out in solution with N-N, dimethylformamide which acted as catalyst and swelling agent. The ease and rates of reaction were compared and the compounds are ranked as SA > ASAC12 > ASAC17 > ASAC20. Above 30% loading for all reagents, the wood cell wall began to degrade. Proof of SA and ASAC17 reaction was confirmed using Infrared Spectroscopy, and comparison of increase in wood volume with treatment and the calculated volume of added chemical.

Succinic anhydride and ASAC17 modified samples were subject to a 12 week soil block test to test the efficacy of the modifications against attack by the Basidiomycete fungi *Coniophora puteana* and *Gloeophyllum trabeum.* Results indicate that there is a good correlation between increased reagent loading and an increase in effectiveness, paralleled by a marked reduction in wood moisture content. ASAC17 was the more successful treatment in all cases.

Copper was fixed to ASAC17 modified wood in aqueous solution. Copper content was determined by gain in weight and Atomic Absorption Spectroscopy. The treated blocks were subject to a 12 week soil test. Results showed that the modified wood was completely resistant to decay by *C. puteana* at levels of combined reagent loadings that would individually be ineffective, ASAC17 loadings as low as 0.4% in combination with 0.4% copper proved effective.

Leaching the blocks in water after modification gave a variance of weight losses in blocks that were subsequently attacked by *C. puteana*

Table of Contents

Chapter 1

Introduction

Wood has been used by man for thousand of years. Its has many advantages, both in structural load-bearing situations, having a very high weight to strength ratio, and where aesthetic appearance is of importance. Wood is cost-effective. It is a recyclable material and once the initial costs of planting a tree, and the end costs of harvesting are absorbed, the energy and nutritional inputs required to make it grow come from the sun, rain, and the soil. Wood is a stable material, composed of the most abundant polymers of the biosphere: cellulose, hemicelluloses and lignin. The way in which these polymers are structured give the living tree the ability to withstand the stresses that the environment places upon it. Wood is an anisotropic material, which means that it swells and shrinks to a different extent in the three anatomical directions: longitudinal (vertical direction), tangential (parallel to the annual growth rings) and radial (perpendicular to the annual growth rings. The longitudinal cell system gives the tree very high tensile strength and flexibility along its length.

The moisture and nutrient cell transport system which is ideally suited to the living tree can be a disadvantage when the wood is transformed to an end-product for our use. Wood has a pore structure and cell cavities that can provide agents of degrade such as fungi with an entrance into the wood and spaces in which to initiate the decay process. When many species of wood are machined to produce building products such as window frames they are vulnerable to attack when in an untreated state. When wood products are attacked in storage or in service the economic losses can be enormous. Wood degradation in service can also be extremely dangerous.

Despite its high stability, wood is susceptible to biological decay. There are many factors that can bring about this decay. Decay fungi can be divided into three types: brown rot, white rot and soft rot. Wood can also be decomposed by insects, crustaceans and bacteria. It is flammable and it can degenerate during exposure to ultra-violet light and both acid

and base in aqueous media. Wood can undergo significant dimensional change with varying moisture content. Wood in service is assigned to a Biological Hazard Class according to its' siting when in use (EN 335 - 1. Part 1. 1992), (Appendix 1.1).

Most of the negative changes in wood structure are brought about by chemical reaction between degenerative environmental agents and the reactive elements in the wood substrate. Some species of softwood are used extensively in the construction industry and the immediate need for timber in bulk has led to the introduction of quickly grown plantation crops with a high proportion of sapwood. Generally speaking the sapwood from softwoods contains marginally more lignin and cellulose and less extractive material than heartwood. In a narrow sense the term extractives is used in wood analysis to cover a large number of different compounds which can be extracted form the wood by means of polar and non-polar solvents. Several woods contain extractable substances which are toxic or a deterrent to fungi, bacteria and termites. Thus a fast grown timber with a high sapwood content has less natural protection and is more susceptible to attack by fungi and other decay agents.

There is a need to protect products manufactured from such timber and a preservation industry has been established with the aim of lengthening product life. Conventional preservation treatments consist of introducing toxic chemicals into the wood to kill the agents of decay. The least effective way of introducing a preservative involves short-term dipping or surface treating by brushing or spraying. A more efficient method is to impregnate under increased pressure which results in higher loadings and deeper penetration. This work is summarised in many texts. For example, Dinwoodie (1981) discusses three preservative types: tar oil types, of which coal-tar creosote is the most important because of its natural toxicity and water repellency; water-borne types, the most effective are traditional formulations containing copper, chromium and arsenic salts; organic solvent types based on metallic soaps or other organic biocides, these are applied by vacuum impregnation and/or immersion treatments. Small quantities of timber can be

treated by a diffusion process. The timber must be in the green state and the preservative must be water soluble. The preservative, commonly disodium octaborate (Timbor) is applied to the surface in the form of a concentrate and over a period of weeks it diffuses into the green timber.

Many of these substances have an appreciable level of mammalian toxicity, many are poisons and are easily leached out into the environment. Some compounds, widely used in the past are barred from current use.

This work investigates an alternative to introducing a poison into the wood. It looks at a novel method of protecting wood by introducing a non-toxic chemical compound into the wood which could modify the substrate by reacting with it at a molecular level. This system would work by making the wood substrate unrecognisable as a food source by fungi.

Chapter 2

Literature Review

2.0 Wood Cell Wall Polymer Composition

2.0.1 Carbohydrate Content of Softwood

As far as the chemical components of wood are concerned there is a distinction between the main macromolecular cell wall components cellulose, hemicelluloses and lignin, which are present in all woods and the minor low-molecular-weight components such as extractives and mineral substances, which are generally more related to species in type and amount.

Figure 2.1 is a diagrammatic representation of the general scheme of chemical wood components. This work is mainly concerned with those carbohydrate and lignin substances defined as macromolecular.

WOOD

Low-molecular-weight-substances	Macromolecular substances
Organic matter, Inorganic matter	Polysaccharides, Lignin
Extractives, Ash	Cellulose, Hemicelluloses

Figure 2.1 General scheme of chemical wood components

The wood cell wall consists mainly of cellulose, hemicelluloses and lignin. A simplified picture is that cellulose forms a skeleton which is surrounded by other substances functioning as a matrix (hemicelluloses) and encrusting (lignin) materials. The cellulose fibres provide tensile strength and the hemicelluloses and lignin provide cross-linking, binding the structure together (Reid, 1995).

Most of the wood carbohydrates and lignins are to be found in the secondary wall of the wood cell, particularly in the S_2 layer. Figures 2.2 and 2.3 represent a simplified structure of the wood cell wall and a transverse section of softwood tracheids, both with the main cell wall features indicated.

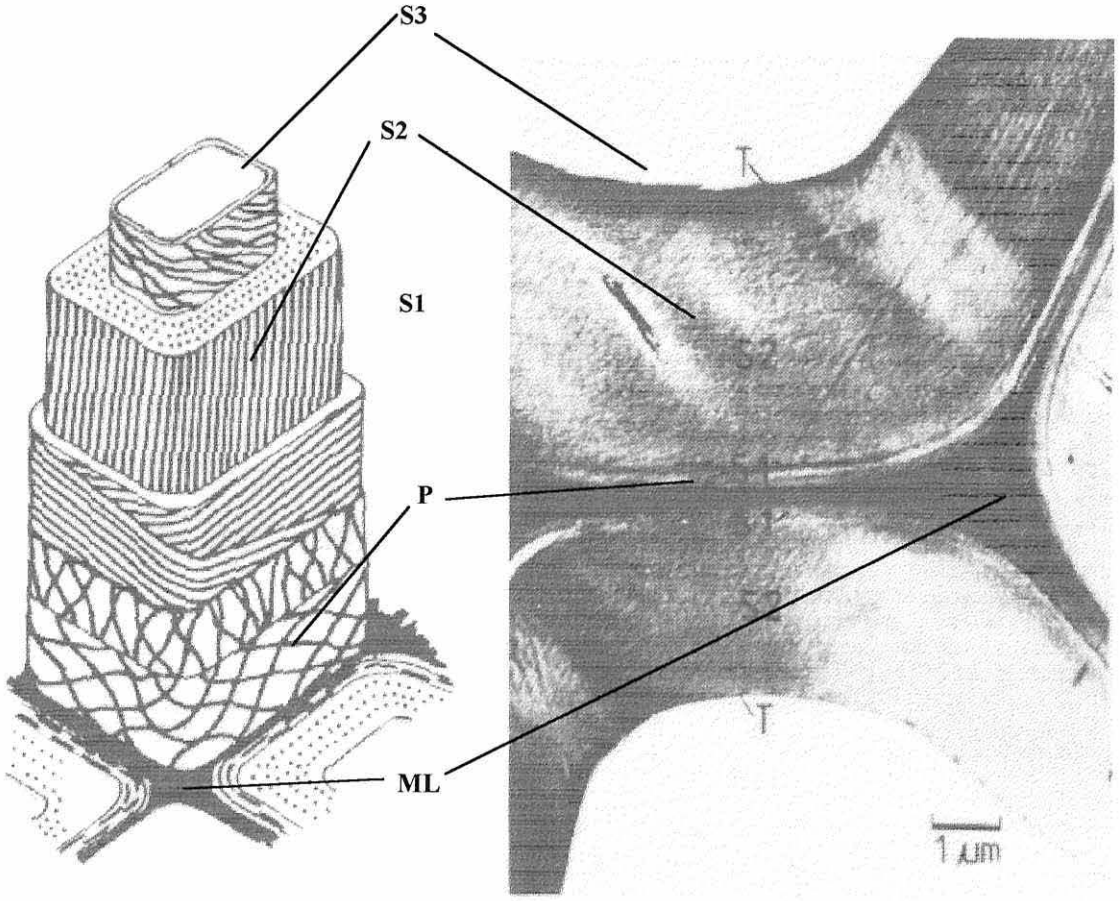


Figure 2.2 Figure 2.3

Figure 2.2 Simplified structure of the wood cell wall showing the cell wall layers: ML, Middle Lamella; P, Primary Wall; S1, The outer layer of the secondary wall; S2, The middle and thickest of the cell wall layers; S3, A very thin layer that may not always be present. The orientation of cellulose fibres are indicated.

Figure 2.3 Transverse Section of the cell wall of softwood tracheids (*Picea abies*). T represents Tertiary Wall, the alternate name for the S3 layer See Figure 2.2

The polysaccharides are organised within the cell wall as described below, (Dinwoodie, 1981).

ML, The middle lamella. This is a thin layer between the individual cells. This layer is a lignin-hemicellulose complex, in principle free of cellulose. The transition from the middle lamella to the adjacent cell wall layers is not very clear, so that for the middle lamella and both adjacent primary walls the term **compound middle lamella** is used.

P, The primary wall. The cellulose microfibrils are loosely packed. Little lamellation is present. The amount of cellulose in the primary wall is very limited.

S_1, The outer layer of the secondary wall. Characterised by having about 4 to 6 lamellae, the microfibrils spiral alternately to left and right.

S_2, The middle and thickest of the cell wall layers. The microfibrils exhibit a similar orientation in a steep right hand spiral. Changes in the angle and differences in the packing of the fibres results in a strong lamellar structure.

S_3, Also known as the **Tertiary layer**. This may not be present in certain timbers. It is very thin with only a few lamellae. The fibres have a gentle slope and are not in a strict parallel order. This layer has a higher concentration of non-structural substances.

Table 2.1 Chemical composition of timber (Dinwoodie, 1981).

	Weight (%)	Polymeric State	Function
Cellulose	40 - 50	Crystalline	Fibre
Hemicelluloses	20 - 30	Semi-crystalline	Matrix
Lignin	25 - 30	Amorphous 3-D molecule	Matrix
Extractives	0 - 10	Some polymeric others not polymeric	Extraneous

These are approximate values. Proportions are for timber in general and slight variations can occur between timbers of different species.

Meier (1964) defines the percentage of polysaccharides in the cell wall of Scots pine (*Pinus sylvestris.* L) as:

Table 2.2 The average percentage of the polysaccharides in the cell wall of *Pinus sylvestris* (Meier, 1964).

	ml + p	S1	S2 Outer	S2 Inner + S3
Galactan	20.1	5.2	1.6	3.2
Cellulose	35.5	61.5	66.5	47.5
Glucomannan	7.7	16.9	24.6	27.2
Arabinan	29.4	0.6	0	2.4
Glucuronoarabinoxylan	7.3	15.7	7.4	19.4

2.0.1.1 Cellulose

Cellulose is the structural basis of all plant cells and is responsible for most of the structural characteristics of wood. In wood, cellulose is accompanied by and intimately associated with hemicelluloses and lignin. It constitutes about 40 - 50% of the dry substance in most wood species and is located predominantly in the secondary cell wall (Figures 2.2 and 2.3).

Cellulose is a homopolysaccharide composed of β-D-glucopyranose units which are linked together by β-1-0-4- glycosidic bonds (Figure 2.4). The repeating unit of the cellulose chain is a cellobiose unit (Figure 2.4). Buswell and Odier (1987) estimated the degree of polymerisation of wood cellulose to be about 8,000 to 10,000 and possibly as high as 15,000. A degree of polymerisation of 10,000 is equivalent to a chain length of about 5 μm.

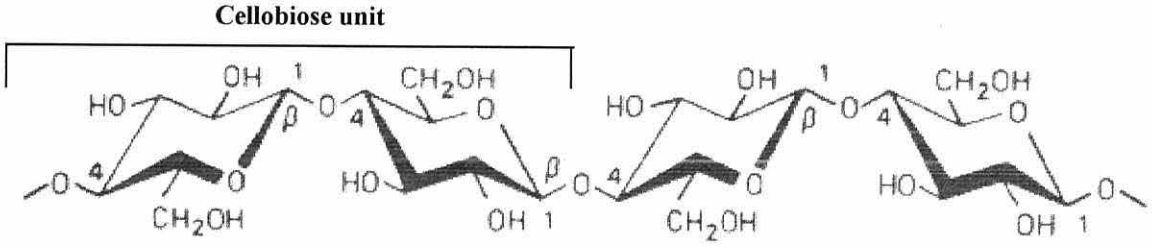


Figure 2.4 Structure of cellulose. The β-D-glucopyranose chain units are in chair formation ($^4C_1$) and the substituents HO-2, HO-3 and CH_2OH are oriented equatorially.

Cellulose molecules are linear and of indeterminate length. They have a strong tendency to form intra- and intermolecular bonds. In a sense, the surface of cellulose chains are studded with hydroxyl (-OH) groups. These groups are not only responsible for the supramolecular structure but also for the chemical and physical behaviour of the cellulose. The -OH groups are able to interact with each other forming a particular linkage, the hydrogen bond (H-bond). The H-bond takes place by the approach of an -OH group to the lone electron pair of another oxygen atom. In the linked state the hydrogen atom is co-ordinately divalent. Hydroxyl groups of cellulose molecules are able to form two types of H-bond depending on their site in the glucose units. Intramolecular linkages are H-bonds between -OH groups of adjacent glucose units in the same cellulose molecule.

This gives a certain stiffness to the single chains. Intermolecular linkages occur between -OH groups of adjacent cellulose molecules (Figures 2.5 and 2.7). These linkages are responsible for the formation of the supramolecular structures.

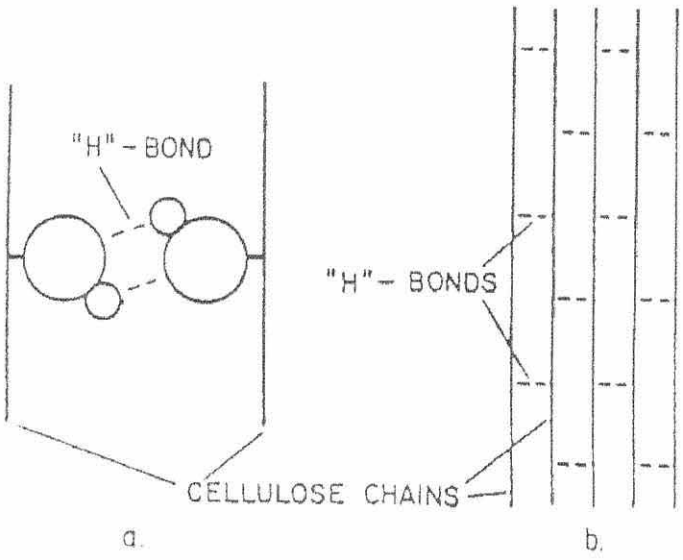


Figure 2.5 Hydrogen bonds (broken lines) between cellulose molecules: a. A single bond pair between adjacent segments; b. Series of hydrogen bonds linking several molecules in a crystalline region

The primary structure formed by intermolecular H-bonds are crystalline cellulose strands, the microfibrils. In the cellulose microfibrils there are highly ordered regions (crystallite) which alternate with less ordered (amorphous) regions. It is believed that less ordered paracrystalline regions of cellulose exist on the outside of and between the microfibrils and that matrix and encrusting materials are associated with these regions. These microfibrils build up into fibrils and finally into cellulose fibres. The fibrous structure and strong H-bonds give the cellulose structure a high tensile strength. The unit cells which make up the crystalline core of native cellulose consists of five glucose residues (Figure 2.6). Four are parallel at the corners of a rhomboid and the fifth runs antiparallel in the centre.

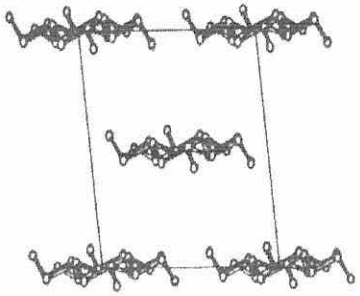


Figure 2.6 Axial projection of the structure of native cellulose showing four chains at the corner of a rhomboid with a fifth running anti-parallel at the centre. (Kolpak, Weih and Blackwell, 1978)

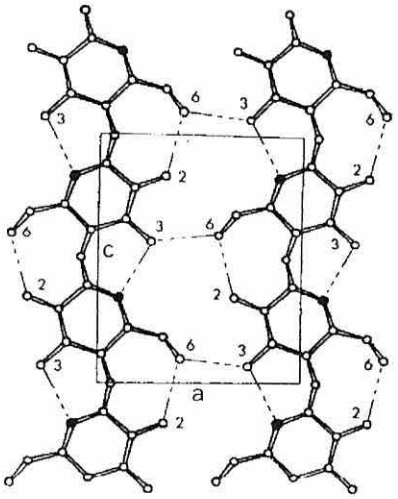


Figure 2.7 Adjacent cellulose chains showing the hydrogen bonding network and the numbering of the molecules (Gardener and Blackwell, 1974)

In the chain direction the repeating unit is a cellobiose residue and every glucose residue is turned 180^{0} with respect to its neighbours. This gives the cellulose chain a two-fold axis. All chains in native cellulose microfibrils are parallel. There are two H-bonds within each cellulose chain, from O-6 in one glucose residue to O-2-H in the adjacent glucose and also from O-3-H to the ring oxygen (Figure 2.7). The chains form a layer where they are held together by H-bonds from O-3 in one chain to O-6-H in the other. There are no H-bonds between these layers, only weak van der Waal's forces. Native cellulose has both a chain lattice and a layer lattice (Gardner and Blackwell, 1974).

It is now generally accepted that the cellulose microfibrils have a crystalline core which may run undisturbed for up to 50 nm in the longitudinal plane and that at some point chain dislocation will occur with some measure of disorder resulting. (Figure 2.8).

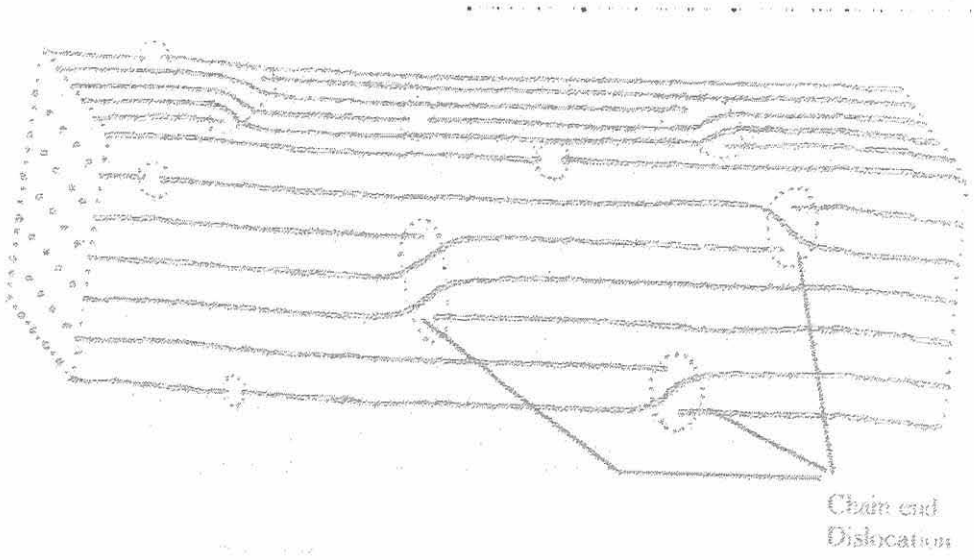


Figure 2.8 Sectiion of cellulose microfibril showing chain end dislocation.

2.0.1.2 Hemicelluloses

In contrast to cellulose which is a homopolysaccharide, hemicelluloses are heteropolysaccharides. Like cellulose most hemicelluloses function as supporting material in the wood cell walls. They differ from cellulose by being a composition of various sugar units, having shorter molecular chains (most hemicelluloses have a degree of polymerisation of only about 200) and by branching of the chain molecules. The sugar units (anhydro-sugars) making up the hemicelluloses can be subdivided into groups such as pentoses, hexoses, hexuronic acids and deoxy-hexoses. Hemicelluloses constitute between 20 - 25% of the dry weight of wood.

Galactoglucomannans are the principle hemicelluloses in softwoods (about 20-25%). Their backbone is linear (Hoffmann and Timell, 1970) or possibly a slightly branched chain built up by 1-4-linked β-D-glucopyranose and β-D-mannopyranose units (Figure 2.9). Hoffmann and Timmell (1970) give evidence that part of the galactoglucomannans are linked to lignin

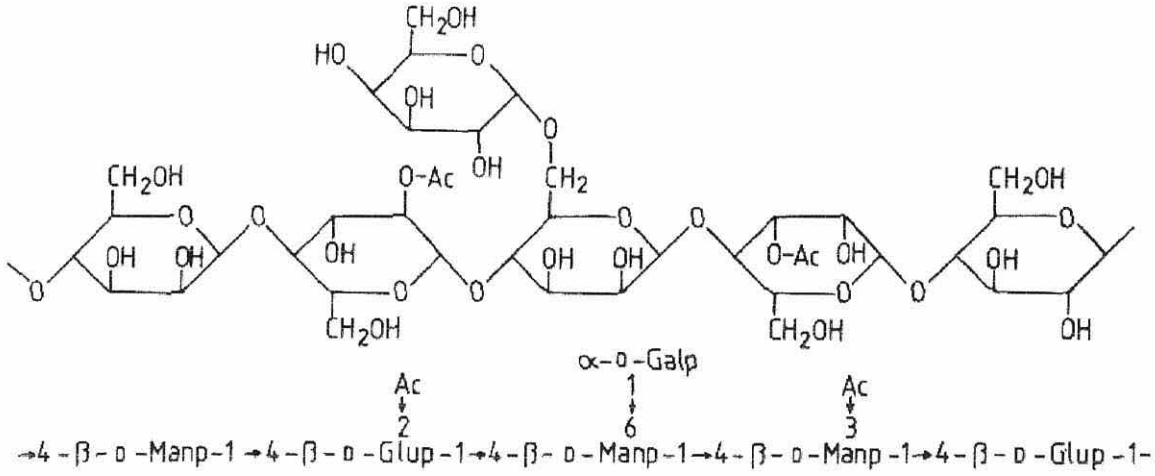


Figure 2.9 The partial chemical structure of galactoglucomannan from softwood

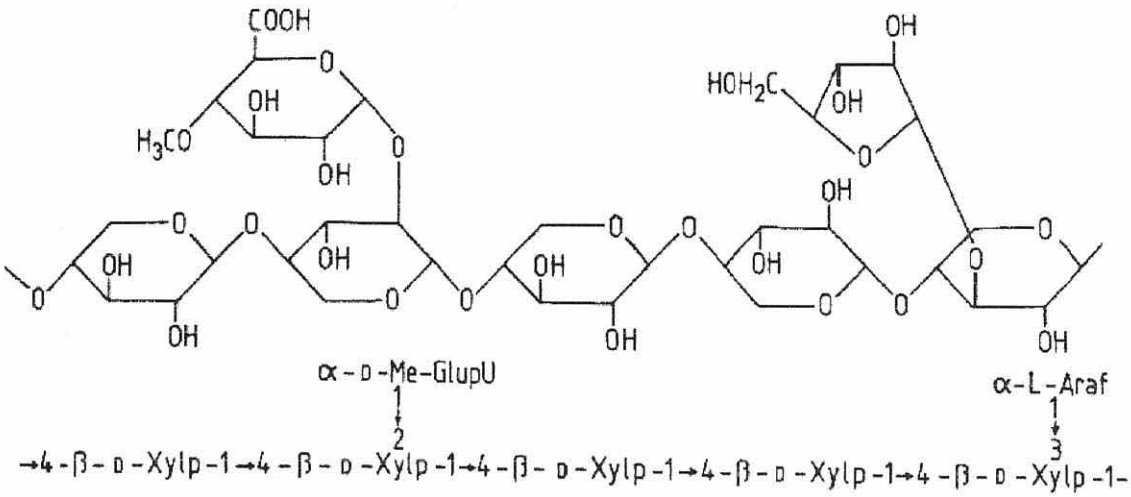


Figure 2.10 The partial chemical structure of arabinoglucuroxylan from softwood

In addition to galactoglucomannans, softwoods contain an **arabinoglucuronoxylan** (10 - 15%). It is composed of a framework containing 1-4-linked β-xylopyranose units which are partially substituted at C-2 by 4-O-methyl-α-glucuronic acid groups (Figure 2.10).

Besides galactoglucomannans and arabinoglucuronoxylan, softwoods contain other polysaccharides, usually present in minor quantities. They are built up dominantly by units of arabinose, glucuronic and galactouronic acids and include starch and pectins.

2.0.1.3 Lignin

Dinwoodie (1981) says that with hemicelloses lignin can be regarded as cementing material contributing to the structural integrity of wood and also to its stiffness. Fengel and Wegener (1984) describe it as one of the most difficult natural polymers with regard to its structure and heterogeneity. Its synthesis results in polyphenolic branched polymers which lack regular and ordered repeated units such as in the case of cellulose. They continue to say that the growing lignin molecules are forced to fill up the spaces between the preformed polysaccharide fibrillar elements of the cell wall. Sjöström (1981) describes lignin as polymers of phenylpropane units and discusses the lignin precursors, p-coumaryl, coniferyl and sinapyl alcohols as being formed from glucose by a variety of enzymatic reactions involving oxidations, reductions, aminations, deaminations and decarboxylations. The functional groups of major influence on the reactivity of lignin are given as phenolic hydroxyl, benzylic hydroxyl and carbonyl groups. He says that covalent links exist between lignin and wood polysaccharides and that the hemicellulose components (xylan and galactoglucomananns in softwood) are bound to lignin mainly through arabinose, xylose and galactose moieties.

Reid (1995) gives a comprehensive description of lignin formation. He states that lignin is the material that confers the qualities of rigidity and durability that makes wood plants "woody" and that it makes up about 30% of softwood. It is biosynthesised by the polymerisation of three propanoid precursors, differing in the number of methoxyl groups on the aromatic ring. Softwood lignin contains mostly guaiacyl (monomethoxy) units.

Reid (1995) agrees with Fengel and Wegener (1984) in saying that lignin is polymerised in the wood cell walls after the polysaccharides have been deposited and goes on to say that is initiated by enzymatic oxidation of the precursors to phenoxy radicals.

These radicals can couple with each other and with the growing lignin polymer in numerous ways to form a complex cross-linked network. He says that the most common inter-unit linkage is an ether bond formed between the middle carbon of a propanoid side chain and the phenolic group of another unit (ß-O-4 bond). He describes direct C-C bonds between various

ring positions and suggests that ether bonds to sugars in hemicelluloses are also present.

The lignin concentration is high in the middle lamella and low in the secondary wall. However because of the thickness of the secondary wall, at least 70% of the total lignin in the secondary wall of earlywood cells and over 80% in the latewood cells of softwoods is located there.

The distribution of lignin in the secondary wall shows that there is about 27 - 30% lignin in each of the S^1 and S^2/S^3 layers (Eaton and Hale, 1993)

There are difficulties in isolating native lignin from wood without degradation (Fergus and Goring, 1970) and this leads to problems in accurately determining a realistic molecular weight. They give a molecular weight of about 20,000.

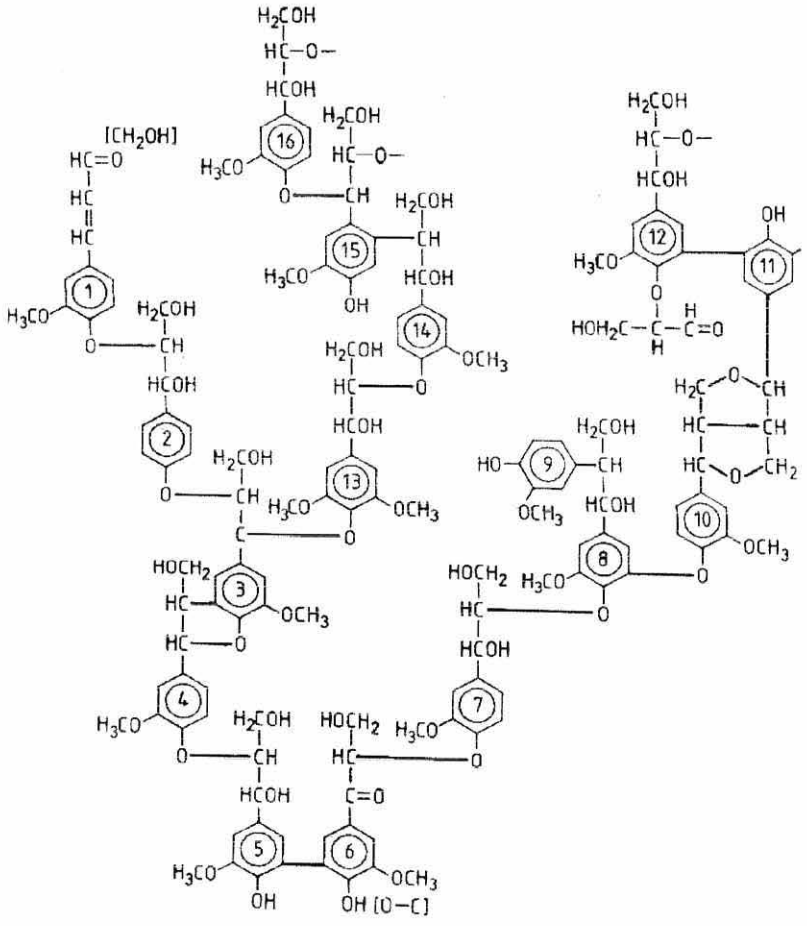


Figure 2.11 Diagrammatic representation of the lignin molecule (Adler, 1977)

2.0.2 The Internal Structure of the Cell Wall

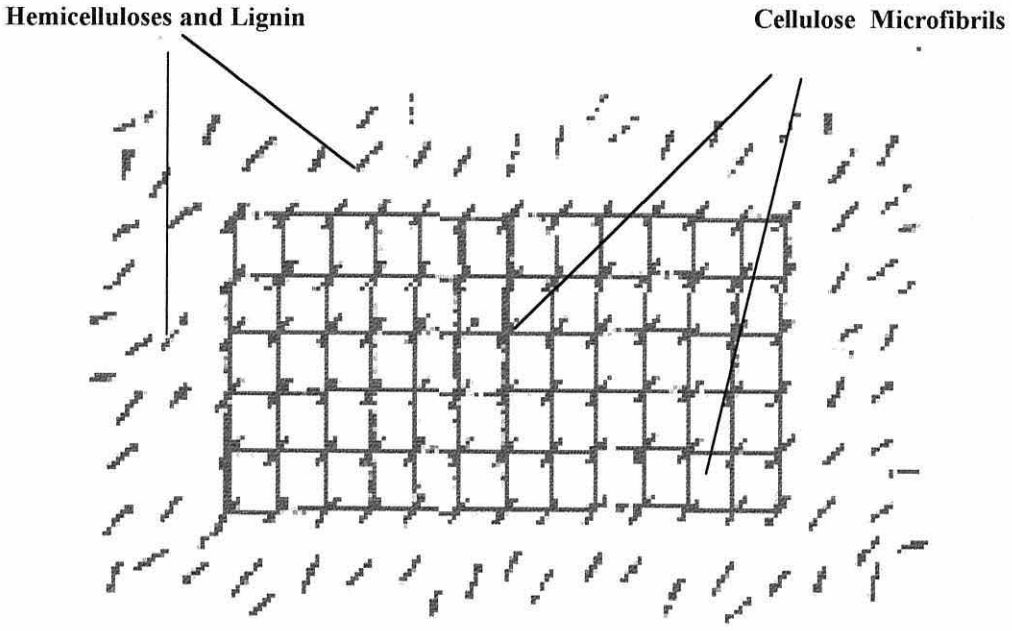


Figure 2.12 The association of cell wall materials according to Preston (1962)

Various models of the wood cell wall structure have been proposed. Preston (1962) shows the cellulosic fibrils enclosed by less ordered cellulose, hemicelluloses and lignin (Figure 2.12). Marchessault (1964) arranged the hemicelluloses and lignin molecules as slack sheaves between the cellulose fibrils.

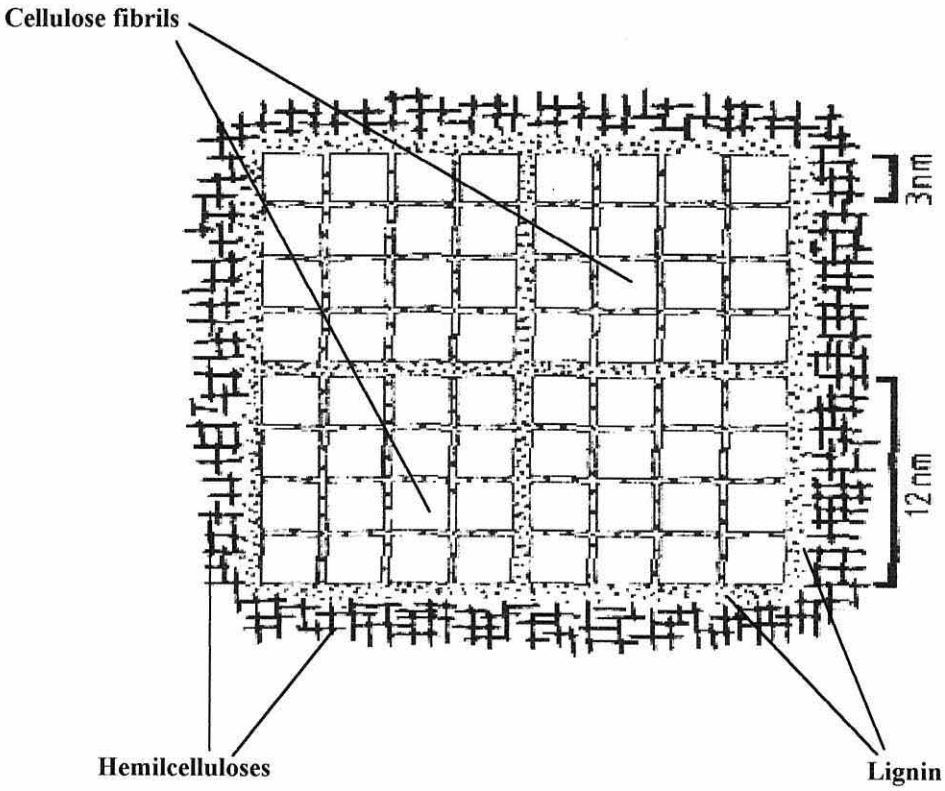
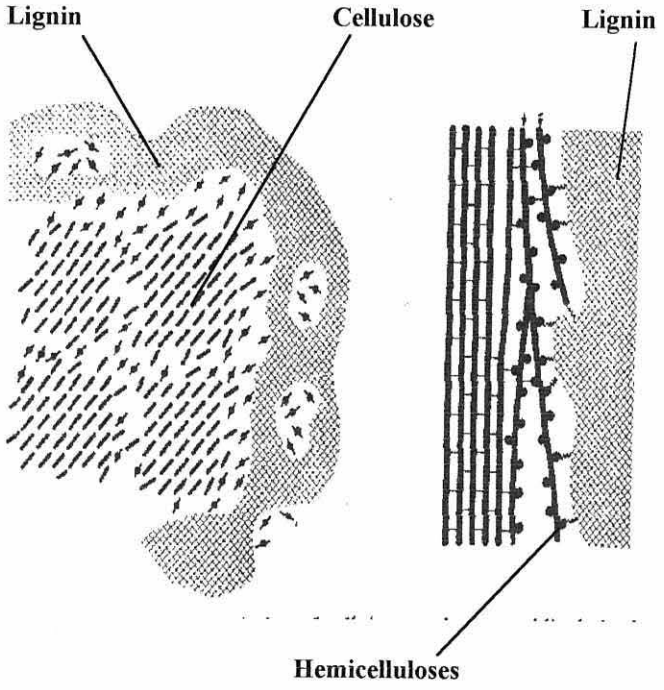


Figure 2.13 Model of the association of cell wall components according to Fengel (1970)



Figue 2.14 Model of the association of cellulose, hemicelluloses and lignin shown in transverse and longitudinal aspects. The longitudinal view shows bonding between the three components (Fengel and Wegener, 1984)

Fengel's (1970) model describes the smallest fibrillar units of cellulose (3nm in diameter) separated from each other by monomolecular layers of hemicelluloses. The largest units(25nm in diameter) are enclosed by hemicelluloses and lignin (Figure 2.13). Figure 2.14 shows the model proposed by Fengel and Wegener (1984).

2.1 Water in Wood

Water in wood may be present in two forms (Skaar, 1972) as free water in the cell cavities in the form of vapour or liquid and as bound or hygroscopic water in the cell wall. The moisture content at which the cell wall is fully saturated but the cell cavity is empty of liquid water is designated the fibre-saturation point. Usual values are from 25 - 35% of the dry wood weight. Wood is hygroscopic, it is able to remove water from the atmosphere and maintain a moisture equilibrium with the water vapour in the air. It is hygroscopic primarily because of the -OH groups which exist throughout its structure. These groups can attract and hold water molecules by the mechanism of H-bonding (Dinwoodie, 1981).

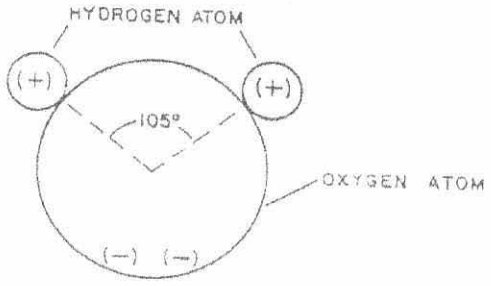


Figure 2.15 Diagrammatic representation of a water molecule

Hydrogen bonding can occur because the hydrogen atom is very small compared with the oxygen atom. In the water molecule (Figure 2.15) the atomic arrangement of the two smaller hydrogen atoms attached to the larger oxygen atom results in a polar complex, the side of the oxygen atom on the opposite side from where the hydrogen atoms are attached becoming electronegative. The hydrogen side of the wood -OH group has a positive charge and the oxygen side has a net electronegative charge. There is potential for OH_2 - OH bonding. This potential is not strong relative to other primary chemical bonds but it is very important in water-containing materials such as wood. Hydrogen-bonding of water molecules to -OH groups in wood is important. In one glucose anhydride unit in a cellulose chain there are 6 -OH groups available for H-bonding. Each half-unit contains 3 -OH groups. When the cellulose unit is fully hydrated there are three water molecules per unit of $C_6H_{10}O_5$

giving the hydrated formula of $C_6H_{10}O_5.3H_2O$. One mole of dry cellulose weighs 162g and three moles of water weigh 54g giving a moisture content for fully hydrated cellulose of 33%. However not all of the cellulose -OH groups are accessible to water since many are in an inaccessible crystalline form (section 2.0.1.1). Here the hygroscopic -OH groups are mutually satisfied by the formation of H-bonds between adjacent cellulose molecules (Figure 2.5). In the crystalline regions of cellulose the chains form in a parallel uniformly spaced arrangement. These chains are bound together by hydrogen bonds between -OH groups. Here the -OH groups are not available for bonding water.

Water-cellulose-OH bonding is more likely to take place on the surface of the cellulose crystallites or in the amorphous regions, provided the -OH groups are not cross - linked with other -OH groups. Also in wood, the hemicelluloses, lignins and various extractive materials have -OH and oxygen containing sorption sites that may be more accessible to water than some of those in cellulose.

The entrance of water into the cellulose structure means a swelling of the structure. As the water content of the wood increases the OH-OH bond between adjacent cellulosic surfaces (Figure 2.16 A.) can be broken to accommodate a single molecular layer of water (Figure 2.16 B). If the water content continues to increase, other layers of water molecules may be accommodated (Figure 2.16 C) there now being cellulose-OH-OH_2 and H_2O-H_2O H-bonds formed. Dependant on the water content, single molecules or clusters can be linked to cellulose surfaces.

The reverse process of adsorption of water and swelling is the removal of water and shrinkage of the cellulose. The process of drying is continuous but can be resolved into individual stages. Firstly the H-bonds between the water molecules are broken, they are the lowest energy bonds in the system. Part of the water is removed and the cellulose surfaces approach each other. The process continues until there is a monomolecular layer of water. Then the H-bonds between water and the cellulose -OH are broken and the H-bonds between the cellulose surface are formed.

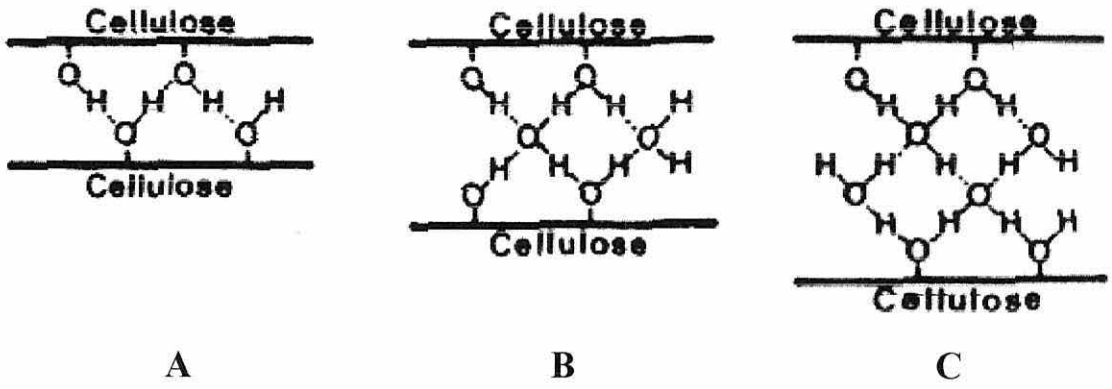


Figure 2.16 Showing the swelling of cellulose caused by water adsorption.

2.2 The Relevance of Hydroxyl Groups

Cellulose, the hemicelluloses and lignin make up the solid phase of wood and all contain in abundance the hydroxyl group, which is the main functional group in wood. It is the most abundant group available for reaction and it may be alcoholic in character, being associated with the long chain polysaccharides and lignin side chains or phenolic, attached directly to lignin aryl rings. Most of the reported derivitisation of wood is based on these functional sites. Banks (1990) says that the only functionality available for non-destructive reaction in cellulose is the -OH group, apart from the very sparse hemiacetal reducing ends of polymer chains.

Because of the high degree of H-bonding between -OH groups distributed along the polymer chain the reactivity of cellulose is relatively low. West and Banks (1987) preparing butyl carbamates of wood using butyl isocyanate found that the level of reactivity increased when the reaction was carried out in the presence of pyridine. The 'swelling effect' brought about by the pyridine exposed previously inaccessible wood polymer -OH groups to reaction. It has been estimated (Stamm, 1964) that 65% of the cellulose in wood is crystalline and probably not accessible (in a non-swollen) state to reactions involving the -OH groups. Rowell (1983) says that the polysaccharide components in wood have more -OH group functionality than lignin but its accessibility is restricted by its partial crystallinity.

The -OH group is that site in wood where the contest between environmental degrade agents

and chemical modification takes place. For a fungus to effectively break down and metabolise the solid phase of wood there has to be sufficient water present in the wood. Decay by the brown rot fungi can occur at about 20% moisture content (based on dry wood weight) but optimum values for decay are at and above fibre saturation point. This optimum moisture content varies with fungal specie but is in the range 28-50% of the dry wood weight for brown rot fungi (Rugevitsa, 1977). It is at the many -OH group sites that water sorption takes place. Browning (1963) says that for *Eucalyptus regnans* the fractional contribution of each cell wall polymer to the total water sorption is cellulose 47%, hemicelluloses 37% and lignin 16%. He interpreted this to be a function of -OH group accessibility and states that lignin (to a small degree), the hemicelluloses (all are non-crystalline in nature), the non-crystalline portion of cellulose and the surface of the cellulose crystallites are responsible for moisture uptake by wood.

The attachment of a protective agent at the -OH group sites in wood could prevent the moisture content of the wood reaching a level suited to fungal attack.

2.3 Fungal Decay of Wood

Blanchette (1994) defines biodeterioration as any undesirable change in the properties of a material caused by the action of degrading organisms. Degradation of the lignocellulose complex in wood varies depending on the organism causing decay. Wood can be decomposed by a variety of different biological organisms. Most species of timber are susceptible to attack by at least one species of insect. Termites can bring about the biodeterioration of most timbers. They cannot flourish in Northern climates and are found principally in the tropics with some species active in the Mediterranean area. In Britain insect attack is usually by the grub or larval stage of certain beetles, notably the furniture, death watch, powder post and longhorn beetles. Marine borers such as the shipworm (*Teredo* sp.) and the gribble (*Limnoria* sp.) are a hazard to timbers in salt water (Dinwoodie, 1981). Certain bacteria are known to degrade the lignified cell walls of wood by tunnelling, erosion and cavitation (Blanchette, Nilsson, Daniel and Abad, 1990; Singh, Nilsson and Daniel, 1990). It is the wood destroying fungi which cause the most widespread and extensive damage to both standing and felled timber and timber in service.

In the natural carbon cycle the fungal organisms which live in or on wood break down the wood macromolecules in order to utilise the organic carbon sources to release energy which is harnessed to drive their metabolic processes. This process, whilst being of immense importance in the forest and global ecosystems, is less desirable when directed at wood or wood products in the service of man. The wood substrate offers a range of carbon sources to organisms and the fungi have developed in various forms which are able to utilise part or all of the wood material. Micro-organisms are able to utilise the monomeric and dimeric carbohydrates and the storage polysaccharides such as amyloglucans. These "available" nutrients are found mainly in the ray parenchyma and axial parenchyma (Cowling, 1965). Glucose and xylose are the most easily utilised wood sugars and as monomers and short chain polymers these are only available in small quantities in wood. However many organisms have developed which are able to degrade large polymeric carbohydrates and lignins to monomers and other carbon macromolecules to soluble forms from whole wood.

In general three categories of fungal degradation are accepted although there may be overlap

between the categories. They are brown rot, white rot and soft rot.

Brown rot fungi are able to degrade the cellulose and hemicelluloses in wood and to modify the lignin. White rot fungi are characterised by their ability to degrade all cell wall components, cellulose, hemicelluloses and lignin. Soft rot fungi can degrade all cell wall components, they are generally but not exclusively associated with wood in wet environments.

This investigative section of this work concentrates on the degradation of wood by fungi, specifically that caused by brown rot.

2.3.1 The Importance of Enzymatic Reactions

The biochemistry of the breakdown of wood by fungi is not yet fully understood. The current hypotheses consist of different degradative pathways involving non-enzymatic and enzymatic routes (Highley and Murmanis, 1985; Eriksson, Blanchette and Ander, 1990; Highley and Illman, 1991; Flournoy, Kirk and Highley, 1991; Blanchette, 1994).

Chemically, enzymes are proteins and the important reactions catalysed by them so far as wood degradation is concerned are hydrolysis of cellulose and hemicelluloses and the oxidation of lignin. The exact mechanisms by which enzymes induce hydrolysis and oxidation are not fully understood. In simple terms the following processes are involved: an enzyme bonds to a complimentary group in a substrate molecule. Reaction then takes place between the substrate, and external substance (e.g. Air or water) and enzyme. The structural bond in the substrate molecule is broken to leave simpler fragments and the enzyme is released to bond to another complimentary group in a substrate molecule.

The insoluble wood components are degraded to soluble products and finally to the simple chemical compounds which are used for the metabolism of the degrading organism. The group of enzymes comprises a large number of various biocatalysts which accelerate and control biochemical reactions. Many of the catalysts involved in the decay of wood are highly specific. Their action is restricted to certain molecules or molecular structures. Green, Clausen, Micales, Highley and Wolter (1989), Eriksson *et al.*. (1990), Blanchette, Obst, and Timell (1994) are amongst those giving summary descriptions of wood degrading

enzyme activity.

Wood decaying fungi are known to produce endocellulases and exocellulases, hemicellulases and oxidases. Endocellulase activity involves random attack along the length of accessible cellulose molecular chains. Exocellulase attack takes place at the ends of cellulose molecules. White rot fungi produce enzymes which under certain conditions are able to take part in the breakdown of lignin. Eaton and Hale (1993) say that a concerted action of enzymes is required to break down the wood cell wall.

Wood has a complex ligno-cellulosic nature and the structural inter-relationships of wood polymers is a strong factor in enzymatic degradation. Enzyme size and wood openings are relevant, there could be a steric hindrance to enzyme movement. It is thought (Cowling and Brown, 1969) that the degrade enzymes of all the fungal decay groups are too large to be able to penetrate the wood cell capillaries and initiate attack deep within the cell walls. Fungal enzymes are ellipsoidal rather than globular in shape (Eaton and Hale, 1993) and even in the smallest dimension enzymes are thought to be too large for penetrating the cell wall. As examples these writers give details of some cellular enzymes from various fungi. The brown rotter *Oligoporus placenta* produces an enzyme that has a molecular weight of 185 kdaltons; *Trametes versicolor*, a white rot fungus produces an enzyme with a molecular weight of 51 kdaltons (equivalent width if an ellipsoid, 3.5nm); *Myrothecium verrucaria*, a soft rot produces enzymes with molecular weights ranging from 30 to 63 kdaltons (equivalent widths if an ellipsoid, from 5.1 to 7.7nm). Murmanis, Highley and Palmer (1987) agree with Flournoy *et al.* (1991) in saying that no proteins with a molecular weight greater than 20 kdaltons can enter into the cell wall even in an already brown rotted wood, they were discussing pine. Flournoy *et al.* (1991) found that a maximum pore diameter in the cell walls of wood severely decayed by brown rot fungi had a molecular radius of probably not greater than 1.5nm. Endoglucanases produced by the brown rot fungi *Polyperus schweinityii* and *Gloeophyllum trabeum* have been purified and partially characterised (Keilich, Bailey, Afting and Liese, 1969). These enzymes have a molecular weight of 45 and 29 kdaltons. respectively. Highley and Illman (1991) isolated a range of cellulases from *O. placenta* which appear to be grouped together in a complex having a molecular weight of about 185

kdaltons.

Srebotnick and Messner (1991) and Flourney *et al.* (1991) support the theory that even in decaying wood the cell wall capillaries are too small to allow free movement of enzymes.

As the enzymes are too large to enter the cell wall and initiate decay it has been suggested that oxidative free radicals are low molecular weight agents that start the decay process by brown rot fungi (section 2.2.4). It is all a question of accessibility of the crystalline cellulose microfibrils to the cellulose degrading enzymes. Current thinking suggests that low molecular weight agents are essential in initiating fungal attack.

Water is essential for the enzymatic degradation of wood. In terms of moisture the ideal situation for the degradative process is one where the cell wall is fully saturated with bonded water (fibre saturation point) and a film of free water is present, lining the cell lumen. This allows fungal enzymes to move around the cell by diffusion whilst also maintaining ample oxygen for respiratory processes to take place. Above fibre saturation point there is an accumulation of free unbonded water in the cell lumen. At this stage gaseous diffusion slows down and oxygen is limiting. Under conditions of lowered oxygen tension there are a number of wood destroying fungi still quite active but when fully anaerobic conditions exist the decay and growth of fungi is arrested (Viitanen and Ritschkoff, 1991). At this stage only very slow progressive breakdown of wood by anaerobic bacteria is observed (Eaton and Hale, 1993).

2.3.2 Polysaccharide Degradation

Eaton and Hale (1993) give three major classes of enzymes which deploymerise cellulose and suggest that a variety of isozymes and cellulases are produced by organisms in different stereospecific ways. 1,4-β-D-glucan cellobiohydrolase (exoglucanase, cellobiohydrolase. exocellulase, C_1) attack crystalline cellulose to result in cellobiose; endo-1,4,-β-D-glucan-4-glucanhydrolase (endoglucanase, β-glucanase, endocellulase, C_x) attack amorphous cellulose to give oligosaccharides; β-D-glucoside glucohydrolase (cellobiase) attack cellobiose to give glucose. Also present may be an additional enzyme, 1,4-β-D-glucan 4-glucohydrolase (glucohydrolase) which can attack cellulose to give glucose oligomers.

Reese (1975, 1977) developed a scheme which recognised, for true cellulytic organisms, a multi-step synerginistic action by two types of cellulase, C_1 and C_x, and β-glycosides. He worked with *Trichoderma reesei*. He explains that the C_1 component disrupts the hydrogen bonds in the cellulose chains to give reactive cellulose. The C_x component hydrolytically attacks this cellulose to give cellobiose which is then attacked by the β-glucosidase to give the glucose monomer.

Jeffries (1987) reviewed the synerginistic action of enzymes. He discusses endoglucanase attack which hydrolyses the non-crystalline regions and regions of less ordered crystalline cellulose, opening up the cellulose chains. Cellobiohydrolase binds the edge of the cellulose crystallite and moves along the chains towards the reducing end, cleaving off cellobiose units from the non-reducing end. Endoglucanases open up various random points of the crystalline regions which are immediately attacked by the cellobiohydrolases to prevent closure of the original scission. Further attack by the cellobiohydrolases releases more cellobiose units. β-Glucosidase converts cellobiose to glucose. The exoglucohydrolase enzymes attack soluble oligomers released from cellulose to yield glucose molecules. Of the brown rot fungi only the Coniophraceae have been found to have the complete cellulase complex (Nilsson and Ginns, 1979). Many have been shown to lack exoglucanases, oxidative mechanisms have been formulated to explain cellulose breakdown. Ayers, Ayers and Eriksson (1978) looked at oxidative enzymes involved in cellulose degradation and discuss cellobiose oxidase, glucose oxidase and an oxidoreductive enzyme, cellobiose dehydrogenase. Eaton and Hale (1993) explain that with *Phanerochaete chrysosporium* the oxidases and dehydrogenases serve to prevent an accumulation of the end products of hydrolysis which if present in too high a concentration and remaining unchecked, cause end product inhibition and repress enzyme synthesis.

Cellulose crystallites are surrounded by a matrix of encrusting materials. The nature of these surrounding materials have a big effect on the breakdown of the wood cell wall as they have to be removed or penetrated before the cellulase enzymes can access the cellulose substrate. The hemicelluloses and the amorphous cellulose can generally be much more easily broken down than lignin. Crystalline cellulose in the S_2 cell wall layer is surrounded by a matrix of

hemicelloses. If cellulose degrading enzymes are to reach the cellulose then this matrix must be degraded or loosened. Exoenzymes and endoenzymes exist which will degrade hemicelluloses. Only endomannanases have been found in a wide range of fungi. Reid (1995) looked at galactanases. He says that the preferential ability of different fungi to degrade susceptible wood species may be related to hemicellulose composition. Eaton and Hale (1993) discuss the importance of the hemicelluloses in the matrix regions of the S_2 layer of the wood cell wall. They say that as with cellulases, endoenzymes are the most frequently found. Reid (1995) points to lignin content and type as being important in hemicellulose degradation.

2.3.3 Lignin Degradation

The white rot fungi are the most efficient lignin degraders. They occur predominantly on hardwoods and in the laboratory they generally decay hardwoods better than softwoods. Highley (1982) says that this is apparently because of the presence of syringyl lignin in hardwood. White rot fungi are able to completely break down both the lignin and carbohydrate components of wood. Some species, simultaneous rots, remove carbohydrate at the same proportional rate. Others, selective rots, remove lignin faster than cellulose (Blanchette, 1991).

Lignin is an insoluble polymer. Reid (1995) claims that the initial steps in lignin biodegradation must be extracellular and that the final steps in lignin mineralisation, culminating in the release of CO_2, are likely to take place inside the fungal hyphae. This, he says, means that the extracellular reactions must break lignin into fragments that are able to diffuse to the hyphae and across the cell membrane. Chen, Chua, Evans and Chang (1982) and Chen and Chang (1985) claim chemical and physiological evidence that lignin degradation is predominantly oxidative. Kirk and Farrell (1987) use the term 'enymatic combustion' to describe the process by which the lignolytic fungi overcome the problem of not possibly being able to produce enzymes that could recognise all the diverse interunit linkages in lignin. This process apparently evolved by the white rot fungi, involves the production of enzymes of low specificity that initiate, but do not direct, oxidative reactions in

lignin. The enzyme activates the lignin to overcome an energy barrier and begin a thermodynamically favourable oxidative fragmentation without further control of the reaction pathway by the enzyme. It appears (Reid, 1994) that lignin degradation does not proceed by an orderly removal of the peripheral subunits as single ring compounds. He says that it also involves oxidation of the aromatic rings and side chains in the interior of the polymer

Hatakka (1994) says that different combinations of the known enzymes, one reductive and three oxidative are commonly found. Reid (1995) describes lignin peroxidase as showing little substrate specificity and reacting with a wide variety of lignin model compounds It is produced by many but not all white rot fungi. Blanchette (1994) describes native lignin as being sequestered in a dense matrix of polysaccharides which means that access by enzymes to the lignin is restricted. Reid (1995) says that during simultaneous decay by white rot fungi, the porosity of the cell wall does not increase enough to allow enzyme penetration into the lignin-polysaccharide matrix but as polysaccharides are removed from the wall at the same rate as lignin, the zone of active lignin degradation remains directly accessible at the surface. In selective delignification the cellulose matrix of the secondary wall remains in place and lignin degradation takes place at a considerable distance from the surface of the lumen. He continues to say that removal of lignin and hemicelluloses creates channels wide enough to allow access of the enzymes to the sites of lignin degradation. Thus, he says, in either case the fungal enzymes can reach their substrate.

2.3.4 Brown Rot Decay

Blanchette (1994) describes the drastic alteration of wood by brown rot fungi. Of the fungal decay organisms brown rot fungi are known to cause extensive damage to timber in buildings. These fungi, classified in the Basidiomycota cause a diffuse attack on cellulose early in the decay process. The decayed wood is brown, it often cracks and checks into cubical fragments. Polysaccharides are removed but lignin degradation is limited to demethoxilation of the aromatic portion and loss of the propane side chain (Crawford, 1981). One distinguishing feature of brown rot decay is their capacity to depolymerize crystalline

cellulose early in the decay process causing substantial loss in wood strength properties before appreciable weight loss (Cowling, 1961; Eriksson *et al*, 1990).

Brown rot fungi initiate attack on wood via the ray structure. The hyphae then penetrate the axial tracheids and fibres. Wilcox (1973) says that in the initial stages of infection single hyphae occupy nearly every cell. The pit tori are destroyed and the hyphae may also penetrate the cell walls by means of bore holes. Eaton and Hale (1993) say that as attack progresses the bore holes may expand but that in severe attack cell wall breakdown is associated with hyphae lying in the lumina and on the luminal surfaces. As decay proceeds, they continue, the cell wall collapses and small splits are seen in walls very close to the S_2 microfibrils. As brown rot decay nears completion only a lignin skeleton remains.

To accomplish decay the fungi must produce degrading agents that penetrate rapidly into the pore structure of wood. However, research has demonstrated (Highley and Illman, 1991; Flournoy *et al.* 1991) that cellulases and hemicellulases so far classified cannot initiate decay because they are too large to penetrate sound wood and diffuse through the cell walls (section 2.2.3). A current hypothesis is that other small chemical species may be involved at the initial stage of attack, when a low molecular weight system is used by the fungi to depolymerise cellulose and the products further processed with endocellulases. Wilcox (1968) has shown that the removal of cellulose occurs in the different cell wall layers in the order S_2, S_1, S_3. This supports the idea that brown rot fungi release a highly diffusible decay system which is able to initiate decay in the cell wall below the luminal surface (Eaton and Hale, 1993). In the early stages of wood degradation it is often observed that the S_2 layer of the cell wall is extensively degraded while the S_3 layer adjacent to the lumen remains virtually unchanged (Highley and Murmanis 1985; Kuo, Stokke and McNabb 1988).

It is suggested by Highley and Illman (1991) that enzymatic and non-enzymatic reactions occur and that they may involve reduced oxygen species. Halliwell (1965) described the depolymerisation of cellulose by Fentons reagent (Fe^{2+} + H_2O_2) which generated a -OH radical or similar oxidant. One current model of how deplolymerization takes place includes the production of -OH radicals from H_2O_2 by the Fenton-Haber-Weiss reaction (Highley and Illman, 1991). Walling (1975) and Winterbourn (1987) state that the reductive cleavage of

H_2O_2 by metal ions or iron complexes is known to generate free -OH radicals in acidic solution. Kirk, Ibach, Mozuch, Conner and Highley (1991) chemically characterised and compared cellulose degradation by the brown rot fungus *O. placenta* and cellulose treated with acid hydrolysis, Fentons reagent and other chemical oxidants and found the Fenton treated cellulose to most closely resemble the brown rotted cellulose.

Enoki, Yoshioka, Tanaka and Fuse (1990) suggest that brown rot fungi produce a one electron oxidation system that is capable of penetrating the wood cell walls. Their one electron system includes reduced oxygen species such as H_2O_2 and -OH radicals. They give an explanation of how this system initiates and results in cellulose breakdown. They say that in the initial degradation of wood cell walls by brown rot fungi, the hyphae in the cell lumina secrete glycopeptides. The glycopeptides are small enough to penetrate the cell wall layers of the wood. They then adsorb Fe^{2+} in the cell walls and catalyse a redox reaction between electron donors and oxygen to produce H_2O_2. This H_2O_2 is reduced by the ferrous iron bound in the peptides. The active O_2 produced during the production of H_2O_2, ascorbate and other unknown reductants reduces ferric iron to the ferrous state. Then the ferrous iron reduces H_2O_2 to form -OH radicals. The -OH radicals transform the cell wall layers and split the cellulose and lignin. They offer no conclusive proof of this hypothesis.

Ritschkoff and Viikari (1991) say that the nature of cellulose breakdown suggests that a small metabolic product may be involved in the initial stage of brown rot attack. They hypothesise that the oxidative degradation of cellulose involves a one electron transfer in the conversion of H_2O_2 in the Fenton reaction. They say that brown rot fungi create a low pH while decaying wood primarily by secreting oxalic acid which reduces the transition state metals existing in wood (Fe^{2+}, Mn^{2+}). The two valenced metal then reacts with H_2O_2 to produce highly reactive -OH radicals which might initiate the oxidative depolymerisation of the cellulose. They point out that H_2O_2, in the presence of Fe^{2+} or other transition state metals, may form highly reactive radicals that initiate the oxidative degradation of the wood carbohydrates. They too found that the structural changes in the wood polysaccharides induced by Fentons reagent is very similar to that of an attack by brown rot fungi.

Highley (1977) and Lundborg (1989) agree that the most important way of the formation of

the -OH radical is via the Fenton reaction. Their research also indicated that the production of H_2O_2 by brown rot fungi is stimulated by low nitrogen and sugar concentrations in growth medium.

Backa, Gierer, Rierberger and Nilsson (1992) deduced that reductive cleavage of H_2O_2, transition metal irons or iron complexes generated free -OH radicals in acid solution.

$$Fe^{2+} + H_2O_2 + H^+ \longrightarrow Fe^{3+} + .OH + H_2O$$

They claim that a Fenton - type reaction is responsible for the generation of -OH radicals and that the observed effects of brown rot fungi is the rapid depolymerisation of the cellulose polymer and the oxidative cleavage of bonds between anhydroglucose units in the cellulose chain and that these phenomena can be explained by the action of -OH radicals on cellulose. They detected -OH radicals in wood inoculated with brown rot fungi using a chemluminescence method. The hydroxylation of phthalic hydrazide produces a 3 - hydroxyphthalic acid which when oxidised has strong chemiluminescence that is detected in a luminometer. Their study showed that -OH radicals were formed during the growth of brown rot fungi and were readily detected in decayed wood. The addition of veratryl alcohol enhanced -OH radical production and they postulate that the presence of lignin compounds may stimulate brown rot decay.

Most brown rot fungi are unable to attack and utilise pure cellulose (Nilsson 1974; Highley 1977; Nilsson and Ginns, 1979). A close spatial association with the other elements in wood appears to be a requirement. Ritschkoff and Viikari (1991) investigated the 'order' of cellulose within the cell wall and the effect that this has on H_2O_2 production. They found that pure highly crystalline cellulose had the most pronounced effect on H_2O_2 production. Amorphous cellulose did not have the same effect. They deduced that the crystalline or ordered, less easily hydrolyzable structure of the substrate might play a key role in the induction of the oxidative H_2O_2 producing metabolism on cellulose degradation. Blanchette (1994) suggests that the lignocellulose matrix appears necessary for the brown rot degradation system to be induced and functional.

Oxalic acid has been proposed as an important precellulolytic factor that possibly acts as a chelator to help mobilise iron, manganese, or other metal ions that could be used during the nonenzymatic process (Green, Larsen, Winady and Highley, 1991). As early as 1955 Shimano characterised brown rot fungi by the abundant accumulation of "free" oxalic acid in culture media with a resultant pH of less than 5. Green *et al.* claim that oxalic acid plays a catalytic role by the direct reduction of iron, supporting the hypothesis that the Fenton reagent ($Fe^{2+} + H_2O_2$) depolymerises cellulose. Bech-Anderson (1987) postulated that oxalic acid is the agent by which brown rot fungi hydrolyse hemicelluloses and increases the accessibility of cellulose to wood decay enzymes. Schmidt, Whitten and Nicholas (1981) demonstrated that oxalic acid, secreted in brown rot fungi in liquid cultures, reduces Fe^{3+} to Fe^{2+}. Shimada, Akamatsu, Ohta and Takahashi (1991) reported that oxalic acid concentrations of 1% and 5% decreased the viscosity of cellulose in Kraft pulp. Grethlein (1985) provides evidence for acid-induced increases in porosity and subsequent penetration by cellulytic enzymes. Shimada *et al.* (1991) propose an alternative role for oxalic acid in cellulose depolymerisation by brown rot fungi. They reported that oxalic acid alone reduces the viscosity of wood pulp to 60% of the original and may be directly involved in the cellulose deploymerising process.

Green *et al.* (1991) did work with *Poria placenta* in which they conclude that the fungus produced sufficient oxalic acid to lower the pH in wood to initiate the brown rot process They say that the early breakdown of carbohydrates by brown rot fungi in situ may be mimicked by oxalic acid in vitro and that the mechanism of strength loss during fungal-induced decay may be accounted for by reductions in pH associated with hemicellulose and cellulose depolymerisation. They claim that their results support the view that acid production is the key to the initial stages of brown rot and that the hydronium ion, H_3O^+, is an initiating low molecular weight decay agent. They conclude that low molecular weight acids are important in the initiation of brown rot decay. The acid-mediated effect acts initially to break off hemicellulose side-chains, providing the fungus access to arabinose, galactose and to a lesser extent, other sugars. After initiation of acid mediated hydrolysis of side-chains, acids begin to rapidly depolymerise and solubilise the hemicellulose backbones and

amorphous cellulose, thus increasing the porosity of the wood structure to the hyphal sheath, decay enzymes, or other low molecular weight decay agents.

Most workers, including Enoki *et al.* (1990), Fekete, Chandhoke and Jellison (1989), and Jellison, Chadhoke, Goodell and Fekete (1991), agree that an array of mechanisms, to date not fully understood, may be acting to generate the necessary components to facilitate depolymerisation.

Polarised light microscopy is a useful tool in confirming brown rot decay, especially in the later stages when a loss of birefringence is apparent, indicating a loss of crystallinity.

2.3.5 White Rot Decay

White rot fungi are a large heterogeneous group of fungi classified in the Basidiomycotena and are characterised by their ability to degrade all cell wall components (Blanchette, 1994). They possess a complete cellulase complex and can also degrade lignin. It is partly the removal of lignin which gives 'white-rotted' wood its whitened appearance although oxidative bleaching reactions may also cause whitening.

Cowling (1961) describes the physical mechanism of white rot decay as beginning with the fungus inhabiting the wood cell lumen where the hyphae begin to penetrate the cell wall through holes made by the fungus or through openings in the pits. The degradation of the cell wall proceeds from the cell lumen outwards toward the compound middle lamella with a gradual thinning of the cell wall as the cell wall components are metabolised by the fungus.

White rot fungi can be classified in two ways. 'Simultaneous rot' (Liese, 1975) is characterised by more or less simultaneous degradation of cellulose, hemicelluloses and lignin. Blanchette (1991) discusses 'non-selective' white rotters as causing cell wall erosion wherever hyphae are present. Eroded zones in cell walls are described as coalescing as decay proceeds forming larger voids that often become filled with hyphae. 'Non-selective' types of white rot fungi remove lignin without appreciable loss of polysaccharides leaving areas of white delignified wood (Otjen and Blanchette, 1982). Eaton and Hale (1993) say however that the distinction is not widely made between the two types as both may occur together. They say that culture conditions (presence of inorganic or organic nitrogen sources) may

influence the type of decay produced. Blanchette (1991) says that white rot fungi always either selectively or non-selectively attack wood and that there are many examples where one fungus produces both types of attack on the same substrate. He describes the ratio of lignin to cellulose that is decayed by an isolate as being variable on one substrate.

The relative amounts of lignin and carbohydrate fractions that are removed by the different white rot fungi and the rate of removal of the different components are dependant on both wood species and the type of fungus. (Kirk and Highley, 1973). Blanchette (1991) says that the concentration and type of lignin affects decay by white rot fungi. Syringylpropyl units are preferentially degraded whereas guaiacypropyl units are more resistant to degradation. Blanchette (1994) claims that in many situations one strain of fungus causes different types of attack within a substrate. He says that the reason for this is unclear but that it is most likely related to the different oxidative enzymes produced by the fungus. Srebrotnik and Messner (1991) say that there is a tremendous variability among white rot fungi in how selectively they dilignify wood. Some species for example, can cause selective removal of lignin at one location and simultaneous removal of both lignin and polysaccharides at another location in the same wood sample. They say that even different strains of a single species can show considerable variation in the type of attack. Blanchette, Nilsson, Daniel and Abad (1990) claim that some white rot fungi seem to be able to switch from selective to simultaneous degradation over a period of time.

Most investigative work has been carried out on *Phanerochaete chryosporium*. Ayers *et al.* (1978) say that *P. chryosporium* produces all three types of hydrolytic enzymes for the degradation of cellulose and cellulosic residues, endoglucanases, exoglucanases and glucosidases. He also reports cellobiose oxidase and cellobiose:quinone oxidoreductase from *P. chryosporium*. Eaton and Hale (1993) say that a range of xylanases and glucomananases have been isolated from this fungus. The hemicelluose degrading enzyme system has been less extensively studied but it appears that these hemicellulases are of the endo- type, their mechanism being random. It is possible that the less-ordered structure of hemicelluloses does not require the synerginistic action of exo- and endo- enzymes, one enzyme type being sufficient.

A range of white rot fungi have been studied over a number of years to investigate the lignin degrading process, notably *T. versicolor* and latterly *P. chryosporium.* The breakdown of lignin is difficult to elucidate because of its complicated structure, it lacks the regular repeating units found in wood polysaccharides. Chang, Chen and Kirk (1980) and Chen *et al.* (1982) studied the degradation of whole wood. Hatakka (1994) working with *P. chryosporium* found that carbohydrates such as glucose are necessary for the degradation of lignin. It seems that lignin-degrading fungi require carbohydrates as a carbon and energy source. Lignin is unable to provide these.

Lignin breakdown requires a variety of enzymes (Kirk and Farrell, 1987). Amongst these are lignin-peroxidase, laccase, Mn-dependant peroxidases, demethoxylases, H_2O_2-generating enzymes and the monomer degrading enzymes, cellobiose dehydrogenase, vanillic acid hydroxylase and trihydroxybenzene dioxygenase. Eaton and Hale (1993) say that lignin-peroxidase, Mn-dependant peroxidases and laccase are the most important. Brown, Glenn and Gold (1990) working with *P. chryosporium* say that manganese, calcium and other cations from wood may facilitate the degradatative processes. They confirm that Mn-dependant peroxidases have important roles during lignin degradation. They say that some white rot fungi have mechanisms that enable manganese and other elements to be released from wood cell walls.

Blanchette (1994) notes that during the early stages of degradation by Ceriporiopsis, copper and manganese is seen to accumulate around the hyphae and wood surfaces. He says that as decay progresses, large concentrations of copper oxalate and zones of manganese dioxide accumulate.

2.3.6 Soft Rot Decay

Soft rot fungi are able to break down and utilise all of the wood carbohydrates but are not as effective as white rot fungi in degrading lignin. The rate of lignin degradation is much slower. Savory (1954) first used the term soft rot to describe an attack of wood in wet environments by fungi classified in the Ascomycota and Deuteromycota. He described the edges of the wet, decayed wood as having a brown, soft appearance and that it cracked and checked when dry. He was describing the appearance of wood exposed in warm, very wet situations such as industrial cooling towers and other aquatic environments.

White rot fungi grow mainly in the wood cell lumina. In the early stages of decay the hyphae may be numerous but as some hyphae autolyse, in the later stages of decay fewer hyphae may be seen. In hardwoods initial colonisation is via ray parenchyma and vessels while in softwoods, ray parenchyma and resin canals are seen to be colonised.

There is considerable variation in decay by different soft rot fungi and differentiation is difficult without considerable ultrastructural or chemical analysis. Nilsson (1988) has suggested that soft rot should include decay by all fungi in the Ascomycota and Deuteromycota. Blanchette (1994) says that soft rot appears to predominate in environments too severe for white rot and brown rot, i.e. excessively wet or dry sites, and in substrates that do not favour the growth and development of other types of fungi. Although soft rot in general is associated with wood in wet environments. Blanchette (1994) found it to occur in relatively dry sites. Soft rot has also been found in wood with high concentrations of naturally decay-resistant compounds and woods preserved with various chemicals (Nilsson and Henningsson, 1978). Elevated temperatures and elevated concentrations of soluble nitrogen may also favour soft rot (Eaton and Hale, 1993). Daniel and Greaves (1989) found that soft rot caused serious decay in the sapwood of some preservative treated hardwood species at the ground-line of stakes and poles. The wood appeared reasonably sound but when broken it broke with 'carroty', short fractures.

Two forms of soft rot are generally recognised (Blanchette, 1994). One type, type I, forms distinct biconical or cylindrical cavities within secondary walls. The other type, type II, gradually degrades the entire secondary wall but leaves the middle lamella intact. Blanchette

says that this is possibly because of the high concentrations of the guaiacylpropane units in the middle lamella giving resistance to decay by soft rot fungi. Type I fungi are common on coniferous wood. Fine hyphae penetrate the secondary walls from the lumina and cause chains of cylindrical cavities , often with pointed ends, within the cell walls (Eaton and Hale, 1993). Eriksson, Blanchette and Ander (1990) describe the cavities as being aligned along the microfibrillar axis of the secondary wall layers.

Nilsson (1974) showed that many but not all soft rot fungi had detectable cellulases, xylanases and mannanases. *Trichoderma viride* produced a complete cellulase complex of endo- and exoglucanases enabling it to degrade crystalline cellulose. He detected exocellulases in *Myrothecium verracaria* and also in *Phialophora malorum.* Bailey, Liese, Rösch, Keilich and Afting (1969) working with *Chaetomium globosum* detected cellulases with a molecular weight of 30 kdaltons. They suggested that the 30 kdalton component would be too large to diffuse freely in the wood cell wall. They reasoned that with cavity type and some erosion types of soft rot attack decay is restricted to the wood surface.

Levi and Preston (1965) say that the rate of cellulose breakdown is governed by the rate of lignin modification, inferring that the quantity of lignin present affects the rate of cellulose degradation. Buswell and Odier (1987) working with hardwoods and softwoods confirmed that the quantity of lignin is a relevant factor in the degradation of cellulose by soft rotters and also showed that the type of lignin was important. They exposed the hardwoods alder and poplar to soft rot fungi and found that the carbohydrates were utilised ahead of the lignin. However when pine was exposed to Paecilomyces species and *Thielavia terrestris* the lignin was removed faster than the carbohydrates. Haider and Trojanowski (1980) using ^{14}C-labelled lignin-models found that species of *Chaetomium Preussia* were able to convert the aromatic ring, side chain and methoxyl groups to CO_2. Leary, Morgan and Newman (1986) using ^{13}C-labelled lignin-model compounds found that lignin methoxyl groups are depleted by soft rot fungi .

2.4 The Chemical Modification of Wood

As long ago as 1909 there was an awareness that wood could be chemically altered to give a product that could have enhanced physical properties. In that year Leo Bakeland developed a 'wood-plastic alloy'. His product was 'Bakelite', manufactured by reacting wood with phenol formaldehyde. It was extensively used for manufacturing telephones and in products where electrical insulation was required. Stamm (1943) working for the U.S. Forest Products Laboratories developed the wood based sheet materials 'Impreg' and 'Compreg'. They were made by impregnating wood with a low molecular weight water-dispersing phenolic resin that was allowed to diffuse into the wood cell walls. The product was dried to remove water and cured by heating. The resulting modified thin veneers could be layered to form aeroplane propellers, machine parts and automobile die models. 'Papreg' was manufactured in a similar way by Erickson and Boiler (1945). They reacted layers of cellulose-rich paper with a phenolic resin to form a counter and table top laminate. 'Arboneeld' products were made from wood impregnated with dimethylol urea (Anonymous, 1946). Goldstein (1955) reacted wood with furfuryl alcohol monomer containing a dissolved catalyst to produce an alkali resistant wood which was used to manufacture certain storage tanks and filter presses. Talbot (1959) produced a modified wood similar to Stamm's 'Impreg' and 'Compreg', he called it 'Flapreg'. Clermont and Bender (1957) reacted wood with acetic anhydride, Stamm (1962) did similar work but found the process to be too costly for commercial use. McMillin (1963) reacted wood with ethylene oxide in vapour form. Wood was effectively reacted with the derivatives of ethylenimine by McClure (1964), he presumed that the reaction was directly with cellulose. Kenega, Fennessey and Stannett (1962) worked on giving wood added dimensional stability by introducing non-polar resins such as styrene as a cell wall bulking agent. Stamm (1964) wrote that chemicals introduced into wood could reduce its hygroscopic nature and give added dimensional stability by modifying the wood's -OH groups. Chemicals bulked into the cell wall he reasoned would keep the wood in a partly swollen state. Research at this stage was mentioned by Bryant (1965) as ushering in a 'new age of wood'

Rowell (1983) states that "chemical modification is defined as a chemical reaction between

some reactive part of a wood component and a simple, single chemical reagent, with or without catalyst, to form a covalent bond between the two". He points to the fact that historically, the bulk of work directed at the chemical modification of wood has been aimed at improving either biological resistance or dimensional stability.

Wood is capable of being biologically degraded because organisms recognise the chemical conformation of the polysaccharide polymers in the cell wall. They have very specific non-enzymatic and enzymatic systems (section 2.2.1) that are able to hydrolyse these polymers into digestible units. The chemical modification of wood for biological resistance therefore presumes the theory that the decay initiating low molecular weight agents that may initiate attack and the fungal enzymes (e.g. cellulases) that degrade the cell wall polymers must directly contact the substrate. The fungal degrade systems have developed to be highly specific. In order to begin the degrade process they must be able to recognise the cellulosic substrate. If the substrate is chemically changed the highly selective degrade reactions may not be able to take place. Such modification can also change the hydrophilic nature of the wood and as water is necessary for decay organisms to function, moisture exclusion from biological sites could be an important part of this protective system.

The major advantages that this method of preservation has over the more traditional processes previously described are that microbial toxicity is unnecessary since the substrate becomes unrecognisable as a food source and the system remains effective over long time periods. Exclusion of water from the cell wall improves dimensional stability by occupying those reactive sites on the cell wall polymers that would normally be taken by water (section 2.1.3). Rowell, Gutzmer, Sachs and Kinney (1976) found that the volume of wood increases with chemical added to about 25 percent weight gain (compared to the dry wood weight) at which point the treated volume is approximately equal to green volume. Should this bulked wood come into contact with water very little additional swelling can take place. This briefly describes the mechanism for the effect of bulking treatments on dimensional stability. Rowell and Ellis (1979) used multicycle water-swelling tests to compare the effectiveness of treatments with propylene oxide, butylene oxide, acetic anhydride, acrylonitrile and methyl

isocyanate. They found that acetylation with acetic anhydride gave the most consistent results.

This enhanced property, together with a permanent lowering of the wood moisture content could go a long way to extending the service life of utilised wood when exposed to wood destroying fungi.

2.4.1 Cell-Wall Bulking Treatments

The process of putting chemical into the wood cell wall is known as 'bulking'. Rowell and Banks (1985) consider three classes of bulking treatments: non-bonded and non-leachable, non-bonded and leachable, and bonded and non-leachable. It is the latter system involving reagents covalently bonded into the wood cell wall which is considered in this work.

Chemical loadings given are calculated as a percentage of the dry wood weight.

2.4.1.1 Bonded, Non-Leachable Treatments

The most effective method of dimensional stabilisation and moisture content control involves the chemical bonding of an organic chemical reagent into the cell wall. This bulking chemical cannot be leached out by water and the hygroscopicity of the wood is reduced. This is the approach followed in this study. Rowell (1983) makes the following general statements about the chemical modification process. The modifying chemicals are chosen to satisfy certain criteria in order to be practically, environmentally and aesthetically acceptable. They must be capable of reaction with wood -OH groups under neutral or mildly alkaline conditions at temperatures up to or little in excess of 120°C. The chemical system, which may need a catalyst, should be simple and capable of sufficiently swelling the cell wall to allow reagent penetration and subsequent reaction. Reaction should be quick and should form products which are stable over time with no toxic side effects. As well as imparting the desired enhancements the treatment should leave the desirable natural properties of wood not adversely affected.

Two classes of reaction may be identified: single-site addition, here the reaction is with a single -OH group and polymerising addition where polymerisation takes place after reagent -OH group reaction.

2.4.1.1.1 Single-Site Addition

2.4.1.1.1.1 Esterification

Most work has been carried out using acetic anhydride. Reaction is easy to achieve, one successful system being without catalyst in xylene as solvent (Goldstein, Jeroski, Lund, Neilson and Weater, 1961):

$$\text{WOOD-OH} + \text{CH}_3\text{-CO-O-CO-CH}_3 \longrightarrow \text{WOOD-O-CO-CH}_3 + \text{CH}_3\text{-CO-OH}$$

Loadings up to 25% are easily attained giving Anti-Swelling Efficiencies (ASE) up to 70%. (Tarkow, Stamm and Erickson 1950; Koppers' Acetylated Wood 1961; Rowell 1982b). ASE is a measure of dimensional stability given to wood as a result of a treatment. Goldstein *et al.* (1961) found that loadings over 17% gave wood resistance to *Coniophora puteana* in a 90 day soil block test. Twelve months weathering of Southern pine treated by this process brought about a slight drop in acetyl content and a fall in ASE. from 78% to 64% (Koppers' Acetylated Wood, 1961). This reaction has the inherent problem that acetic acid is produced as a by-product resulting in up to 50% loss of reagent. Maximum useful reagent loading appears to be about 25%.

Goldstein *et al.* (1961) reacted propionic and butyric anhydrides without catalyst and found the reaction to be slower than that with acetic. Arni, Gray and Scougall (1961) obtained only surface modification with trifluoroacetic anhydride in the esterification reaction. Phthalic anhydride was found to react readily in solution with dimethylformamide (Risi and Arseneau, 1958).

Isocyanates react to form a nitrogen containing ester. Mono isocyanates mainly give single-site reaction:

$$\text{R-OH} + \text{R}^1\text{-N=C=O} \xrightarrow{\text{Base}} \text{R-O-C-N-R}^1$$

2.4.1.1.1.2 Carboxyesterification

Matsuda, Ueda and Masanori (1984) showed that is was possible to modify wood by esterification with cyclic carboxylic acid anhydrides. They used dimethylformamide and dimethylsulfoxide as catalysts/swelling agents and were successful in producing esterified wood, containing pendant carboxyl groups. Their products had an enhanced ability to resist moisture ingress. Matsuda, Ueda and Murakami (1984) found that without a solvent, above 60 $^{\circ}$C, maleic and succinic anhydrides reacted to give carboxyl-group-containing esterified wood. Matsuda and Ueda (1984a) reacted maleic, succinic and phthalic anhydrides to give a product with reduced hygroscopicity. In a later work Matsuda and Ueda (1985) moulded anhydride-modified wood chips into water resistant sheet form. Dunningham and Parker (1992) similarly treated wood with succinic anhydride and were able to attach copper to the product. This modification gave the wood enhanced resistance to decay by brown rot fungi.

Carboxyesterification of wood is potentially an effective method of helping wood products to resist fungal attack. Such modification may, by attachment at and obscuration of wood cell wall polymer -OH groups, bulk the cell wall to such an extent that the water needed by fungal enzymes for the degradation process is denied access. A modifying reagent that is a large molecule may also sterically hinder the highly specific fungal enzymes and prevent their attachment at a potential nutrient source, ie., the long chain polysaccharides, within the cell wall. It may also be possible to use the modification to fix a fungi-toxic metal into the protective system. In this system it could be feasible to fix the metal ion by chelation which would give it a stability that would not be found in a simple metal salt preservative.

The investigative section of this work looks at the possibility of reacting a succinic anhydride derivative with the -OH groups on the wood cell wall polymers and the further possibility of chelating copper, which is fungi-toxic, into the system .

2.4.1.1.1.3 Etherification

Rudkin (1950) successfully reacted wood with dimethyl sulphate and methyl iodide but found reaction rate was very low and repeat reactions were needed to achieve loadings of 20%.

dimethyl sulphate

$$\text{WOOD-OH} + CH_3I \longrightarrow \text{WOOD-O}CH_3 + HI$$

Alkyl chlorides react with wood reactive sites but the hydrochloric acid produced during the reaction causes a great deal of wood degradation. Kenega (1957) and Kenega and Sproull (1951) reacted allyl chloride in pyridine and initially gained a high degree of dimensional control. Risi and Arseneau (1957) did similar work but found that the stabilising effect was lost on drying and re-soaking. The proposed reaction is analogous to that with alkyl iodide, ie.

$$\text{WOOD-OH} + \text{R-Cl} \longrightarrow \text{WOOD-O-R} + HCl$$

Acrylonotrile reacts with wood in the presence of an alkaline catalyst to give a cyanoethylated product:

$$\text{WOOD-OH} + CH_2{=}CH\text{-}CN \longrightarrow \text{WOOD-O-}CH_2CH_2CN$$

Goldstein, Dreher and Jeroski (1959) found that wood treated in this way gave resistance to *G. trabeum* at 25% weight gain in soil block tests.

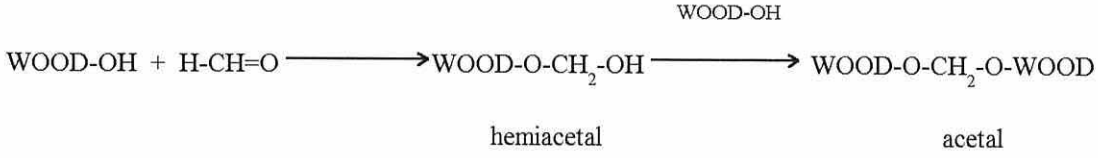
2.4.1.1.1.4 Cross-Linking with Aldehydes

Several workers have investigated the "acetal" reaction between wood hydroxyls and aldehydes. For example, Tarkow and Stamm (1953) reacted acetaldehyde and benzaldehyde using nitric acid or zinc chloride as catalyst. Weaver, Nielson and Goldstein (1960) reacted benzaldehyde but achieved only 40% ASE. These workers also achieved reaction with glyoxal, glutaraldehyde and hydroxyadipaldehyde using zinc chloride, magnesium chloride, phenyl dimethylammonium chloride and pyridinium chloride as catalysts.

Tarkow and Stamm (1953) found the mechanical properties of formaldehyde treated wood

were greatly reduced. The reaction between wood hydroxyls and formaldehyde is a two step reaction.

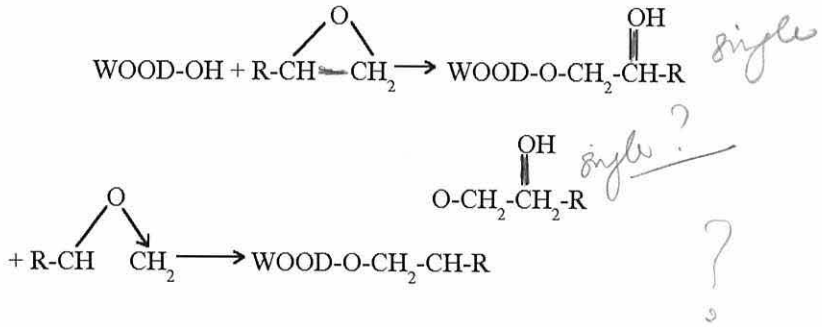
Where the two -OH groups involved are on different cellulose chains the reaction is called cross-linking:



2.4.1.1.2 Polymerising Addition

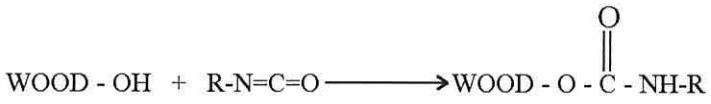
Catalysed in mildly basic conditions, epoxides are found to react with wood with the creation of a new -OH group.

This polymerises by reacting with another epoxide group, etc.. (Rowell and Gutzmer 1975).



Rowell *et al.* (1976) found that at reagent loadings of about 25% the cell wall started to rupture as further reaction occurred within the cell wall. Beyond this point the ASE. was drastically reduced. McMillin (1963) obtained an ASE. of 60% by reacting ethylene oxide using triethylamine as catalyst. Rowell and Banks (1985) state that a new -OH group is formed during the reaction which can then react with another reagent epoxide group and polymerisation can occur.

Aliphatic isocyanates react easily with alcoholic hydroxyl under homogenous conditions (Rowell and Ellis, 1979). In the reaction of wood hydroxyls with isocyanates a nitrogen containing ester is formed:



Baird (1967) easily reacted phenylisocyanate in dimethylformamide as solvent/catalyst. Clermont and Bender (1957) reacted ethyl-, n-butyl- and t-butyl- isocyanate in `dimethylformamide to give weight gains of up to 30%. Rowell and Ellis (1979) reacted methyl isocyanate without catalyst with no destructive effects to 30% weight gain. Above this loading they observed cell wall rupture.

Phenyl isocyanate, n-propyl isocyanate, tolylene-2,4-diisocyanate and n-butyl isocyanate were reacted with 35% dimethylformamide (Rowell and Ellis, 1979). The specimens showed better volume and dimensional stability than unmodified samples.

West and Banks (1986) reacted butyl-isocyanate with swollen and non-swollen wood. Reactions were carried out with and without catalysts. Swelling agents toluene, acetone and methyl acetate were used and the reactions catalysed by triethylamine and pyridine. In the non-catalysed reaction the increased rate of reaction observed in the absence of a catalyst is limited when only swelling agents were used. In the base-catalysed reaction, where there is increased reaction, the reaction is strongly influenced both by the accessibility of functional groups in the wood (the swelling effect) and by chemical (here base) catalysis. There is a strong suggestion that effective reaction requires the wood to be swollen and a chemical catalyst to be present for maximum reaction rates to be achieved.

Baechler (1959) produced cyanoethylated cellulose by reacting acrylonitrile with cotton in sodium hydroxide. Reaction was easy to achieve but in the presence of large amounts of water it is thought that the ether linkage was hydrolysed with the reformation of a -OH group. He successfully treated pine sapwood with acrylonitrile and ammonia but the ammonia was found to be very difficult to handle.

2.5 Modification for Resistance to Fungal Decay

The acetylation reaction with wood has resulted in a product that gives wood an enhanced resistance to decay. Goldstein *et al.* (1961) found that reagent loadings of 17% and over gave resistance to the brown rot fungus *C. puteana* after a 90 day soil block test. Rugevitsa (1977) and Svalbe, Ozolina, Truskne and Vitolins (1978) confirmed these findings.

Stamm and Baechler (1960) suggest that the decay resistance of chemically modified wood is explained on the basis of the inability of the wood cell walls to absorb the moisture needed by decay fungi. Decay was eliminated in acetylated wood when the acetyl content was above 30%. When 50% of the optimum acetylation had occurred or when an estimated 50% of the wood -OH groups had been replaced, there was complete protection against fungal attack. These workers state that acetylation first occurs in the amorphous regions of cellulose and on the surface of the crystallites. It has been estimated (Stamm and Tarkow, 1947) that 50% of the -OH groups in wood occur in these accessible regions and that half of these may satisfy themselves in inter-crystallite hydrogen bonding. This reasoning means that only 25% of the -OH groups are available for reaction.

Acetylation treatments (Peterson and Thomas, 1978) at loadings of 15% and up gave excellent protection to yellow poplar and loblolly pine when attacked by *G. trabeum* and *Coriolus versicolor* over a six week test period. There was no ultrastructural evidence of cell wall decomposition. It was observed that the modifying reagent was not directly toxic as the fungi continued to grow into agar solution when sections of infected wood were removed and placed in that medium. Bore holes were found at the only at the lower levels of modification. There was no gelatinous sheath around the hyphal tips and little or no enzyme activity in the bore hole areas.

Gjovik & Davidson (1973) tested cyanoethylated stakes and found that they had an average life of 7.8 years in ground contact compared with 3.9 years for untreated material.

Kalnins (1982) reacted allyl isocyanate, methyl isocyanate, allyl isothiocyanate and phenylhydrazine at 100°C using pyridine as a swelling agent. Decay agents in test of the products were the brown rot fungi *G. trabeum* and *Lentinus lepideus,* and the white rot fungus *C. versicolor.* Allyl isocyanate and methyl isocyanate were both found to react with

the wood polymers to give the carbamate (urethane) derivatives. Resistance to decay was found to be superior to that shown by wood modified by acetylation. With allyl isothiocyanate the main reaction appeared to be one of homopolymerisation only, leaving the isothiocyanate groups intact. There seemed to be no chemical bonding with the wood. Treating with phenylhydrazine gave very limited decay resistance with no uniform protection against the various fungi. The following reagents were also reacted: methyl isothiocyanate, benzyl isothiocyanate, phenyl isocyanate and phenyl isothiocyanate. Weight gains of several percent were recorded but addition to the wood could not be confirmed by Infrared spectroscopy. Lutomski (1975) reacted beech wood with a diisocyanate to 50% weight gain and found that the weight loss after 6 weeks exposure to the brown rot fungus *C. puteana* was only 4.5 - 8.1%.

Goldstein *et al.* (1959) reacted wood with acrylonitrile in sodium hydroxide and achieved weight gains up to 30%. At 25% loading there was no weight loss in soil block tests with *C. puteana*. Baechler (1959) investigated this modification system. He leached the modified wood and found that the leachate was non-toxic. He concluded that the decay resistance was a function of a mechanical bulking of the cell wall pore structure.

Epoxides have been used to modify wood in order to increase its resistance to decay. In two month soil burial tests Pihl and Olssen (1968) found that epichlorohydrin- or dichlorohydrin treated specimens did not decay at all. Rowell (1982a) reacted wood with butylene oxide to 20% loading and found that the modified wood resisted attack by ground organisms after 7 years. In soil block tests Rowell (1982a) found that butylene oxide modified wood loaded to 17% and over was resistant to decay by the brown rot fungus *C. versicolor* and the white rotter *C. versicolor*. An interesting modifying reagent used by Goldstein *et al.* (1959) was β-propiolactone which when loaded in wood to 25% saw weight losses of less than 2% after attack by the brown rot fungi *C. versicolor*, *G. trabeum* and *C. puteana*. Research was discontinued when β-propiolactone was classed as a carcinogen.

Mallari, Fukuda and Morohoshi (1989) modified wood chips of Giant ipil-ipil (*Leucaena leucocephala*) with propylene oxide and obtained weight gains of 3% to 18% with triethylamine as catalyst. Reductions in weight loss after attack by the white rot fungus *C.*

versicolor were observed at weight gains as low as 5%. Attack by the brown rot fungus *Tyromyces palustris* was totally prevented at reagent loadings of 10% and greater. Weight gain of reagent was found to be proportional to reaction time but was more effective when triethylamine was added progressively. Mallari, Fukuda, Morohoshi and Haraguchi (1989) reacted Giant ipil-ipil wood with acetic anhydride, maleic anhydride glycerol and propylene oxide triethylamine. All modifications were successful in combating decay by the brown rot fungi *G. trabeum,* and *T. palustris,* the white rot fungi *C. versicolor* and *Pycnoporus coccineus* and the soft rot fungus *C. globosum.* They found that many of the sample blocks were deformed after reaction, possibly because of over reaction. Acetic anhydride was biologically effective at a loading of about 16%, decay weight loss of 1% being observed after a 16 week soil test. They worked exclusively on particleboards and results were inconclusive. It is suggested that the presence of modifying reagent interfered with the action of the binding resins.

Rowell & Ellis (1979) assessed the fungal resistance of wood reacted with ethyl isocyanate, n-propyl isocyanate, n-butyl isocyanate, isopropyl isocyanate, 1,6-diisocyanatohexane, isophorone diisocyanate, phenyl isocyanate, p-tolyl isocyanate and tolylene-2,4-diisocyanate. All reagents were reacted at 120°C in a nitrogen atmosphere at 150 psi. The decay fungus in test was the brown rotter, *G. trabeum.* Catalysts/solvents, dimethylformamide, dimethylsulfoxide and triethylamine were used. With up to 5% catalyst present, phenyl isocyanate, p-tolyl isocyanate, 1,6-diisocyanatohexane, isophorone and tolylene-2,4-diisocyanate mostly formed non-bonded polymers in the wood voids. The resultant non-bonded lumen filling polymers did not increase dimensional stability or resistance to decay. Ethyl isocyanate, n-propyl isocyanate and n-butyl isocyanate reacted with the wood with no solvent or catalyst. The modified wood showed 30 - 50% better dimensional stability and a high resistance to decay. Wood reacted with n-butyl isocyanate using 35% dimethylformamide as catalyst showed 30 - 35% weight gains. Specimens were 70% more dimensionally stable and showed a high resistance to decay, losing only 2% in decay tests with *C. puteana.* They concluded from the levels of methyl isocyanate required to obtain decay resistance (about 20%) that the mechanism of effectiveness was due to non-toxic

substrate modification not toxicity. Free methyl isocyanate they say, is toxic but once reacted the urethane is not.

In conclusion. Work so far has shown that chemically modifying wood can give it enhanced resistance to decay by fungi. A successful protection system has been seen to affect a chemical modification of the substrate that may render it unrecognisable as food to the fungi and also to bulk the wood cell wall, preventing the access of water which is required by fungi for successful attack. Most of the research in the area of chemical modification has been concerned with the reaction of a chemical reagent and the -OH groups that are abundant on cellulose, hemicelluloses and lignin. The -OH groups are also the attachment point for water molecules. Thus modification of the -OH group is essentially the modification of wood.
The investigative part of this work will continue the investigation of the wood modification process and of the possible resistance to decay that chemical modification can bring about.

Chapter 3

Chemical Modification Reactions

3.0 Selection of Reagents

The reagents used to modify the wood to test for increased biological effectiveness were succinic anhydride, here referred to as SA, and an alkenyl derivative of this compound. Succinic anhydride, a 4-carbon cyclic saturated compound is the simplest carboxy esterifying agent. The derivative, a more complex unsaturated compound, included a 16 to 18 carbon aliphatic chain having a carbon = carbon bond with an average chain length of 17 carbons. This derivative will be described as ASAC17. The long carbon chain was included to import the hydrophobic effect, the implications of which would be investigated in a later bio-assay (Chapter 4). It is used as a size in the manufacture of paper (Figure 3.0.

Succinic anhydride was chosen for a number of reasons. It has been shown to react readily with wood by other workers including Matsuda and Ueda (1984b) where it was successful in reducing substrate hygroscopicity. It is a readily available and relatively inexpensive compound and is easy to handle. It particularly suited this work as derivatives, ASAC12 and ASAC20 (see below) were available free of charge.

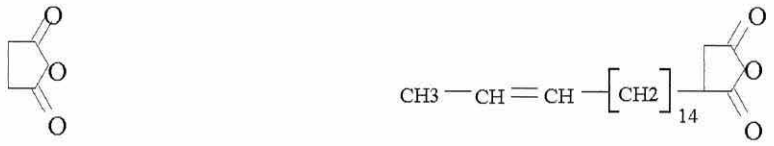


Figure 3.0 The structure of SA and ASAC17 respectively

Succinic anhydride derivatives with different carbon chain lengths, though not normally commercially available were synthesised for this work. Those having aliphatic carbon chain lengths of 12 and 20 carbons were used to modify wood samples to observe the effect that the different chain lengths would have on the rate and amount of reaction. These will be referred to as ASAC12 and ASAC20. They were not be used in subsequent biological testing.

3.1 Selection of Solvent

Ideal solvents for use in reactions with wood need to have particular properties. They should swell the wood structure sufficiently to allow ingress of the modifying chemical system and where necessary have a catalytic effect on the reaction. Ashton (1973) is one of many workers who have investigated the swelling of wood in various solvents and he observed that the rate and amount of swelling is both species and density dependant. He says that the swelling of wood by water results from the hydration of -OH groups in the hemicelloses and lignin, on the surface of cellulose crystallites and in the amorphous regions of the cellulose chains. He describes one approach to greater reactivity as swelling the wood so that more functional groups are available to the modifying reagents. Water is a good swelling reagents but will of course also react with reagents that react with wood -OH groups. He discusses the swelling properties of dimethyl sulfoxide, dimethylformamide and N-methyl pyrrolidone. He found that dimethylformamide caused the most rapid swelling of wood in most cases.

N-N, dimethylformamide (DMF) was used throughout this work as it has both the swelling and catalytic properties necessary to drive the reaction. Clermont and Bender (1957) used DMF as catalyst and swelling agent in the acetylation of cellulose and whole wood in a liquid phase procedure. Rowell and Hart (1981) report a volumetric swelling coefficient of 12.8 to DMF in relation to a water swell value of 10.0. Matsuda, Ueda and Masanori (1984) used DMF in esterification reactions with various carboxylic acid anhydrides and comment on its suitability as a swelling agent and catalyst.

3.2 Experimental

3.2.1 Selection of Wood Species and Specimen Geometry

Scots pine (*Pinus sylvestris* L.) was chosen as being readily available, permeable and easily biodegradable. The timber was free from decay, straight-grained and without knots or visible concentrations of resin. It was exclusively sapwood and the average rate of growth was of 2.5 to 8.0 annual rings per centimetre; the proportion of latewood in the annual rings did not exceed 30% of the whole. Growth ring pattern was uniform between samples and they were end-matched to be as similar in structure as possible. Specimens were machined to the following dimensions: 20 mm tangential, 20 mm radial and 5 mm longitudinal. The short longitudinal dimension was chosen to ensure easy penetration of reagent throughout the cellular structure. Prior to reaction the specimens were lightly sanded to remove loose fibres.

3.2.2 The Anhydride Reaction

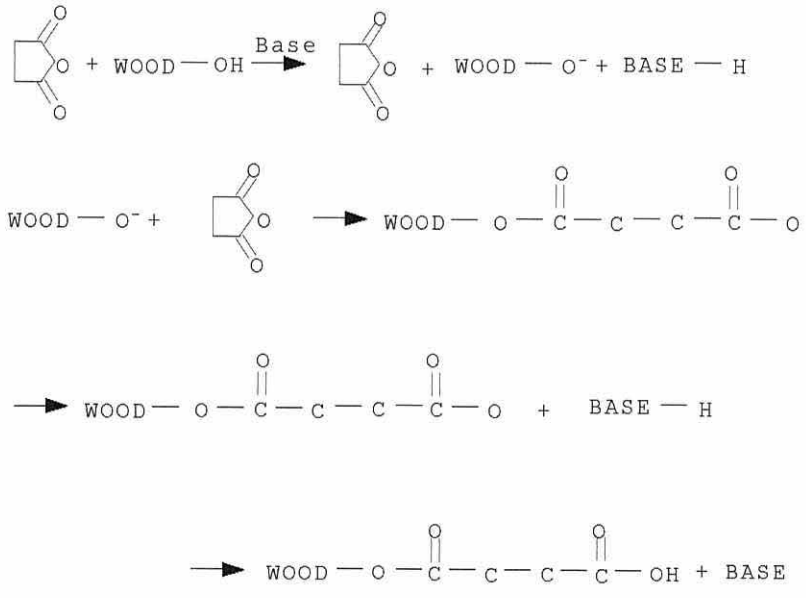


Figure 3.1 The base catalysed reaction of SA with wood hydroxyl groups

The anhydride adds to the wood by ring-opening of the anhydride to give esterified wood-bearing pendant carboxyl groups (Figure 3.1). The product has a carboxylic acid group that may be used as an active attachment point in further reactions.

3.2.3. Reaction Procedure

Prior to reaction the wood extractives were removed by refluxing sets of 40 samples for 4 hours in a mixture of toluene, ethanol and acetone in the ratio 4:1:1 v:v. This ensured that any reagent reaction would be with the required cell wall components and not with the extractive material. In commercial processing it would be unrealistic to attempt to remove extractives but at this experimental level it was thought worthwhile to exclude any possible side reactions from the equation.

The blocks were then placed in a fume cupboard for about two hours to allow the solvents to evaporate. When ostensibly free of solvents they were oven dried to constant weight at 105°C to ensure that all solvents were removed. Finally the samples were stored over anhydrous phosphorous pentoxide until required. Rowell (1982a) found that the amount of water present in the wood at the time of reaction was important and that 2 - 5% was best for modification. Above this level the moisture hydrolises the anhydride to the acid causing a loss of reagent therefore it was essential to keep the blocks dry prior to reaction.

The dry blocks were numbered and their weights recorded. This is referred to as the "initial dry weight" against which reagent loadings were calculated.

A selection of specimens were carefully measured in the longitudinal, radial and tangential directions and their volumes calculated and recorded. After reaction and drying the volume of these blocks were re-calculated and comparisons were drawn between the volume increase and the theoretical volume of chemical added.

Batches of 20 blocks were placed into a 500 ml beaker containing a molar solution of reagent in DMF and held below the surface with nylon gauze and glass weights. The beaker was put into a vacuum dessicator (Figure 3.2) and impregnated with the modifying solution for 20 minutes under vacuum. The vacuum was released and the blocks were allowed to soak for

one hour at atmospheric pressure. This procedure was to ensure that the modifying chemicals had thoroughly penetrated the wood before reaction was attempted at higher temperatures.

Sets of 20 samples were placed into a round bottomed reaction flask containing an excess of modifying reagent in molar solution with DMF. The inert gas argon was continually introduced into the flask using a rubber reservoir and a hollow tube and needle inserted into the neck of the flask. This is to ensure that reaction took place in a dry inert atmosphere. After sealing, the reaction vessel was securely held in a pre-heated oil bath in a fume cupboard (Figure 3.3). The temperature and duration of reaction were varied to give a range of reagent loadings in the region of 5 - 30%.

After reaction the samples were removed from solution and cleansed of any residual reagent and solvent by refluxing in acetone for 2 hours. Unreacted chemicals remaining in the cell wall could lead to misleading results and the clean-up procedure is to overcome this problem. After oven-drying to constant weight at 105°C, which took about 4 hours, the samples were allowed to cool over a desiccant and re-weighed. The weight gain was calculated as a percentage of the initial dry weight and this was taken to be the amount of reagent that had been taken up by the wood block.

This procedure was repeated for SA, ASAC12, ASAC17 and ASAC20. In order to determine the effects of the solvent on the reaction, a set of ten blocks was subject to the same preparation and clean-up process but was reacted in DMF only.

Figure 3.2 Reagent Impregnation Apparatus

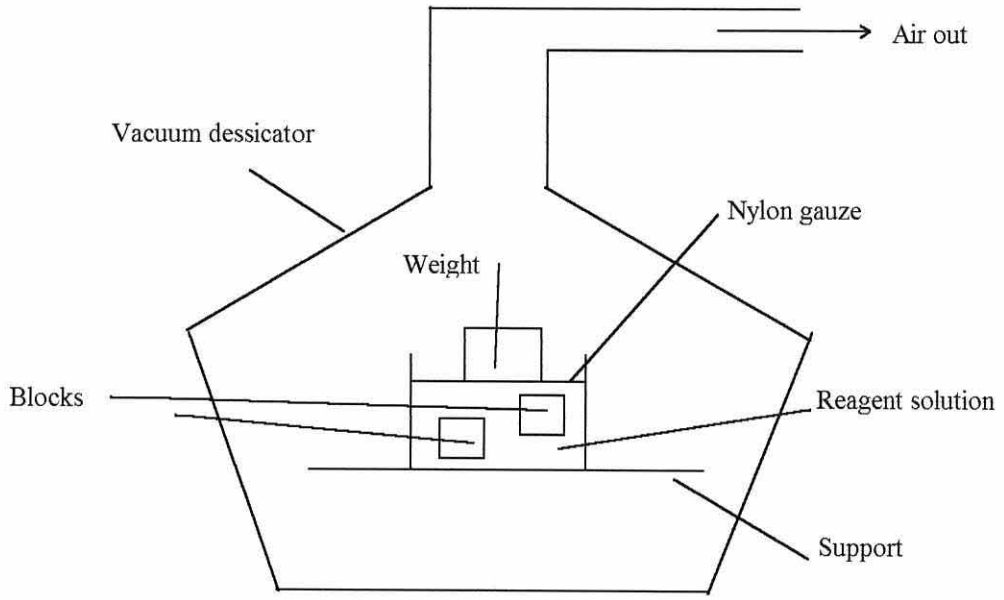
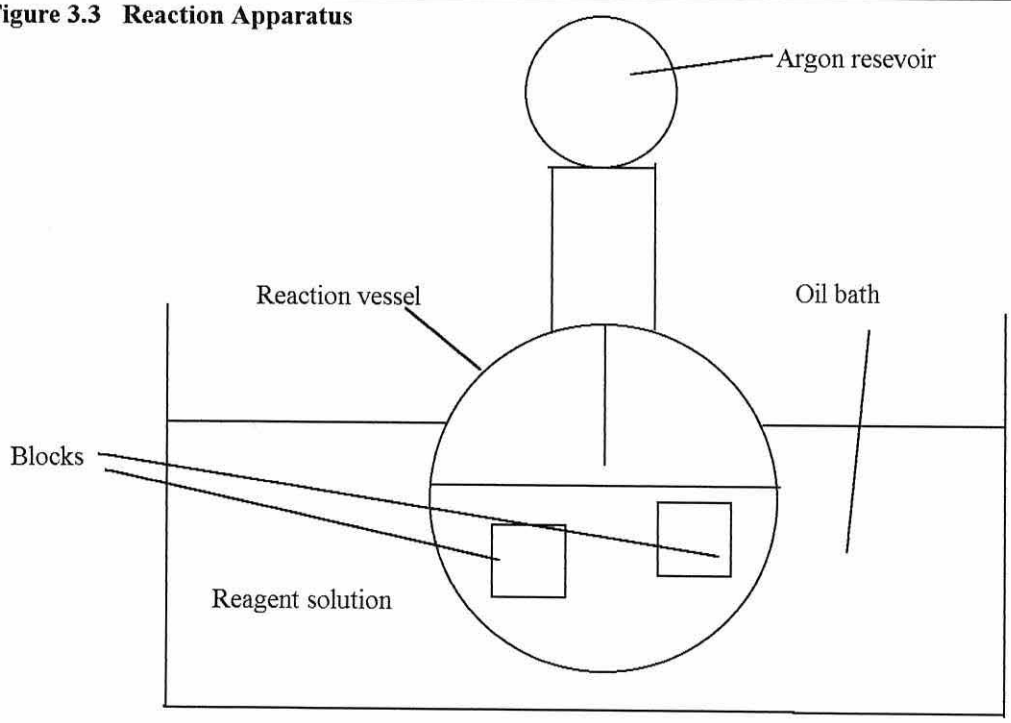


Figure 3.3 Reaction Apparatus



3.3 Determination of Extent of Reaction

3.3.1 Weight Gain

The gain in weight of individual samples after reaction was calculated by subtracting the initial dry weight from the dry weight at the end of reaction. This weight gain was taken to be the amount of reagent remaining in a bonded state within the wood cell structure. Infrared analysis (section 3.5.3) helped to confirm that the required reaction had taken place. The weight gain was calculated as a percentage of the initial dry weight as follows:

$$\text{Weight Gain} = \frac{\text{Dry weight after reaction - Initial dry weight}}{\text{Initial dry weight}} \times 100\ \%$$

3.3.2 Degree of Substitution (DS)

This is an estimate of the amount of wood hydroxyl groups that have been substituted by a modifying reagent in a single site reaction. The calculation of this value is here based upon the approximate composition of pine being 40% cellulose, 30% hemicelluloses and 30% lignin and all hydroxyl groups being available for substitution. It equates to 1.5×10^{-2} moles of hydroxyl groups per gram of wood. The calculation of this value is given in Appendix 3.1.

For each individual sample it is calculated as:

$$\frac{\text{Weight gain during reaction}}{\text{Molecular wt. of reagent x Initial dry weight x Estimated moles of hydroxyl groups in 1 gram of wood}}$$

3.3.3 Infrared Analysis

Infrared spectroscopy (Marchessault, 1962; Chou, 1972) is a useful technique for chemical analysis. The complexity of molecular vibrations virtually assures that two different molecules or substituent functional groups cannot produce identical infrared spectra. In

wood modification analysis various workers have made use of this technique to help confirm the success of the modification process (Rowell, 1982a, 1983a; Matsuda, 1987).

Here the analysis was done on a Beckman IR-20A Double Beam Infrared Spectrophotometer, obtaining normal scans between 4000 and 250 $^{cm-1}$.

Potassium bromide pellets were prepared by thoroughly mixing 6% of dry modified wood flour into the dried I.R. grade KBr. This mixture was made into a transparent disc approximately 1 mm thick and 1 cm diameter by compressing under vacuum at 10,000 psi. for 2 minutes. Pellets were prepared from wood modified to various reagent loadings for SA and ASAC17.

3.4 Water Leaching

A selection of specimens were leached in water to test the permanence of the modification in moist conditions. Only samples modified with SA and ASAC17 were leached, since only wood modified with these two reagents were subject to bio-assay (Chapter 4) where the stability of the modifying system in wet conditions is important. Ten samples were chosen for each of the two reagents covering a loading range of approximately 5 - 30%. A control set of 10 unmodified blocks were similarly leached .

The leaching was carried out according to the European Standard prEN 84. Edition 1, March 1978. The selected specimens were conditioned for two weeks at 65% RH. and 22 ^{0}C. In batches of 5, they were then placed in a 500 ml glass beaker. Sufficient deionised water was introduced to completely cover the blocks which were held below the surface by a plastic mesh and glass weights. The beaker was placed in a vacuum dessicator and a vacuum was applied for 20 minutes before release. The test specimens were left immersed at atmospheric pressure for 2 hours to ensure complete saturation. The water was emptied from the beaker and the beaker was refilled with fresh water to a ratio of about 5 volumes of water to 1 volume of wood. The specimens did not need any ballast as they did not float after the impregnation procedure. The blocks remained immersed in water for 14 days at room temperature with 9 changes of water as follows: the water was changed after days 1 and 2

and changed regularly for the remaining time at a minimum interval of 1 day and a maximum interval of 3 days. They were then removed from the water, superficially dried with absorbent paper and placed on their narrow sides on a glass plate leaving enough space between them to allow a free flow of air. They were allowed to stand for 2 weeks at 65% RH. and 22°C when they were weighed and any weight change recorded as a percentage of the dry weight before leaching. It is not correct to assume that all or part of any weight loss during leaching can be attributed fully to loss of bonded reagent as water soluble extractive material could equally well be removed. This factor could have been eliminated by leaching the unmodified wood in water prior to chemical modification. However the blocks used were very small and it is thought that after leaching in toluene, ethanol and acetone at an elevated temperature for four hours before reaction and acetone for two hours after reaction (section 3.2.3) any non-bonded material would have been leached out. Pre-leaching however, in any future work could help eliminate this problem.

3.5 Results and Discussion

3.5.1 Weight Increase

3.5.1.1 Reaction of SA with the Wood Hydroxyl Groups in Scots Pine Sapwood

Table 3.1 and Figure 3.4 shows that it was possible to accomplish loadings of up to about 26% by controlling the duration and temperature of reaction.

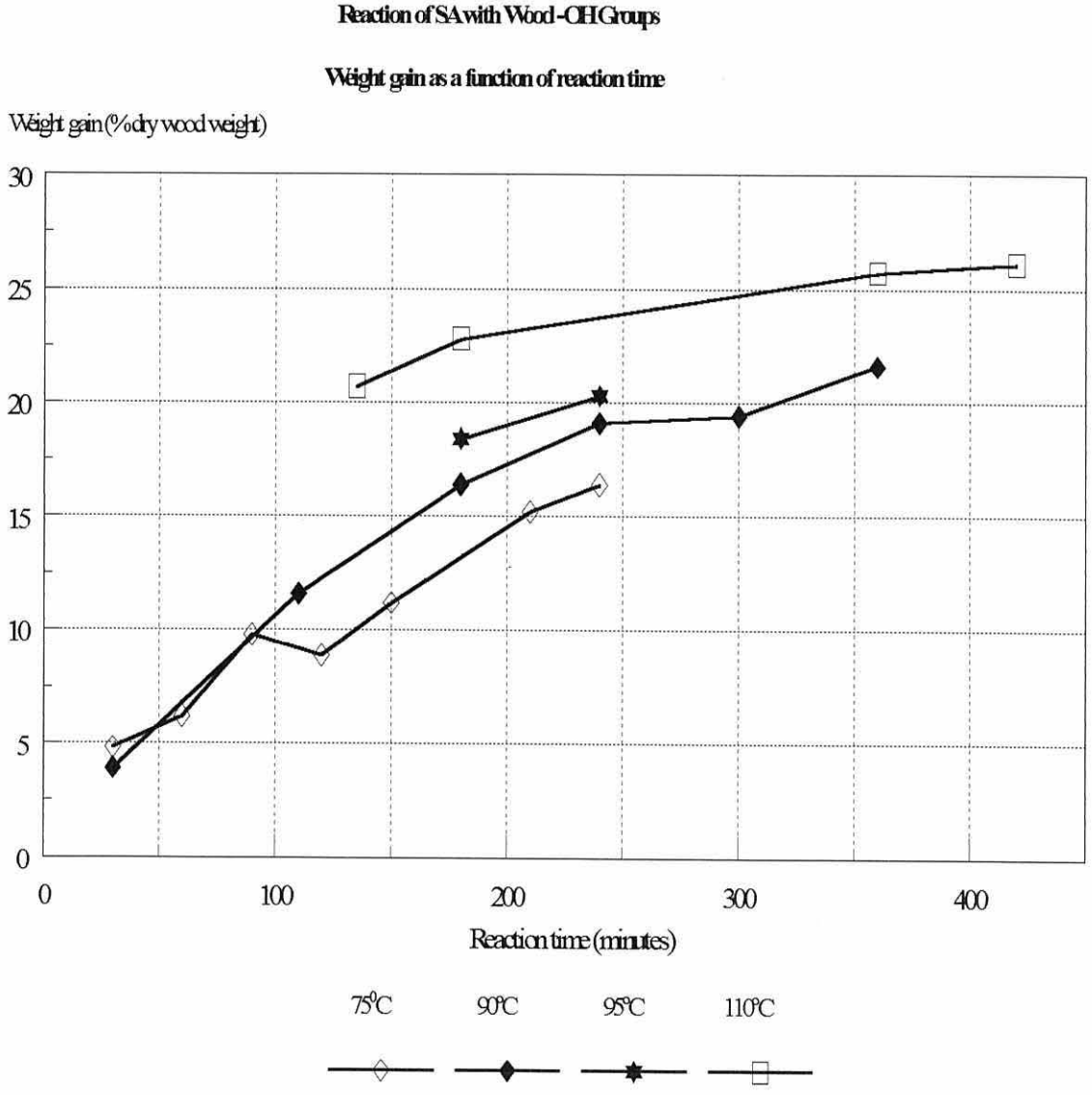


Figure 3.4 (Table 3.1)

Table 3.1 indicates weight gain of SA as a function of reaction time and temperature. the weight gain is calculated as a percentage of the initial oven dry weight of the wood and the degree of substitution is a measure of the number of substituted hydroxyl groups in the wood (Appendix 3.1). Degree of substitution 1 indicates that all hydroxyl groups have been substituted. Each item is the average of a batch of 5 samples.

Reaction Time (mins)	Weight Gain (%)	Standard Deviation	Degree of Substitution
Temp. 75oC			
30	4.8	2.3	0.04
60	6.2	0.5	0.05
90	9.8	0.8	0.07
120	8.9	1.5	0.07
150	11.2	1.5	0.09
210	15.2	0.3	0.12
240	16.4	0.6	0.13
Temp 90oC			
30	3.9	0.9	0.03
110	11.6	0.9	0.09
180	16.4	0.9	0.13
240	19.1	0.6	0.16
300	19.4	0.9	0.16
360	21.6	1.2	0.19
Temp. 95oC			
180	18.4	1.4	0.16
240	20.3	0.9	0.17
Temp. 110oC			
135	20.7	0.3	0.18
180	22.8	0.4	0.2
360	25.7	0.6	0.24
420	26.1	0.5	0.24

Increasing the temperature and reaction period had a marked positive effect on the amount of reagent inducted into the wood.

The reaction system appears adequate for loading this reagent as loadings of up to 26% were attained. Various workers including Clermont and Bender (1957) Ashton (1973) Rowell and Hart (1981) and Matsuda, Ueda and Masanori (1984) have found DMF to be a successful swelling agent/catalyst in chemical modification reactions. Whilst in this work reagent-only

reactions were not carried out it can be assumed that DMF is effective as a catalyst for the reaction. The maximum loading was 26% and this took 420 minutes at 110°C to achieve. Extending the reaction time and increasing the temperature beyond these parameters did not significantly raise the loading above this level. Samples from the reaction at these increased parameters were inspected using a light microscope and some cell wall checking was apparent. This concurs with Rowell (1982b) who found that with acetylation reaction, increased reaction time did not result in continual chemical reaction and that there was subsequent cell wall rupture. Possibly the cell wall can accommodate no further reagent without losing its structural integrity. Rowell and Ellis (1981) observed this with methyl isocyanate at loadings of 25 - 30% when they observed splitting in the tracheid wall. Regardless of reaction time they found that a reagent weight gain of 30% seemed to be the upper limit of the chemical reaction before cellular disruption occurs. The fibre saturation point of wood is about 25 - 35% of the dry wood weight which closely parallels the observed useful reagent upper loading limit for wood modification. It appears that the wood cell wall is able to be bulked either with water or with chemical compound only up to about 30%. Thereafter, certainly with chemical reagent, physical deterioration occurs.

Previous workers have had little problem in reacting SA with cell wall polymers. Matsuda and Ueda (1985) found that SA entered into the esterification reaction with hydroxyl groups even at room temperature. The loadings they attained were with wood flour and the increased surface area of wood in this state probably accounts for the higher loadings achieved. They observed 42.2% weight increase based on dry wood weight, reacting at 20°C for 15 hours. They found that reacting at 60°C for 3 hours gave a weight gain of 18.6% and that above 80°C the progress of the reaction was "remarkable". Modifying reagent is not able to penetrate the solid wood specimens used here with similar ease.

Loading was reasonably constant between blocks at each level of loading over the whole of the weight gain range. The only high variation was for the set which averaged 4.8% loading (30 mins. @ 75°C., SD. 2.3). This appears to confirm that the selection process designed to

produce physically uniform samples works well. Small density differences between blocks can result in a significant difference in reagent loading.

As significant weight increases were observed after the clean-up process which involved refluxing the modified samples in acetone it is reasonable to assume that the reagent had been bonded into the wood structure. Rowell (1983) states that if the chemical that caused the cell wall to swell to accommodate it is bonded to the cell wall polymers then subsequent solvent extraction would not be able to leach it out. If the SA in this experiment had been non-bonded then the weight increase on drying would have been minimal, but no experiments with unreacted reagents were made to confirm this.

The Degree of Substitution is a useful comparative indication of loading but as this value is based on all of the estimated hydroxyl groups in the wood being available for reaction then the actual amount of hydroxyl substitution is probably higher than those stated in Table 3.1. It has been estimated (Stamm, 1964) that 65% of the cellulose in wood is crystalline and therefore probably not accessible for reactions involving these hydroxyl groups. To give more precise hydroxyl group substitution values would require precise analysis of hydroxyl group substitution sites in all of the cell wall components for Scots pine. This is outside the scope of this work.

The wood became darker during reaction but not necessarily in proportion to increased reagent loading. Rowell (1982b) observed a similar colour change in pine wood when using DMF as solvent.

3.5.1.2 Reaction of ASAC12, ASAC17 and ASAC20 with the Wood Hydroxyl Groups in Scots Pine Sapwood

Although ASAC17 was to be the only aliphatic anhydride to be used in biological testing, the results from the ASAC12 and ASAC20 reaction are included as it is interesting to see the effect that the increasing chain length has on the rate and amount of reaction.

It was relatively easy to react the three alkenylsuccinic anhydrides into the cell wall (Table 3.2) although as the aliphatic carbon chain length increased it became increasingly more

difficult to react equal amounts of reagent at the same reaction parameters. To bring about higher loadings required the reaction to be carried out at higher temperatures and for extended reaction times. The ease of loading relative to aliphatic chain length is ranked as ASAC12 > ASAC17 > ASAC20. Figure 3.5 compares weight gain of reagent as a function of reaction time, the graph for SA is included indicating that it reacts more easily than the bulkier reagents. There are a limited amount of hydroxyl groups available for reaction in the cell wall and the smaller molecules may be able to gain access to more of these sites without the extra energy and swelling of the cell wall structure required by the larger compounds.

Table 3.2 indicates weight gain of ASAC12, ASAC17 and ASAC20 as a function of reaction time and temperature. The weight gain is calculated as a percentage of the initial oven dry weight of the wood and the degree of substitution is a measure of the number of substituted hydroxyl groups in the wood (Appendix 3.1). Degree of substitution 1 indicates that all hydroxyl groups have been substituted. Each item is the average of a batch of 10 samples apart from the data for ASAC20 which are averages of batches of 5 samples.

Reaction Time (mins)	Weight Gain (%)	Standard Deviation	Degree of Substitution
ASAC12			
120oC			
120	18.4	0.6	0.05
180	23.7	0.4	0.06
240	29.3	0.9	0.08
300	34.1	0.6	0.09
ASAC17			
110oC			
60	4.4	0.6	0.01
120	7.2	0.5	0.02
180	9.3	1.5	0.02
240	11.8	0.8	0.02
120oC			
60	5.3	1.1	0.01
120	10.3	0.6	0.02
180	16	1.1	0.03
210	18.9	1.5	0.04
240	19	1.1	0.04
130oC			
270	27.2	0.8	0.06
360	31.4	1.5	0.07
420	34.1	1.8	0.07
480	35.4	1.5	0.07
135oC			
150	25.9	2.1	0.05
270	33.4	2.1	0.07
300	34.3	3.6	0.07
360	36.9	2.7	0.08
ASAC20			
120oC			
60	5.2	0.5	0.01
120	8.8	0.4	0.02
180	12.8	1.5	0.03
240	14.5	1.5	0.03

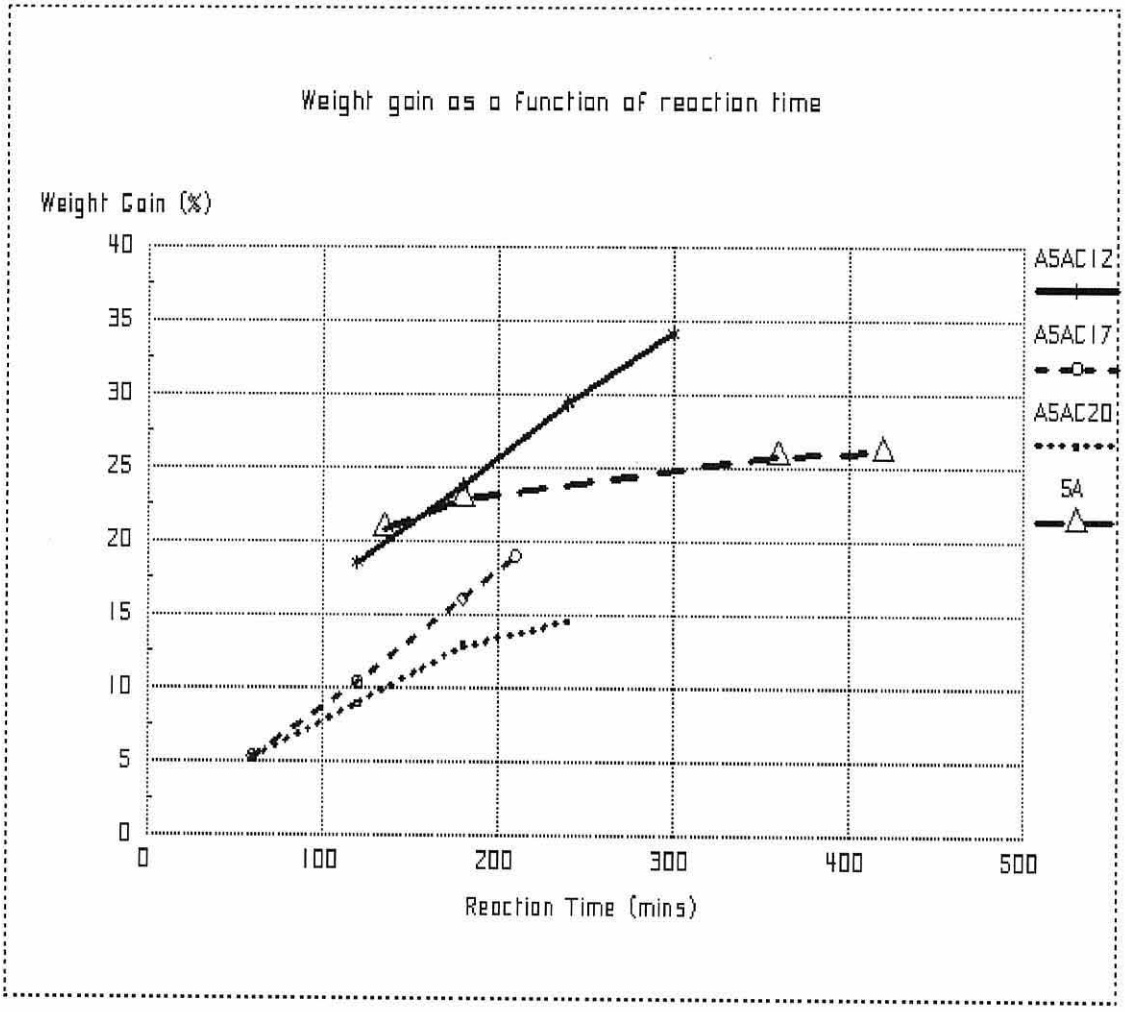


Figure 3.5 Graph comparing the rates of reagent uptake in wood modified with SA, ASAC12, ASAC17 and ASAC20. The three aliphatic anhydrides were reacted at 120°C and the SA at 110°C.

The ASAC17 and ASAC20 molecules are large in comparison with some other treating agents, the molecular weight of SA is 100, ASAC17, 336 and ASAC20, 378. A low degree of hydroxyl group substitution suggests that ASAC17 and ASAC20 could be self-limiting, as reaction at one site may mean that adjacent attachment points are obscured. Table 3.2 shows that the degree of substitution decreases with the increasing size of the modifying compound at similar reagent loadings. At a weight gain of 27.2% ASAC17 gives an estimated substitution value of 0.06 while SA substitutes at 0.24 at a loading. of 26.1%.

Although it was possible to get loadings of 35% and 36% with ASAC17 the reaction temperatures were 130°C and 135°C respectively and the reaction times 8 and 6 hours.

Visual observation revealed that the wood surface had lost some of its "woody" appearance and texture. Light microscopy revealed that at these loadings and after such severe reaction conditions the wood was very dark and appeared to be in a stressed condition with small checks evident in the cell walls. These checks were in the secondary wall and appeared to run in the direction of the cellulose fibrils. The solvent only control specimens showed almost no weight change at all, there was no evidence of reaction. It is safe to assume that DMF does not react with the cell wall polymers.

3.5.2. Volume Change after Modification

Table 3.3 Volume changes in Scots pine sapwood resulting from chemical modification by SA. Each set of data is the average of a set of 5 samples. The specific gravity of SA is taken as 1.503.

Weight Gain (%)	Standard Deviation	Increase in Wood Volume with Treatment (%)	Standard Deviation	Calculated Volume of Chemical Added (%)	Standard Deviation
8.1	1.5	7.6	2.6	6.2	1.5
9.8	0.1	8.2	2.4	6.6	0.7
13.9	1.4	8.9	3.9	9.1	1.6
16.7	0.6	11.4	3.3	10.3	0.3
18.6	0.3	11.3	2.8	12.4	1.7
21.4	0.2	12.2	3.1	13.8	1

Table 3.4 Volume changes in Scots pine sapwood resulting from chemical modification by ASAC17. Each set of data is the average of a set of 5 samples. The specific gravity of ASAC17 is taken as 1.405.

Weight Gain (%)	Standard Deviation	Increase in Wood Volume with Treatment (%)	Standard Deviation	Calculated Volume of Chemical Added (%)	Standard Deviation
7.5	0.2	5.9	1.3	5.4	0.2
9.7	0.3	8.9	1.9	7.2	0.3
11.6	0.7	9.4	1.2	8.2	0.4
16.4	0.6	9.5	.0.5	11.7	0.5
19.1	1.5	11.4	2.9	13.5	1.4
26.3	2.5	17.1	3.1	18.1	1.5

Tables 3.3 and 3.4 compare the oven dry volume of wood samples after modification with the theoretical volume of SA and ASAC17 added to bring about that modification. In most cases the two amounts are almost the same and this is strong evidence that the modifying chemicals have ended up in the cell wall. In some cases, mainly at the higher loading levels, there is a greater volume of chemical added than there is an increase in wood volume. This could mean that not all of the chemical is in the cell wall and some of it is in the wood cavities. Whilst these results indicate that the required reactions have taken place they do not indicate whether or not the chemical is bonded.

3.5.3 Infrared Analysis

Any new trace peaks noted or any variation to existing peaks are thought to be indicative of changes to the substrate brought about by the chemical modification process. These are discussed as a comparison between traces taken from similar wood samples before and after the modification process.

Figures 3.6 and 3.7 show spectra of an SA specimen modified to 30.5% weight gain and an ASAC17 specimen modified to 29.7% weight gain. Both spectra are shown compared with a trace of an unmodified sample.

In both there is a strong absorption band in the 3400/3500 $^{cm-1}$ area. This is due to hydroxyl group vibration. Because chemical substitution is never high enough to eliminate all hydroxyl groups these bands are always present. In both spectra however there is a slight increase in absorption in this area in the modified samples compared with the unmodified which could indicate a substitution of some hydroxyl groups.

The spectrum of the SA modification shows a strong absorption band at 1730 $^{cm-1}$ in comparison with the control spectrum. This is due to the C=O group in the acetyl bond. Rowell (1982c) observed this strong band with acetic anhydride modification and Matsuda (1987) indicates a similar strong band working with succinated wood flour.

Infrared Spectra

Figure 3.6 Infrared spectrum of succinic anhydride block modified to a loading of 30.53 % compared with the spectrum of an unmodified specimen.

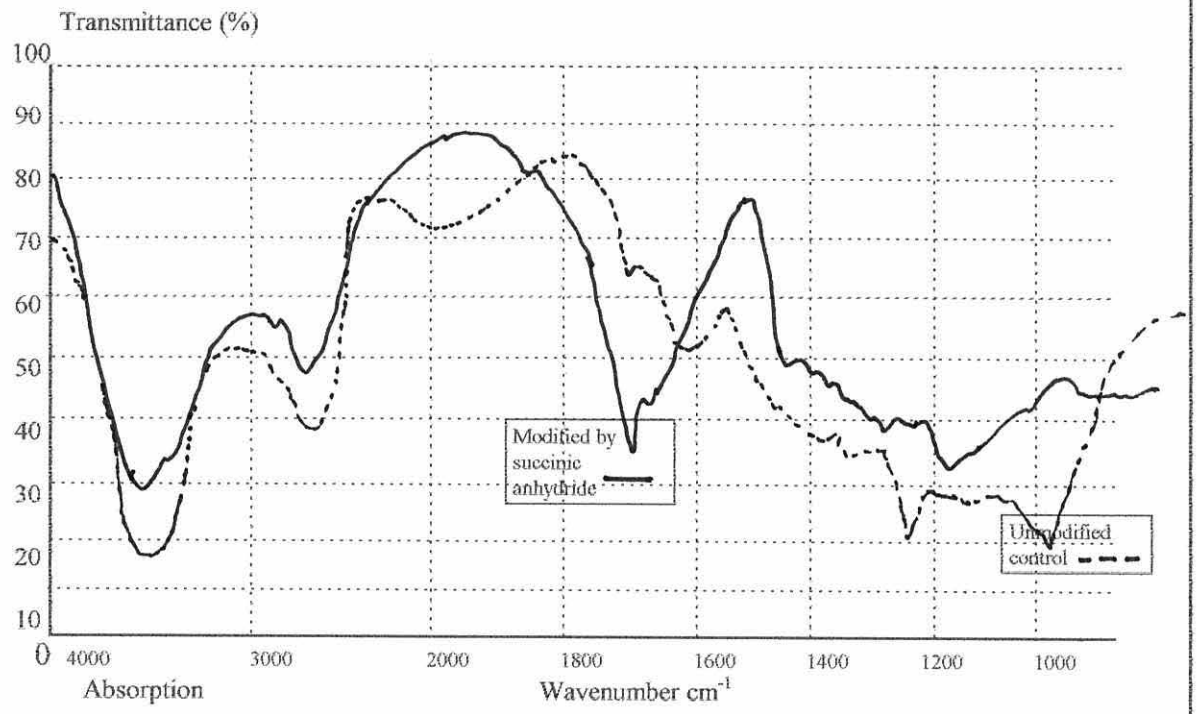
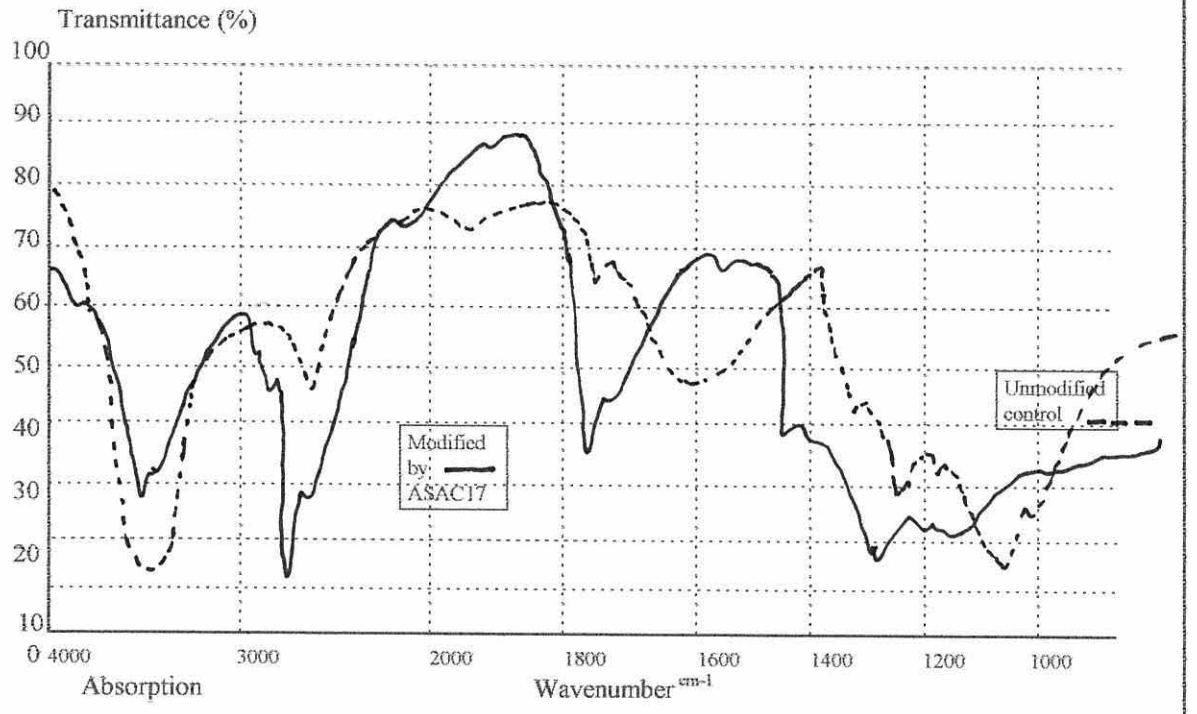


Figure 3.7 Infrared spectrum of ASAC17 block modified to a loading of 25.69 % compared with the spectrum of an unmodified specimen.



The ASAC17 spectrum exhibits a similar increase in absorption in the C=O vibration area. A greatly increased C-H stretch absorption at about 2800 $^{cm-1}$, relative to the hydroxyl group band for ASAC17 modification is strong evidence of the presence of the long aliphatic chain which is part of the structure of this compound.

In Table 3.5 the bands in the C=O absorption area for SA and ASAC17 modification and the C-H band for ASAC17 modification are given relative to the hydroxyl group absorption band which although slightly altered after modification is sufficiently constant to allow comparison.

Table 3.5 Comparison of the hydroxyl absorption band and the relevant bands affected by chemical reaction.

	Absorption Levels			
	Modified		**Unmodified**	
	-OH	C=O	-OH	C=O
Succinic	70	63	87	40
ASAC17	87	67	87	40
	-OH	C-H	-OH	C-H
ASAC17	87	79	87	60

In comparison with the relatively constant hydroxyl group absorption band the carbonyl group absorption increased by 40% after SA modification and 27% after ASAC17 modification. The aliphatic C-H stretch band increased by 19% after ASAC17 reaction. These are strong indications that the required reactions have occurred.

3.5.4 Effect of Water Leaching Wood Modified with SA, ASAC17 and Control Samples

The results below indicate the weight lost by sample blocks during the 14 day water leaching procedure described in Section. 3.4.

Table 3.6 Indicates the weight loss of samples modified with SA. Figures at each level of reagent loading are the average of sets of 5 blocks (Appendix 3.3)

Reagent Loading (%)	Standard Deviation	Weight Loss During Leaching (%)	Standard Deviation
4.9	0.6	1.2	0.8
10.9	1.1	1.4	0.6
15.3	0.6	1.6	0.8
20.4	0.8	2	1
25.8	1.3	1.7	0.9

Table 3.7 Indicates the weight loss of samples modified with ASAC17. Figures at each level of reagent loading are the average of sets of 5 blocks (Appendix 3.4)

Reagent Loading (%)	Standard Deviation	Weight Loss During Leaching (%)	Standard Deviation
5.1	0.8	1.7	0.9
11	0.6	2.3	1.2
15.6	1	1.9	0.7
20.7	0.7	2.2	1.1
23.9	1.6	1.9	0.7

Table 3.8 Indicates the weight loss of unmodified control samples. Figures are the average of a set of 10 blocks (Appendix 3.5)

Average	Standard Deviation
0.4	0.3

Table 3.6 shows that throughout the average SA loading range of 4.9% to 25.8% the weight loss during the leaching procedure resulted in average losses of 2% or less. The losses in the ASAC17 modified wood given in Table 3.7 show a maximum of 2.3%. These figures are in

line with those of Rowell (1983) who reports losses of 1% and 1.2% in acetylated wood blocks at 16.3% and 22.5% weight gain respectively after a 7 day water soak. Leaching in water prior to chemical modification could have helped ensure that these results relate to reagent loss only (section 3.4).

Matsuda (1987) found the esterification reaction in succinated wood to be unstable at high relative humidities when de-esterification was induced. This does not seem to be so here. However he was working with wood flour and it is possible that the reaction sites are far more accessible to moisture when the wood is in this condition. Rowell (1983) found that acetylated ground wood lost 12.2% of an initial 22.5% loading after 24 hours soxhlet extraction with water and attributes this increase in loss to there being a greater amount of internal cell surface when the wood is not solid.

The operational control loss (Table 3.8) was 0.4%. In the light of this figure the overall weight losses from both the SA and ASAC17 modified wood over the whole loading range are very low and considering the maximum average loadings were 25.8% for SA and 23.9% for ASAC17 then these chemical modification systems can be considered stable in the presence of water.

Chapter 4

Biological Testing

4.0 Pilot Studies

Having established a chemical system and a laboratory method that would bring about the required modification of the wood cell walls it was necessary to define a method to biologically test the modification. In order to arrive at a suitable procedure three pilot studies were carried out. Each involved Scots pine sapwood being exposed to the brown rot fungi *Coniophora puteana* (Schum.:Fr) Karst, strain FPRL 11E and *Gloeophyllum trabeum* (Pers.:Fr) Murril, strain BAM 109, over a twelve week period. One test used chemically modified strips of wood and investigated loss of tensile strength after fungal attack. The other two tests used small blocks of modified wood and used weight loss after fungal attack as a judge of the efficacy of the modification process. Each set used unmodified control samples. Chemical modification in each test was similar to that described in section 3.2.3 using the apparatus given in Figures 3.2 and 3.3.

The first method looked at changes in tensile strength in small strips of wood after fungal attack (Henningson, 1967; Bravery and Grant, 1971). Using a microtome, the strips, measuring 10 x 1cm x 100mm, were cut from a block of water-saturated wood from which extractives had been removed. Sixty strips were modified with SA and ASAC17 in a range of loadings of about 5 - 30% before being exposed to *C. puteana* in petri dishes over 2% malt agar. The strips were supported in the petri dishes on pre-infected Scots pine sapwood feeder strips measuring 3 x 10 x 60mm. Each petri dish contained three feeder strips supporting 10 modified test strips. After sealing the petri dishes with laboratory film they were incubated at 22°C and 65% relative humidity. It was intended that the samples should remain on test for 42 days but 30 days into the test it was observed that almost all of the strips appeared very wet and there was very little fungal growth after an initial period of healthy propagation. Some samples were removed from test, weighed wet and after drying to constant weight. The average moisture content was calculated to be 70 - 80% and weight losses were negligible. This water content is too high for growth or decay by *C. puteana* which has an

optimum decay moisture content of 50 - 60%. There was insignificant weight loss in unmodified control samples. Moisture relations of wood undergoing fungal decay are complex because of the dynamic nature of the system. The process of decay itself leads to the generation of considerable quantities of water due to respiration.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6H_2O + 6CO_2$$

One mole of glucose (170g) yields 6 moles of water (108g). Hence the water produced is equivalent to about 64% of the cellulose consumed. Thus in a closed system as here, the moisture content increases considerably as decay proceeds. It is possible that small capillaries in the feeder strips or the fungal hyphae may have transported an excess of moisture from the agar into the strips or that the strips once moist lost their structural integrity and touched the agar at any unsupported parts which was observed on some samples. The test strips were very thin and even a small amount of water would have been enough to increase their moisture content to a level at which the fungi would be unable to metabolise.

A mini-block method (Bravery, 1987; Brown, Caswell and Williams, 1991) was then investigated. Blocks were machined to 10 x 10 x 3mm and chemically modified with SA and ASAC17 in a range of loadings of approximately 5 - 30%. After sterilising using gamma irradiation they were then exposed to *C. puteana* over 2% malt agar. There were four blocks in each petri dish and they were supported on a fine nylon mesh. The purpose of the mesh was to keep the blocks from touching the agar surface and help prevent water-logging of the wood which might depress fungal growth and decay. Again fungal growth was very weak and the subsequent weight losses in modified blocks and unmodified controls were negligible. In all of the blocks tested the moisture contents were too high for the test results to be valid.

It may have been expected that the chemical modification would have prevented the moisture content of the wood from reaching such high levels but this was not the case. With such small and ultimately fragile sections, strips and blocks, it is possible that the physical

disruption of the samples brought about by their preparation and chemical modification could have rendered the wood more susceptible to moisture ingress in these tests. Here, testing over agar was unsuccessful in both of these tests.

The third test proved successful. Blocks were machined to 20 x 20 x 5mm and exposed to fungal attack in an infected soil test based on ASTM D 1413 - 76 (1986), (sections 4.1.2 and 4.1.3) This test did not bring about the excessive moisture levels as did the previous two and control blocks were heavily decayed. The results of these initial investigations are not reported quantitatively.

4.1 Experimental

4.1.1 Preparation for Inoculation

4.1.1.1 Selection of Wood Species and Specimen Geometry

Scots pine sapwood was machined to 20 x 20 x 5mm in the tangential, radial and longitudinal directions. The preparation for chemical modification was as described in Chapter 3, section 3.2.1.

4.1.1.2 Chemical Modification and Water Leaching

The chemical modification process is detailed in Chapter 3, section 3.2.3. Succinic anhydride and ASAC17 were the modifying agents and the reactions took place in solution with DMF. One hundred and sixty blocks were modified in loadings ranging from approximately 3 - 35% of initial dry wood weight.

Half of these samples were leached in water according to the prEN 84, the procedure for which is described in Chapter 3, section 3.4.

Both the water leached and non-leached samples were to be subject to biological attack. The dried modified blocks were stored over the desiccant anhydrous phosphorus pentoxide until required for biological testing.

4.1.1.3 Control System

All control specimens underwent similar weighing, drying and dry storage procedures as did those modified by SA and ASAC17. Half of all control blocks were water leached according to the prEN84.

To test the effect of the solvent 24 specimens were solvent extracted, oven dried and reacted for 6 hours in DMF only. No attempt was made to clean the blocks by reflux after reaction.

To investigate whether or not removing extractives from the wood has an effect on the biological protection process 24 blocks were solvent extracted. These samples were not reacted with reagent or solvent.

A further 24 blocks were oven dried only. These would be used to observe the effect that the test fungi have on wood in an untreated state.

Operational controls to monitor any weight change brought about by the uninfected test system (sterile soil) were two sets of 20 blocks, each loaded with SA and ASAC17 respectively in the approximate range of 3 - 25%.

Table 4.1 summarises the test design.

Table 4.1 Test design. Described in sections 4.1.1.2 and 4.1.1.3

Chemically modified specimens	**Not water leached after treatment (number of samples)**	**Watere leached after treatment (number of samples)**
Test fungus : C. puteana		
SA	20	20
ASAC17	20	20
Test fungus: G. trabeum		
SA	20	20
ASAC17	20	20
Control specimens		
No reagent modification		
Test fungus: C. puteana		
DMF only	6	6
Extracted only	6	6
Oven dried only	6	6
Test fungus: G. trabeum		
DMF only	6	6
Extracted only	6	6
Oven dried only	6	6
Operational		
SA modified	10	10

4.1.1.4 Test Organisms

Cartwright and Findlay (1958) suggest that test fungi should be chosen on the basis of their economic importance, resistance to disinfectants, growth and decay rates and ease of laboratory cultivation. With these factors in mind, two fungi were chosen for the bio-assay. They were the Basidiomycetes *C. puteana,* strain FPRL 11E and *G. trabeum,* strain BAM 109. *C. puteana* is commonly called the wet rot fungus though it is not the only wet rot fungus. 'Wet rot' usually refers to the decay of wooden structures occurring within buildings or in external joinery, cladding etc. In the U.K. this type of decay is often caused by *C. puteana.* This fungus is said to be the cause of 90% of the wet rot decay in buildings in the U.K. and is thus of great economic importance. It rapidly decays a range of softwood and hardwood species but is more common on softwood sapwood. The importance of *C. puteana* is due to its' frequent occurrence in buildings, its' rapid decay of wood and its' ability to attack wood treated with several different preservatives (Eaton and Hale, 1993). *G. trabeum* is rarely seen inside buildings and is found mainly out of doors. It occurs mainly on softwoods, only sometimes on hardwoods. It is found throughout Europe, North America, South Africa and Australia. It is less common in the U.K. but is frequently used as a test fungus.

The reasons for using brown rot fungi in this investigation were that the end-use for modified wood is seen as for small softwood internal and external building joinery such as window and door frames, cladding and soffits. Here brown rotters are economically very important and these fungi are commercially acceptable as test fungi. *C. puteana* and *G. trabeum* have been found to cause rapid decay in softwood sapwood in the laboratory and whilst white rot fungi could have been used they tend to be less destructive to softwoods in a given period of time. Both fungi are stated as obligatory fungi in the ASTM D 1413 - 76 (1986) on which this biological test was based and they can both be easily grown in the laboratory where growth is rapid and controllable on malt agar medium.

For wood not in contact with the ground, softwoods are more susceptible to attack by brown rot than are hardwoods. Brown rot is quite often the cause of decay in items such as window

joinery and eaves boards made from pines, hemlocks and spruce. Hardwoods in service are more susceptible to white rot attack than are softwoods and thus brown rot is more commonly associated with softwoods. But both types of rot may infect both types of wood especially in ground contact where alternative sources of nutrient are available.

4.1.1.5 Culture Medium

The culture medium was based on malt agar with the following composition: 50g concentrated malt extract containing 0.9% nitrogen and 30g agar containing approximately 0.3% total nitrogen and causing no inhibition of fungal growth. This was made up with deionized water to 1000 ml. The medium was prepared by warming the mixture in a boiling water bath and stirring until completely dissolved. Sufficient was placed in each petri dish to a depth of 3 to 4mm with the dish laid flat. The dishes were sealed with a lid and plastic tape and sterilised in an autoclave for 20 minutes at 120°C.

The dishes were inoculated with the two fungi and put on wooden slats in an incubation room maintained between 25 and 27°C and a relative humidity between 65 and 75%. It took about 2 weeks for the cultures to have grown sufficiently to be used in test.

4.1.2 Inoculation Procedure

This section describes the procedure followed in setting up the biological test necessary to assay the effectiveness of the chemical modification process (Chapter 3). It is based on ASTM D 1413 - 76 (1986). A preliminary test described in section 4.0 proved this method to be satisfactory in testing SA and ASAC17 modification against attack by *C. puteana* and *G. trabeum*. A strong advantage of this test is that decay is rapid by these fungi on softwood sapwood and results can be obtained in the relatively short time of 12 weeks, the intended test time span. The test was easy to set up with readily available materials. Eaton and Hale (1993) say that recent thinking in this type of investigation favours soil tests which use an unsterile soil-bed system as a medium in which to incubate treated samples. An advantage is that a simulated field situation is created whereby test samples are exposed to a natural microflora which can include all types of decay flora and bacteria.

All inoculation was carried out in aseptic conditions at a laminar flow bench.

The culture bottles used for the test were French Cylindrical, 450 cm^3 (16 oz.) (Figure 4.1). They were fitted with a metal screw top with an 18mm central aeration hole which was tightly plugged with cotton wool. Three hundred grams of John Innes Number 2 compost soil was measured into each jar and lightly compacted. In order to bring the soil to a growth optimum moisture content it was necessary to determine its water holding capacity. This term is defined as "the ability of a sample of test substrate to retain water against the pull of a vacuum pump", (BRE. Garston, 1990). In order to determine the water holding capacity of the soil used in this test the following procedure was carried out. A small amount of water was added to 25 grams of the test soil. This was mixed well and water addition repeated until the soil particles had a crumb-like structure and were just beginning to aggregate. Twenty five millilitres of water was added and the sample was allowed to stand for 1 hour. The soil was then evenly spread on a moistened filter paper in a Buchner funnel and this was evacuated until no more water came from the sample. After drying to constant weight at 105 $^{\circ}$C the weight of the dry sample of the soil was noted. The moisture content of the original soil was determined and this enabled the correct volume of water to be added to each test jar to bring the soil up to the required moisture content. In this test this was 50%. In the next stage a 50mm square of filter paper was placed on top of the soil in each jar to act as a carbon source for the growing fungal culture. The lids were lightly screwed on to the jars and these were sterilised in an autoclave for 2 hours at 120 $^{\circ}$C. After cooling, 2 pieces of the inocula (from the malt agar culture) were placed on opposite corners of the filter paper. The jars were tightly sealed with the screw lid and laboratory film and placed on slatted shelves in an incubation room maintained at between 25 and 27 $^{\circ}$C and a relative humidity between 65 and 75%. After 3 weeks the fungal growth was sufficiently advanced to enable the test blocks to be introduced.

4.1.3 Exposure Procedure

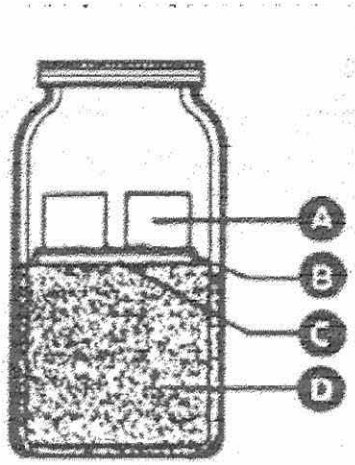


Figure 4.1 Culture Jars

A test blocks. B Nylon mesh. C Filter paper. D soil. (ASTM D 1413-76, 1986)

A 50 x 50mm piece of sterilised nylon mesh was placed on top of the filter paper in each jar to support the test samples during the test. Two samples, each of a similar test designation (Figure 4.1) and previously sterilised by gamma irradiation were placed in each jar. The jars were tightly sealed and returned to the incubation room for 12 weeks. The test required that 272 samples blocks should be used and this meant that 136 culture jars were prepared as detailed. At the end of the exposure period the blocks were removed from the test jars, cleaned of superficial debris and weighed in a moist state. After drying to constant weight in an oven at 105 $^{\circ}$C they were allowed to cool over a desiccant and re-weighed. Calculation determined the moisture content of the blocks at the end of the test period and the amount of weight lost.

4.2 Results and Discussion

In this section the terms NWL and WL refer respectively to blocks not leached in water and leached in water after initial modification (Chapter 3, section 3.4). Biological threshold values are those reagent loadings at which decay protection is estimated to be complete and are calculated as a percentage of the initial dry wood weight. Weight loss and moisture content (m.c.) are those at the end of the test period.

ASAC17 modification was more effective than SA treatment in preventing decay in Scots pine sapwood by both *C. puteana* and *G. trabeum* (Table 4.2.). Increasing the loading of both reagents saw decreasing levels of decay by both fungi and this was generally accompanied by a lowering of the moisture content of the decayed blocks (Tables 4.4 and 4.5, Figures 4.2 to 4.9), (Appendices 4.1 to 4.8). This was true in different degrees for NWL and WL blocks in attack by both fungi. Blocks modified with ASAC17 had a lower moisture content at the end of the bio-assay than did those modified with SA.

Leaching ASAC17 modified blocks in water after initial chemical modification had much less of an effect on weight losses during the bio-assay than similarly leaching blocks modified with SA. The control specimens were extensively decayed by both fungi at the end of the bio-assay.

Table 4.2. gives biological threshold values extrapolated from data in Tables 4.4 and 4.5 and accounting for operational control losses, all of which were below 2% (Table 4.6), (Appendices 4.9 and 4.10), for NWL blocks ASAC17 is estimated to give complete protection from decay by *C. puteana* at a loading of 31% (Figure 4.6); the equivalent SA loading is 44% (Figure 4.2). Against *G. trabeum* comparable values are 28% for ASAC17 (Figure 4.8) and 60% for SA (Figure 4.4). In WL blocks ASAC17 is estimated to give complete protection against *C. puteana* at 32% (Figure 4.7) which is 24% lower than that

Figure 4.2 (Table 4.4). Weight Loss and Moisture Content as a Function of Reagent Loading SA C. puteana. NWL

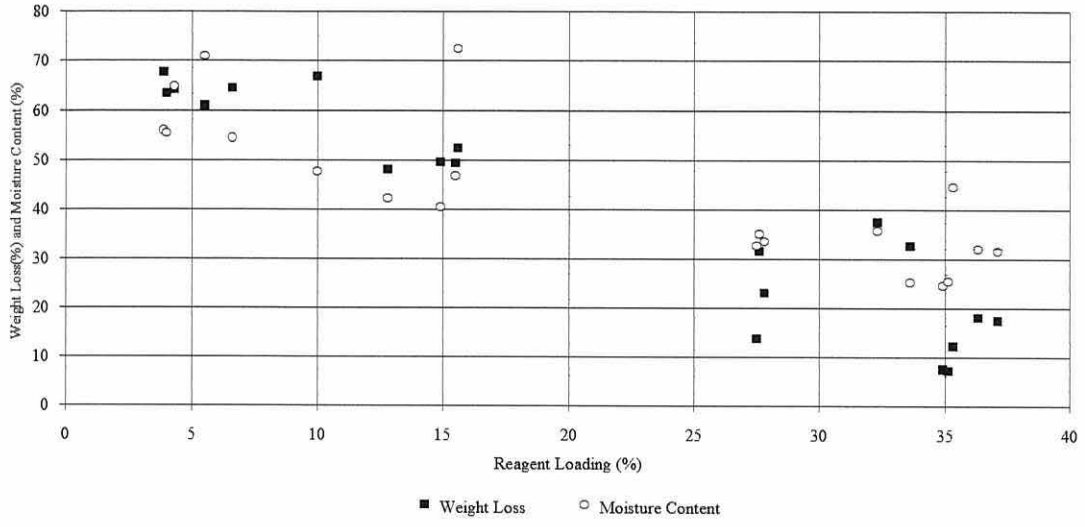


Figure 4.3 (Table 4.4). Weight Loss and Moisture Content as a Function of Reagent Loading SA C. puteana. WL

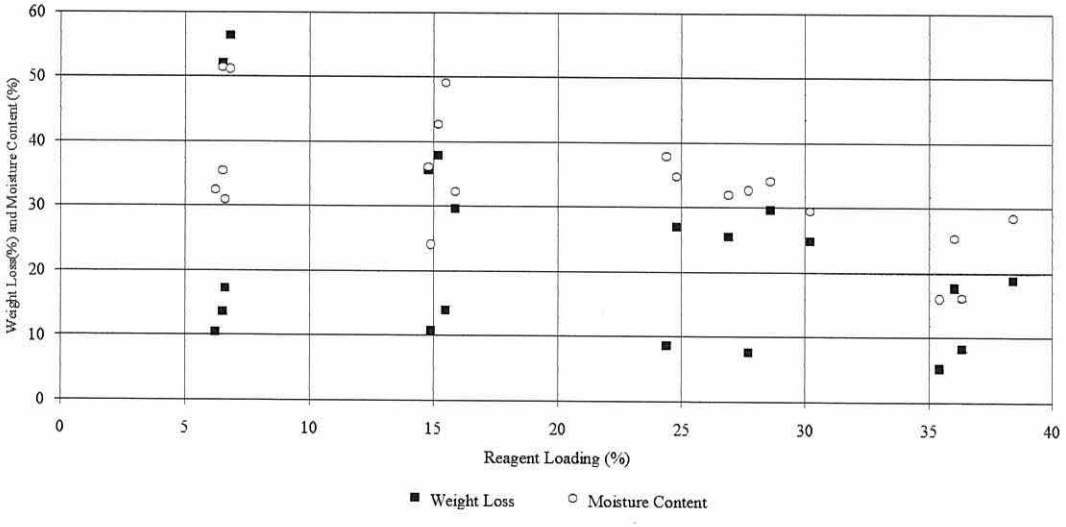


Figure 4.4 (Table 4.5). Weight Loss and Moisture Content as a Function of Reagent Loading SA G. trabeum. NWL

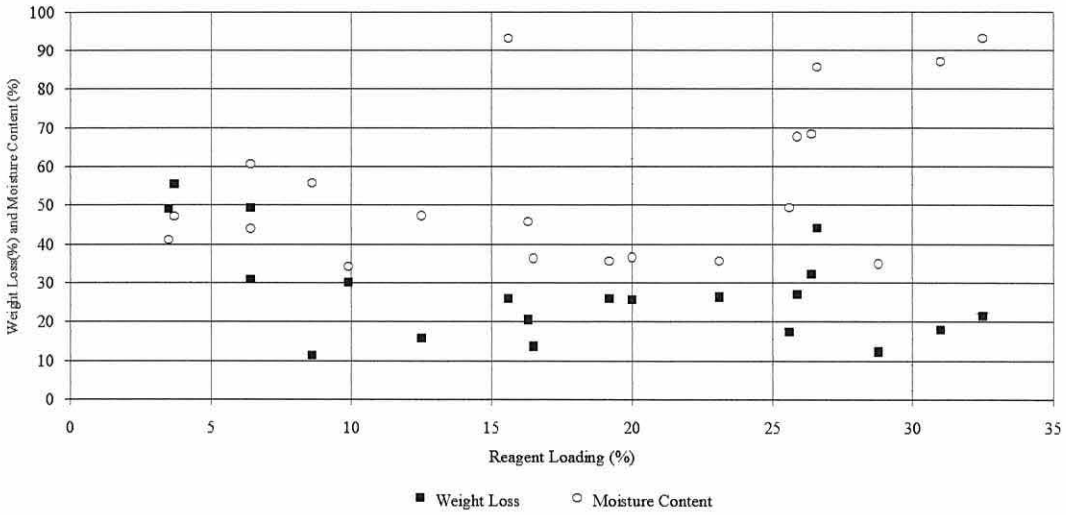


Figure 4.5 (Table 4.5). Weight Loss and Moisture Content as a Function of Reagent Loading SA G. trabeum. WL

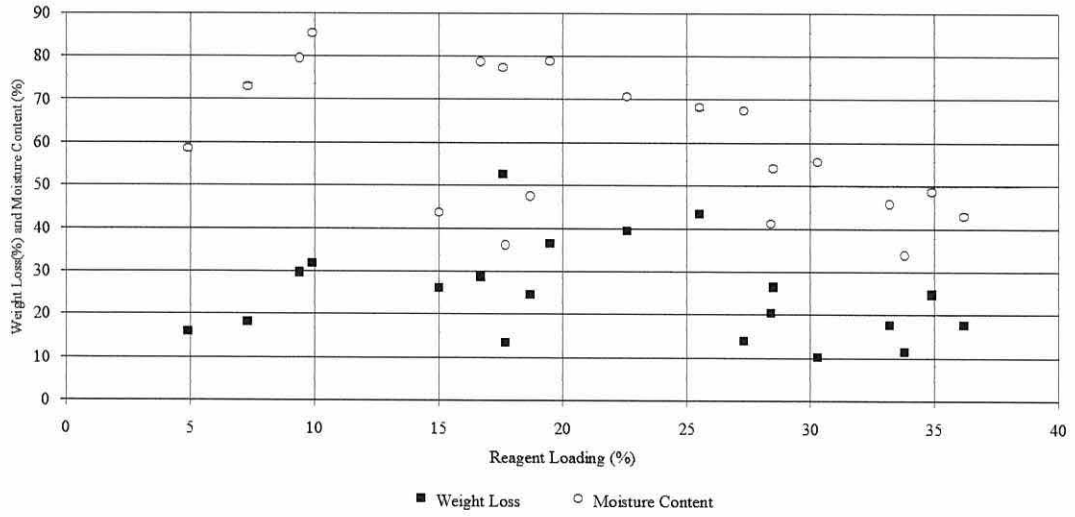


Figure 4.6 (Table 4.4). Weight Loss and Moisture Content as a Function of Reagent Loading ASAC17 C. puteana. NWL

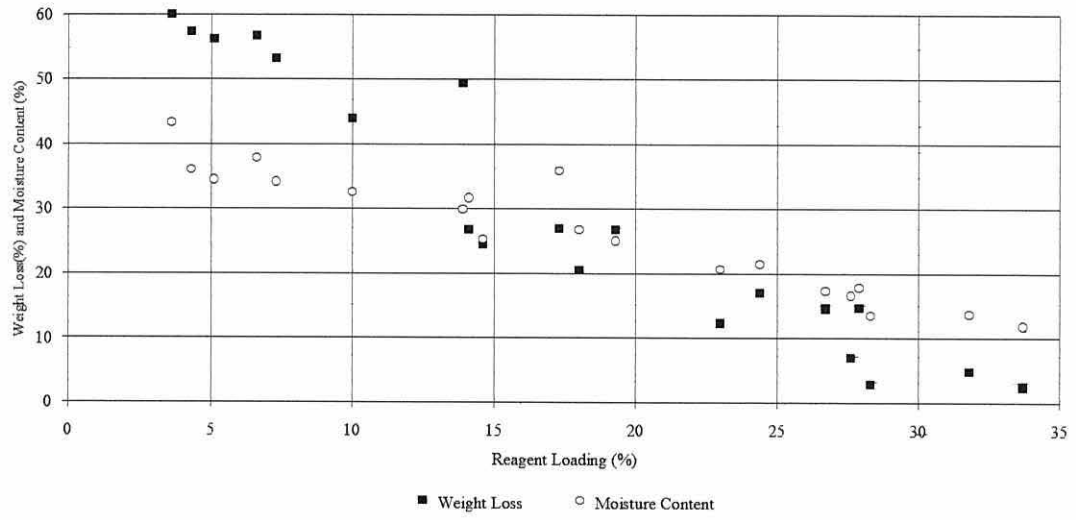


Figure 4.7 (Table 4.4). Weight Loss and Moisture Content as a Function of Reagent Loading ASAC17 C. puteana. WL

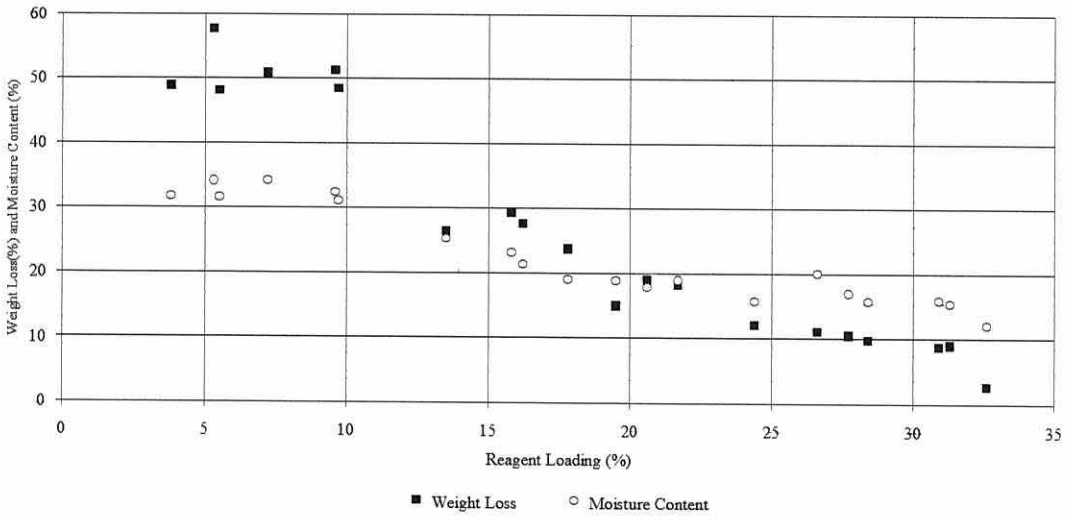


Figure 4.8 (Table 4.5). Weight Loss and Moisture Content as a Function of Reagent Loading ASAC17 G. trabeum. NWL

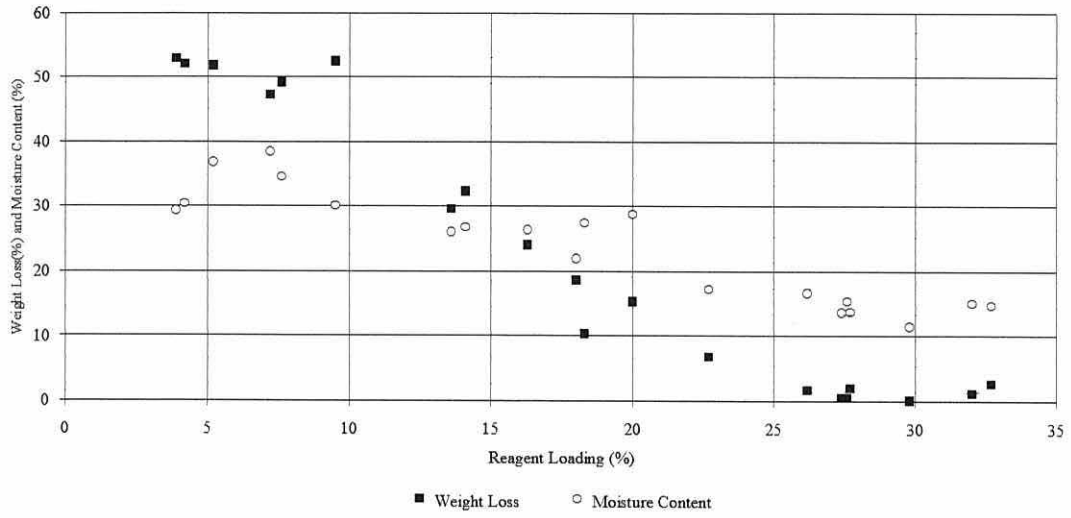
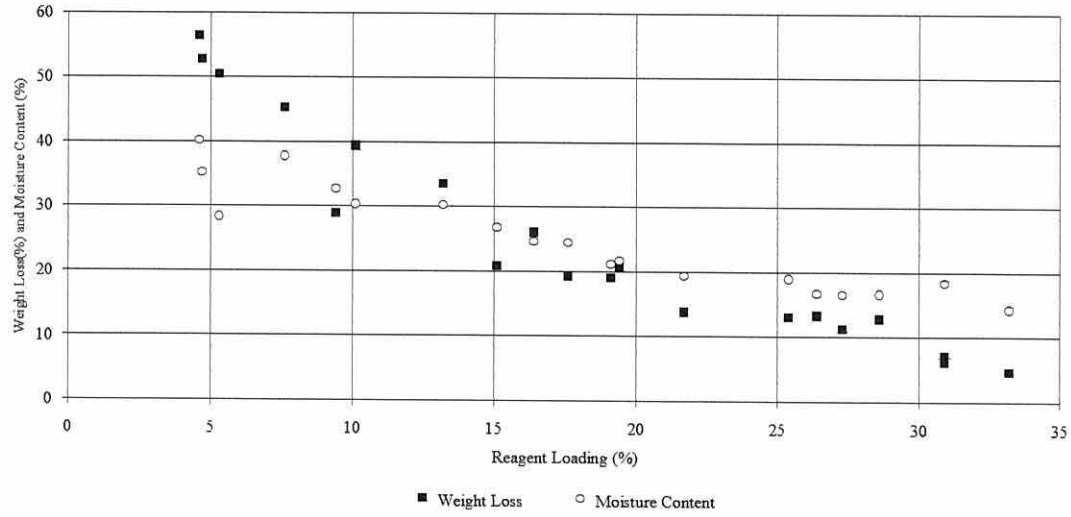


Figure 4.9 (Table 4.5). Weight Loss and Moisture Content as a Function of Reagent Loading ASAC17 G. trabeum. WL



required by SA modification (Figure 4.3). Against *G. trabeum* the advantage of ASAC17 modification is even more apparent. Water leached threshold value for ASAC17 treatment is 33% (Figure 4.9) and 69% for SA modification (Figure 4.5).

It is very difficult to ascribe threshold values by extrapolation and this is especially apparent when looking at the data for weight loss against SA loading in this test when the degree of lineal relationship is almost non-existent. When operational losses are accounted for, the extrapolated threshold values as drawn from the data in this test, have no significant impact on the given values.

Table 4.2 Biological threshold values calculated by extrapolation from experimental data given in Tables 4.4 and 4.5 The concluding figures are those estimated reagent loading values at which complete biological protection would be afforded in this test.

Reagent		Reagen loading range (%)	Loading at which biological protection is complete (%)
Fungus: C. puteana			
SA	**NWL**	3.9 - 37.1	44.5
	WL	6.2 - 38.4	56.5
ASAC17	**NWL**	3.6 - 33.7	31.5
	WL	3.8 - 32.6	32.5
Fungus: G. trabeum			
SA	**NWL**	3.5 - 31.0	60.1
	WL	4.9 - 36.2	68.5
ASAC17	**NWL**	3.9 - 32.7	28
	WL	4.6 - 33.2	33.1

Table 4.3 shows that for ASAC17 modification there is a significant negative linear relationship between reagent loading and weight loss. All correlation values (Appendix 4.12) are greater than -0.90 (-1.0 signifies a perfect inverse correlation).

Table 4.3. Correlation data indicating the extent of linear relationship (Appendix 4.12) between reagent loading and weight loss, and reagent loading and moisture content at the end of the infection period. Loading indicates the percentage weight gain of specimens after modification.
Figures relate to sets of 20 blocks.

Reagent	Factors		r
Fungus: C. puteana			
SA	NWL	loading / weight loss	-0.89
		loading / m.c.	-0.82
	WL	loading / weight loss	-0.4
		loading / m.c.	-0.62
ASAC17	NWL	loading / weight loss	-0.92
		loading / m.c.	-0.97
	WL	loading / weight loss	-0.93
		loading / m.c.	-0.95
Fungus: G. trabeum			
SA	NWL	loading / weight loss	-0.46
		loading / m.c.	-0.44
	WL	loading / weight loss	-0.55
		loading / m.c.	-0.23
ASAC17	NWL	loading / weight loss	-0.97
		loading / m.c.	-0.91
	WL	loading / weight loss	-0.95
		loading / m.c.	-0.93

As reagent loading increased the loss in weight caused by decay became less. With SA, NWL modification against *C. puteana* there is a high correlation of -0.89 which indicates that the modification was successful and increased loading resulted in lower weight losses. However when similar samples were leached in water after chemical modification then this correlation drops to-0.40 for WL samples. The efficacy of the modification in this case was reduced by the leaching process. Correlation values for SA modification against *G. trabeum* are less significant than those for *C. puteana*, -0.46 for NWL blocks and -0.55 for WL.

The following tables relate weight loss and moisture content at the end of the infection period to reagent loading for *C. puteana* and *G. trabeum*. Each reagent loading level (Appendices 4.1 to 4.8) is the average (standard deviation) of a set of 5 blocks. Weight loss and moisture content are given as a range of values for a set. (Figures 4.2 to 4.9).

Table 4.4 Fungus: *C. puteana*

	Weight loss (%)		Moisture content (%)	
Reagent loading (%)	Low (%)	High (%)	Low (%)	High (%)
SA. NWL				
4.9 (1.2)	60.9	67.8	54.4	70.9
13.8 (2.4)	48.0	66.8	40.4	52.5
29.8 (3.0)	13.6	37.3	13.6	37.3
35.7 (0.9)	7.2	18.0	24.6	44.5
SA. WL				
6.5 (0.2)	10.4	56.3	30.8	51.4
15.3 (0.5)	10.8	37.8	24.1	49.1
27.6 (2.0)	7.6	29.6	29.4	34.7
36.1 (1.5)	5.2	17.6	16.1	37.9
ASAC17. NWL				
5.4 (1.6)	53.2	59.9	34.1	43.2
14.0 (2.6)	24.5	49.3	25.3	35.9
22.3 (3.6)	12.3	26.7	17.3	26.7
29.9 (2.7)	2.4	14.7	11.8	17.8
ASAC17. WL				
6.3 (2.2)	48.1	57.7	31.5	34.2
14.6 (3.1)	23.7	48.4	19.1	25.3
22.6 (2.9)	11.1	18.2	15.7	20.1
30.2 (2.1)	2.6	10.5	12.1	16.0

Table 4.5 Fungus: *G. trabeum*

Reagent loading (%)	Weight loss (%)		Moisture content (%)	
	Low(%)	High (%)	Low (%)	High (%)
SA. NWL				
5.7 (2.1)	11.3	55.4	41.4	60.5
14.2 (2.9)	13.6	25.9	34.2	93.1
22.8 (3.1)	17.4	27.1	35.4	67.6
29.1 (2.7)	12.2	44.1	34.8	92.9
SA. WL				
9.3 (3.8)	15.8	31.7	43.6	85.4
18.1 (1.1)	13.3	52.6	36.1	78.8
26.5 (2.5)	13.9	43.4	41.1	70.5
33.7 (2.2)	10.1	24.7	33.8	55.4
ASAC17. NWL				
5.6 (1.7)	47.3	52.9	29.3	38.4
14.3 (3.2)	18.6	52.4	21.9	30.1
22.9 (3.9)	0.5	15.3	13.6	28.7
30.0 (2.4)	0.1	2.7	11.5	15.4
ASAC17. WL				
6.3 (2.1)	28.9	56.3	28.3	40.2
14.5 (2.9)	19.2	39.4	24.5	30.3
22.4 (3.4)	13	20.6	16.7	21.6
30.2 (2.3)	4.6	12.8	14.3	18.4

Table 4.6 Controls
Data are averages of sets described in section 4.1.1.3. Appendices 4.9 and 4.10 give data for individual blocks. Weight loss and moisture content (m.c.) are those at the end of test.

	Not water leached				Water leached			
Control	Weight loss (%)	Std.Dev.	m.c. (%)	Std. Dev.	Weight loss (%)	Std. Dev.	m.c. (%)	Std. Dev.
Fungus: C. puteana								
DMF	70.2	2.9	71.8	1.1	68.6	1.5	64.4	11.8
Extracted	65.5	5.0	53.0	1.7	65.6	2.9	55.3	0.8
O.D.	64.2	1.9	45.1	4.0	66.2	1.2	50.4	4.6
Fungus: G. trabeum								
DMF	41.7	5.9	44.3	2.6	36.0	11.9	57.8	7.4
Extracted	41.3	13.3	56.3	11.8	41.0	12.6	47.0	2.1
O.D.	50.1	4.8	39.9	6.0	36.0	8.6	58.1	22.1
Operational (sterile soil)								
SA	1.6	0.2			1.4	0.4		
ASAC17	1.9	0.5			1.7	0.5		

Table 4.3 shows that for ASAC17 modified wood the correlation between reagent loading and moisture content is very high, all values are greater than -0.90 for both NWL and WL against both *C. puteana* and *G. trabeum* attack. Increasing the reagent loading resulted in a lower moisture content after fungal attack by both fungi. SA modification was not as successful in controlling moisture content. The correlation values for SA are not as significant for *C. puteana* attack and are even less significant for *G. trabeum* attack.

Selected blocks were split open using a sharp blade and examined under a light microscope. At the lower levels of modification by SA and ASAC17, less than about 8%, mycelial strands of *C. puteana* were widely distributed throughout the tracheid structure and in the rays. Pit membrane removal and bore holes were seen. At the mid-loading levels of about 20% the only accumulation of hyphae was in the cell lumina. At the highest loading levels, approaching 30% only isolated mycelial strands were seen in the rays. The observations

were similar for attack by *G. trabeum* but generally the attack was not as severe.

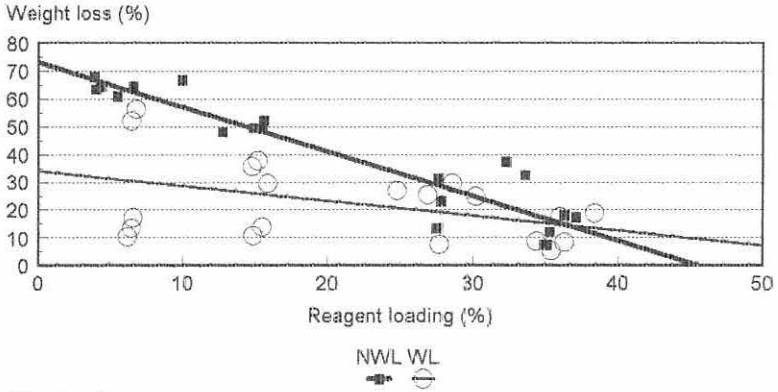
Figures 4.10 to 4.13 show the effect on decay, of leaching blocks in water after the initial chemical modification. Figures 4.12 and 4.13 for ASAC17 treatment show that there is a minimum difference between NWL and WL blocks during attack by both *C. puteana* and *G. trabeum.* This is not true for SA modified samples. Figure 4.10 indicates a reasonable linear correlation between reagent loading and weight loss for NWL blocks attacked by *C. puteana* but for WL blocks this relationship is very tenuous. Figure 4.11 shows that the protection given by SA modification against attack by *G. trabeum* is minimal for both NWL and WL samples. Previous discussion states that the SA modification was relatively unsuccessful against *G. trabeum* and subsequent leaching of the samples thus had little comparable effect on an already unstable protection system.

Work in Chapter 3, section 3.5.4 showed that SA and ASAC17 are initially stable in water and it is proposed that ASAC17 could give a greater degree of dimensional stability than does SA modification. Hydrolysis could eventually occur with SA treatment. The SA treated blocks may have swollen above their green volume during the soaking process and whilst reagent may not have been leached out (this has not been determined by leachate analysis) the soaking may have opened the modified wood structure sufficiently to disrupt the protection system. It is also possible that any free anhydride remaining after the clean up process could have been hydrolysed and released after water leaching.

Table 4.6 indicates extensive weight losses in all controls. The solvent, DMF, reaction gave no protection against either fungi and the effect of leaving extractives in the wood did not prevent the fungi from extensively degrading those blocks. There was no apparent effect on the moisture content of the blocks during fungal attack. *C. puteana* caused a

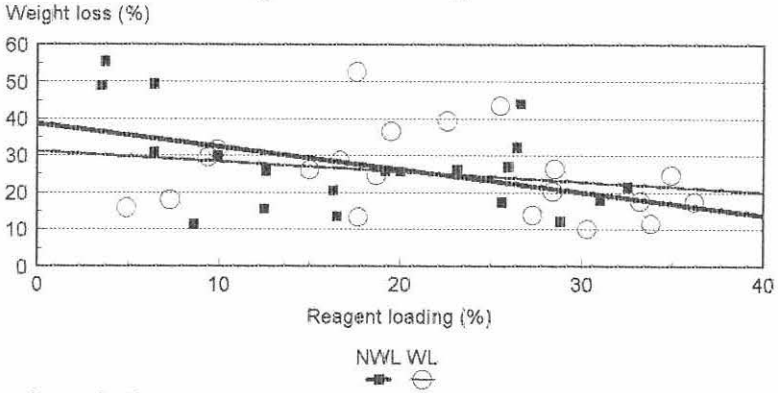
Comparison of weight loss between NWL and WL samples
Weight loss as a function of reagent loading
Appendices 4.1 to 4.8

Figure 4.10. Reagent : SA



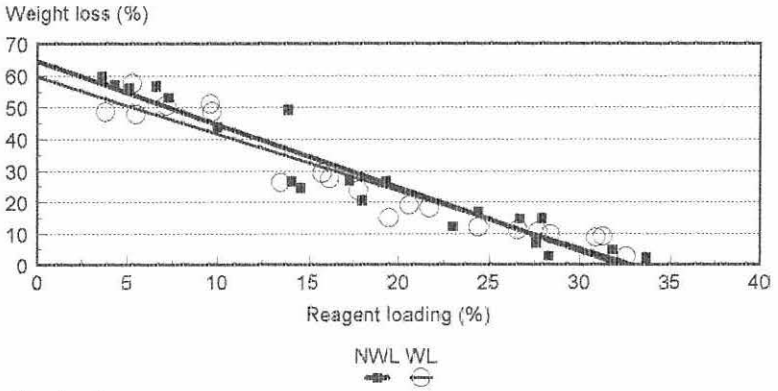
Coniophora puteana

Figure 4.11. Reagent : SA



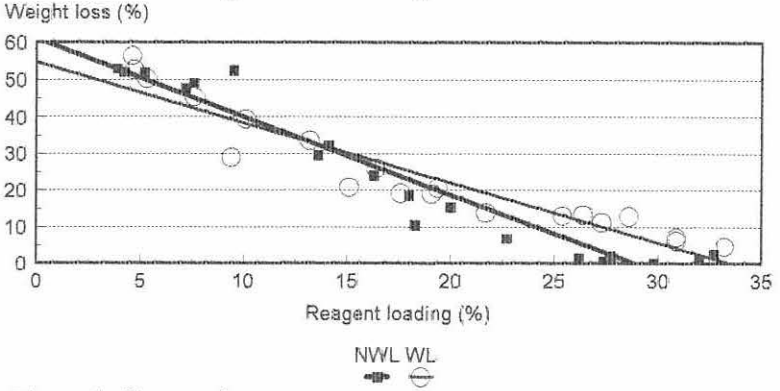
Gloeophyllum trabeum

Figure 4.12. Reagent : ASAC17



Coniophora puteana

Figure 4.13. Reagent : ASAC17



Gloeophyllum trabeum

greater amount of decay than *G. trabeum*. Operational weight losses resulting from the uninfected bio-assay system were very low at under 2% for both SA and ASAC17 modification indicating that the weight losses brought about by the uninfected test system were insignificant.

Leaching in water did not have a significant effect on the control specimens. Succinic anhydride and ASAC17 modify wood by single-site reaction with the available cell wall hydroxyl groups, there is no polymerisation. Any biological protection afforded by these two reagents might be attributed to a number of factors:

i) The -OH sites to which water molecules would H-bond could have been made unavailable by reagent substitution at these sites thus denying the fungi the moisture essential for attack.

ii) The low molecular weight agents suggested as initiating brown rot attack (section 2.3.4) and the highly specific fungal enzymes may have been sterically hindered by the attached reagent molecules.

iii) Substrate modification may have made the potential nutrient source of wood polysaccharides unrecognisable to the fungal decay agents.

It is possible that unreacted reagent and solvent may have been occluded in the wood after chemical modification but the severe solvent leaching process after reaction makes this possibility unlikely.

Whilst the fungal hyphae in this test may have been able to make an initial intrusion into the wood it is at the later critical stage of bore hole formation and subsequent enzyme diffusion at which chemical modification by SA and ASAC17 may be effective. The observed decrease in the amount of decay and lowering of the wood moisture content (Tables 4.4 and 4.5) (Figures 4.2 to 4.9) may point to increased reagent substitution at some of the reactive -OH and other oxygen containing sites within the wood cell wall. It is probable that the chemical modification reaction is occurring in the more amorphous regions of the cell wall with the hemicelluloses, at the lignin hydroxyl groups and on the surface of the cellulose crystallites. Stamm (1952) estimated that 50% of the -OH groups occur in these accessible regions and reasoned that after extensive reaction it is improbable that any readily available -

OH groups remain in the wood to be attacked by decay organisms. This implies that modification reactions in these areas may be sufficient for adequate protection. The non-crystalline celluloses and hemicelluloses are more hygroscopic than lignin and are mainly responsible for moisture uptake. Substitution reactions in these regions might deny the fungi the moisture essential for growth. Rowell (1983) suggests that lignin substitution does not contribute significantly to the overall protection of wood and points to the importance of substitution in the hemicelluloses. Substitution in the hemicelluloses is a strong factor in the preservation process but it also seems likely, considering the numerous reaction sites in the lignin molecule that substitution here could have a significance effect. Physiological tests by Hunt and Garrett (1953) showed that a moisture content of at least 20% is necessary to support decay by these fungi. With sufficient added chemical it may be that insufficient moisture is "available" at the critical zones to support decay. In this work SA modification was not able to bring the moisture content of the wood to below this lower optimum growth level. ASAC17 is seen to be more effective in this respect and was able to control the moisture content to levels below or not greatly exceeding 20% where decay is inhibited.

There is a problem in accurately assessing the moisture content of decayed wood during fungal attack (section 4.0). There is an assumption that the moisture content during the test does not reach significant highs and lows in a fluctuating manner and that the final moisture content at the end of the assay is the highest reached during the test. When the wood cell wall is modified using a chemical reagent then the fibre saturation point is lowered, some of the -OH groups not being available for water molecule attachment. This could mean that a recorded moisture content at the end of bio-assay reflects an increased quantity of water in the cell lumen. This water, during the test could prevent the fungal hyphae which are prominently on the cell wall, in the lumen, from attacking the wood cell wall. Decay by the destructive Basidiomycete brown rot decay fungi can occur at about 20% moisture content but significant decay generally occurs at and above fibre saturation point which is approximately 28 - 35%. Under these conditions of moisture, decay by most destructive fungi is optimal although decay occurs at higher moisture contents but generally by less

destructive fungi and bacteria (Hale, 1976. Unpublished).

ASAC17 is a large molecule and substitutes at far fewer -OH groups than a smaller molecule such as SA. It may have a greater moisture repelling effect in the wood cell wall and thus lead to increased lumen water during and at the end of the biological test, resulting in a possible error factor in the recorded moisture content. This point would need to be investigated in any future work.

The bulk of the ASAC17 molecule could be one of the reasons why this modification is more biologically efficient than that using SA. The ASAC17 molecule, attaching at one reaction site could obscure neighbouring sites and deny access to water molecules and fungal enzymes, even though these sites may be unsubstituted. ASAC17 may be self-limiting inasmuch as reaction at one site might prevent attachment by other ASAC17 molecules in the immediate vicinity by steric effects. This may explain why ASAC17 starts to become effective at a lower degree of substitution than SA.

Cell lumena are adequately large to accommodate the movement of fungal enzymes but at a micro level, porosity and microstructure are critical in determining the accessibility of fungal decay agents to wood's polymeric substrate. The physical structure of the tissue and the microstructure of the components naturally deters the ingress of fungal enzymes and here ASAC17 could be succeeding as its bulk could help block the pathways needed for fungal intrusion, although it could be argued that it would require an accumulation of substituted sites at pore entrances to bring about this type of blocking. This could also be true of SA but the effect of ASAC17 may be more apparent because of its greater molecular size and its non-polar hydrophobic "tail".

SA is seen to be reasonably successful in preventing decay by *C. puteana* but the estimated loadings required to combat *G. trabeum* attack are very high. ASAC17 was able to prevent decay by both *C. puteana* and *G. trabeum* at levels of loading that did not damage the wood. The next stage of this work looks at the feasibility of attaching a metal ion to the carboxyl group resulting from the ASAC17 - wood interaction and the biological effectiveness of the product.

Chapter 5

Reaction of Carboxy Esterified Wood with Copper

5.0 Introduction

Chapters 3 and 4 show that ASAC17 reacts with the cell wall polymers in Scots pine sapwood to give a robust covalently bonded esterified structure that imparts to the wood an enhanced ability to withstand decay by *C. puteana* and *G. trabeum*. The level of reagent loading required to bring about complete protection of the altered product is about 30% of the original dry weight of the modified wood. This, in commercial practice would be seen as an expensive method of protection and this protective chemical scheme would be more acceptable if the threshold loading of the modifying reagent could be brought down to a lower level.

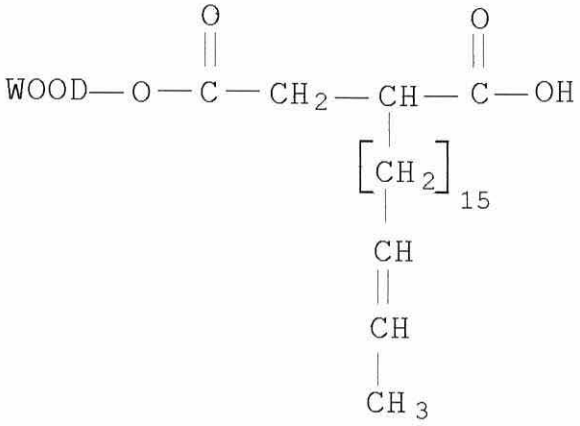


Figure 5.1. Suggested structure of wood modified with ASAC17.

The product of the modification has a newly formed carboxylic acid group (Figure 5.1). If a fungi-toxic element could be attached at this point then it may be possible that the combination of the various aspects of protection involved in this scheme, i.e., the inability of the fungal enzymes to recognise the altered substrate and the increased hydrophobicity of the substrate keeping moisture levels to below that required by fungi for the initiation of the decay process. The bulk of the attached compound may inhibit decay by the above processes. The toxic effect of the adducted element, could result in a biologically successful chemical system requiring a lower and thus more economic level of reagent loading. This

section of work describes the addition of copper as a proposed fungi-toxic agent to wood previously modified with ASAC17 and the subsequent biological testing of the product against the brown rot fungus *C. puteana.*

Copper has been used as a fungitoxic agent in wood and agricultural crops for over a century. As early as 1830 (Wilkinson, 1979) a sap-displacement technique was used to introduce copper sulphate along with zinc chloride and mercuric oxide into wood. Whilst these salts were effective biocides, being water soluble they were easily leached out in wet situations. In 1838 Boucherie treated green unseasoned timber with copper sulphate. The solution readily penetrated wet wood but was easily leached out in wood in soil contact or wet situations. Early this century the German worker Heinrich Bruchner found that normally-soluble metal salts could be made insoluble, or fixed inside wood, by the addition of large amounts of chromium. Baechler and Roth (1964) describe a Double Diffusion treatment of green peeled timber. The treatment sequentially treats wood with two preservative solutions. Many solutions are described but the most effective is that using copper sulphate and sodium arsenate. The solutions are cold and the timber is soaked for two days in each solution of 10% concentration. Today numerous copper compounds are used commercially in wood preservation. Copper pentachlorophenate (copper PCP) is more toxic to fungi than PCP alone (Findlay, 1962); copper naphthenate, copper oxide and copper-8-hydroquinolinate are successful preservative compounds. Copper/Chrome/Arsenate (CCA) has been extensively used in dry crystalline form, as pastes or as a liquid concentrate (Wilkinson, 1979). It is likely that the fungicidal properties of these compounds are dependent on the efficacy of the cupric ion, Cu^{2+} for different chemical groups in the fungal cell, particularly thiol groups, which may result in the non-specific denaturisation of proteins and enzymes (Eaton and Hale, 1993).

The object of this part of the work was to discover the lowest levels of ASAC17 and attached copper that would give the wood protection against decay by the Basidiomycete *C. puteana* and to see if the two reagents working in combination were more biologically effective than the individual substances.

Dissolving anhydrous copper sulphate in water results in the hexaquacopper(II) ion, a cationic complex. Figure 5.2 shows the proposed resultant structure.

$Cu^{2+} + 6H_2O$

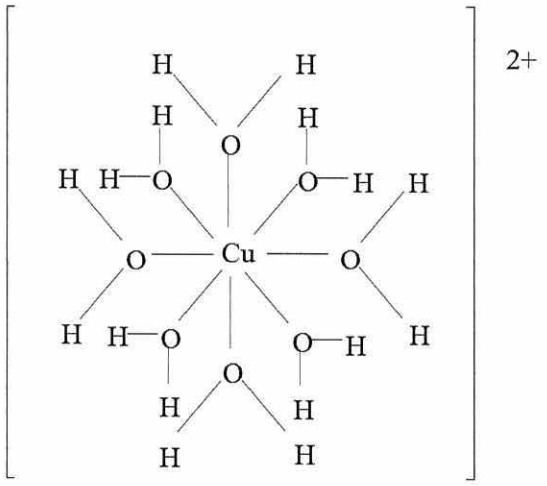


Figure 5.2. The suggested cationic complex formed when anhydrous copper sulphate is dissolved in water.

Each water molecule donates a pair of electrons to form a co-ordinate (dative) bond.

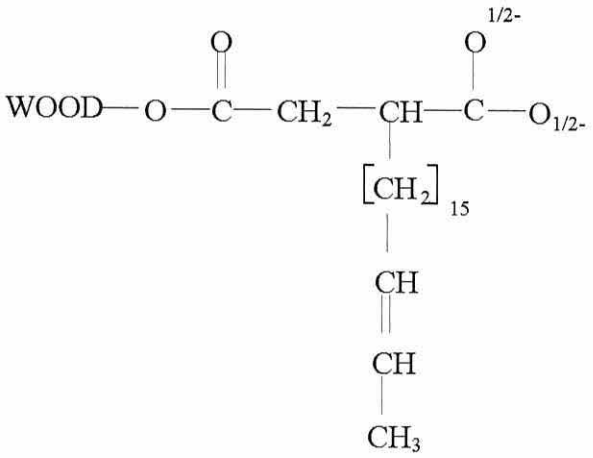


Figure 5.3. The resulting carboxylate ion after the removal of a proton from ASAC17 modified wood.

The ASAC17 modified wood structure has a carboxylic acid group which is capable of manipulation. Raising the pH removes the proton from this group to leave the carboxylate ion (Figure 5.3).

If the pH is allowed to go too high while the carboxylate ion is forming, copper hydroxide is formed as a pale blue precipitate according to the following equation:

$$[Cu(H_2O)_6]^{2+} + 2OH^- \qquad [Cu(H_2O)_4(OH)_2] + 2H_2O$$

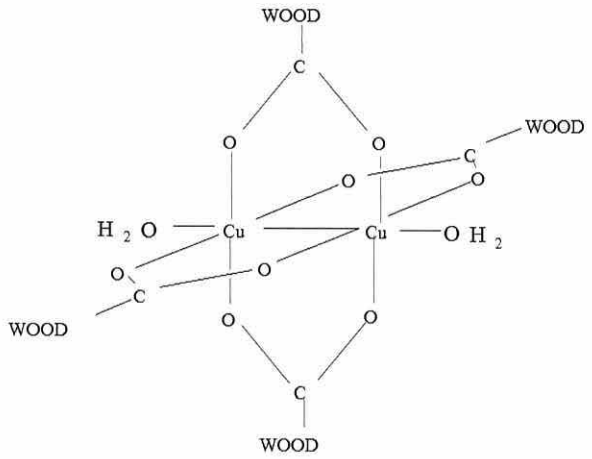


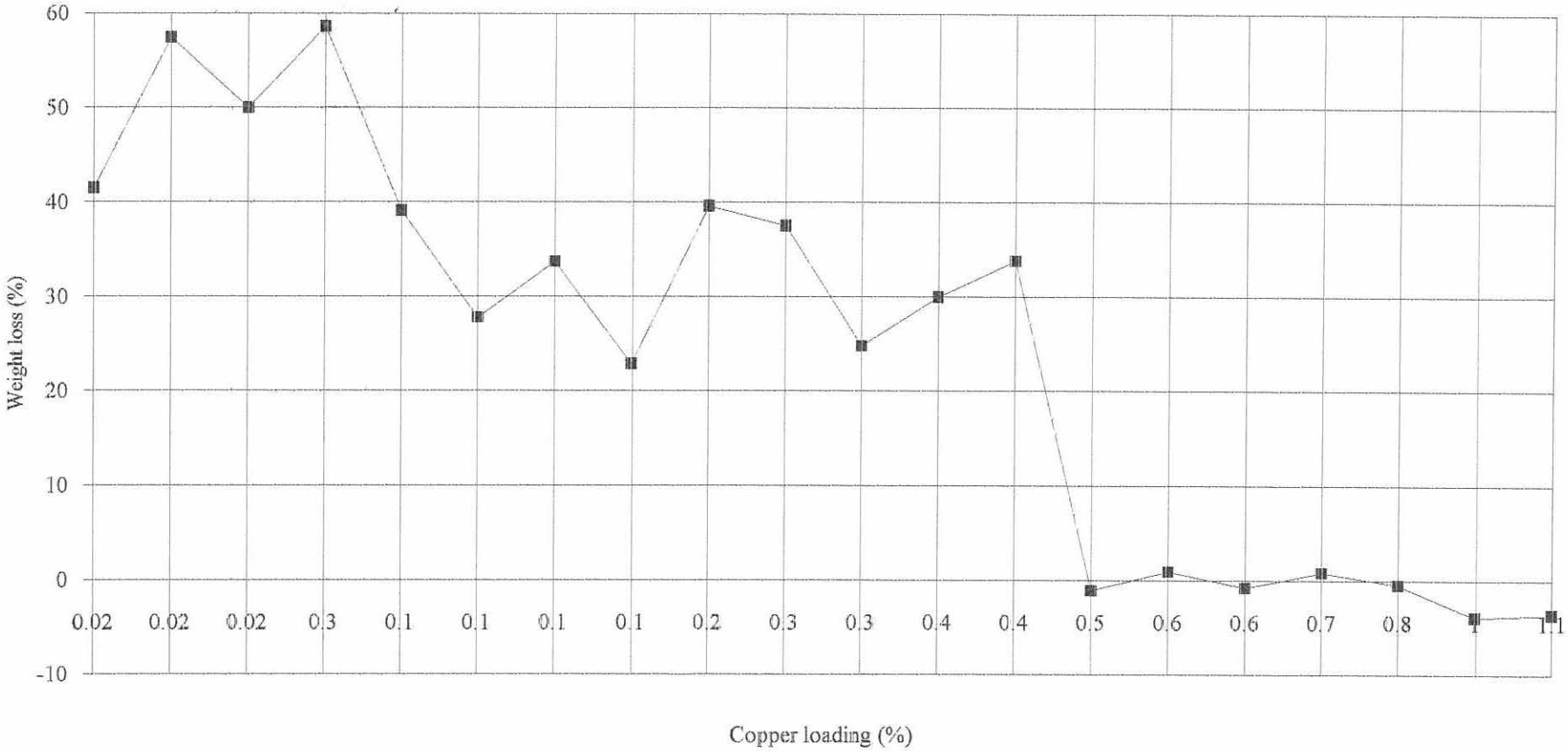
Figure 5.4. Idealised product formed after the addition of copper to ASAC17 modified wood.

The ideal product would be as shown in Figure 5.4 where the copper is fixed into the wood structure by chelation.

It is unlikely that the rigid structure of the cellulose chains would facilitate the formation of such a system but it could form in the less structured hemicelluloses and lignins. Water molecules would probably be more in evidence as ligands. It is possible that bonding to the hemicelluloses may be easier because of the increased degree of flexibility.

Commercially acceptable copper loadings are in the range 0.1 - 1.0% (0.55 to 5.50 kg/m^3), (Williams, 1994). Figure 5.5 indicates that copper loadings at and in excess of 0.5% (of the initial dry weight of wood) are effective in combating decay by this fungus and results from Chapter 4 (Table 4.2) shows that ASAC17 loaded to about 30% of the dry wood weight

Figure 5.5. Control blocks treated with copper only
Samples exposed to 12 weeks attack by C. puteana
Each data point represents one block



completely protect scots pine sapwood from attack by *C. puteana.* Considering the significant amount of time that it takes to modify wood chemically and complete a full bio-assay it was decided that mututal loadings of ASAC17 and copper should be investigated over that loading range where ASAC17 modification alone may not have been successful and a range of copper loadings covering loading levels of expected biological failure to success. The projected loadings, in combination, were to be 1 - 18% for ASAC17 and 0.1 - 1.5% for copper.

5.1 Experimental

5.1.1 Chemical Modification Reactions

5.1.1.1 Selection of Species and Specimen Geometry

Scots pine (*Pinus sylvestris* L.) sapwood was machined to the following dimensions: 20mm tangential, 20mm radial and 5mm longitudinal. The wood extractives were removed by organic solvent reflux . A full description of specimen preparation is given in Chapter 3, sections 3.2.1 and 3.2.3.

5.1.1.2 Reaction Procedure

5.1.1.2.1 ASAC17 Modification

The wood was modified using ASAC17 in solution with dimethylformamide as described earlier in this work (Chapter 3). The test design (Table 5.1) required 212 blocks to be treated with this reagent. After reaction the dry blocks were stored over anhydrous phosphorous pentoxide until required.

5.1.1.2.2 Copper Modification

Solutions of 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0M pentahydrate copper sulphate were prepared and the pH of each adjusted upwards to just over 4.0. Small amounts of 2.0M sulphuric acid and 0.5M sodium hydroxide were used for this adjustment. It was possible to bring an initial pH of 3.50 to about 4.02 before copper hydroxide formed as a precipitate. Blocks previously modified to a range of loadings with ASAC17 (Table 5.1) in sets of 12, were held below the surface of aqueous solutions of copper sulphate and vacuum impregnated with the salt solution for 20 minutes (Chapter 3, section 3.2.3, Figure 3.2). The vacuum was released and the blocks were washed with de-ionised water by vacuum / soak impregnation, 20 minutes under vacuum followed by 30 minutes soak. The water was drained and replaced and the procedure repeated three times. This was to remove any non-fixed copper. The blocks were then oven dried to constant weight at 105^{o}C. They were re-weighed to calculate the amount of copper uptake (see following section 5.2.1.1.1 for discussion of analysis of copper content). Copper loading for a block was calculated as the

Cu ion taken up by the sample block and expressed as a percentage of the initial dry wood weight. Using different solution strengths enabled the required copper loadings to be achieved.

Further reactions were required to produce bio-assay control specimens and samples for copper content analysis, these aspects are detailed in the following section.

All blocks were stored over anhydrous phosphorous pentoxide until required.

5.1.1.2.3 Control System

ASAC17 + copper control specimens were prepared by first loading with ASAC17 and impregnating with copper sulphate in aqueous solution adjusted to pH 4.0 as described in the preceding section 5.1.1.2.2. Copper only specimens were prepared in similar pH 4.0 aqueous solution. All control specimens underwent similar weighing, drying and dry storage procedures as did those samples designated for bio-assay. Copper loadings were similar to those in chemically modified samples.

20 copper only control blocks were implanted, each of which was partnered in an infected culture jar with an untreated sample.

The method used to load samples with copper only was a technique of vacuum impregnating and soaking blocks in pre-determined aqueous solution strengths of copper sulphate using a process devised by Hicksons Timber Products Ltd.

The following equation was used to arrive at that solution strength needed to give the required reagent loading:

$$\% \text{ solution concentration (w/w)} = \frac{R \times Bv}{P \times 10}$$

where: R is that weight of copper required to be retained in the final oven dried block (kg/m^3),

: Bv is the mean wet volume of the batch of blocks, here in sets of 6 (cm^3),
: P is the average weight of solvent (here water) retained, determined in a pre-test (g),

This method gives copper loadings as kg/m^3 which for the purpose of this work were converted to a percentage of the initial dry block weight.

To test the effect of the solvent, 24 specimens were oven dried and reacted for 6 hours in DMF only. No attempt was made to clean the blocks by reflux after reaction. A further 12 blocks were oven dried only. These were used to observe the effect of the test fungus on wood in an untreated state.

Operational controls to monitor any weight change brought about by the uninfected test system (sterile soil), were 12 blocks modified with ASAC17 + copper, 20 loaded with copper only, and 12 reacted in DMF only.

Table 5.1. outlines the test design.

In the following table outlining the design of the bio-assay, all specimens but the operational controls were exposed to *C. puteana*. Operational controls were set in sterile soil only and not exposed to fungal attack. Loadings are given as a percentage of the initial dry wood weight.

Table 5.1. Test design. Blocks modified with ASAC17 + copper

WL refers to blocks leached in water after reagent modification, ASAC17 + Cu (following section, 5.1.3.). NWL refers to those blocks not leached.

	Range of ASAC17 loadings (%)	Range of copper loadings (%)	Number of samples
NWL	1.0 to 18.0	0.1 to 1.5	100
WL	1.0 to 18.0	0.1 to 1.5	100
Operational controls			
NWL	1.0 to 18.0	0.1 to 1.5	6
WL	1.0 to 18.0	0.1 to 1.5	6

Other controls

Copper only			
	Copper loading (%)		
NWL	0.01 to 1.0 *		20
NWL	Untreated *		20
NWL	0.01 to 1.0	Operational	20
Solvent (DMF) only. No loading designation			
NWL			6
NWL	Operational		6
WL			6
WL	Operational		6
Untreated (oven dried only)			
NWL			6
WL			6

* 20 infected jars containing 1 treated and 1 untreated sample

5.1.2 Water Leaching

Other than those control blocks loaded with copper only, half of all sets of blocks were water leached after modification according to the prEN 84 procedure (Chapter 3, section 3.4). Blocks treated with ASAC17 + copper were leached after the introduction of copper to the ASAC17 modified samples. Blocks that were treated with copper only were not leached in water as pilot investigations (not quantitatively reported) showed that almost all copper leached out during this process.

5.1.3 Determination of Extent of Reaction

5.1.3.1 Weight Gain

ASAC17 loading was reported as weight gained during chemical reaction and expressed as a percentage of the initial dry wood weight (Chapter 3, section 3.3.1).

It was thought that this method of loading appraisal would be suited to copper modification but the complexity of the modification system and the unknown sorption effects raised doubts as to the validity of loadings calculated in this way. Copper loading for all ASAC17 + copper modified blocks was calculated approximately, using weight gain as the criterion and then adjusted where necessary using data obtained from Atomic Absorption Spectroscopy analysis of selected modified blocks (section 5.1.3.2).

5.1.3.2 Atomic Absorption Spectroscopy (AAS.)

The amount of copper added to ASAC17 modified blocks was calculated from the (dry) weight increase after loading. It was thought that this method was insufficiently precise and that analysis by AAS. would give more accurate data.

AAS. analysis is a destructive one so random samples, chosen from the population sets, were tested. Twenty six blocks which were not leached in water after modification were analysed by AAS. for copper content; weight gain loadings were in the ranges, ASAC17, 1.3 - 16.9% and copper, 0.1 - 1.7%. Twenty two samples leached in water after treatment were similarly analysed, they were in the weight gain ranges: ASAC17, 1.2 - 15.8%, and copper, 0.1 - 1.9%.

A process of "Wet Ashing" was required for this analysis to be carried out, this is described below.

Blocks were chipped into small pieces using a guillotine and then ground into a very fine powder in a rotary grinder. The resultant wood flour was dried in an oven overnight at 105°C and after cooling was weighed. 0.3 to 0.4g of the flour was put into a dry conical flask which was put onto an electric hot plate in a fume cupboard. Drops of concentrated sulphuric acid were carefully added which reduced the wood to a black charred state. The solution was oxidised by carefully adding hydrogen peroxide solution. After heating for about ten minutes most of the wood had been dissolved. Sufficient drops of concentrated nitric acid were then added to the brown solution until all of the wood residue had been dissolved and the solution was clear. After cooling, the residue was made up to 100 ml of aqueous solution and this was analysed for copper content. Knowing the amount of wood used to prepare this solution contained enabled the resulting AAS. figure, given in parts per million of copper, to be translated to a percentage copper loading for that whole sample. This was repeated for a selection of wood blocks, both those leached in water and those not leached. The set average figure could then be compared with that previously obtained using weight gain as the loading criterion.

5.1.4 Biological Testing

The biological test was that described in Chapter 4, section 4.1. The twelve week assay was a soil block test based on ASTM D.1413-76 (1986) using the Basidiomycete *C. puteana,* strain FPRL 11E. This fungus resists zinc and cadmium compounds but is only partially tolerant to copper at higher reagent concentrations. At the end of the test period the blocks were removed from test, cleaned of superficial deposits and weighed in a wet, and later oven-dried state. The moisture content at the end of the test period and overall weight loss for each block was calculated.

5.2 Results and Discussion

5.2.1 Chemical Reactions

5.2.1.1 Copper Modification

5.2.1.1.1 Analysis of Copper Content in ASAC17 + Copper Modified Blocks

As the blocks were thoroughly washed in de-ionised water after copper had been added to the ASAC17 modified substrate it is assumed that any copper remaining was fixed into the wood structure.

Assessing copper content by weight gain in this type of work is considered unreliable and values obtained can only be considered approximate. AAS. analysis (section 5.1.3.2) is a more precise analytical method. In order to carry out this analysis the test specimens have to be destroyed thus it is not possible to analyse the blocks to be used in future bio-assay. In this test two sets of blocks (NWL and WL) were prepared, additional to those designated for biological testing and approximating the loading ranges of ASAC17 + copper of the bio-assay sets. These data were used to adjust where necessary, the weight gain figures of the sets designed for biological testing.

Table 5.2 shows that for NWL. samples the average values by both methods were close enough for the weight gain values to be considered valid. The average AAS. value for WL samples was lower than that determined by weight gain. To reflect this, the copper weight gain values of the 100 blocks to be biologically tested were proportionately adjusted. The effects of leaching are discussed later in section 5.2.2 of this chapter.

In any future work this process could be assisted by rigorous analysis of all leachate during the washing and soaking processes.

Table 5.2. Comparison of weight gain method and Atomic Absorption Spectroscopy analytical method of determining the copper content of ASAC17 + copper modified blocks. NWL and WL refer to blocks not leached in water and leached in water after copper modification (Chapter 3). All loading values are given as a percentage of the initial dry wood weight.

	Range of ASAC17 loading (%)	Copper content by weight gain (%)		Copper content by AAS analysis (%)		No. of samples analysed by AAS
		Average	SD	Average	SD	
NWL	1.3 to 16.9	0.9	0.4	0.9	0.3	26
WL	1.2 to 15.8	0.8	0.5	0.5	0.2	22

5.2.1.1.2 Copper Content

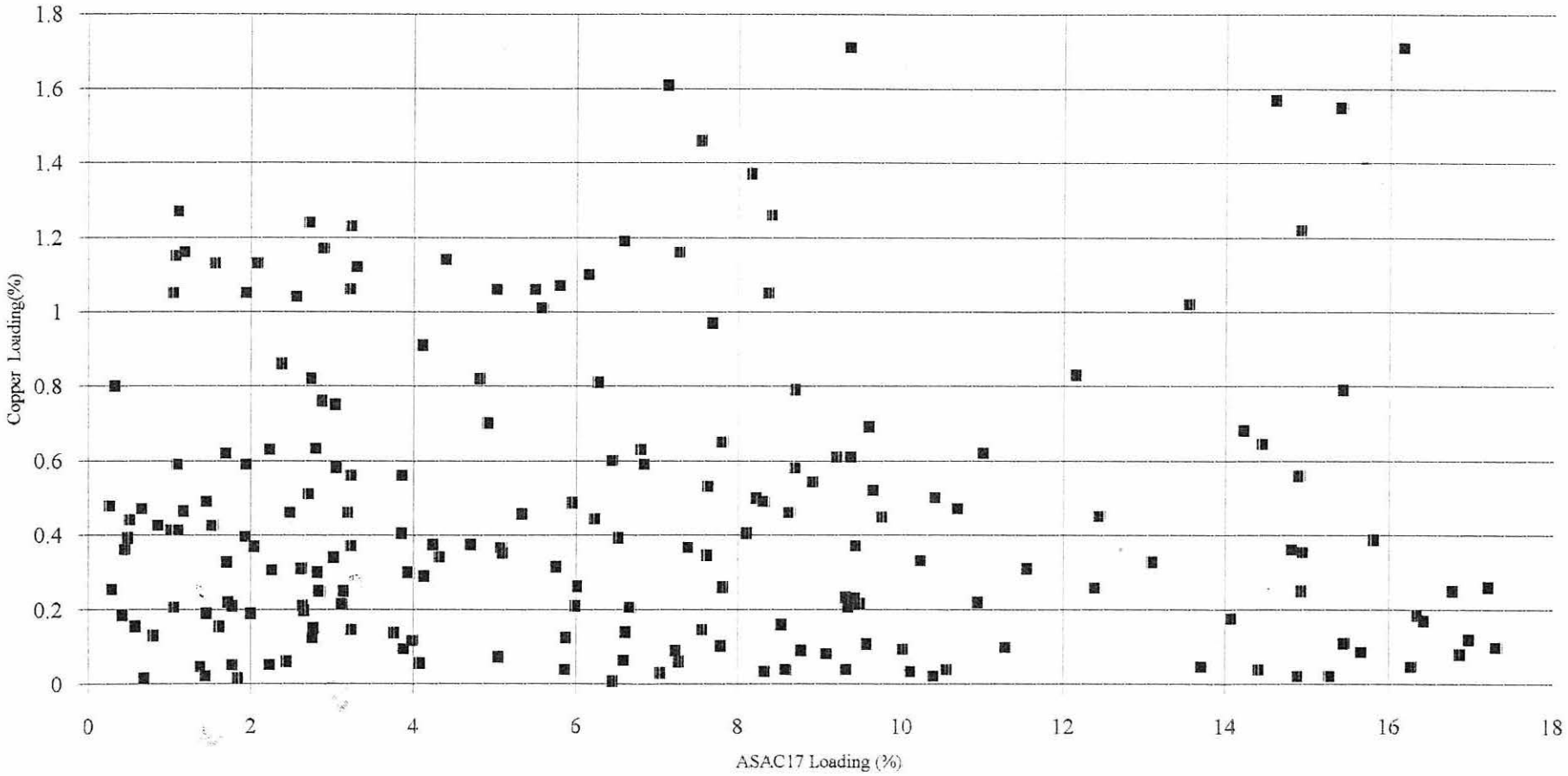
The range of copper loadings that it was possible to achieve in ASAC17 treated samples (loadings up to 17.3%) was 0.1 - 1.9% of the initial dry wood weight. Dunningham and Parker (1992) found that with blocks of Radiata pine sapwood succinated to over 30% they were unable to get copper loadings over 3%. A pilot investigation during this work (not quantitatively reported here) indicated a maximum copper loading of 2.7% at ASAC17 levels approaching 30%. This is broadly in line with the data of Dunningham and Parker mentioned above.

5.2.1.1.3 Copper Uptake as a Function of ASAC17 Loading

Although there was a large increase in copper sorption ASAC17 loading levels had no significant systematic effect on copper uptake (Figure 5.6). Data give a correlation value, r, of -0.1.

At any one treating copper salt solution concentration level an increase in the initial amount of bonded ASAC17 in the wood did not result in a corresponding increase in copper uptake. All that can be said with any certainty is that for any one loading level of ASAC17 the higher copper loadings were in blocks treated with a greater copper salt

Figure 5.6. Copper Uptake, Assessed by Weight Gain, as a Function of ASAC17 Loading (Section 5.1.1.2.2, Appendices 5.1 and 5.2). Data are for 200 Blocks.



concentration. There is some evidence that there may be a degree of positive correlation at the 0.5 and 1.0 M concentrations of copper solution but the results were not sufficiently marked to confirm this. The determining factor was the concentration of the treating copper salt solution. Increasing the concentration of the copper solution did result in a corresponding increase in copper uptake by the carboxylated substrate. It is possible that steric hindrance and increased hydrophobicity of the substrate brought about by the initial reagent modification (here ASAC17) prevents copper loadings above the levels found here. Observations at Hicksons Timber Products Ltd. (Williams, 1994) and work not quantitatively reported here saw copper introduced to untreated pine almost completely leached out after two weeks leaching in water.

The samples treated at the higher copper salt concentrations had a much stronger green colouration than did those treated at lower levels. Selected samples were split open using a sharp blade and it was obvious from the internal appearance that copper penetration was complete in blocks treated with high concentrations of copper. This effect was not apparent at low copper levels other than in the outer regions of a sample. It is possible that at the lower concentrations of treating copper solutions the copper ions in the solution may be depleted by attachment in the outer regions of the wood blocks leaving an insufficient concentration of ions to add on in the centre of the sample. This is very likely the reason for lack of correlation between ASAC17 and copper loadings. The biological implications of this are discussed later (section 5.2.3).

It is difficult to ascertain the distribution of carboxylate ions in the succinated substrate but it is probable that they occur only in the more amorphous regions of the cell wall, the crystalline areas remaining inaccessible in this respect. If this method of copper attachment is successful then it is in these succinated areas that the copper may be found.

5.2.2 Effect of Water Leaching Wood Modified with ASAC17 + Copper

Table 5.3. Summary of the change in weight of 100 ASAC17 + Cu modified blocks after 14 days leaching in water (Appendix 5.4). The average figure is based on change in weight of dry modified blocks. A negative value indicates a weight gain.

Range of loadings		Weight loss during leaching (%)	
ASAC17 (%) calculated before leaching	Copper (%) calculated after leaching	Average	Range
0.3 to 17.3	0.1 to 0.6	0.6	0.1 to 1.8

Table 5.3 (Appendix 5.4) details the weight lost from the 100 blocks designed for subsequent bio-assay after the 14 day leaching period described in section 5.1.2 of this chapter.

The prime objective at this stage of the investigation was to prepare 100 blocks for subsequent bio-assay. The copper content of the individual blocks was not calculated (by weight gain and AAS. adjustment (section 5.2.1.1.1) until after the blocks had been leached and thus it is not possible to give a comparison of pre- and post-leach copper content values. What the summarised values in Table 5.3 do tell us is that after the leaching process the blocks retained copper at levels of 0.1 - 0.6% of the initial dry wood weight and that the overall weight losses, averaging below 1% were very small. Any unreacted, adsorbed copper is lost during leaching and it is probable that the remaining copper is fixed into the wood by the ASAC17. Here a system of weight gain and AAS. analysis to determine the copper content of treated, water leached blocks was used to help give copper content after leaching. It is felt that in future work this aspect should be more rigorously addressed. It is suggested that weight gain and AAS. analysis should be used to determine copper content immediately after chemical modification and the same procedures carried out after the 14 day leaching period. This would help to more accurately determine the amount of copper fixed into the wood and the amount of unfixed or poorly fixed copper that is affected by leaching.

5.2.3 Biological Testing

Figures 5.7 and 5.9 (Appendices 5.1 and 5.2) show that modification of the Scots pine sapwood by ASAC17 + copper was effective in preventing decay by *C. puteana* in this test. The treatment was less efficient in blocks leached in water after the chemical modification process (later section 5.2.2). All unmodified controls (Table 5.11) were extensively decayed. Operational control losses (Tables 5.10, 5.11, 5.12, 5.13) were minimal.

There are two distinct sub-sets of post - bio-assay test specimens within both those not leached in water after chemical modification and those leached. In all test blocks the levels of ASAC17 modification alone is not high enough to ensure biological protection (maximum 17.3%). The copper loading however ranges from 0.1 - 1.7%. The controls in this test (Figure 5.5) show that wood loaded with copper at 0.5% gives complete protection. The two sub-sets therefore, are those where copper content is 0.5% and over and protection against decay would be expected and those where copper levels are below 0.5% and decay would be expected. This discussion addresses the bio-assay test results as a whole with sections specifically referencing those results from blocks loaded with expected biologically ineffective loadings of ASAC17 and copper in combination.

5.2.3.1 Samples Not Leached in Water after Chemical Modification

5.2.3.1.1 Weight Loss

5.2.3.1.1.1 ASAC17 Factor

Figure 5.7 diagrammatically summarises the results of the complete set of 100 blocks. Tables 5.4 and 5.5 present the data in two sets. In the first, copper was present in amounts at and above its estimated individual reagent biological threshold level and in the second present in amounts below this threshold. In both sets ASAC17 was present in its sub-biological individual threshold level, estimated at about 32% (Chapter 4).

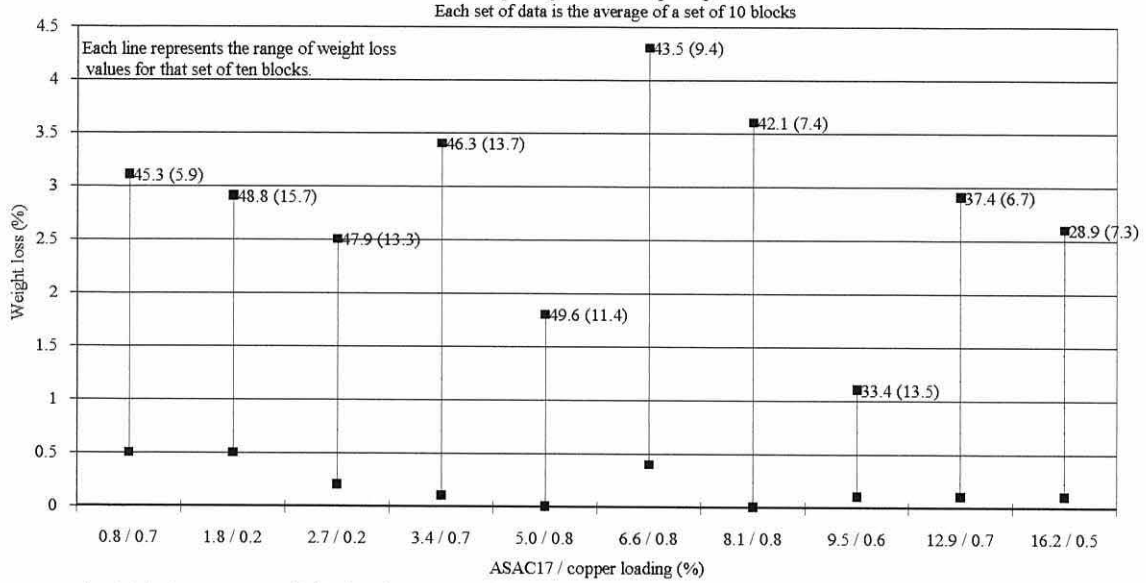
Table 5.4. Details of 66 of the 100 blocks NWL after chemical modification and subject to 12 week attack by *C. puteana*. These 66 blocks did not have levels of ASAC17 that would protect the wood. The copper levels were at 0.5% and over, where complete biological protection by copper alone would be expected. (Appendix 5.1). Percentage values are relative to initial dry wood weight. Although separate correlation values are given for each reagent it is not known to what extent the reagents are chemically combined within the wood structure. (Appendix 4.12)

	ASAC17 (%)	Copper (%)
Range	0.3 16.2	0.5 - 1.7
Mean	6.5	1
Standard deviation	4.2	0.6
Moisture content at end of test (%)		
Range	30.7 - 49.7	
Mean	45.8	
Standard deviation	4.2	
Weight loss at end of test (%)		
Range	0.1 - 4.3	
Mean	1.4	
Standard deviation	0.9	
Extent of linear correlation		
Factor	r	
ASAC17 vs moisture content	-0.4	
ASAC17 vs weight loss	-0.2	
Copper vs moisture content	0.1	
Copper vs weight loss	-0.1	
Moisture content vs weight loss	0.1	

Table 5.5. Details of 34 of the 100 blocks NWL after chemical modification and subject to 12 week attack by *C. puteana*. These 34 blocks did not have levels of ASAC17 that would protect the wood and copper levels where biological protection would not be expected by copper alone. (Appendix 5.1). Percentage values are relative to initial dry wood weight. Although separate correlation values are given for each reagent it is not known to what extent the reagents are chemically combined within the wood structure. (Appendix 4.12)

	ASAC17 (%)	Copper (%)
Range	0.4 17.2	0.1 - .4
Mean	6.8	0.3
Standard deviation	5.7	0.3
Moisture content at end of test (%)		
Range	20.1 - 77.9	
Mean	37.1	
Standard deviation	13.7	
Weight loss at end of test (%)		
Range	0.1 - 2.9	
Mean	0.8	
Standard deviation	0.6	
Extent of linear correlation		
Factor	r	
ASAC17 vs moisture content	-0.6	
ASAC17 vs weight loss	-0.3	
Copper vs moisture content	0.1	
Copper vs weight loss	-0.3	
Moisture content vs weight loss	0.1	

Figure 5.7. Weight loss as a function of reagent loading. NWL
Samples exposed to Coniophora puteana
Each set of data is the average of a set of 10 blocks



Annotated values are average (StdDev) moisture content of the set of samples, calculated at the end of test.

ASAC17 is biologically successful in combination with copper (Figure 5.7) at levels at which it would be expected to fail as the sole modifying agent. It is effective in combination at average loading levels of 0.8 - 16.2%, ranging from 0.3 - 17.2% (Appendix 5.1).

Table 5.4 details 66 of the blocks that were loaded with ASAC17 at what would be expected to be biologically ineffective levels. Weight losses were negligible, 0.1 - 4.3%. The correlation between ASAC17 loading and weight loss is very low at -0.2 inferring that increased ASAC17 loading had little effect on decay levels. Table 5.5 gives details of the 34 blocks out of the 100 assayed where both the ASAC17 and copper levels were below those where biological protection would have been expected. Weigh losses are again negligible, 0.1 - 2.9%. Increasing ASAC17 loading is statistically seen to have a limited effect on weight loss with a correlation value of -0.3.

5.2.3.1.1.2 Copper Factor

Copper is seen to be effective at the lowest levels of loading (down to 0.1%), (Appendix 5.1) in combination with ASAC17. Figure 5.7 shows it to be effective at average levels of 0.2 - 0.8%, ranging from 0.1 - 1.7%. Success would be expected at the higher levels but not at those below 0.5%. Figure 5.5 shows that copper alone in this test was not effective until loaded to 0.5%.

Table 5.4 gives details of the 66 blocks where copper levels were relatively high at 0.5 - 1.7% and decay levels were very low, 0.1 - 4.3%. As ASAC17 is ineffective at the levels to which it was loaded it is reasonable to assume that here copper was the determining factor in preventing fungal decay. In Table 5.5 copper is at individually biologically ineffective levels (0.1 - 0.4%) yet in combination with ASAC17 it is very effective in preventing decay. At this stage it is not certain whether or not the successful effect that copper has on the system is one of synergism or more simply that of addition.

When looking at the given relational correlation values for weight loss it should be born in mind that the ASAC17 and copper are at least partially combined. The statistics relate to each as a separate entity. It is not possible to statistically assess the two separately when they are present in chemical combination but comparisons are useful.

5.2.3.1.1.3 Combined Factors

ASAC17 combined with copper (Figure 5.7), (Tables 5.4 and 5.5) is a protective system which is seen to be very effective according to this work. The combined system is efficient at levels at which neither reagent is effective as the sole reagent.

Figure 5.5 shows that in control blocks, copper alone gives complete biological protection at a loading of 0.5% of the initial dry wood weight. Blocks modified with ASAC17 and copper, having a copper content of 0.5% and up, would therefore be sufficiently protected by the amount of copper present to prevent decay, given that the amount of ASAC17 present, as discussed above, is insufficient to give a significant amount of biological protection.

The interest in this work lies in those modified blocks where each reagent is present in amounts below the toxic threshold levels for individual components and weight losses are

very low or nil and this is the set detailed in Table 5.5. In these blocks there cannot be enough of either reagent present to completely penetrate and modify the accessible wood polymers throughout the blocks and yet in this test they are undecayed. It is proposed that in all of the above detailed blocks there is probably only sufficient chemical to give a superficial or surface modification of the wood cell wall.

Tables 5.4 and 5.5 show that weight losses are insignificant when operational control losses of average 3.2% (1.5 SD), (Table 5.10) are considered. ASAC17 certainly gives no protection at all at the lowest weight gains. Even at the higher levels, approaching 17.2% there is little protection. In Chapter 4 (section 4.2) it is shown that when ASAC17 alone is loaded at an average of 14% (2.6 SD) weight losses range from 24.5 - 49.3%. The success of copper at less than 0.5%, the biological threshold defined in this test, would not be expected.

Moisture content in this set has not been controlled to below the approximate minimum 20% that *C. puteana* needs for decay (Hunt and Garrett, 1953). The average of 33.4% (9.7 SD) for the 100 blocks assayed compares with the operational control moisture level of 28.8 % (1.7 SD), (Table 5.10). The moisture content of most of the blocks was above 20% and thus it seems that this system is not working solely by excluding moisture from the available hydroxyl groups in the blocks. In the absence of other control factors decay would be substantial at moisture contents from 28 - 50%.

The sample blocks were machined to 20 x 20 x 5mm with the axial tracheids aligned in the 5mm direction. This was primarily to make reagent penetration easy during chemical modification but it also gives the fungal mycelia ready access to the wood during the bio-assay along with penetration through the ray tissue. Figure 5.8 shows diagrammatically the configuration of axial tracheids in a modified block. The tracheids are typically 2 to 4 mm in length. It would be expected that the modifying solution would fill all of the cell lumen and penetrate fully the cell wall at all levels of ASAC17 solution concentration. However the very small amount of ASAC17 loaded into some blocks, as low as 0.3%, would probably only have been sufficient to modify a small superficial area as suggested in Figure 5.8., partitioning being at the cell wall diffusion stage. It is proposed that there would have been

insufficient ASAC17 to carry the modification process through to the inner region of the cell wall. The copper introduced would then have had only this zone of ASAC17 modification into which to be fixed. Any unfixed copper which had been taken in solution to the centre of the wood cell wall would have been leached out by the subsequent washing in de-ionised water. At the very low solution concentrations of copper it is probable that there would have been insufficient copper ions to progress into the centre as they would have been preferentially fixed by ASAC17 at the outer areas of the tracheid walls.

This envelope of treated material may be sufficient to protect the inner untreated parts of the wood cell wall. Time did not allow further analytical work at this stage of the investigation but an analytical technique such as Fine Trace Infra-Red scanning would help to define the distribution of this protection system within the wood cell wall.

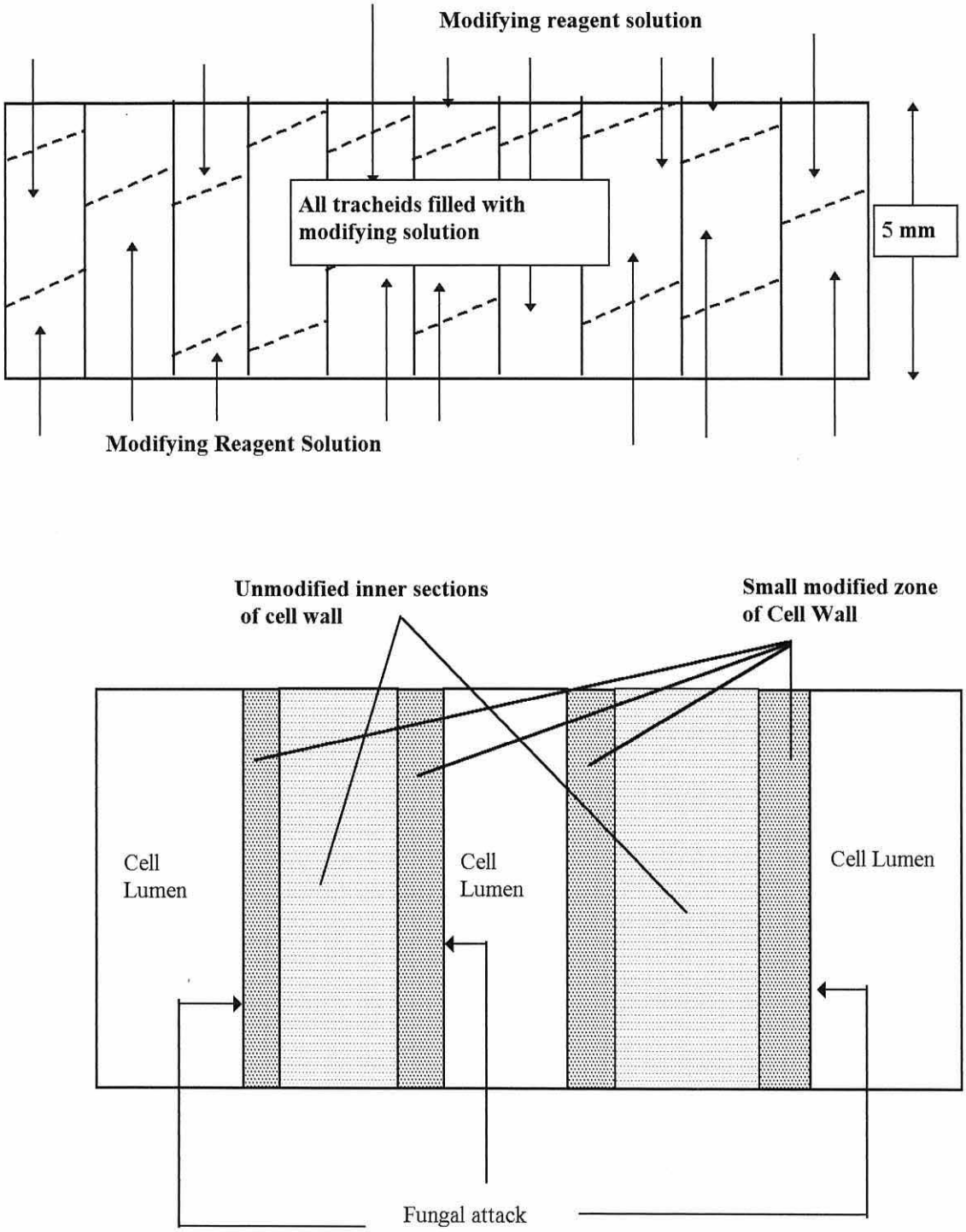


Figure 5.8. Diagrammatic representation of axial tracheid system in a block modified with ASAC17 + Copper. The first diagram shows the modifying solution filling the cell lumen and penetrating the cell wall. The second diagram shows the superficial modification of the cell wall that could result from very low amounts of modifying reagent.

5.2.3.1.2 Moisture Content Effects

The optimum moisture content for decay by *C. puteana* is about 50 - 60% with substantial decay observed above 28%. Figure 5.7 shows that the average moisture content of the full set of 100 blocks at the end of test ranged from 28.9 - 49.6% and that it was not until ASAC17 levels averaged 9.5% that there was any lowering of moisture in the blocks. At the highest average ASAC17 level of 16.2% the average moisture content was 28.9% whilst up to ASAC17 loadings of 9.5% the moisture contents were in the range 30 - 50% and decay would have been expected had moisture content been the only factor affecting decay control. Table 5.11 shows that untreated controls averaging 45.1% moisture content lost an average of 64.2% in weight. Copper loading does not appear to have had a direct effect on moisture levels. Correlation values for the complete set of 100 blocks give relationships of $r = -0.5$ for ASAC17 and 0.4 for copper. The statistics indicate that ASAC17 could be having the greater effect on moisture control, increasing loading resulting in lower moisture contents.

A chemical protection system can work in several ways (Chapter 4, section 4.2). Should this system prove to be fungitoxic then it should be realised that once the invading fungus has been killed by a chemical protection system any moisture entering the wood thereafter is limited to simple diffusion from the soil into the block. In this test the samples were separated from the soil by a nylon mesh so it is thought that this factor would be minimal. This would result in a low moisture content even though the protective system may not be hydrophobic. This could be the case here where the levels of reagent loading are too low to render the wood more hydrophobic.

5.2.3.2 Samples Leached in Water after Chemical Modification

5.2.3.2.1 Weight Loss

5.2.3.2.1.1 ASAC17 Factor

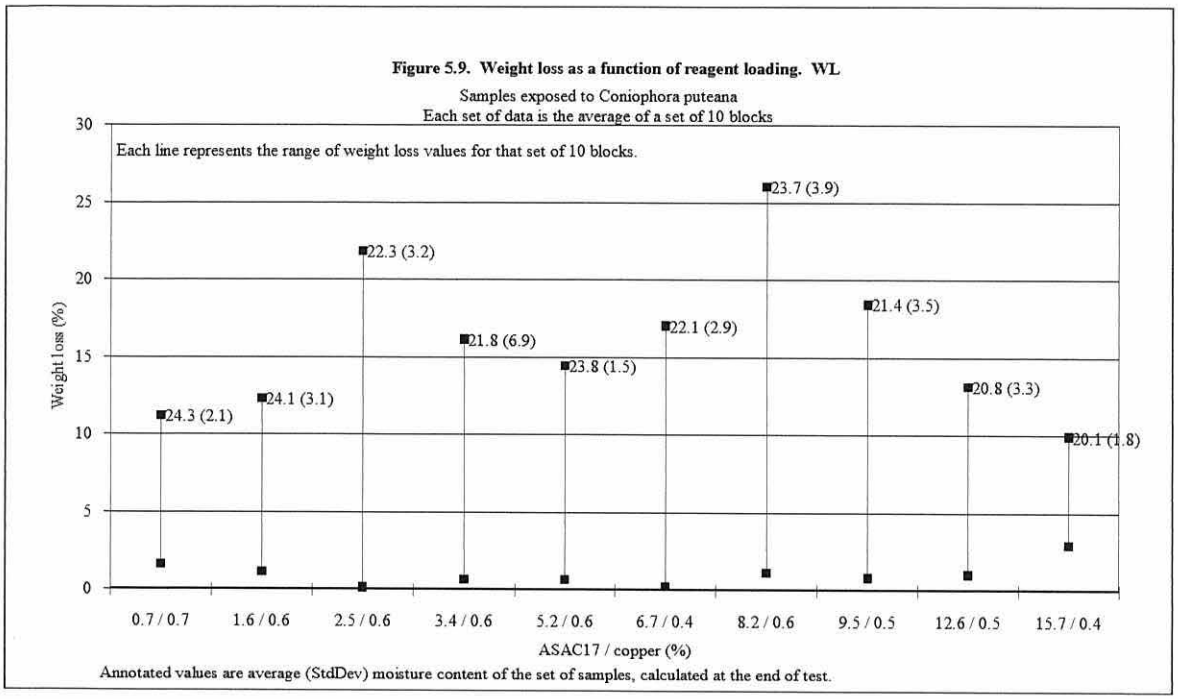
Table 5.6. Details of 13 of the 100 blocks WL after chemical modification and subject to 12 week attack by *C. puteana*. These 13 blocks did not have levels of ASAC17 that would protect the wood. The copper levels are such that biological protection would not be expected by copper alone. (Appendix 5.2). Percentage values are relative to initial dry wood weight. Although separate correlation values are given for each reagent it is not known to what extent they are chemically combined within the wood structure. (Appendix 4.12.)

	ASAC17 (%)	Copper (%)
Range	0.3 - 14.9	0.5 - 0.6
Mean	6.5	0.54
Standard deviation	4.5	0.005
Moisture content at end of test (%)		
Range	21.2 - 31.2	
Mean	23.2	
Standard deviation	2.5	
Weight loss at end of test (%)		
Range	3.7 - 26.0	
Mean	10.6	
Standard deviation	6.3	
Extent of linear correlation		
Factor	r	
ASAC17 vs moisture content	0.1	
ASAC17 vs weight loss	-0.2	
Copper vs moisture content	-0.1	
Copper vs weight loss	-0.2	
Moisture content vs weight loss	0.7	

Table 5.7. Details of 87 of the 100 blocks WL after chemical modification and subject to 12 week attack by *C. puteana*. These 87 blocks did not have levels of ASAC17 that would protect the wood and copper levels are such that biological protection would not be expected by copper alone. (Appendix 5.2). Percentage values are relative to initial dry wood weight. Although separate correlation values are given for each reagent it is not known to what extent they are chemically combined within the wood structure. (Appendix 4.12)

	ASAC17 (%)	Copper (%)
Range	0.3 - 17.3	0.1 - 0.4
Mean	6.6	0.2
Standard deviation	4.7	0.1
Moisture content at end of test (%)		
Range	14.8 - 32.1	
Mean	22.6	
Standard deviation	3.1	
Weight loss at end of test (%)		
Range	0.1 - 23.8	
Mean	6.5	
Standard deviation	5.4	
Extent of linear correlation		
Factor	r	
ASAC17 vs moisture content	-0.5	
ASAC17 vs weight loss	-0.1	
Copper vs moisture content	-0.2	
Copper vs weight loss	-0.1	
Moisture content vs weight loss	0.7	

Increasing the average levels of ASAC17 loading in the presence of copper (Figure 5.9) statistically had no significant effect on weight loss. Table 5.6 details 13 of the 100 blocks WL after chemical modification with ASAC17 and copper where copper would be the determining factor in decay prevention, loaded to 0.5 - 0.6%. ASAC17 is seen individually to have a statistically limited effect on weight loss at the end of the test. The individual correlation value of -0.2 is very low, yet again ASAC17 is successful at levels at which it would not be as the sole reagent, weight losses were 3.7 - 26.0%. Table 5.7 details the 87 blocks in which ASAC17 alone was not present in sufficient quantity to prevent decay yet weight losses were limited to 0.1 - 23.8%.



5.2.3.2.1.2 Copper Factor

Copper modification is successful in combination with ASAC17 at levels at which it would not normally be so. Statistically as an individual entity it is seen to have a very low impact on weight loss with a correlation value (r) for the complete set of 100 blocks, of -0.1. Tables 5.6 and 5.7 show that individually it's statistical contribution to decay prevention is very small.

5.2.3.2.1.3 Combined Factors

Generally this system is biologically successful even after a 14 day leaching process.

In 87% of the blocks (Table 5.7) both ASAC17 and copper levels were below those at which decay would be arrested if both reagents were present as the sole modifying agent. In this set of 87 blocks the mean weight loss was 6.5% (5.4 SD).

There are some anomalies, examples are given below in Table 5.8.

Table 5.8. Details of blocks were decay has occured in blocks were the levels of copper loading would have been expected to prevent decay.

ASAC17 (%)	Cu (%)	Moisture content (%)	Weight Loss (%)
0.3	0.5	22.8	11.2
2.8	0.6	21.9	14.3
3.1	0.6	25.3	16.1
6.3	0.5	23.0	14.4
8.2	0.5	22.4	15.4
8.6	0.5	31.2	26.0

Decay would not be expected at these levels of copper loading yet some decay has occurred. It is possible that the copper present in these blocks was poorly fixed by the ASAC17 and there is also the possibility that the blocks were physically damaged during the chemical modification process. In either case the protective properties of the system would be diminished.

It is possible that, as is proposed and discussed later, this system may work by giving a superficial or surface-type of treatment to the tracheid wall. If this is true then it may be that with some samples this surface treatment is weak and can be disrupted by the severe leaching process to which this set of blocks was exposed. Once the proposed fungi-toxic barrier is broken down the fungus could gain access to the unmodified interior of the cell wall with subsequent increased levels of decay.

5.2.3.2.2 Moisture Content Effects

In almost all of the blocks the moisture content at the end of test was restricted to a level at which little or no decay would be possible. Average values (Appendix 5.2) ranged from 20.1 - 24.3%. Decay was observed in some blocks and this is discussed in the previous section 5.2.3.2.1.3.

Statistically ASAC17 is seen to be making a greater contribution to this effect, r = -0.1 for 13 of the blocks which is insignificant but this rises to -0.5 for the remaining 87 blocks. This latter set is that in which the copper content is below its' biological threshold level. Figure 5.9 shows that the average moisture content values at the end of test varied little and appeared unaffected by increasing levels of both reagents over the range tested, from 20.1 -

24.3%, although statistically ASAC17 is more significant as discussed above.

The moisture content of these blocks are generally lower than those for the non-leached ones. Moisture contents are probably all too low for significant decay to have occurred. With a longer test period all of the blocks would probably have failed. It can only reasonably be said that the decay was suppressed during the test period. In most of the blocks the protective system remained stable in the presence of water but in others it seems to have broken down. It would appear that a number of the blocks, as detailed below (Table 5.9) were too dry for decay to have occurred and yet the blocks were degraded.

Table 5.9. Details of blocks were decay has occured at moisture levels at which decay would not have been expected.

ASAC17 (%)	Cu (%)	Moisture content (%)	Weight Loss (%)
15.8	0.4	17.9	6.1
16.3	0.1	18.3	9.4
11.6	0.3	19.7	7.8
14.9	0.1	19.9	7.3

The key here is probably moisture content. At low moisture contents but relatively high reagent loadings the wood cell wall -OH group is not available for water attachment. This could also be due to changes occurring in test due to changes in the equilibrium moisture content behaviour of the blocks after chemical modification.

5.2.3.3 Controls

Operational controls for ASAC17 + copper modification (Table 5.10) show minimal weight losses for both NWL and WL blocks.

Table 5.11 shows that copper alone in unleached blocks is successful in preventing decay. Figure 5.5 indicates a biological threshold of 0.5%. Goldstein *et al.* (1961) found that unleached Southern Yellow pine treated with copper sulphate (0.25% Cu calculated) lost an average of 2.7% of its weight when exposed to brown rot in a soil test. They tested 4 replicates. Dunningham and Parker (1992) claim no weight loss in copper only samples of Radiata pine sapwood treated to an average of 2.6% loading and exposed to brown rot attack by *C. puteana*. They do not say whether or not this is a lower limit of effective loading. According to this work this level is very high and decay would certainly not be expected at such a high loading. There are no data for leached blocks for reasons previously discussed.

The solvent DMF. offered no biological protection at all (Table 5.13). Weight losses in both leached and unleached samples were very high. Untreated samples were extremely decayed.

Table 5.10. Operational controls. Blocks modified with ASAC17 + copper. Data are average (Standard Deviation) for sets of 6 blocks. (Appendix 5.3.)

	NWL	WL
Weight Loss (%)	3.2 (1.5)	4.3 (1.0)
Moisture Content (%)	28.8 (1.7)	32.4 (2.5)

Table 5.11. The set of 20 copper modified and 20 unmodified blocks, one of each per culture jar and 20 operational controls (Appendix 5.3.). A negative value indicates a weight gain.

	Range of copper loading (%)	Weight change (%)	Moisture content	Correlation values	
				Copper loading vs weight loss (r)	Copper loading vs Moisture content (r)
Modified with copper. Exposed to C. puteana	0.02 to 1.1	-3.9 to 74.0	21.9 to 76.1	-0.8	-0.7
Unmodified. Exposed to C. puteana		57.2 to 72.0	27.8 to 56.7		
Modified with copper. Operational control	0.03 to 1.1	0.5 to -2.8	22.9 to 37.8		

Table 5.12. ASAC17 + Copper modified operational controls . Average and Standard Deviation relate to sets of 6 blocks. (Appendix 5.3.).

Range of ASAC17 loading (%)	Range of copper loading (%)	Weight loss (average %)	Standard Deviation	Moisture content (average %)	Standard Deviation
0.4 to 15.2 WL	0.1 to 1.0	3.2	1.5	28.8	1.7
0.7 to 16.5	0.1 to 1.1	4.3	1	3.2	2.5

Table 5.13. Solvent (DMF) and untreated controls. Exposed to *C. puteana* and operational. Figures relate to sets of 6 blocks. (Appendix 5.3.).

	Weight loss (average %)	Standard Deviation	Moisture content (average %)	Standard Deviation
NWL				
DMF only exposed to CP	70.6	2.9	71.7	11.1
DMF operational	1.0	0.3	29.7	1.0
WL				
DMF only exposed to CP	68.9	1.3	64.6	11.8
DMF operational	1.3	0.7	25.8	1.6
Untreated exposed to CP				
NWL	64.2	1.9	45.1	4.0
WL	65.2	1.2	50.4	4.6

5.4 Conclusion

It is difficult to envisage the way in which such small amounts of ASAC17 and copper in chemical combination could be so much more effective than would be similar amounts of each when used as sole modifying agents. It was postulated in Chapter 4 that ASAC17 being a large molecule may work by obscuration of hydroxyl sites rendering them unavailable for moisture and fungal enzyme attachment and by mechanical blocking of the woods micro-pore structure. However, here it is not present in quantities that would be expected to succeed in this way. Moisture content levels in non-leached samples were within a range where decay would be expected, approximately 20 - 50%. There was a falling off in moisture levels at the higher loadings but decay levels were low at the levels of reagent modification much lower than those required to significantly effect moisture content. A similar pattern is observed in the leached set but here the decay levels were higher in a lot of blocks and moisture levels lower. Copper too, in combination with ASAC17, gave protection at a lower level than would have been expected of it as the sole reagent.

The ASAC17 + copper modification at low levels may be sufficient to take place only at the surface of the cell wall and thus give a modified layer of chemically altered wood. This may afford an "envelope" of protection at the tracheid cell wall level, where the fungal hyphae are blocked by ASAC17 and the fungitoxic copper, the internal regions remaining unattacked. Should this be the case then the higher decay levels seen in the blocks which had been extensively leached in water could be explained by the removal of some reagent (ASAC17 or copper) from the ASAC17 fixing system allowing the fungus access to the inner unmodified region. This has not happened in all water leached samples. In a lot of blocks the system appears to have remained intact. Copper is susceptible to being leached out by water and it is possible that in many cases the copper was poorly fixed by ASAC17.

This may be a function of the type of treatment.

There are two scenarios:

i) Treatment of relatively low loaded ASAC17 blocks with a high concentration copper solution.

ii) Treatment of relatively high loaded ASAC17 blocks with low concentration copper solution.

In the first case the ASAC17, being present in small amounts would probably only modify the available -OH sites at the cell wall surface. A high copper concentration would be sufficient to bond copper to all available carboxylate sites. The resultant modification would be that of ASAC17 + copper at the tracheid surface only with the interior unmodified by ASAC17 or copper. Any non-fixed copper in the centre of the cell wall would be washed out by the wood subsequently being cleansed with de-ionised water. In the latter case there would be sufficient ASAC17 to modify vulnerable sites throughout the cell wall but only sufficient copper ions to bond at the surface of the cell wall.

Thus in the second case the wood would be less vulnerable than that in the first. Should copper be stripped away from the cell surface by water then there would still be an element of modification in the centre by the presence of a certain amount of ASAC17.

In future work of this nature it would be useful to accurately define the areas of decay within the wood blocks and thus indicate those areas of the cell wall which are protected by the chemical modification process. Amongst the analytical methods now being used to study wood decay are ELISA, EDXA, and FTIR spectroscopy.

The enzyme - linked immunoabsorbant assay (ELISA), a quantitative serological assay has been used to detect and quantify wood degrading fungal metabolites (Kim, Jellison, Goodell, Tracy and Chandhoke, 1991). These workers found that ELISA was a useful technique for the detection and quantification of decay fungi in culture and in wood. They used the technique to quantify various lignocellulose degrading fungal metabolites and to evaluate the sensitivity and specificity of antibodies produced to the white rot fungi *C. versicolor* and *Lentinus edodes* and the brown rot fungi *P. placenta* and *T. palustris*. They conclude that

ELISA is a useful diagnostic tool in the detection of wood decay, in culture, down to a dilution of 1 : 10,000.

Electron -microscopy is increasingly used in wood biodegradation research and is seen as an important compliment to biochemical and chemical approaches (Daniel, 1994). EM X-ray microanalysis (EDXA) can be used for labelling and detecting wood components (Cellulose, hemicelluloses and lignin) and studying areas of biodegradation.

Changes in coniferous wood due to the growth of fungi may be indicated as a result of applying FTIR spectroscopy (Korner, Faix and Weinhaus, 1992).

Chapter 6

Concluding Discussion

6.0 Chemical Modification

6.0.1 SA and ASAC17

It was relatively easy to react both SA and ASAC17 with the wood cell wall. Scots pine sapwood, in the size and orientation chosen was suitable for modification and the reflux clean up method after modification appeared to have removed excess reagent and solvent effectively. This latter fact was confirmed by the reaction appraisal methods of weight gain, volume change in relation to volume of chemical added and infrared analysis.

Succinic anhydride was easier to react with wood than ASAC17 and this is almost certainly a function of the relative sizes and shapes of the reagent molecules, ASAC17 having a long aliphatic carbon chain. Loading of both reagents only appears useful up to a limit. At loading levels in excess of 30% the wood began to show signs of physical deterioration at a microscopic level. This agrees with Rowell (1982a) who reports about 30% as the maximum useful loading before the onset of cell wall degradation. At a visible level both SA and ASAC17 modification approaching 30% loading brought about a darkening of the wood and a loss of surface integrity. The wood is noticeably harder to the touch with no loose surface fibres.

The maximum SA loading achieved was 26% and this took 7 hours at 110°C. This considerable time at an elevated temperature brought about a lot of cell wall checking. Rowell (1982b) found that with acetylation reaction, increasing the reaction after about 30% weight gain time did not bring about continuing useful reaction. Rowell and Ellis (1981) observed similar cell wall disruption at loadings of 25 - 30% with methyl isocyanate. The results reported here from work with SA and ASAC17 are in accord with these findings.

Proof of bonding by both SA and ASAC17 was confirmed by the significant weight increases observed after acetone refluxing the modified samples. Should either reagent have not been bonded then the weight increases on drying would have been minimal as the four hour

acetone reflux would have removed any unbonded chemical.

The alkenylsuccinic anhydride (ASAC17) is a much larger molecule than SA, molecular weight 336 as against 100 and it would be expected (Chapter 3, section 3.5.1.2) that it would be more difficult to react ASAC17 with the cell wall to equal levels at similar reaction parameters. This proved to be the case. The ASAC17 reacted to 16% weight gain in 180 minutes at 120°C whilst SA reacted to 16.4% in 180 minutes at 90°C; ASAC17 gave rise to 19% loading after 240 minutes at 120°C whilst SA brought about 19.1% after 240 minutes at 90°C.

There are a limited amount of -OH groups available for reaction in the cell wall. Stamm (1964) estimates that 65% of those in cellulose are unavailable for chemical modification reactions, being involved in inter-crystalline bonding. Succinic anhydride may be able to bond to more -OH sites than ASAC17 by being a smaller molecule. ASAC17 may be self limiting in reaction in as much as reaction at one -OH site probably limits reaction at neighbouring sites by steric hindrance. Succinic anhydride, because of its smaller molecular size is able to react at more sites, thus to achieve equal loadings, as outlined above lower reaction parameters are required.

6.0.2 ASAC12 and ASAC20

Modification using these two reagents served to compare the effect of different aliphatic chain lengths / molecular size on the extent and rate of reaction with the wood cell wall.

It is suggested that as the size of the reagent molecule increased, ie. ASAC12 to ASAC17 to ASAC20 it would become increasingly difficult to react equal amounts of reagent at the same reaction parameters. This proved to be the case as the summary Table 6.1. below shows.

Table 6.1. Weight gain and Degree of substitution as a function of reagent loading. Comparison of ASAC12, ASAC17 and ASAC20.

Reagent	Reaction time (mins)	Reaction temp (oC)	Weight gain (%)	Degree of substitution
		120.0	18.4	0.05
	180.0	120.0	23.7	0.06
ASAC17	120.0	120.0	10.3	0.02
	180.0	120.0	16.0	0.03
ASAC20	120.0	120.0	8.8	0.02
	180.0	120.0	12.8	0.03

Reagent loading and degree of substitution becomes less as the size of the anhydride molecule increases.

It is proposed that following reaction at one -OH site, when reacted with these relatively large molecules, sites local to this reacted site may be physically obscured thus rendering them sterically unavailable for further reagent reaction. It is logical to assume that increasing molecule size would increase this obscuration effect and the results obtained seem to confirm this.

The two reagents biologically tested cover a reasonable range of molecular size to assess the impact of -OH substitution and the hydrophilic property and bulkiness on fungal resistance.

6.0.3 ASAC17 + Copper

It was possible to load ASAC17 with the wood samples from 1% to 17.3% which approximated the projected loadings for the bio-assay. Copper was loaded into the ASAC17 modified wood at levels of 0.1% to 1.9%. These loadings were chosen, as previous work (Chapter 4) has shown that these levels of ASAC17 are not biologically effective and it is at these levels that there is interest in the combined ASAC17 + copper effect. It was not expected that copper loaded to under 0.5% alone would prevent fungal decay (Williams, 1994).

The pentahydrate copper sulphate in aqueous solution was adequate for introducing copper into the ASAC17 modified wood. The loading determining factor was the copper salt solution strength. ASAC17 levels had no significant effect on copper uptake. It was shown for example that for any one level of ASAC17 loading the greater copper loadings were achieved in blocks treated with solutions of greater copper salt concentration. At ASAC17 loadings of about 10%, treatment with a 1.0M copper salt solution resulted in a 0.5% copper loading whereas treatment with a 0.0625M solution resulted in copper loadings in the region of 0.1% and 0.2%.

Water washing the ASAC17 + copper modified blocks after modification will remove any unbonded copper. As there was a detectable amount of copper remaining in the wood, as proved by AAS. analysis, it is reasonable to assume that it was fixed in the wood. It is suggested that this occurs by interaction between the carboxylate ion formed on the modified wood and the copper cation.

The weight gain method of estimating copper loading was considered insufficiently sensitive. Here a method was developed based on AAS. analysis of a subsample and adjustment of the weight gain data, based on this subsample data.

6.1 Biological Efficiency

6.1.1 SA and ASAC17

6.1.1.1 Biological Threshold

The biological threshold of a modification system is that level of loading at which the wood is completely protected from fungal attack in that test. The twelve week soil test was successful in appraising the biological effectiveness of these reagents. ASAC17 modification was the more effective treatment against both *C. puteana* and *G. trabeum*. Increasing loadings of both reagents resulted in decreasing levels of decay with an accompanying decrease in the moisture content of the test blocks at the end of the test period.

6.1.1.2 Weight Loss and Moisture Content

The bio-assay was carried out on two sets of blocks for each reagent modification. One set underwent a 14 day leach in water (WL) and the other did not (NWL).

The alkenylsuccinic anhydride has biological threshold levels of 31.5% against *C. puteana* (NWL) and 28% against *G. trabeum* (NWL); 32.5% against *C. puteana* (WL) and 33% against *G. trabeum* (WL) (Chapter 4, section 4.2). Statistical correlation values, r, for reagent loading against weight loss are very strong, all at -0.92 and over; -1.0 being perfect correlation (Chapter 4, section 4.2). Increasing the loading had a strong inversely proportional effect on weight loss. This reagent modification had an equally strong effect on the moisture content of the blocks. Increasing loading brought about a marked lowering of moisture in the blocks as measured at the end of the bio-assay. Correlation values, r, for moisture content as a function of reagent loading are all at -0.91 and over.

Threshold values for SA are 44.5% against *C. puteana* (NWL) and 60% against *G. trabeum* (NWL); 56.5% against *C. puteana* (WL) and 68.5% against *G. trabeum* (WL). Statistical correlation values, r are very strong against *C. puteana* (NWL) at -0.89, for weight loss and -0.82 for moisture content. In all other respects the correlation is much weaker (Chapter 4, section 4.2).

Because of its greater size ASAC17, for any given weight gain, molar substitution is less with

ASAC17 than SA. It is difficult to achieve high degrees of substitution with ASAC17. This could point to an obscuration effect of the ASAC17 molecule reacting at one site and because of its size, obscuring neighbouring unreacted sites. ASAC17 loaded to 27.2% gives an estimated substitution value of 0.06 while SA has a substitution value of 0.24 at 26.1%. Figures 6.1 and 6.2 illustrate the difference between the biological effectiveness of the two reagents used in this test, using molar substitution and weight gain as criteria. ASAC17 is seen to be much more effective using the molar substitution criterion but there is less of a difference when results are compared using weight gain. This could indicate ASAC17 being more effective in two ways: the reactive-site shielding effect of the larger molecule and the increased hydrophobicity imparted to the substrate. Popper and Bariska (1975) found that phthalic anhydride modification of pine was effective in combating brown rot decay and attributed this success in part to the obscuration effect of the added reagent.

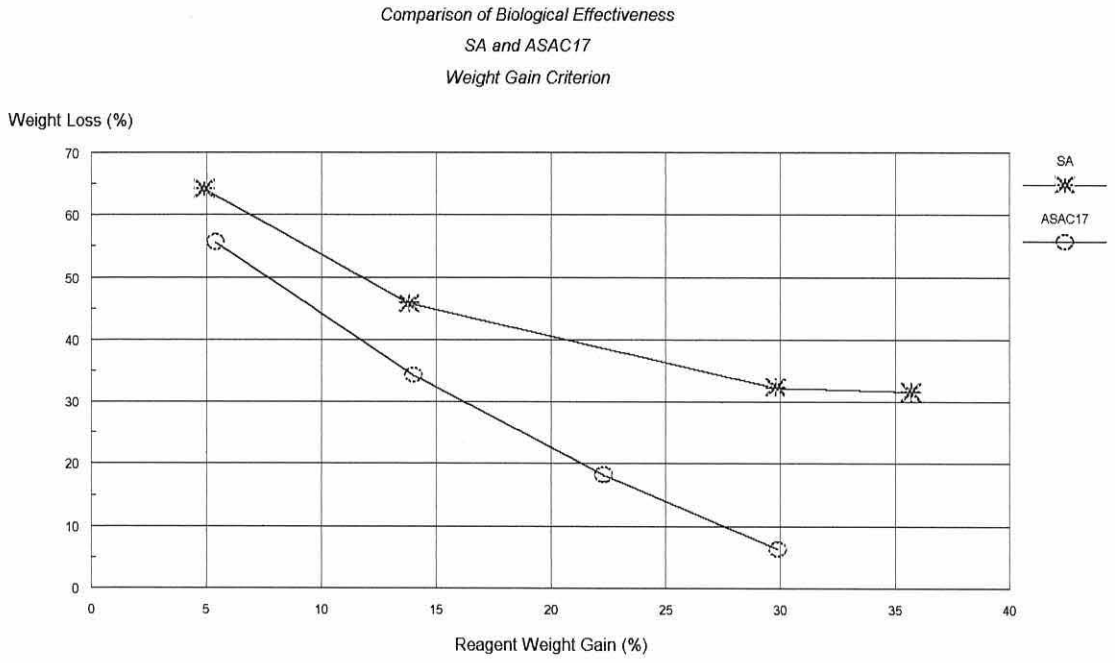


Figure 6.1

Figure 6.1. Weight loss in SA and ASAC17 modified samples using weight change as loading criterion.

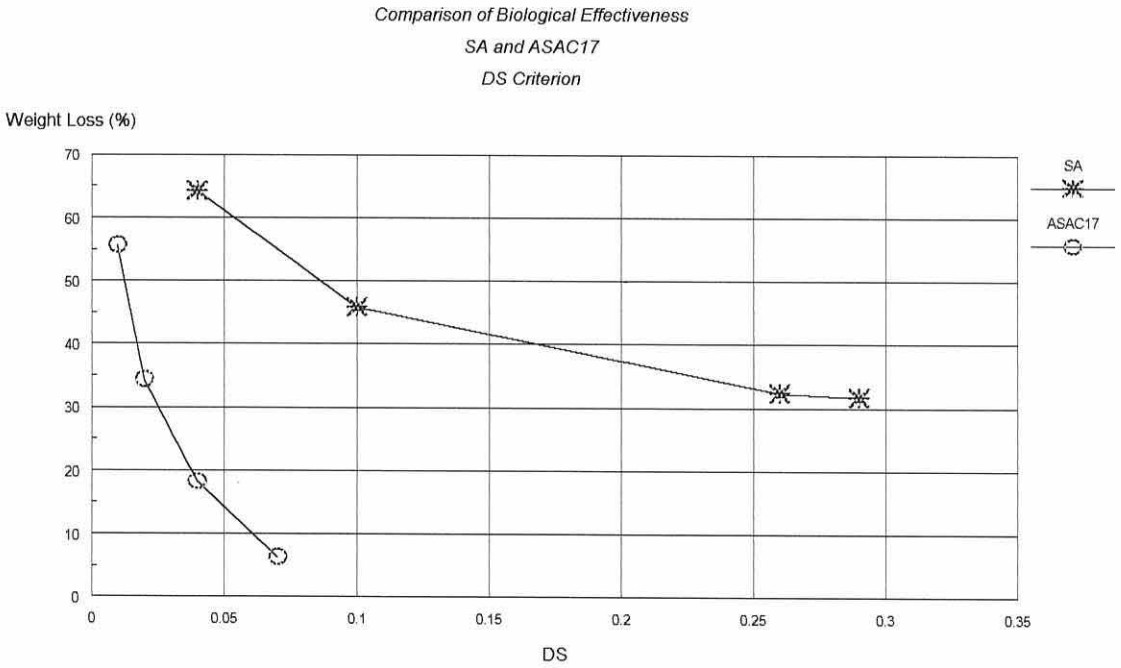


Figure 6.2

Figure 6.2. Weight loss in SA and ASAC17 modified samples using D.S. as loading criterion.

With wood undergoing fungal decay it is always difficult, because of the dynamic nature of the system, to determine precise moisture relations, as discussed in Chapter 4, section 4.0. One mole of glucose yields 6 moles of water and in a closed system moisture content increases considerably as decay proceeds. For this reason it is difficult to measure precisely an optimal moisture content for fungal decay. The moisture content reported in this work is that measured at the end of the twelve week soil test. This is assumed to be the highest reached within a block over that period as it is unlikely that a block gets drier during the later stages of attack, although this can happen. It has been estimated that wood destroying Basiodiomycetes require a moisture content of at least 20% for the decay process, with an optimum of 50 - 60%.

Water is essential for the enzymatic degradation of wood. In terms of moisture the ideal situation for the degradative process is one where the cell wall is fully saturated with bonded water (fibre saturation point) and a film of free water is present, lining the cell lumen. This allows fungal enzymes to move around the cell by diffusion whilst also maintaining ample oxygen for respiratory processes to take place. Above fibre saturation point there is an accumulation of free unbonded water in the cell lumen. At this stage gaseous diffusion slows down and oxygen is limiting. Under conditions of lowered oxygen tension there are a number of wood destroying fungi still quite active but when fully anaerobic conditions exist the decay and growth of fungi is arrested (Viitanen and Ritschkoff, 1991). At this stage only very slow progressive breakdown of wood by anaerobic bacteria is observed (Eaton and Hale, 1993).

ASAC17 treatment is seen to be more successful in controlling moisture content than that based on SA. Table 6.2. below summarises the average moisture content at the end of the bio-assay and the average reagent loading associated with that value.

Table 6.2. Average moisture content of blocks modified with SA and ASAC17 and associated average reagent loading. Moisture content is that recorded at the end of the bio-assay. Figures are the average of sets of 5 (Appendices 4.1 to 4.8).

	SA		ASAC17	
	Reagent loading (%)	Moisture content (%)	Reagent loading (%)	Moisture content (%)
C. puteana NWL	35.7	31.6	29.9	14.7
C. puteana WL	36.0	24.7	30.2	15.2
G. trabeum NWL	22.8	44.9	30.0	14.1
G. trabeum WL	33.7	45.2	30.2	16.8

In all cases ASAC17 modification results in a moisture content below the 20% minimum required by the fungus for optimal decay. This points to a strong hydrophobic or water exclusion effect in the ASAC17 treatment.

6.1.1.3 Effect of Leaching

Wood in exterior service is subject to wet conditions, often severe, and thus it is appropriate to simulate these conditions when testing chemically modified wood.

The leaching process in this investigation, involving half of the modified samples and controls, was quite rigorous, involving a vacuum impregnation and soak and a 14 day leach in de-ionised water. During this process the modification by both SA and ASAC17 appeared to have been stable. Of the SA modified blocks those averaging 25.8% weight gain lost only 1.7% in weight. All of the blocks in an average loading range of 4.9% to 25.8% had an average weight loss of 2% and under. SA modification was marginally more stable than ASAC17. The maximum average weight loss in ASAC17 modified blocks was 1.9%, in specimens loaded to an average of 9%. Weight losses due to leaching in this set were at or below an average of 2.3% in a loading range from 5.1% to around 30%. Leaching caused no additional weight loss.

Rowell (1984) found acetic anhydride modified wood equally resistant to leaching of added chemical. In this work blocks reacted to a 16.3% weight gain lost 1% and those loaded to

22.5% lost 1.2%, both after a 7 day leach in water.

The effects of leaching on subsequent bio-assay weight loss results were varied. Leaching SA modified blocks increased the biological threshold against *C. puteana* attack by 12% (44.5 - 56.5%) (Table 4.2). The corresponding result for ASAC17 is 1% (31.5 - 32.5%) (Table 4.2). Against *G. trabeum* the SA threshold increased by 8.5% after leaching (60 - 68.5%) (Table 4.2) and ASAC17 by 5% (28 - 33%) (Table 4.2). There is a wide spread of weight loss values after fungal attack recorded in those blocks modified by SA and leached in water after modification (Table 6.3). This is true after attack by *C. puteana* and *G. trabeum*. There is not the same variance in blocks modified by ASAC17 and similarly treated. Figures 4.6 to 4.9 in Chapter 4 show this clearly.

Table 6.3. Gives examples of the wide variance in weight loss values for SA modified samples after fungal attack. Figures are averages for sets of 5 blocks.

Reagent / Fungus / State	Loading (%)	Weight Loss (%)	Standard Deviation
SA *C. puteana* WL			
	6.5	29.9	22.3
	15.3	25.5	12.5
	27.6	22.9	8.7
	36.0	11.7	6.1
SA *G. trabeum* WL			
	18.0	31.1	14.6
	26.5	28.7	12.5
	33.7	16.3	5.8

Leaching the SA treated material does not appear to have had the same effect on all blocks. It appears that ASAC17 modification could be marginally more stable in water than SA. Although no proof is offered here, it is possible that ASAC17 gives a greater degree of dimensional stability than does SA. Some of the SA treated blocks may have swollen above their green volume during the soaking process and while reagent may not have been leached

out, the soaking may have opened the wood structure sufficiently to disrupt the protection system.

6.1.2 ASAC17 + Copper

6.1.2.1 Biological Threshold

Extrapolation of experimental data (Chapter 4, section 4.2) gives an ASAC17 biological threshold value of 31.5% for NWL samples and 32.5% for WL samples. The copper threshold is shown in Chapter 5 (section 5.2.3) to be 0.5%. Interest lies in those blocks where ASAC17 loading is less than about 32% (all those treated), the copper content is less than 0.5% and where there is little or no decay. Of the 200 non-control blocks assayed after ASAC17 + copper modification, 34 of the NWL blocks and 87 WL fall into this category.

In this section the 121 blocks are considered, where the levels of ASAC17 and copper in the blocks would be too low to give biological protection were they individually present as the sole modifying reagents.

6.1.2.2 Cell Wall Bulking: Dimensional Stabilisation and Moisture Content

The external volume of wood increases as modifying reagent is introduced into the cell wall until the treated volume is about equal to green volume. When wood bulked in this way comes into contact with water little additional water may be able to penetrate the cell wall. Since water is needed at polymer sites for decay to occur, this is an obvious advantage when attempting to protect wood from decay. In this work ASAC17 was not present in sufficient quantity (maximum weight gain 17.3%) to occupy all water sites. The wood was thus able to take in water and there were free -OH groups available to the fungi for the decay process to occur.

In the NWL set of blocks the average moisture content at the end of the bio-assay was 33.4%, The modification did not control moisture content to below 20%, at which level little or no decay can occur. In the WL set the moisture content of 15 blocks (ASAC17 loadings of 10.4 - 17.3%) were at or below 20%. Results show (Appendix 4.6) that an average ASAC17

content of 14.6% resulted in a post-bio-assay moisture content of about 24% so it can be assumed that ASAC17 had some effect in preventing moisture uptake in these blocks.

In the absence of copper, the ASAC17 levels have been shown to be at sub-toxic levels. Not enough enzyme binding sites were modified to render the substrate unrecognisable. Yet a significant number of blocks remained undecayed.

6.1.2.3 Decay Prevention Mechanism and the Effects of Leaching

Baechler (1959) says that the conversion of cell wall polysaccharides on or near the surfaces of cell walls might be adequate for decay protection even though the degree of chemical modification may not dimensionally stabilise the substrate. This principle is suggested, in part, for the success of the ASAC17 + copper modification.

The amounts of ASAC17 and copper fixed in this test, individually were not present in sufficient quantity to bring about complete biological protection. The low concentrations of each reagent separately could only give rise to a modest reduction in decay rates and yet 121 out of 200 blocks were completely protected.

The often very small concentrations of modifying ASAC17 solutions could only result in a superficial modification of the wood cell wall. In these small concentrations it is unlikely that there is sufficient reagent to carry the modification through to the centre of the cell wall. Thus it is only in this outer region of the axial tracheids that copper is able to be fixed, the carboxylate ion formed on the anhydride modification being the point of attachment for the copper ion. Any copper ions in solution penetrating further into the cell wall would not have ASAC17 modified wood on which to fix and would be leached out in the washing process that follows impregnation. It is suggested that the resulting protection is given by modified cell wall surfaces surrounding unmodified and vulnerable cell wall interiors.

The results (previous section) show that this proposed layer could be successful in preventing decay. During the initial stages of the bio-assay there was abundant fungal mycelia on and around most of the test specimens but in those where there was no decay there could have been no penetration into the blocks. Cardias and Hale (1990) discuss a similar "envelope"

effect in the successful isocyanate treatment of blocks of Corsican pine which was protected against attack by *C. puteana*. Their discussion differs inasmuch as the suggested envelope of treated wood is more gross than microscopic in aspect.

To date the proposed superficial layer of modified material in this work has not been substantiated. Part of future work would need to be directed towards analysis of the modified cell wall using procedures such as ELISA, EDXA and FTIR spectroscopy. In order to determine the sites of ASAC17 + copper modification.

Leaching in water had an effect on the modification system in some blocks but not in all. The system was not stable in all cases in the presence of excess moisture. It should be noted however that vacuum impregnation with water and the subsequent leaching process is quite severe.

6.2 Future Work

The concept of the effectiveness of cell wall surface modification needs to be verified. Any future work involving ASAC17 chelating copper into the wood structure would require a greater degree of reaction site monitoring to see exactly where in the wood the reaction was taking place at such low levels of loading and with what wood fractions. ELISA, EDXA and FTIR spectroscopy could be used. The methods of accurately determining the copper content of individual sample blocks prior to bio-assay needs to be investigated.

The effect of water leaching on all three modification systems needs to be more thoroughly investigated. Whilst ostensibly leaving the modification system intact there is a real effect on the results of subsequent biological testing.

Some quantitatively unreported work involving ASAC17 remaining unbonded in the wood voids was carried out during the course of this research and it could be worthwhile looking at the biological efficiency and permanence of such a system.

The effectiveness of this modification against other Brown Rot fungi and species of White- and Soft Rot fungi should be looked at.

At a laboratory investigation level it was relatively easy to impregnate the small wood blocks used and to bring about a chemical modification of the wood. Treating commercial sizes of timber, window and door frames for example, would require substantial treating equipment and this would need a great deal of investigation.

Appendix 1.1

The Definition of Biological Hazard Classes for wood used in Europe (EN 335 - 1. Part 1. 1992).

Hazard Class	Situation in Service	Description of Exposure in Service	Wood Moisture Content (%)
1	Above ground (dry)	Permanently dry	<18
2	Above ground, covered (risk of wetting)	Exposed to occasional wetting	Occasionally >20
3	Above ground, not covered	Exposed to frequent wetting	Frequently >20
4	In contact with ground or fresh water	Permanently exposed to wetting; in contact with ground or fresh water.	Permanently >20
M	In salt water	Permanently exposed to wetting by salt water	Permanently >20

Appendix 3.1

Calculation of the amount of hydroxyl groups in pine.

The result of this calculation forms the basis of the degree of substitution calculations used in Tables 3.1, 3.2.

Approximate composition of pine:

Cellulose	40%
Hemicelluloses	30%
Lignin	30%

Cellulose: Estimated amount in 1 gram 0.4g

$$\frac{0.4}{162} \times 3 \text{ moles of -OH} = 7.4 \times 10^{-3} \text{ moles of -OH}$$

Hemicelluloses A 20% galactoglucomannans
10% arabinoglucuronoxylans ------ 8% xylose B
..... 2% glucoronic acid C

A $\frac{0.2}{162} \times 3$ moles of -OH $= 3.7 \times 10^{-3}$ moles of -OH

B $\frac{0.08}{147} \times 2$ moles of -OH $= 1.1 \times 10^{-3}$ moles of -OH

C $\frac{0.02}{207} \times 3$ moles of -OH $= 2.9 \times 10^{-4}$ moles of -OH

Lignin $\frac{0.3}{211} \times 1.5$ moles of -OH $= 2.1 \times 10^{-3}$ moles of -OH

$\therefore$ Estimated moles of -OH in one gram of pine wood $= 1.5 \times 10^{-2}$

NB. The denominator in the above fractions is the molecular weight and the multiplicand is the mean number of -OH groups per monomer unit. Average molecular weights and number of -OH groups are used for hemicelluloses and lignin.

Appendix 3.2

Effect of 14 day water leaching on modified wood blocks. The data are for individual blocks. Summarised in section 3.5.4

Reagent: succinic anhydride

	Reagent loading (%)	Dry weight after modification (g)	Dry weight after leaching (g)	Weight change (%)	Reagent loading (%)	Dry weight after modification (g)	Dry weight after leaching (g)	Weight change (%)
	4.8	1.015	1.003	-1.2	11.3	1.018	1.005	-1.3
	5.1	1.056	1.045	-1.0	10.5	1.025	1.003	-2.2
	3.9	1.063	1.037	-2.5	9.8	1.005	0.989	-1.6
	5.2	0.995	0.992	-0.3	12.6	0.983	0.968	-1.5
	5.4	1.003	0.995	-0.8	10.2	1.121	1.115	-0.5
Average	4.9			-1.2	10.9			-1.4
SD	0.7			0.8	1.1			0.6
	16.1	0.985	0.975	-1.0	20.4	1.121	1.105	-1.4
	14.6	1.113	1.103	-0.9	19.6	1.019	0.987	-3.1
	15.2	1.054	1.034	-1.9	21.6	1.005	0.988	-1.7
	15.7	1.031	1.002	-2.8	19.9	1.201	1.166	-2.9
	14.9	1.104	1.087	-1.5	19.7	0.985	0.978	-0.7
Average	15.3			-1.6	20.2			-2.0
SD	0.6			0.7	0.8			1.0
	24.6	0.986	0.978	-0.8				
	24.9	0.995	0.985	-1.0				
	27.8	0.948	0.936	-1.3				
	26.5	1.156	1.125	-2.7				
	25.1	1.002	0.976	-2.6				
Average	25.8			-1.7				
SD	1.3			0.9				

Appendix 3.3

Effect of 14 day water leaching on modified wood blocks. The data are for individual blocks. Summarised in section 3.5.4

Reagent: ASAC17

	Reagent loading (%)	Dry weight after modification (g)	Dry weight after leaching (g)	Weight Change (%)	Reagent loading (%)	Dry weight after modification (g)	Dry weight after leaching (g)	Weight Change (%)
	5.1	1.023	1.019	-0.4	10.9	1.019	1.007	-1.2
	4.8	1.156	1.132	-2.1	10.4	1.053	1.017	-3.4
	3.9	1.015	0.986	-2.9	11.2	0.998	0.978	-2.0
	5.6	1.072	1.054	-1.7	11.9	1.038	1.026	-1.2
	6.1	1.032	1.016	-1.6	10.5	1.042	1.005	-3.6
Average	5.1			-1.7	11.0			-2.3
SD	0.8			0.9	0.6			1.2
	17.2	1.019	0.998	-2.1	21.5	1.025	1.015	-1.0
	15.6	1.019	1.002	-1.7	20.6	1.036	0.998	-3.7
	14.9	1.015	0.987	-2.8	20.8	0.993	0.973	-2.0
	14.8	0.986	0.978	-0.8	19.7	1.032	1.018	-1.4
	15.6	1.081	1.061	-1.9	21.1	1.071	1.039	-3.0
Average	15.6			-1.9	20.7			-2.2
SD	1.0			0.7	0.7			1.1
	25.3	1.001	0.986	-1.50				
	22.4	0.968	0.956	-1.24				
	23.8	1.024	1.008	-1.56				
	25.8	1.023	0.998	-2.44				
	22.4	1.042	1.013	-2.78				
Average	23.94			-1.91				
SD	1.59			0.67				

Appendix 3.4

Effect of 14 day water leaching on unmodified wood blocks. The data are for individual blocks. Summarised in section 3.5.4

Reagent: none

Initial dry weight (g)	Dry weight after leaching (g)	Weight change (%)
1.125	1.118	-0.6
1.132	1.127	-0.4
1.026	1.027	0.1
0.987	0.986	-0.1
0.988	0.979	-0.9
1.023	1.019	-0.4
1.034	1.028	-0.6
1.006	1.002	-0.4
1.083	1.084	+0.1
1.022	1.021	-0.2
Average		-0.4
SD		0.3

Appendix 4.1

Bio-assay data summarised in section 4.2, Table 4.4
Reagent: Succinic anhydride. Fungus: *Coniophora puteana.*
The data are for individual blocks. The average and standard deviation are for sets of five blocks as indicated.

Samples not water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	3.9	67.8	55.9
	4.0	63.4	55.3
	4.3	64.4	64.8
	5.5	60.9	70.9
	6.6	64.5	54.4
Average	4.9	64.2	60.3
StdDev	1.2	2.5	7.3
	10.0	66.8	47.5
	12.8	48.0	42.1
	14.9	49.5	40.4
	15.5	49.3	46.7
	15.6	52.3	72.5
Average	13.8	53.2	45.8
StdDev	2.4	7.8	4.8
	27.5	13.6	32.5
	27.6	31.4	34.9
	27.8	23.0	33.4
	32.3	37.3	35.6
	33.6	32.5	25.2
Average	29.8	27.6	32.3
StdDev	3.0	9..4	32.3
	34.9	7.5	24.6
	35.1	7.2	25.4
	35.3	12.1	44.5
	36.3	18.0	31.9
	37.1	17.3	31.5
Average	35.7	12.4	31.6
StdDev	0.9	5.2	8.0

Appendix 4.2

Bio-assay data summarised in section 4.2, Table 4.4.
Reagent: Succinic anhydride. Fungus: *Coniophora puteana.*
The data are for individual blocks. The average and standard deviation are for sets of five blocks as indicated.

Samples water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	6.2	10.4	32.4
	6.5	52.0	51.4
	6.5	13.5	35.3
	6.6	17.2	30.8
	6.8	56.3	51.1
Average	6.5	29.9	40.2
StdDev	0.2	22.3	10.2
	14.8	35.6	36.0
	14.9	10.8	24.0
	15.5	13.9	49.0
	15.2	37.8	42.6
	15.9	29.5	32.2
Average	15.3	25.5	36.8
StdDev	0.5	12.5	9.6
	24.8	26.9	34.7
	26.9	25.4	31.9
	27.7	7.6	32.6
	28.6	29.6	34.0
	30.2	24.8	29.4
Average	27.6	22.9	32.5
StdDev	2.0	8.7	2.1
	34.4	8.6	37.9
	35.4	5.2	15.9
	36.0	17.6	25.3
	36.3	8.3	16.0
	38.4	18.8	28.4
Average	36.0	11.7	24.7
StdDev	1.5	6.1	9.2

Appendix 4.3

Bio-assay data summarised in section 4.2, Table 4.5
Reagent: Succinic anhydride. Fungus: *Gloeophyllum trabeum*
The data are for individual blocks. Average and standard deviation are for sets of five blocks as indicated.

Samples not water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	3.5	48.9	41.1
	3.7	55.4	47.0
	6.4	49.4	43.9
	6.4	30.8	60.5
	8.6	11.3	55.5
Average	5.7	39.2	49.6
StdDev	2.1	18.1	8.1
	9.9	30.0	34.2
	12.5	15.6	47.2
	15.6	25.9	93.0
	16.3	20.5	45.7
	16.5	13.6	36.2
Average	14.2	21.1	51.3
StdDev	2.9	6.9	24.0
	19.2	25.8	35.4
	20.0	25.7	36.5
	23.1	26.3	35.4
	25.6	17.4	49.4
	25.9	27.0	67.6
Average	22.8	24.4	44.9
StdDev	3.1	4.0	14.0
	26.4	32.2	68.2
	26.6	44.0	85.6
	28.8	12.2	34.8
	32.5	21.6	92.9
	31.0	17.9	87.0
Average	29.1	25.6	73.7
StdDev	2.7	12.6	23.6

Appendix 4.4

Bio-assay data summarised in section 4.2, Table 4.5
Reagent: Succinic anhydride. Fungus: *Gloeophyllum trabeum*
The data are for individual blocks. Average and standard deviation are for sets of five blocks as indicated.

Samples water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	4.9	15.8	58.4
	7.3	18.0	72.8
	9.4	29.6	79.4
	9.9	31.7	85.1
	15.0	26.1	43.6
Average	9.3	24.2	67.9
StdDev	3.8	7.0	67.9
	16.7	28.6	78.6
	17.6	52.6	77.3
	17.7	13.3	36.0
	18.7	24.6	47.4
	19.5	36.5	78.8
Average	18.0	31.1	63.6
StdDev	1.1	14.6	20.4
	22.6	39.3	70.5
	25.5	43.4	68.1
	27.3	13.9	67.4
	28.4	20.3	41.1
	28.5	26.4	54.0
Average	26.5	28.7	60.2
StdDev	2.5	12.5	12.5
	30.3	10.1	55.4
	33.2	17.6	45.7
	33.8	11.5	33.8
	34.9	24.7	48.5
	36.2	17.5	42.8
Average	33.7	16.3	45.2
StdDev	2.2	5.8	7.9

Appendix 4.5

Bio-assay data summarised in section 4.2, Table 4.4
Reagent: ASAC17. Fungus: *Coniophora puteana.*
The data are for individual blocks. The average and standard deviation are for sets of five blocks as indicated.

Samples not water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	3.6	59.9	43.2
	4.3	57.3	36.0
	5.1	56.1	34.4
	6.6	56.7	37.8
	7.3	53.2	34.1
Average	5.4	55.6	37.1
StdDev	1.6	2.4	3.7
	10.0	43.9	32.5
	13.9	49.3	29.8
	14.1	26.7	31.6
	14.6	24.5	25.3
	17.3	26.9	35.9
Average	14.0	34.3	31.0
StdDev	2.6	11.5	3.9
	18.0	20.5	26.7
	19.3	26.7	25.0
	23.0	12.3	20.7
	24.4	17.0	21.5
	26.7	14.6	17.3
Average	22.3	18.2	22.2
StdDev	3.6	5.6	3.7
	27.6	7.0	16.6
	27.9	14.7	17.8
	28.3	2.8	13.5
	31.8	4.8	13.7
	33.7	2.4	11.8
Average	29.9	6.3	14.7
StdDev	2.7	5.0	2.5

Appendix 4.6

Bio-assay data summarised in section 4.2, Table 4.4
Reagent: ASAC17. Fungus: *Coniophora puteana.*
The data are for individual blocks. The average and standard deviation are for sets of five blocks as indicated.

Samples water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	3.8	48.8	31.7
	5.3	57.7	34.1
	5.5	48.0	31.5
	7.2	50.8	34.2
	9.6	51.2	32.4
Average	6.3	51.3	32.8
StdDev	2.2	3.8	1.3
	9.7	48.4	31.0
	13.5	26.4	25.3
	15.8	29.3	23.1
	16.2	27.5	21.3
	17.8	23.7	19.0
Average	14.6	31.1	23.9
StdDev	31.4	9.9	4.6
	19.5	14.9	18.8
	20.6	19.0	17.8
	21.7	18.2	18.9
	24.4	12.0	15.7
	26.6	11.1	20.0
Average	22.6	15.0	18.2
StdDev	2.9	3.6	1.6
	27.7	10.5	16.9
	28.4	9.7	15.7
	30.9	8.7	15.8
	31.3	9.0	15.4
	32.6	2.6	12.0
Average	30.2	8.1	15.2
StdDev	2.1	3.2	1.9

Appendix 4.7

Bio-assay data summarised in section 4.2, Table 4.5
Reagent: ASAC17. Fungus: *Gloeophyllum trabeum.*
The data are for individual blocks. The average and standard deviation are for sets of five blocks as indicated

Samples not water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	3.9	52.9	29.3
	4.2	52.0	30.4
	5.2	51.8	36.7
	7.2	47.3	38.4
	7.6	49.1	34.5
Average	5.6	50.6	33.9
StdDev	1.7	2.3	3.9
	9.5	52.4	30.1
	13.6	29.6	26.0
	14.1	32.3	26.7
	16.3	24.0	26.3
	18.0	18.6	21.9
Average	14.3	31.4	26.2
StdDev	3.2	12.9	2.9
	18.3	10.3	27.4
	20.0	15.3	28.7
	22.7	6.8	17.1
	26.2	1.6	16.6
	27.4	0.5	13.6
Average	22.9	6.9	20.7
StdDev	3.9	6.2	6.9
	27.6	0.5	15.4
	27.7	2.0	13.7
	29.8	0.1	11.5
	32.0	1.1	15.1
	32.7	2.7	14.7
Average	30.0	1.3	14.1
StdDev	2.4	1.1	1.6

Appendix 4.8

Bio-assay data summarised in section 4.2, Table 4.5
Reagent: ASAC17. Fungus: *Gloeophyllum trabeum.*
The data are for individual blocks. The average and standard deviation are for sets of five blocks as indicated

Samples water leached after chemical modification

	Reagent loading (%)	Weight loss at the end of the infection period (%)	Moisture content at the end of the infection period (%)
	4.6	56.3	40.2
	4.7	52.6	35.2
	5.3	50.3	28.3
	7.6	45.3	37.7
	9.4	28.9	32.7
Average	6.3	46.7	34.8
StdDev	2.1	10.7	4.6
	10.1	39.4	30.3
	13.2	33.5	30.2
	15.1	20.8	26.7
	16.4	26.0	24.6
	17.6	19.2	24.5
Average	14.5	27.8	27.3
StdDev	2.9	8.6	2.9
	19.1	19.0	21.1
	19.4	20.6	21.6
	21.7	13.8	19.3
	25.4	13.0	18.9
	26.4	13.3	16.7
Average	22.4	15.9	19.5
StdDev	3.4	3.6	2.0
	27.3	11.2	16.5
	28.6	12.8	16.6
	30.9	7.1	18.4
	30.9	6.1	18.4
	33.2	4.6	14.3
Average	30.2	8.4	16.8
StdDev	2.3	3.5	1.7

Appendix 4.9

Bio-assay control data. Summarised in section 4.2, Table 4.3. Data are for individual blocks. Exposed to *Coniophora puteana*

Extractives removed and reacted in DMF only

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples water leached after reaction		Samples not water leached after reaction	
	50.2	66.3	60.3	70.8
	58.3	68.0	62.5	70.7
	58.5	69.2	65.2	70.1
	63.7	69.3	72.4	66.1
	74.8	70.6	86.1	75.1
	81.9	69.4	84.2	70.7
Average	64.4	68.6	71.8	70.2
StdDeviation	11.8	1.5	11.1	2.9

Extractives removed. No reaction

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples water leached after reaction		Samples not water leached after reaction	
	54.1	66.7	50.4	58.8
	54.7	65.2	51.7	69.5
	55.0	66.2	53.1	69.0
	55.8	67.1	53.9	68.1
	55.9	60.1	54.3	59.3
	56.2	68.2	54.8	68.2
Average	55.3	65.6	53.0	65.5
StdDeviation	0.8	2.9	1.7	5.0

Oven dried only. Not solvent extracted

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples water leached after reaction		Samples not water leached after reaction	
	42.6	65.5	37.8	64.9
	48.3	64.0	43.0	65.3
	50.5	63.9	46.9	66.2
	52.4	64.4	47.0	64.6
	52.7	66.2	47.7	63.5
	55.8	66.9	47.9	60.8
Average	50.4	66.2	45.1	64.2
StdDeviation	4.6	1.2	4.0	1.9

Appendix 4.10

Bio-assay control data. Summarised in section 4.2, Table 4.3.
Data are for individual blocks.
Exposed to *Gloeophyllum trabeum*

Extractives removed and reacted in DMF only

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples water leached after reaction		Samples not water leached after reaction	
	44.5	40.3	46.0	43.1
	56.4	58.4	44.5	47.1
	56.3	29.9	47.3	41.7
	62.5	30.1	39.8	36.9
	65.4	30.2	43.2	33.1
	61.5	27.2	45.1	48.3
Average	57.8	36.0	44.3	41.7
StdDeviation	7.4	11.9	2.6	5.9

Extractives removed. No reaction

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples water leached after reaction		Samples not water leached after reaction	
	44.3	21.5	58.5	36.6
	50.2	29.6	44.6	53.7
	47.9	49.8	64.1	27.7
	47.2	48.5	74.6	45.2
	45.1	44.0	45.1	58.1
	47.4	52.7	51.1	26.2
Average	47.0	41.0	56.3	41.3
StdDeviation	2.1	12.6	11.8	13.3

Oven dried only. Not solvent extracted

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples water leached after reaction		Samples not water leached after reaction	
	51.6	35.4	44.5	47.8
	36.9	25.2	48.2	56.5
	79.7	33.4	31.3	51.1
	91.4	30.6	36.4	43.1
	44.5	41.4	39.5	53.8
	44.4	49.7	39.2	48.1
Average	58.1	36.0	39.9	50.1
StdDeviation	22.1	8.6	6.0	4.8

Appendix 4.11

Sterile controls. Summarised in section 4.2, Table 4.3.
Data are for single blocks.

Reagent: Succinic anhydride.

Samples not water leached after modification		Samples water leached after modification	
Reagent loading (%)	Weight loss (%)	Reagent loading (%)	Weight loss (%)
3.6	1.6	4.1	1.2
4.2	1.9	4.5	1.1
6.5	1.5	6.2	1.1
8.8	1.7	7.9	0.9
9.7	1.6	10.4	0.8
12.6	1.4	12.8	1.5
16.1	1.3	14.7	1.7
16.5	1.2	15.6	1.2
19.8	1.7	20.4	2.1
22.4	1.7	21.9	1.9
Average	1.6	Average	1.4
StdDeviation	0.2	StdDeviation	0.4

Reagent: ASAC17.

Samples not water leached after modification		Samples water leached after modification	
Reagent loading (%)	Weight loss (%)	Reagent loading (%)	Weight loss (%)
4.2	1.8	3.7	1.9
4.7	1.7	5.2	2.0
6.8	2.2	5.9	1.9
9.1	1.6	7.8	1.9
10.4	2.3	12.5	1.5
12.5	.2.0	13.0	2.1
17.6	3.0	16.4	1.7
19.6	1.3	20.5	1.7
20.1	1.1	20.3	1.6
22.6	2.0	23.5	0.5
Average	1.9	Average	1.7
StdDeviation	0.5	StdDeviation	0.5

Appendix 4.12

Statistical Analysis

The Pearson Product Moment Correlation Coefficient

r : a dimensionless index that ranges from -1.0 to 1.0 inclusive. It reflects the extent of a linear relationship between two data sets.

The **r** value of the regression line is :

$$\mathbf{r} = \frac{n\left(\sum XY\right) - \left(\sum X\right)\left(\sum Y\right)}{\sqrt{\left[n\sum X - \left(\sum X\right)_2\right]\left[n\sum Y - \left[\sum Y\right]_2\right]}}$$

It is very important to realise that correlation is calculated between two sets of *numbers*. A significant value of r indicates that there is a linear association between two sets of numbers X and Y. It does not indicate that one variable is caused by another. Correlation does not imply causation. The best that significant correlation can do is to suggest that there may be a connection between events. Absence of correlation indicates that there is no linear relationship. This does not mean that there is no relationship at all.
Values of r given in this work should be regarded in this light ie., they are indicative of the extent of linear relationships.

The data points (0,0) are used in this work when calculating r.

Appendix 5.1.

Bio-assay data for individual blocks modified with ASAC17 and copper exposed to *C.puteana*. Blocks not water leached after modification. Summarised in section 5.2.3.

	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)
	0.3	0.8	35.8	1.1
	0.4	0.4	44.7	1.3
	0.5	0.4	40.6	1.2
	0.7	0.5	38.4	1.0
	0.8	0.1	44.1	1.2
	1.0	1.1	45.7	0.5
	1.1	1.2	54.1	2.3
	1.1	0.6	47.8	1.5
	1.1	1.3	52.4	1.6
	1.2	1.2	49.0	3.1
Average	0.8	0.7	45.3	1.5
StdDev	0.3	0.4	5.9	0.7
	1.5	0.5	45.6	0.7
	1.5	0.2	26.5	1.0
	1.6	1.1	56.8	1.7
	1.7	0.6	45.7	1.0
	1.7	0.2	44.4	0.8
	1.8	0.2	44.9	1.1
	1.9	1.1	41.0	2.9
	2.0	0.2	34.4	0.5
	2.1	1.1	70.6	1.3
	2.1	1.1	77.9	2.0
Average	1.8	0.6	48.8	1.3
StdDev	0.2	0.4	15.7	0.7
	2.2	0.6	37.7	1.2
	2.4	0.9	68.7	2.3
	2.6	1.0	45.7	1.4
	2.6	0.2	37.6	0.2
	2.7	0.5	43.3	1.4
	2.7	1.2	46.6	2.4
	2.7	0.8	43.4	1.1
	2.8	0.3	34.7	0.5
	2.9	0.8	47.1	2.2
	2.9	1.2	74.7	2.5
Average	2.7	0.8	47.9	1.5
StdDev	0.2	0.3	13.3	0.8

	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)
	3.0	0.8	56.3	1.6
	3.2	0.5	75.0	1.7
	3.2	1.1	58.0	2.8
	3.2	1.2	38.1	3.4
	3.2	0.6	36.3	0.8
	3.2	0.4	28.8	1.0
	3.3	1.1	46.3	2.5
	3.9	0.6	44.1	1.2
	3.9	0.3	34.3	0.1
	4.1	0.9	45.8	1.2
Average	3.4	0.7	46.3	1.6
StdDev	0.4	0.3	13.7	1.0
	4.1	0.3	68.5	0.0
	4.3	0.3	38.1	1.2
	4.4	1.1	50.2	1.8
	4.8	0.8	41.4	1.1
	4.9	0.7	41.4	1.4
	5.0	1.1	56.1	1.6
	5.1	0.4	33.2	0.6
	5.5	1.1	64.9	0.9
	5.6	1.0	52.2	0.3
	5.8	1.1	49.7	0.5
Average	5.0	0.8	49.6	0.9
StdDev	0.6	0.3	11.4	0.6
	6.0	0.2	36.2	0.7
	6.2	1.1	63.3	1.5
	6.3	0.8	53.7	0.4
	6.4	0.6	41.1	0.5
	6.6	1.2	38.3	2.4
	6.6	0.1	38.0	0.4
	6.8	0.6	42.9	1.0
	6.8	0.6	43.6	1.1
	7.1	1.6	30.7	4.3
	7.3	1.2	47.1	2.4
Average	6.6	0.8	43.5	1.5
StdDev	0.4	0.5	9.4	1.3
	7.5	1.5	37.2	3.6
	7.6	0.5	43.8	0.5
	7.7	1.0	49.2	1.7
	7.8	0.7	44.4	1.6
	7.8	0.3	31.1	0.4
	8.2	1.4	47.6	1.6
	8.4	1.1	44.2	1.8
	8.4	1.3	48.0	1.3
	8.5	0.2	28.2	0.0
	8.7	0.6	47.7	1.0
Average	8.1	0.8	42.1	1.3
StdDev	0.4	0.5	7.4	1.0

	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)
	8.7	0.8	42.9	0.3
	9.2	0.6	42.1	0.4
	9.4	1.7	0.5	1.1
	9.4	0.6	46.4	0.3
	9.4	0.2	24.8	0.4
	9.4	0.4	30.5	0.4
	9.6	0.7	43.1	0.2
	9.6	0.5	34.7	1.0
	10.2	0.3	29.7	0.1
	10.4	0.5	39.6	0.3
Average	9.5	0.6	33.4	0.4
StdDev	0.5	0.4	13.5	0.3
	10.7	0.5	32.3	0.2
	10.9	0.2	28.2	0.2
	11.0	0.6	38.2	0.1
	12.2	0.8	40.6	0.1
	12.4	0.5	32.5	0.5
	13.6	1.0	43.6	0.8
	14.2	0.7	38.7	0.2
	14.6	1.6	43.7	2.9
	14.8	0.4	28.9	1.1
	14.9	1.2	47.7	0.9
Average	12.9	0.7	37.4	0.7
StdDev	1.7	0.4	6.7	0.9
	14.9	0.3	25.2	0.4
	15.4	1.6	36.7	2.6
	15.4	0.8	39.3	0.1
	15.5	0.1	24.8	0.4
	16.2	1.7	39.8	2.1
	16.4	0.2	31.7	0.1
	16.8	0.3	20.1	0.2
	16.9	0.1	23.0	1.4
	17.0	0.1	24.6	1.2
	17.2	0.3	23.6	1.0
Average	16.2	0.5	28.9	0.9
StdDev	0.8	0.6	7.3	0.9

Appendix 5.2.

Bio-assay data for 100 individual blocks modified with ASAC17 and copper, exposed to *C. puteana*. Blocks water leached after modification by both ASAC17 and copper (section 5.2.2.). Loadings for ASAC17 are given as a percentage of the initial dry wood weight. Copper loading is the initial weight gain value adjusted to reflect the more accurate Atomic Absorption Spectroscopy analysis . (Summarised in section 5.2.3.)

	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)
	0.3	0.5	22.8	11.2
	0.3	0.3	26.7	2.0
	0.4	0.2	26.0	4.4
	0.5	0.4	25.6	3.0
	0.6	0.2	22.4	4.5
	0.7	0.1	26.5	5.4
	0.9	0.4	22.8	3.0
	1.0	0.4	20.8	3.1
	1.1	0.2	23.9	1.6
	1.1	0.4	25.9	3.5
Average	0.7	0.3	24.3	4.2
StdDev	0.3	0.2	2.1	2.7
	1.2	0.5	22.7	3.7
	1.4	0.1	25.2	1.4
	1.4	0.1	23.9	1.1
	1.5	0.4	22.7	6.2
	1.6	0.2	22.1	1.9
	1.7	0.3	22.5	9.4
	1.8	0.1	32.1	7.6
	1.8	0.1	25.6	10.7
	1.9	0.4	23.0	12.3
	1.9	0.6	21.4	5.0
Average	1.6	0.2	24.1	5.9
StdDev	0.3	0.2	3.1	4.0
	2.0	0.4	23.6	9.5
	2.2	0.1	24.5	11.2
	2.3	0.3	23.0	2.9
	2.4	0.1	29.9	21.8
	2.5	0.5	17.2	2.8
	2.6	0.3	22.2	2.6
	2.7	0.2	22.1	2.8
	2.8	0.1	20.7	0.1
	2.8	0.2	23.4	19.7
	2.8	0.6	21.9	14.3
Average	2.5	0.3	22.9	8.8
StdDev	0.3	0.2	3.2	7.8

	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)
	2.8	0.2	23.6	15.2
	3.0	0.3	24.4	14.1
	3.1	0.6	25.3	16.1
	3.1	0.2	26.8	15.1
	3.1	0.2	24.0	1.2
	3.2	0.1	22.2	2.1
	3.8	0.1	22.7	4.0
	3.9	0.4	25.7	6.1
	3.9	0.1	21.0	0.6
	4.0	0.1	22.4	5.6
Average	3.4	0.3	21.8	8.0
StdDev	0.4	0.2	6.9	6.4
	4.1	0.1	23.8	3.9
	4.2	0.4	21.0	3.3
	4.7	0.4	24.6	11.8
	5.0	0.1	25.1	0.6
	5.1	0.4	22.7	5.7
	5.3	0.5	23.0	14.4
	5.8	0.3	24.6	13.2
	5.9	0.1	24.9	5.2
	5.9	0.1	26.2	13.7
	6.0	0.5	22.4	8.2
Average	5.2	0.3	23.8	8.0
StdDev	0.7	0.2	1.5	5.0
	6.0	0.3	23.0	3.2
	6.2	0.4	21.2	8.1
	6.5	0.1	24.3	2.7
	6.5	0.4	21.5	2.9
	6.6	0.1	27.5	17.0
	6.7	0.2	25.6	14.8
	7.0	0.1	18.5	1.6
	7.2	0.1	18.6	0.2
	7.3	0.1	20.5	1.6
	7.4	0.4	20.9	2.7
Average	6.7	0.2	22.1	5.5
StdDev	0.5	0.2	2.9	5.9
	7.6	0.1	20.2	3.8
	7.6	0.3	28.8	23.8
	7.8	0.1	24.7	11.6
	8.1	0.4	18.7	2.5
	8.2	0.5	22.4	15.4
	8.3	0.5	21.4	8.3
	8.3	0.1	25.6	10.2
	8.6	0.1	20.6	1.1
	8.6	0.5	31.2	26.0
	8.8	0.1	23.9	3.6
Average	8.2	0.3	23.7	10.6
StdDev	0.4	0.2	3.9	8.8

	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)
	8.9	0.5	21.1	7.1
	9.1	0.1	24.6	16.8
	9.3	0.2	21.1	6.2
	9.3	0.1	23.3	8.5
	9.3	0.2	19.0	1.2
	9.5	0.2	17.7	0.8
	9.6	0.1	21.4	7.6
	9.8	0.4	14.8	2.2
	10.0	0.1	26.2	18.4
	10.1	0.1	24.7	7.8
Average	9.5	0.2	21.4	7.7
StdDev	0.4	0.2	3.5	6.0
	10.4	0.1	18.7	1.6
	10.6	0.1	26.5	13.1
	11.3	0.1	19.7	2.2
	11.6	0.3	19.7	7.8
	12.4	0.3	22.1	7.4
	13.1	0.3	15.5	1.0
	13.7	0.1	19.6	3.3
	14.1	0.2	17.8	4.1
	14.4	0.1	23.8	10.3
	14.5	0.6	24.1	3.9
Average	12.6	0.2	20.8	5.5
StdDev	1.6	0.2	3.3	4.0
	14.9	0.1	19.9	7.3
	14.9	0.6	22.2	3.6
	15.0	0.4	22.4	9.9
	15.3	0.1	20.5	4.1
	15.3	0.1	21.3	7.6
	15.7	0.1	21.4	3.5
	15.8	0.4	17.9	6.1
	16.3	0.2	17.6	2.9
	16.3	0.1	18.3	9.4
	17.3	0.1	19.4	3.7
Average	15.7	0.2	20.1	5.8
StdDev	0.8	0.2	1.8	2.6

Appendix 5.3. Bio-assay controls.

Data are for individual blocks.

Reacted with ASAC17 + Copper. Operational controls. Summarised in section 5.2.3.

	NWL				WL			
	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)	ASAC17 loading (%)	Copper loading (%)	Moisture content (%)	Weight loss (%)
	0.4	0.1	27.5	3.2	0.7	0.2	32.5	4.1
	1.5	0.2	29.4	4.1	2.1	0.5	33.0	4.5
	2.7	0.8	26.4	2.5	3.2	0.4	31.5	3.8
	6.2	0.2	28.7	3.2	7.8	0.1	36.9	5.1
	9.4	1.0	31.5	5.2	11.5	1.1	30.8	2.7
	15.2	0.6	29.0	0.8	16.5	0.8	29.8	5.6
High	0.4	0.1			0.7	0.1		
Low	15.2	1.0			16.5	1.1		
Average			28.8	3.2			32.4	4.3
StdDev			1.7	1.5			2.5	1.0

Copper only. NWL. Summarised in section 5.2.3.
Each culture jar contained 1 modified and 1 unmodified block.

Modified blocks			Accompanying Unmodified blocks	
Copper loading (%)	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
0.02	41.5	67.6	43.4	67.9
0.02	67.4	74.0	55.3	69.3
0.02	50.0	70.4	38.7	61.1
0.03	58.6	68.0	44.1	66.2
0.1	53.7	39.1	47.0	65.8
0.1	74.5	27.8	39.0	72.0
0.1	67.2	33.7	54.0	61.4
0.1	76.1	22.9	47.4	69.2
0.2	38.0	59.6	27.8	62.4
0.3	63.9	37.5	37.5	58.4
0.3	27.0	24.8	42.6	59.6
0.4	45.7	30.0	35.0	57.2
0.4	69.5	33.8	39.6	59.4
0.5	21.7	-1.1	34.9	64.0
0.6	21.9	0.9	45.6	61.1
0.6	20.4	-0.8	52.4	69.4
0.7	25.3	0.8	45.6	61.4
0.8	26.1	-0.5	56.7	68.7
1.0	29.7	-3.9	36.5	62.0
1.1	33.1	-3.6	40.8	69.1

Copper only. NWL

Operational controls. Summarised in section 5.2.3. A negative value indicates a weight gain.

Copper loading (%)	Moisture content (%)	Weight loss (%)
0.03	26.8	-1.7
0.03	23.4	-0.9
0.03	22.9	-0.4
0.02	27.1	-0.6
0.1	24.9	0.9
0.1	25.4	1.5
0.1	28.3	0.5
0.1	24.7	-2.8
0.4	29.2	0.6
0.4	22.9	0.6
0.3	29.1	0.8
0.4	23.2	1.1
0.7	29.1	1.7
0.5	29.6	0.9
0.6	28.8	0.7
0.4	29.3	1.1
0.9	38.7	0.2
1.2	35.3	-0.4
1.0	36.1	-0.8
1.1	37.8	-0.4

Reacted in DMF only. Exposed to *Coniophora puteana*. Summarised in section 5.2.3.

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples not water leached after reaction		Samples water leached after reaction	
	60.3	70.8	50.1	66.8
	62.2	70.7	58.3	68.0
	65.2	70.1	58.5	69.2
	72.4	66.1	63.7	69.3
	86.1	75.1	74.8	70.6
	84.0	70.7	82.0	69.4
Average	71.7	70.6	64.6	68.9
StdDeviation	11.1	2.9	11.8	1.3

Reacted in DMF only. Operational controls. Summarised in section 5.2.3.

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples not water leached after reaction		Samples water leached after reaction	
	30.0	0.8	24.8	2.2
	29.5	0.5	26.8	2.0
	30.0	1.3	26.5	0.3
	30.2	0.8	26.2	1.2
	27.7	1.1	27.4	0.9
	30.5	1.2	23.0	0.9
Average	29.7	1.0	25.8	1.3
StdDeviation	1.0	0.3	1.6	0.7

Untreated (oven dried only) Exposed to *Coniophora puteana*. Summarised in section 5.2.3.

	Moisture content (%)	Weight loss (%)	Moisture content (%)	Weight loss (%)
	Samples not water leached after reaction		Samples water leached after reaction	
	37.8	64.9	42.6	65.5
	43.0	65.3	48.3	64.0
	46.9	66.2	50.5	63.9
	47.0	64.6	52.4	64.4
	47.7	63.5	52.7	66.2
	47.9	60.8	55.8	66.9
Average	45.1	64.2	50.4	65.2
StdDeviation	4.0	1.9	4.6	1.2

Appendix 5.4.

Effect of 14 day water leaching on wood blocks modified with ASAC17 and copper.
The data are for individual blocks. Copper loading is that calculated after leaching; it is the initial weight gain value adjusted to reflect the more accurate Atomic Absorption Spectroscopy analysis.

ASAC17 loading (%)	Copper loading (%)	Dry weight after modification (g) (W1)	Dry weight after water leaching (g) (W2)	Weight change (% of W1)
0.3	0.5	1.24	1.22	1.1
0.3	0.3	1.10	1.10	0.2
0.4	0.2	1.26	1.26	0.5
0.5	0.4	1.09	1.07	1.4
0.6	0.2	1.08	1.08	0.5
0.7	0.1	1.19	1.19	0.0
0.9	0.4	1.21	1.21	0.4
1.0	0.4	1.25	1.24	0.7
1.1	0.2	1.08	1.08	0.2
1.1	0.4	1.25	1.25	0.5
1.2	0.5	1.27	1.27	0.2
1.4	0.1	1.19	1.19	0.2
1.4	0.1	1.09	1.06	2.7
1.5	0.4	1.27	1.25	1.3
1.6	0.2	1.10	1.09	1.6
1.7	0.3	1.21	1.23	-1.6
1.8	0.1	1.33	1.33	0.2
1.8	0.0	1.28	1.28	0.3
1.9	0.4	1.38	1.37	0.5
1.9	0.6	1.27	1.27	0.0
2.0	0.4	1.20	1.19	0.8
2.2	0.1	1.31	1.29	1.6
2.3	0.3	1.21	1.21	0.2
2.4	0.1	1.35	1.35	-0.1
2.5	0.5	1.29	1.29	0.4
2.6	0.3	1.32	1.32	0.3
2.7	0.2	1.26	1.26	0.2
2.8	0.1	1.28	1.28	0.0
2.8	0.2	1.29	1.28	0.9
2.8	0.6	1.34	1.36	-1.1
2.8	0.2	1.15	1.15	0.3
3.0	0.3	1.34	1.32	2.0
3.1	0.6	1.25	1.24	0.3
3.1	0.2	1.28	1.28	0.0
3.1	0.2	1.17	1.17	-0.3
3.2	0.1	1.11	1.10	0.6
3.8	0.1	1.06	1.07	-1.3
3.9	0.4	1.23	1.23	-0.1
3.9	0.1	1.19	1.19	0.2
4.0	0.1	1.19	1.19	0.0
4.1	0.1	1.26	1.26	0.5
4.2	0.4	1.11	1.12	-0.5
4.7	0.4	1.27	1.27	0.4
5.0	0.1	1.27	1.26	0.7

ASAC17 loading (%)	Copper loading (%)	Dry weight after modification (g) (W1)	Dry weight after water leaching (g) (W2)	Weight change (% of W1)
5.1	0.4	1.25	1.24	0.5
5.3	0.5	1.13	1.13	0.3
5.8	0.3	1.11	1.10	0.9
5.9	0.1	1.20	1.20	0.2
5.9	0.1	1.31	1.31	0.1
6.0	0.5	1.17	1.17	0.4
6.0	0.3	1.28	1.26	1.1
6.2	0.4	1.13	1.12	0.8
6.5	0.1	1.30	1.29	0.6
6.5	0.4	1.19	1.17	1.8
6.6	0.1	1.30	1.29	1.2
6.7	0.2	1.35	1.34	0.7
7.0	0.1	1.28	1.26	1.9
7.2	0.1	1.32	1.32	0.0
7.3	0.1	1.23	1.22	0.7
7.4	0.4	1.09	1.07	2.1
7.6	0.1	1.06	1.05	0.9
7.6	0.3	1.25	1.24	0.7
7.8	0.1	1.42	1.40	1.5
8.1	0.4	1.09	1.09	-0.4
8.2	0.5	1.14	1.14	-0.3
8.3	0.5	1.32	1.33	-0.7
8.3	0.1	1.31	1.31	0.1
8.6	0.1	1.34	1.31	2.5
8.6	0.5	1.26	1.28	-1.5
8.8	0.1	1.35	1.33	1.6
8.9	0.5	1.35	1.33	1.2
9.1	0.1	1.37	1.36	0.7
9.3	0.2	1.38	1.39	-0.8
9.3	0.1	1.35	1.34	1.0
9.3	0.2	1.31	1.30	1.0
9.5	0.2	1.33	1.32	0.7
9.6	0.1	1.24	1.23	0.7
9.8	0.4	1.37	1.39	-1.8
10.0	0.1	1.29	1.29	0.3
10.1	0.1	1.39	1.38	0.7
10.4	0.1	1.30	1.29	0.8
10.6	0.1	1.24	1.22	1.4
11.3	0.1	1.13	1.11	2.0
11.6	0.3	1.13	1.13	0.4
12.4	0.3	1.07	1.07	0.5
13.1	0.3	1.38	1.37	0.9
13.7	0.1	1.36	1.35	0.8
14.1	0.2	1.37	1.37	0.5
14.4	0.1	1.33	1.32	0.5
14.5	0.6	1.36	1.35	0.7
14.9	0.1	1.36	1.32	2.4
14.9	0.6	1.31	1.32	-0.5
15.0	0.4	1.37	1.32	3.4
15.3	0.1	1.34	1.31	2.6
15.3	0.1	1.30	1.30	0.3
15.7	0.1	1.45	1.45	0.2

15.8	0.4	1.37	1.37	0.1
16.3	0.2	1.45	1.47	-1.0
ASAC17 loading (%)	Copper loading (%)	Dry weight after modification (g) (W1)	Dry weight after water leaching (g) (W2)	Weight change (% of W1)
16.3	0.1	1.39	1.38	0.7
17.3	0.1	1.40	1.38	1.5
	Average	1.26	1.25	0.6
	StdDev	0.1	0.1	0.9

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