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The decay resistance of chemically modified softwood.

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Summary

The purpose of this study was to assess the decay resistance of wood modified with a variety of chemicals and to attempt to further understand the mechanism by which chemical modification protects wood from decay. Corsican pine (*Pinus nigra*) sapwood was modified with three cyclic anhydrides; succinic anhydride, alkenyl succinic anhydride (a derivative of succinic anhydride with a 16-18 carbon alkenyl chain) and phthalic anhydride, and with two more widely studied modifying chemicals; acetic anhydride and butyl isocyanate. All reactions were carried out using pyridine as solvent and catalyst.

Modified wood was tested against decay fungi in a pure culture test against basidiomycete fungi (*Coniophora puteana*, *Gloeophyllum trabeum*, *Trametes versicolor* and *Pycnoporus sanguineus*) under different moisture content regimes, an unsterile soil soft rot test, a fungal cellar test and a field trial. Butyl isocyanate proved the most effective modifying chemical at protecting wood from decay, followed by acetic anhydride and then alkenyl succinic anhydride. Uneven distribution of the modifying chemical in wood was evident using each of these three chemicals, particularly in the case of acetic anhydride. Despite its apparent ability to control decay by basidiomycete fungi, alkenyl succinic anhydride was unable to completely protect wood from soft rot fungi. Phthalic and succinic anhydride modifications both proved susceptible to hydrolysis and leaching, and neither were effective as wood protection chemicals. Phthalic anhydride modified wood performed well in the pure culture test, apparently through biocidal action, but was susceptible to decay in unsterile conditions.

The approach made in this study to understanding the mechanism of protection was to analyse physical properties of the modified wood cell wall. This involved the measurement of adsorption isotherms, volumetric swell due to water soak, and cell wall pore size (using the solute exclusion technique). Neither the moisture content of modified wood nor its cell wall moisture content (measured as the fibre saturation point from the adsorption analysis) provided a good explanation of decay resistance. In several cases, the relationship between volumetric swell (due to water soak) and weight loss (to a given fungus in pure culture) was found to be consistent between modification types. From this it is concluded that the extent by which water swells modified wood is important to decay resistance. A reduction in cell wall pore volume was measured using the solute exclusion technique, though no further conclusions could be drawn from this test.

It is proposed that the mechanism of resistance to decay by basidiomycete fungi involves the blocking of cell wall pores, which restricts the access of degradative agents released by decay fungi. The amount by which wood swells is important in this theory since this will determine by how much transient pores in modified wood can open, and whether enough space is created to bypass this blocking effect. The possibility of the role of site substitution in decay resistance is not discounted, and may contribute to decay resistance, particularly against white rot fungi. Pore blocking is not thought to be the mechanism of protection against soft rot fungi. In this case the substitution and shielding of decay susceptible sites are thought to be more important.

List of abbreviations

ASE	Anti-swell efficiency
BET	Brunauer-Emmet-Teller sorption theory
CCA	Copper - chromium - arsenic
DP	Degree of polymerisation
DP_L	Limit degree of polymerisation
e.m.c.	Equilibrium moisture content
FSP	Fibre saturation point
h	Relative vapour pressure
Kd	Equilibrium constant of the dissolved water (in the Hailwood - Horrobin model)
Kh	Equilibrium constant of the hydrates (in the Hailwood - Horrobin model)
Li-P	Lignin peroxidase
m	Total moisture content (in the Hailwood - Horrobin model)
m.c.	Moisture content
md	Water of solution (in the Hailwood - Horrobin model)
mh	Water of hydration (in the Hailwood - Horrobin model)
Mo	Moisture content corresponding to complete polymer hydration (in the Hailwood - Horrobin model)
Mn-P	Manganese dependent peroxidase
NADH	Nicotinamide adenine dinucleotide (reduced form)
R^2	Coefficient of determination
RH	Relative humidity
Vf	Void volume introduced into wood due to modification (free volume)
Vi	Volume occupied by the modifying chemical
WPG	Weight percent gain

Contents

Chapter 1. Introduction

Many of the most widely available timber species are highly susceptible to biodeterioration by fungi, bacteria, insects and marine borers. In the majority of applications, certainly in the UK, the most destructive degraders are the decay fungi. For many applications biocidal chemical preservatives are applied to the wood to increase its service life, the best known of these being creosote and the copper containing water based preservatives such as CCA. Biocides are not the only means of preventing wood decay, and with increasing legislation restricting the use of toxic chemicals more attention is being turned to non-biocidal methods of protecting wood.

Non-biocidal methods of wood preservation rely on the inhibition of the degrading organism by removing or restricting access to one or more of the various factors required by that organism for growth. The main factors required by wood decay fungi are listed below, along with some examples of non-biocidal protection that are used or have been attempted:

Substrate (a carbon and energy source, i.e. wood). Needs to be accessible to and digestible by the fungi and its enzymes or other degradative agents.

Other nutrients. Apart from the carbon source mentioned above, decay fungi require other nutrients such as nitrogen, thiamine and various metals, all available in the wood in much smaller quantities. Baechler (1959) suggested protecting wood by the removal or inactivation of these. His work involved the destruction of thiamine using heat treatment in alkaline conditions. This was moderately successful although Highley (1970) attributed the success to other changes brought about by the treatment. Baechler (1959) also attempted to complex trace metals found in wood using chelating agents, achieving protection against brown and white rot fungi. Suttie, Orsler and Wood (1996) showed

that decay by the brown rot fungus *Coniophora puteana* (Schum.: Fr.) Karst. could be inhibited when particular iron chelators were impregnated into wood to prevent the utilisation of iron by the fungus.

Water. Required by decay fungi for several reasons (Zabel and Morrell, 1992);

1. As a reactant in the hydrolytic degradation of wood polymers.
2. As a diffusion medium for extracellular degradative agents.
3. As a major constituent of fungal hyphae.
4. To swell the wood cell wall, allowing increased access of extracellular degradative agents to cell wall polymers.

Fungal decay can be prevented by drying wood to moisture contents below those required for decay, though the ease with which it can then be rewetted can cause problems in service. For certain end uses, water repellents and end sealers can be applied to the wood to slow or stop the uptake of water.

Oxygen. Decay fungi require oxygen for respiration and for oxidative degradation of wood polymers. This is particularly important when considering the moisture content of the wood, as the wetter wood gets the less oxygen is available to decay fungi. Storing logs in water ("ponding") is used to prevent decay, though in this case wood may still be susceptible to bacterial attack and possibly also to soft rot.

Other factors. Other factors important to fungal growth include temperature and pH. Wood decay fungi will only grow within certain ranges of both of these, though both would be extremely difficult to regulate with regard to protecting wood in service. High pH in wood-cement composites is thought to be a major factor that contributes to their enhanced decay resistance (Goodell, Daniel, Liu, Mott and Frank, 1997a).

One of the most widely studied non-biocidal methods of protection is chemical modification. Chemical modification of wood is defined as a chemical reaction between some reactive part of a wood component and a chemical reagent, with or without catalyst, to form a covalent bond between the two (Rowell, 1975). Among the most commonly tested types of modifying chemical are the anhydrides, particularly acetic anhydride. Wood modification with acetic anhydride has been found by many workers to impart both dimensional stability and resistance to biodeterioration.

Although chemical modification can be used in conjunction with biocides (e.g. work by Dunningham and Parker, 1992; Codd, 1997) or to introduce biocidal functional groups onto the wood (Chen, Rowell and Ellis, 1990; Chen, 1992a, b), its mode of action is not considered biocidal. The means by which chemical modification does protect wood are not fully understood, but most authors cite the two main proposals made by Stamm and Baechler (1960);

(1) "Insufficient water can be taken up to support decay."

(2) "Susceptible hydroxyl groups are removed or blocked so that decay enzymes cannot dissolve the wood components."

In terms of the factors required for decay listed above, these proposals refer to denying the fungus water (1) and/or utilisable substrate (2). These effects will be discussed in more detail later.

The ability of chemical modification to increase the resistance of wood to fungal degradation has been widely studied. Although a large number of tests have been conducted, comparisons between studies are difficult to make because of the wide range of modification reaction conditions used, different biological test methods, and the variety of test fungi. The purpose of the present study was twofold:

Firstly, the study was intended to assess the performance of structurally different modifications against decay fungi in pure culture and in unsterile conditions. Most particular in this part of the study was to assess the performance of three cyclic anhydrides; succinic, phthalic and in particular alkenyl succinic (16 - 18 carbon alkenyl chain) anhydride, and compare these with the more widely studied acetic anhydride and butyl isocyanate. Alkenyl succinic anhydride has been found to afford good decay resistance to wood when used in conjunction with low levels of copper (Codd, 1997). A more thorough investigation on the properties of wood modified with this chemical without copper, particularly its resistance to a range of decay fungi in different conditions, was required.

Secondly. the main part of the study involved the analysis of decay resistance imparted to wood by the varying structures of these modifying chemicals in order to try and better establish the mechanism by which modified wood is protected from decay. For this purpose, further tests were carried out to determine properties considered important in decay resistance; hygroscopicity, swelling and cell wall pore size. A greater understanding of the mechanisms of protection involved could lead to the use of more appropriate modifying chemicals, or to the development of more effective systems of reaction that could increase the commercial viability of chemical modification as a means of conferring biological resistance to wood.

In chapter 2 a literature survey has been conducted on wood structure, the interaction of wood and water, fungal decay, and on the protection of wood through chemical modification.

Chapter 3 describes the modification procedure and experiments to determine the extent and permanence of reaction.

Chapters 4 and 5 describe tests of the physical properties of wood, undertaken with a view to understanding how changes in these properties could be responsible for improving decay resistance. These include a sorption study, measurements of volumetric swell due to water soak, and the measurement of cell wall pore size.

Chapters 6, 7 and 8 describe the decay tests; a pure culture test against basidiomycete fungi under different moisture content regimes (chapter 6), an unsterile soil soft rot test (chapter 7), and a fungal cellar test and field trial (chapter 8).

The relevant results from each of these chapters are brought together and discussed in chapter 9.

Chapter 2. Literature survey

2.1 The composition of wood

The structure and chemical composition of wood has been widely studied and reviewed, for example by Fengel and Wegener (1984).

In softwoods, 90-95% of the wood tissue is made up of longitudinally aligned tracheids. These are long, thin, hollow cells, the central lumen being used for transport of water and minerals up the living tree. The wall thickness and lumen size of these vary with the stage of the growing season at which they were formed; earlywood tracheids having thinner cell walls and larger lumina and latewood tracheids having smaller lumina with thicker cell walls. The remainder of the wood substance is mostly made up of radial or axial parenchyma. These are small brick shaped cells responsible for radial transport and storage of nutrients and various biochemical processes in the sapwood of the tree. Hardwood tissue is more heterogeneous in its structure, the majority being made up of fibres (needle like cells with thick walls and small lumina) and vessels (very large lumina, very thin walls). Some hardwoods also contain large quantities of axially aligned parenchyma.

The cell walls themselves are constructed of layers that vary in composition and structure. These are known as the primary and secondary layers, the secondary layer itself usually consisting of three further layers, the S_1, S_2 and S_3 layers. The primary layer is the outer layer, inside it being the S_1, then the S_2, with the S_3 layer at the lumen surface. The thickest of these, and therefore the one that dominates wood properties, is the S_2 layer. Between cells there is a layer known as the middle lamella. This holds the wood cells together.

2.1.1 Chemical composition

The tissue in these layers is made up of cellulose, hemicelluloses and lignin. The relative quantities of these in the cell wall layers of spruce (*Picea abies* (L.) Karst.) tracheids was calculated by Fengel (1969, 1970a), and is shown in table 2.1. Fengel found the cell wall composition of spruce earlywood and latewood tracheids to be very similar, and only the data calculated for latewood has been included in table 2.1.

Table 2.1 Relative composition of the cell wall layers of spruce tracheids

(Source: Fengel 1969, 1970a)

Wall layer	**Polymer content (percent of the wall layer)**		
	Cellulose	**Hemicelluloses**	**Lignin**
CML*	13.7	27.4	58.9
S_1	34.6	34.6	30.8
$S_2 + S_3$	58.4	14.5	27.1

*CML = Compound middle lamella = Primary layer + middle lamella

The chemical structures of cellulose, hemicelluloses and lignin, and the way they are arranged in the cell wall are of great importance to the way in which fungi decay wood.

Cellulose is an unbranched homopolysaccharide composed of β-D glucose (although technically the repeating unit is the cellobiose dimer) joined by 1-4 glycosidic linkages. Cellulose in wood is thought to have an average degree of polymerisation (DP) of at least 9000 - 10000 (Goring and Timell, 1962).

In wood pulps approximately 60 - 70% of cellulose is in crystalline form (Fengel and Wegener, 1984). Chains of cellulose are held in sheets by inter and intra molecular hydrogen bonds, and these sheets pack together (held through van der Waals forces) to form crystalline cellulose. Crystallographic studies on cellulose from several sources has shown that natural cellulose is not comprised of a single crystalline form but is a

composite of two natural forms, 1α and 1β. The 1β form is dominant in the higher plants and is the more stable of the two forms, while the 1α is more susceptible to hydrolytic treatments (Atalla and VanderHart, 1989).

It was first thought that the primary structure of crystalline cellulose was the microfibril; a long thin filament of 10-30nm in diameter. With improvements in microscopic techniques there is increasing evidence for thinner units (known as elementary fibrils) with a diameter of 2-4nm which aggregate to make up the larger microfibrils, possibly in conjunction with para- or non-crystalline material such as hemicelluloses (Fengel and Wegener, 1984). This is further discussed in section 2.1.2. Para-crystalline and amorphous cellulose is thought to be found on the surface of the crystalline cellulose fibrils. Such a transition from crystalline to non-crystalline material would explain difficulties in measuring the cross section of these elements.

Structure along the length of the crystallite is also in some dispute. When subject to dilute acid, microfibrils are hydrolysed to crystallites of fairly uniform length which are resistant to further hydrolysis. The length of these crystallites is source dependent and the associated degree of polymerisation is known as the limiting degree of polymerisation (DP_L) (reported as being approximately 140 for spruce wood; Fengel and Wegener, 1984). This suggests areas of higher reactivity along the microfibril which are subject to hydrolysis, an early explanation of which was that cellulose chains passed through crystalline and amorphous regions along a microfibril. Using diffraction contrast transmission electron microscopy of cellulose from the alga *Valonia macrophysa* Kutz, Chanzy (1990) showed that such amorphous regions did not exist.

A popular explanation for areas of increased reactivity along a microfibril is that dislocations along the microfibril cause areas of stress in the region of the dislocation

which make that particular region of the microfibril more susceptible to hydrolysis. Such dislocations are thought to be due to individual cellulose chain ends within the microfibril which disrupt the regular crystalline pattern. This model has not been substantiated. For this to hold, cellulose chains would need to be of non uniform length (O'Sullivan, 1997), and in order to explain the reasonably uniform size of the crystallites, dislocations would have to occur at regular intervals along the microfibril. An alternative model could involve the inevitable stresses that would be built up over the length of the microfibril due to the twisting or curvature in the cell wall. This would cause straining of the bonds between and within cellulose chains and could cause the microfibril to be more susceptible to acid hydrolysis. If and when the microfibril is hydrolysed and broken, a relaxation of the twisting stresses could then cause the microfibril to become less reactive. The length at which the crystalline cellulose in the microfibril becomes immune to acid hydrolysis (the reactivity drops below that required) would be the DP_L. Differences in curvature, crystallinity, or polymorph (1α v. 1β) of the cellulose in the microfibril could all theoretically affect DP_L, and could therefore account for the source dependence of DP_L.

The hemicelluloses are mixed polymers of various sugar units with a degree of polymerisation varying between 100 and 400. The component monosaccharides are glucose, xylose, mannose, galactose, arabinose, rhamnose and fucose, and the uronic acids of glucose and galactose (Eaton and Hale, 1993). The structure of hemicelluloses differs between softwoods and hardwoods.

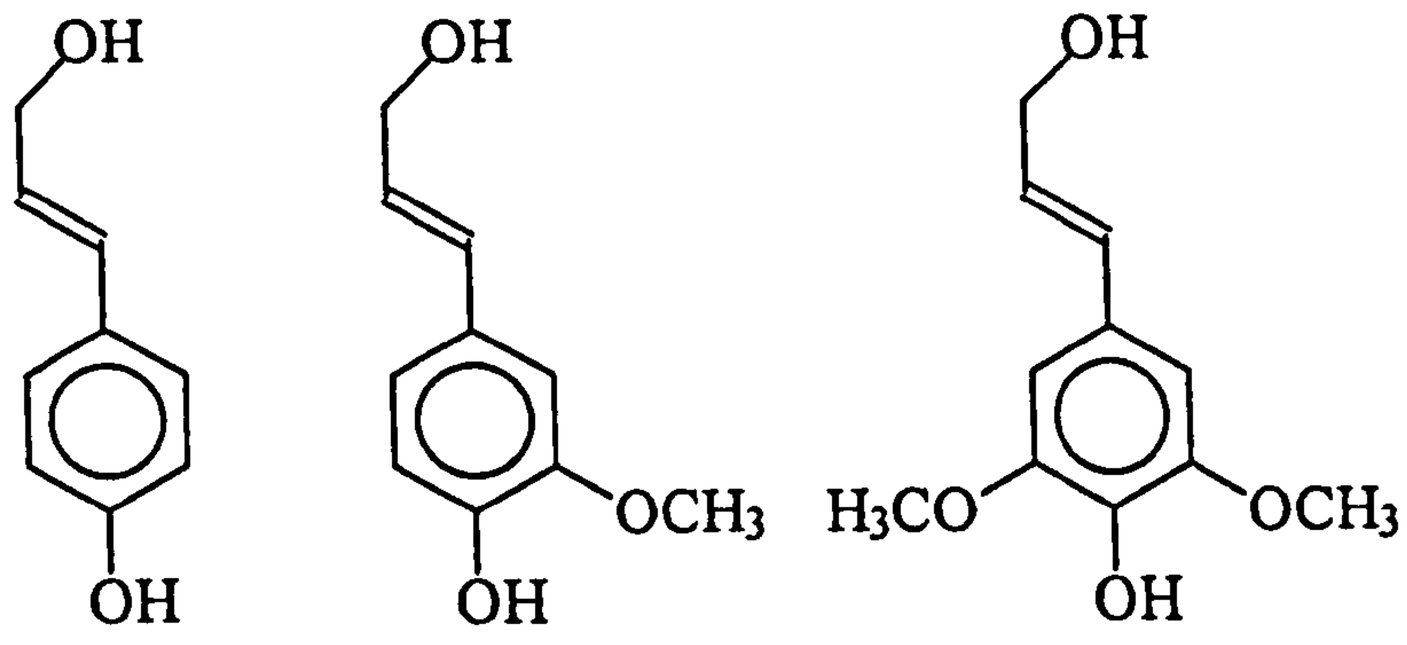
The most common of the hemicelluloses in softwood are the galactoglucomannans. These consist of a backbone built up of mannose and glucose monomers, some of which are acetylated at the C2 or C3 position, joined by β 1-4

glycosidic linkages. Galactose residues are attached onto this backbone chain at various points by α 1-6 linkages. The ratio of galactose : glucose : mannose is approximately 1:1:3, though there is a form of galactoglucomannan with a lower galactose content (ratio approximately 0.1:1:4) usually known as glucomannan (Walker, 1993).

In hardwoods the most common hemicellulose is *O*-acetyl-4-*O*-methylglucuronoxylan. This consists of a backbone of β 1-4 linked xylopyranose units, 40-70% of which are acetylated at the C2 or C3 position. 4-*O*-methylglucuronic acid is linked at regular intervals along the backbone, the ratio of xylose to glucuronic acid units being about 10:1 (Walker, 1993).

Unlike cellulose, hemicelluloses are branched and generally unable to form crystalline material. This makes them more soluble and more susceptible to hydrolysis. It has been suggested that the more linear hemicellulosic polymers such as mannan and to some extent xylan fit more readily around the crystalline cellulose microfibril, while the other hemicelluloses, along with minor amounts of non-crystalline cellulose, are found in the amorphous regions of the cell wall (Walker, 1993).

Lignin makes up about 30% of softwood tissue and about 20% of hardwood. It is synthesised in the cell wall after the holocellulose has been deposited and is considered to be the material that, along with the hemicelluloses, binds the cellulose microfibrils together and causes the cell wall to become rigid (Reid, 1995). Lignin is formed through the polymerisation of three phenolic precursors which differ from each other in the number of methoxyl groups they contain. Softwood lignin contains mostly guaiacyl (monomethoxy) units while hardwoods contain roughly equal amounts of guaiacyl and syringyl (dimethoxy) units. Both contain small quantities of *p*-hydroxyphenyl (no



a. b. c.

Figure 2.1 Lignin precursors

a. *p*-Coumaryl alcohol b. Coniferyl alcohol c. Sinapyl alcohol

Reaction between these monomers can form various inter-unit bonds which cause lignin to be a complex amorphous polymer; there is no repeating pattern. Figure 2.2 shows a theoretical diagram of softwood lignin showing the common linkages. The most common inter-unit link is the β-O-4 linkage, i.e. a bond between the middle carbon on the propyl side chain of one monomer to the phenolic oxygen on the next (Reid 1995).

In addition to those bonds within the lignin shown in figure 2.2 there is evidence of the existence of bonds between lignin and hemicelluloses. These are known as lignin-carbohydrate complexes (LCC's) (Fengel and Wegener, 1984).

Non-structural constituents of wood are present in varying quantities. These are the extractives and ash. Extractives are particularly important when considering natural durability of wood, though they are of little relevance to this work. Inorganic ash includes metal ions such as manganese and iron which are required by decay fungi as described later.

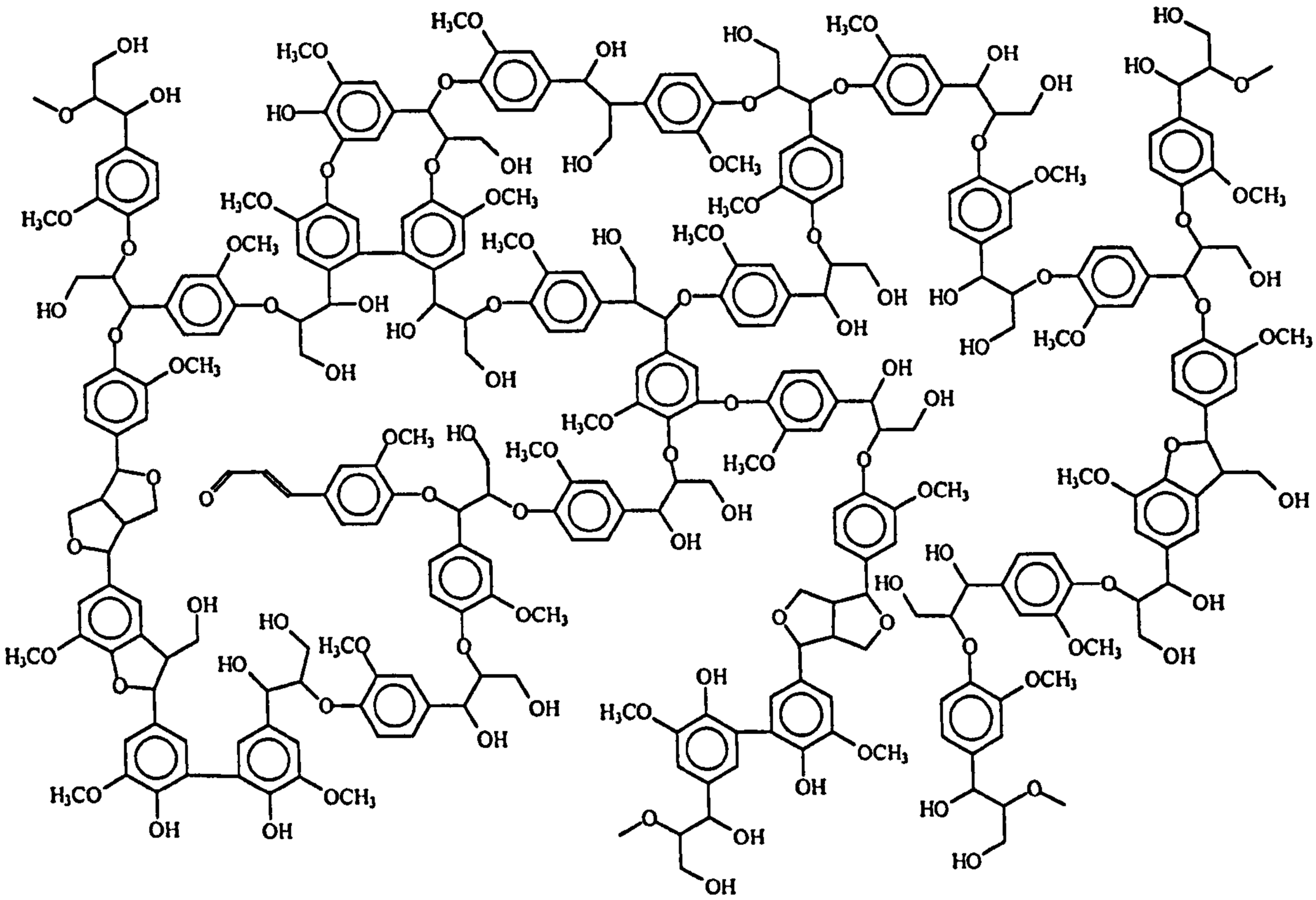


Figure 2.2 Model of softwood lignin (Source: Reid 1995)

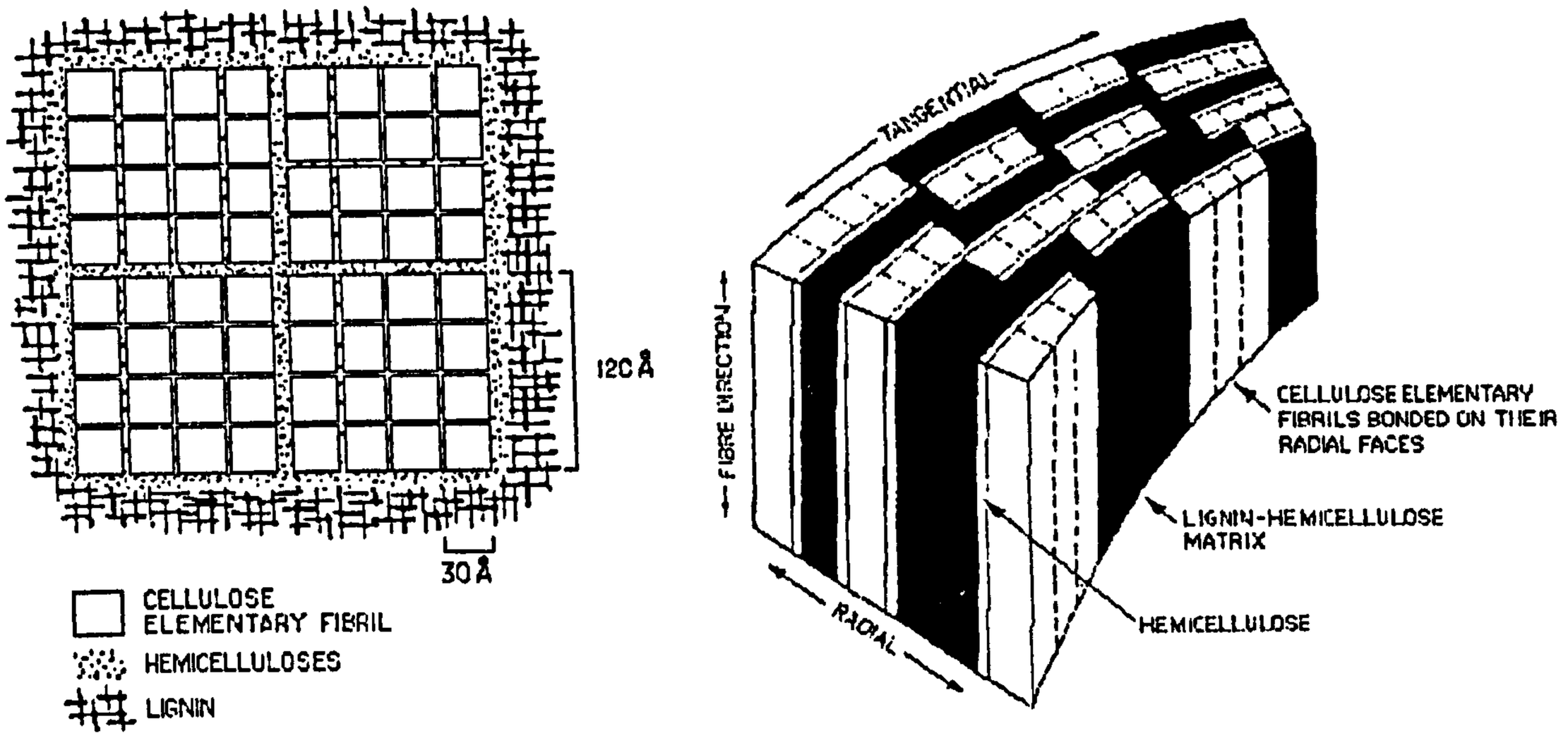
2.1.2 Internal cell wall structure

The way in which cellulose, hemicellulose and lignin aggregate in the wood cell wall is not fully understood, and several models have been proposed.

The alignment of microfibrils making up the wood cell wall varies from layer to layer. The microfibrillar angle is lowest (i.e. microfibrils are closest to being aligned along the axis of the tracheid) in the thickest, S_2 layer. As this is the structurally most dominant of the layers it is this that structural models tend to be based on.

Two of the most well known models for the structure of the wood cell wall are those of Fengel (1970b) and Kerr and Goring (1975).

Figure 2.3a shows the model proposed by Fengel (1970b). This model attempts to explain the various thicknesses of cellulose crystals that have been measured in wood by showing the aggregation of elementary fibrils into larger units, all surrounded by



a. b.

Figure 2.3 Models of the ultrastructural organisation of the wood cell wall

a. Fengel (1970b) b. Kerr and Goring (1975)

hemicelluloses and lignin. Figure 2.3b shows the Kerr and Goring (1975) model developed on the evidence of electron microscopy of permanganate stained transverse sections of softwood tracheids. Here elementary fibrils are joined together at their radial faces to form a system of concentric, interrupted lamellae 3.5nm thick within the cell wall, with some of the hemicellulose directly associated with the cellulose and the rest with the lignin matrix. This is also in agreement with results of Stone, Scallan and Ahlgren (1971) who, from measurements of shrinkage at various stages of delignification, concluded that lignin and cellulose are arranged in concentric layers.

2.2 Water in wood

The presence of water in wood greatly affects many wood properties and is an essential prerequisite for wood decay. As mentioned earlier, one proposed mechanism of wood protection by chemical modification is through the reduction of wood moisture

content. The distribution of water in wood and the requirements of water by wood decay fungi must both be considered.

2.2.1 The fibre saturation point

Prior to a discussion of the role of water in decay it is necessary to clarify a definition for the fibre saturation point (FSP).

Definitions and methods of measuring FSP are discussed in detail by Skaar (1988) and Siau (1996). The common definition of FSP is that it is the moisture content at which wood cell walls are fully saturated while there is no free water in the large permanent voids (i.e. the lumina and pit apertures).

FSP has been measured or estimated using various methods. Recording the heat of wetting (on the basis that adsorbed water will be more closely bound to the wood and will liberate energy as it wets the wood), change in properties (such as strength or electrical resistance) and by recording the point at which shrinkage starts have all led to an estimation of FSP to be 25-30% for many softwoods (Skaar, 1988). Stone and Scallan (1967) measured the FSP of Black spruce (*Picea mariana* (Mill.) B.S.P.) using a polymer exclusion technique and they and Griffin (1977) used discontinuities in the desorption isotherm to estimate FSP (also on *P. mariana*), both obtaining measures of approximately 40%. Siau (1996) argues that these figures are not consistent with shrinkage data and are probably due to the very thin microtome sections or pulp samples used, leading to higher swelling and FSP values than in solid wood due to the lack of mechanical constraint. The existence of small permanent voids could also affect measurements using these methods.

It is conceded in all the literature that FSP is a largely theoretical point. The presence of solutes in parenchyma lead to osmotic pressures that can cause it to have higher moisture contents and some free water before other cells have adsorbed water to

FSP. Additionally, at moisture contents approaching FSP (and corresponding relative humidities) some condensation in the smaller permanent capillaries is inevitable (Siau, 1996). Despite this, FSP is still a useful concept, and is considered to be about 27-30% by most workers for the majority of softwoods.

2.2.2 Water sorption and sorption isotherms

The mechanism by which water attaches to sites in the wood cell wall in response to changes in temperature or relative humidity is known as sorption. When wood is exposed to constant temperature and relative humidity it will reach a moisture content in equilibrium with these conditions. If relative humidity is then increased or decreased, wood will adsorb or desorb water until a new equilibrium moisture content (e.m.c.) is reached.

Sorption can be represented graphically as an isotherm by measuring e.m.c.'s at increasing (adsorption) or decreasing (desorption) relative humidities at constant temperatures. It is necessary to distinguish between adsorption and desorption in such states because e.m.c. at a given relative humidity will differ, depending on whether adsorption or desorption is being measured; the wood adsorption isotherm is lower than the desorption isotherm. This effect is known as hysteresis. Theories of the causes of hysteresis are reviewed elsewhere (Martins 1992).

The water sorption isotherm for wood has a sigmoid shape and is known as a type 2 isotherm. This is from a classification in which Urquhart (1960; cited by Skaar, 1988) described five types of isotherm. Types 1, 2 and 3 are depicted in figure 2.4. The relevance to wood of type 1 and type 3 isotherms to wood is discussed later.

There are several methods that have been used for fitting isotherms to experimental data for relative humidity and e.m.c. Those that have been used for wood

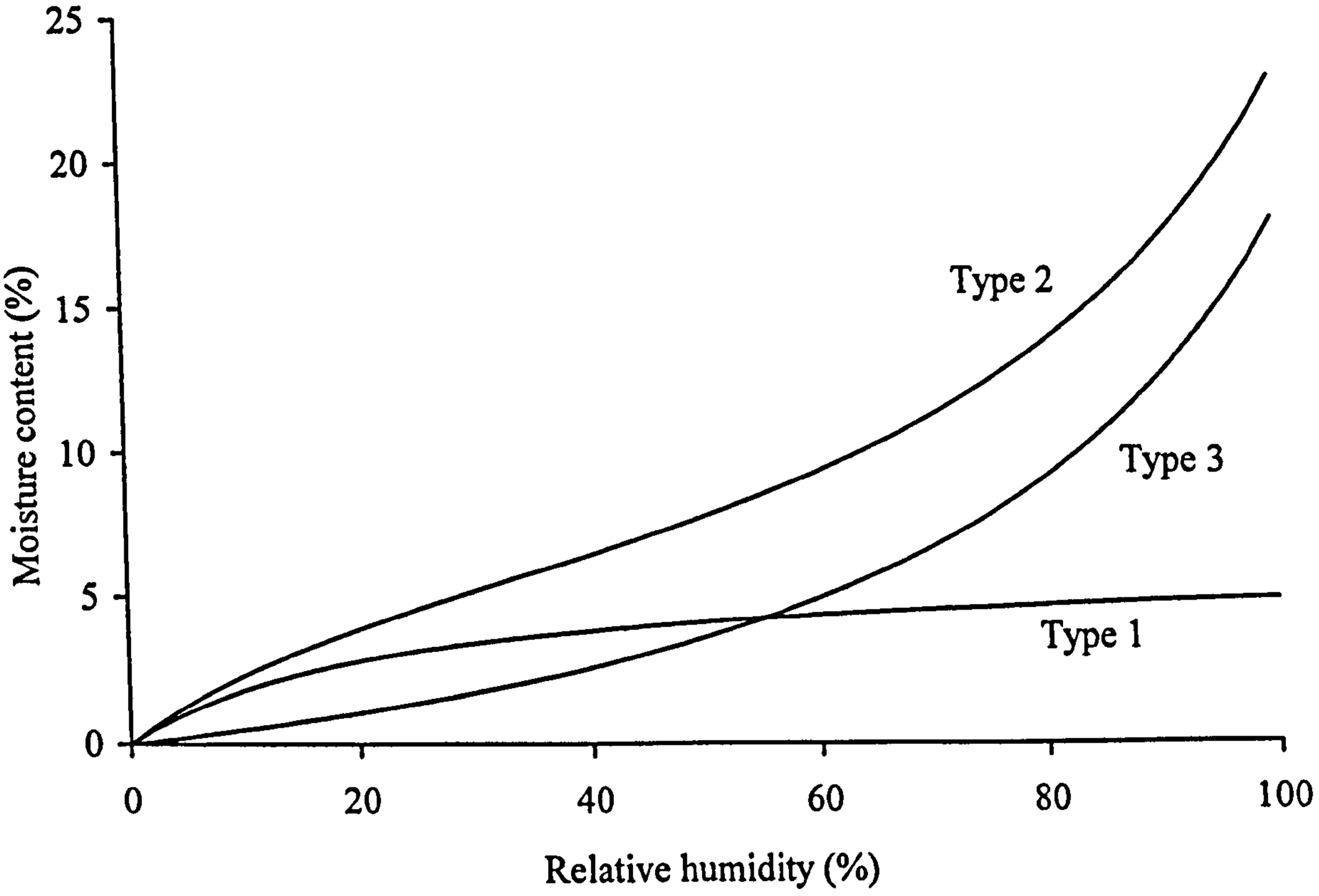


Figure 2.4 Type 1, 2 and 3 adsorption isotherms calculated from data for unmodified Corsican pine (*Pinus nigra* Schneid) (from chapter 4)

have been reviewed by Skaar (1988). Two of the most well known of these models are those proposed by Brunaeur, Emmett and Teller (1938; cited by Skaar 1988) and Hailwood and Horrobin (1946).

Both of these theories consider bound water in wood to be held in two separate ways. These are monomolecular sorption, in which a single layer of water molecules hydrogen bonds to the available sorption sites on the wood polymers, and polymolecular sorption, in which water is sorbed within the wood cell wall but is not in direct contact with the wood sorption sites. The former component is considered to be more strongly bound than the latter component.

Monomolecular sorption forms a type 1 isotherm while polymolecular sorption forms a type 3 isotherm. Adding these two components together forms the overall (type

Monomolecular sorption forms a type 1 isotherm while polymolecular sorption forms a type 3 isotherm. Adding these two components together forms the overall (type 2) isotherm (figure 2.4). At low relative humidities, adsorption at the monomolecular layer is considered the dominant form as an increasing number of sorption sites are exposed (through swelling). As humidity increases, polymolecular sorption becomes increasingly important, becoming predominant at higher levels of relative humidity (Spalt, 1958).

The main difference between the Brunaeur, Emmett and Teller (BET) and Hailwood – Horrobin theories is the way in which they assume that the polymolecular water is held within the cell wall. The BET theory considers polymolecular sorption to take the form of additional layers building up on a first monolayer, bonded to the wood polymers. The Hailwood – Horrobin theory considers monomolecular sorption to take the form of a hydrate of the cell wall polymers while polymolecular water is assumed to be in solution with the wood polymers.

Other sorption theories are often based on either of these two.

The Hailwood – Horrobin model allows for the formation of a multiple hydrate with a single unit of a polymer. Applying the model to wood, Spalt (1958) assumed the formation of a single hydrate, and found this to give a good fit to e.m.c. data for various wood species.

Simpson (1980) compared several of these models for applicability to wood sorption data. While the models tested were unable to accurately predict thermodynamic properties such as heat of sorption, all but the BET model gave an excellent fit to the e.m.c. data. In the present study the Hailwood – Horrobin model was used.

Models and theories for fitting sorption isotherms are based on sets of assumptions, and this leads to weaknesses in the theories. Two such weaknesses in the

Hailwood - Horrobin theory (along with other isotherm fitting models) are that it does not account for capillary condensation and does not predict hysteresis.

In practice, if relative humidities above 99.5 % were obtained then condensation would occur in the microscopically visible capillary structure of the wood after cell walls had become saturated (Stamm, 1964). Equilibrium moisture content at a relative humidity of 100% would be equivalent to complete saturation of the wood. When fitting a Hailwood – Horrobin isotherm to data such as that used in this study (i.e. highest e.m.c. reading at 93% RH) the isotherm forms an extrapolation to 100% RH at a much lower moisture content. The 100% RH value calculated from such an extrapolation is often used as an estimate for FSP (Stamm, 1964).

In order to correctly measure hysteresis using this model it must be fitted separately to e.m.c. data measured in adsorption and in desorption (Spalt, 1958).

2.2.3 Cell wall pore accessibility

When water swells wood, a network of transient pores opens up in the wood cell wall. The size or accessibility of these has been studied for several reasons, one of which is that these pores form pathways for some of the fungal degradative agents to get into the cell wall to degrade wood polymers.

Various attempts have been made to measure the diameters and volume of the transient pores of natural celluloses, wood and wood pulps, many using the solute exclusion technique. This involves soaking swollen samples in solutions of different size probes. The solution then becomes diluted as probes move into the sample to an extent depending on the accessibility of the water in the sample to that particular size probe. Stone and Scallan (1968) used this technique on never dried Black spruce samples and found the maximum cell wall pore diameter to be 36Å, though the median pore size (i.e.

the pore size at which half the cell wall pore volume is made up from pores less than that size) was 12Å. Using the same technique on previously dried sweetgum (*Liquidambar styraciflua* L.) Flournoy, Paul, Kirk and Highley (1993) estimated the maximum pore size to be just 20Å.

The solute exclusion method has been criticised as a means of measuring pore diameter and volume. Alince (1991) argued that the concentration of a probe within a narrow pore would not be the same as that in bulk solution. He further stated that such an effect may account for the distributions measured by solute exclusion and that the porosity of cellulosic materials may be closer to monodisperse than is often believed. Alince (1991) also discusses problems that could affect solute exclusion measures such as osmotic pressures or the "ink-bottle" effect, where narrow pores could exclude probes from larger pores beyond. Walker (1993) suggests that solute exclusion may give a smaller than actual measure of pore diameter because the initial hydrogen bonded monolayer of water may not be accessible to probe molecules. These are all criticisms of the ability of the method to measure actual sizes, whereas the importance for many studies, including this one, is in the accessibility of the cell wall to these probes, as this will give an indication of the ease of accessibility to extracellular degradative agents used by wood decay fungi.

2.3 Fungal decay of wood

Wood decay fungi are classified into three main groups depending on the way in which they degrade wood; white rot, brown rot and soft rot. The first two of these are groups of basidiomycete fungi while the soft rot fungi are members of the ascomycetes and the deuteromycetes.

White rot fungi degrade both the polysaccharide and lignin components in wood. They do this either simultaneously or they decay the lignin and hemicellulose first in preference to the cellulose (simultaneous and preferential forms of white rot decay). A typical pattern of simultaneous decay is the formation of erosion troughs in the wood cell wall directly associated with the fungal hyphae. These troughs widen as decay progresses, leading to cell wall thinning. Erosion can also occur more uniformly around the whole of the cell wall surface. During the preferential form of decay, lignin and hemicelluloses are removed from within the cell wall, starting at the lumen surface and culminating with the degradation of the middle lamella and the separation of the now largely cellulosic fibres. Degradation of the cellulose then follows.

Brown rot fungi degrade the holocellulose while leaving the lignin modified though largely intact. The decay is diffuse within the wood cell wall, at a distance from the hyphae, leading to collapse of the cell wall later in decay rather than a progressive erosion.

Soft rot fungi, like the brown rot fungi, are primarily degraders of holocellulose. They do have a limited capability to degrade lignin, in general removing it to a greater extent than brown rot but more slowly than white rot fungi. Soft rot decay usually follows two patterns; it is generally characterised by hyphal growth and cavity formation within the S_2 layer of the cell wall (type 1 decay), but can also be in the form of an erosion type attack at the lumen surface (type 2 decay).

The preference of these forms of decay for different wood components is demonstrated in table 2.2.

The biochemical methods used to break down the wood polymers to products of a size that can be taken into the hyphae are not fully understood, and can vary even between fungi causing the same form of decay. Enzymes which have the ability to

Table 2.2 Lignin and wood sugars within wood after advanced decay by different micro-organisms. (Source: Blanchette, 1995)

Type of decay	Percent composition after decay			
	Lignin	Cellulose	Xylose	Mannose
White rot				
Simultaneous	30	47	6	13
Preferential	1	97	1	1
Brown rot	60	20	1	2
Soft rot	61	13	2	2
Sound (pine)	28	48	6	16

degrade wood components have been isolated from fungi, as have various other chemicals thought to have an important role in degradation or its regulation. These have been widely studied and reviewed (e.g. Eriksson, Blanchette and Ander, 1990; Eaton and Hale, 1993; Blanchette, 1995).

2.3.1 White rot mechanisms of decay

White rot fungi degrade cellulose largely through the synergistic action of three main types of enzyme. Endo-1,4-β-glucanases hydrolyse cellulose chains at random along the length of the crystallite creating new chain ends upon which exo-1,4-β-glucanases can act. The exo-glucanases bind onto the non-reducing chain ends and move along the chain towards the reducing end, cleaving off cellobiose units. 1,4-β-glucosidases hydrolyse cellobiose and larger glucose oligomers to glucose. In addition to these hydrolytic enzymes, oxidative enzymes such as glucose oxidase, cellobiose oxidase and cellobiose dehydrogenase participate in cellulose degradation. These prevent a build up of the end products of cellulose hydrolysis which could lead to repression of the hydrolytic system (see Eriksson *et al.*, 1990; Eaton and Hale, 1993).

Enzymatic decay of hemicelluloses is similar to that of cellulose in that despite the lack of problems relating to crystallinity, enzymes operating endo- and exo- to the

various hemicelluloses are used by the fungi. However, due to the heterogeneity of hemicelluloses a greater variety of enzymes is required.

The white rot fungi are the only wood decay fungi with fully developed ligninolytic capability. Several enzymes have been identified that are thought to have a role in lignin degradation, though complete mechanisms of degradation and what initiates it are not understood. This is partly due to the complexity of the polymer itself; the enzymes are necessarily non-specific due to the lack of regular bonding patterns as found in the polysaccharides. The enzymes (or groups of enzymes) considered of greatest importance in lignin biodegradation are briefly discussed below (see Eriksson *et al.*, 1990; Reid, 1995).

Lignin peroxidase (Li-P) is thought to be responsible for the majority of lignin degradation as its high redox potential enables it to oxidise non-phenolic as well as phenolic groups within lignin. Li-P oxidises aromatic nuclei to form cation radicals, which initiate cleavage reactions within the polymer including Cα-Cβ, β-O-4 and Cβ-Cγ cleavage. Li-P returns to its reactive form through oxidation by hydrogen peroxide.

Manganese dependent peroxidase (Mn-P) is a phenoloxidase, its lower redox potential makes it unable to oxidise non-phenolic aromatic groups in lignin. Like Li-P it is dependent on hydrogen peroxide, but it also requires manganese to function. It is thought that Mn-P oxidises manganese from Mn^{2+} to Mn^{3+}, and that it is the latter that is responsible for oxidising lignin phenolics to phenoxy radicals. These phenoxy radicals then initiate lignin depolymerisation reactions.

Laccase is another phenoloxidase, oxidising lignin phenolics to phenoxy radicals as in the case of Mn-P, though it is not hydrogen peroxide (or manganese) dependent.

As mentioned briefly above, these enzymes vary in their ability to oxidise different lignin types. This depends on the redox potential of the enzyme and on that of

the particular lignin aromatic unit. The susceptibility of a lignin unit to oxidation depends on its chemical structure, and is affected for example by a carbonyl at the Cα position (increases resistance to oxidation), an unsubstituted phenolic hydroxyl, and the number of methoxyl groups. The higher the number of methoxyl groups, the more susceptible the lignin monomer is to oxidation. This causes guaiacyl type lignin to be more resistant to degradation than syringyl lignin (see Eriksson *et al.*, 1990).

The relative importance of each of the three ligninolytic enzymes mentioned above is unclear. White rot fungi vary in their compliment of ligninolytic enzymes, and an individual strain may not produce all three. A further complication is the fact that lignin degradation takes place within the cell wall, at a distance from the hyphae. Even in the case of simultaneous decay it seems that lignin is degraded ahead of the wall erosion, cellulose being attacked as it becomes accessible after the lignin is removed (Eriksson *et al.*, 1990). Evans, Gallagher, Atkey and Wood (1991) found that enzymes from white rot fungi only enter the cell wall during later stages of decay, and are too big to enter and degrade sound wood. Blanchette, Abad, Farrell and Leathers (1989) found that Li-P and Mn-P could penetrate decay zones in areas of simultaneous decay created by *Trametes versicolor* (L.: Fr.) Pilat, and throughout the walls of wood preferentially delignified by *Phanerochaete chrysosporium* Burds. or *Phellinus pini* (Fr.) A. Ames, but that these enzymes could not penetrate sound wood. Flournoy *et al.* (1993) measured the increase in pore width and volume of sweetgum decayed by *P. chrysosporium*. They found that the simultaneous removal of components in this case increased the void volume of the cell wall only modestly (as would be expected if the only section of the cell wall which was subject to attack was that slightly ahead of wall erosion), though the maximum pore size increased to a significant extent; to a size greater than that required to admit a small enzyme but insufficient to allow access to ligninolytic enzymes. Lignin degradation,

certainly in initial stages of decay, has therefore been attributed to low molecular weight mediators capable of penetrating the cell wall and degrading lignin at a distance from the hyphae. Veratryl alcohol, manganese ions and radicals from hydrogen peroxide have all been attributed with degradation of wood polymers.

Veratryl alcohol is produced by many white rot fungi during lignin degradation. Its exact role is debated, but one suggestion is that it is a mediator for lignin peroxidase. It is proposed that Li-P oxidises veratryl alcohol to a cation radical, which then diffuses into the wood cell wall and oxidises aromatic components in lignin. This may not be possible as there is doubt as to whether the lifetime of this radical is long enough to let it diffuse into the wood (Evans, Dutton, Guillén and Veness, 1994; Schick Zapanta and Tien, 1997).

Reviewing work on Mn-P, Schick Zapanta and Tien (1997) concluded that chelation of Mn^{2+} and Mn^{3+} is necessary for Mn-P activity and that oxalate has a significant role in this. They further stated that chelated Mn^{3+} could act as a diffusible oxidant to remote areas of lignin. The production of oxalate by white rot fungi and its role in decay is not understood. Several purposes have been suggested including a role in the regulation of lignin degradation (Shimada, Akamatsu, Tokinatsu, Mii and Hattori 1997).

Evans *et al.* (1991) suggested that holocellulose as well as lignin degradation by white rot fungi is initiated by low molecular weight degradative agents, proposing that free radicals from hydrogen peroxide initiate decay of polysaccharides, with subsequent decay by glucanases occurring in partially degraded areas of the cell wall. Backa, Gierer, Reitberger and Nilsson (1993) suggested that the hydroxyl radical is responsible for initial decay of all cell wall polymers, with cellulolytic and ligninolytic enzymes responsible for further breakdown of fragments produced. Tanaka, Itakura, Hirano and Enoki (1996)

isolated a low molecular weight iron-containing substance from the white rot *P. chrysosporium* similar to ones previously isolated from the brown rot fungi *Gloeophyllum trabeum* (Pers.: Fr.) Murr. and *Tyromyces palustris* (Berk. et Curt.) Murr. (Enoki, Hirano and Tanaka, 1992; Hirano, Tanaka and Enoki, 1995). They claim this is responsible for the production of hydroxyl radicals and that these are involved in initial stages of holocellulose and lignin decay by white rot fungi. Hydroxyl radical involvement in decay is described in more detail for brown rot decay.

2.3.2 Brown rot mechanisms of decay

The pattern of brown rot decay differs considerably from that of white rot. During brown rot decay holocellulose is rapidly depolymerised and DP is reduced significantly while weight loss is still low. Crystalline cellulose is depolymerised to its DP_L crystallites while white rot fungi cause little change in average DP of cellulose as decay progresses (Cowling and Brown, 1969). Brown rot decay is diffuse within the cell wall at a distance from the fungal hyphae. Several studies have found decay to start in the S_2 layer of the cell wall, initially leaving the S_3 layer at the lumen surface undecayed (Highley, Murmanis and Palmer 1985; Eriksson *et al.*, 1990). Cowling and Brown (1969) compared data for the size of wood cell wall pores and the size of cellulolytic enzymes, and found the latter too large to enter the wood cell wall. Also, although brown rot fungi do produce cellulases and hemicellulases, many lack exoglucanases, and so do not have the full compliment of cellulolytic enzymes required for depolymerisation of crystalline cellulose (see Eriksson *et al.*, 1990).

These observations led to the theory that non-enzymatic low molecular weight compounds were at least in part responsible for decay by brown rot fungi. Cowling and Brown (1969) suggested Fenton's reagent as a possible decay mechanism. This involves

the reaction of hydrogen peroxide and the ferrous ion, Fe^{2+}, to form the highly reactive hydroxyl radical, which would then react with the holocellulose:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + {}^{-}OH$$

The hypothesis that Fenton's reagent is responsible for the cellulose degradation by brown rot fungi has become a popular, if not wholly accepted one, since Koenigs (1974) showed that such a system could depolymerise cellulose in wood and proposed that this was possible at concentrations of iron equivalent to those likely to be encountered in wood. Koenigs (1974), Highley (1977) and Kirk, Ibach, Mozuch, Conner and Highley (1991) all observed close similarities between brown rotted wood or cellulose and that exposed to Fenton's reagent.

Fenton's reagent involvement has still been questioned. Brown rot fungi would require a mechanism to deliver such a highly reactive species as the hydroxyl radical to the polysaccharide cleavage sites while leaving the lignin virtually intact (Flournoy, 1994). This would need to be at a distance from the hyphae, which would otherwise themselves be damaged. In addition there is little evidence of hydrogen peroxide production by brown rot fungi, and the mechanism by which iron is cycled back to the reduced ferrous form has not been established (Flournoy, 1994; Green and Highley, 1997). Another much debated issue is the role in decay of oxalic acid which is produced and accumulated by brown rot fungi in wood.

More recent theories of Fenton chemistry involvement that addresses some of these questions have been proposed by Hyde and Wood (1997) and Goodell and Jellison (1997).

Studying the brown rot *C. puteana*, Hyde and Wood (1995, 1997) suggested that a pH gradient is set up within the wood cell due to the production of oxalic acid by the hyphae. Chelated FeIII (oxalate is assumed as the chelating species) is reduced to FeII by

cellobiose dehydrogenase (found to be produced in cultures of *C. puteana*) and diffuses into the cell wall. The higher pH within the cell wall enables the reduction of molecular oxygen by two FeII complexes to form hydrogen peroxide, which can then react with further FeII present to form the hydroxyl radical. This system helps explain how hydroxyl radicals are formed at a safe distance from the hyphae (and why the S_3 layer may seem resistant to attack early in decay) and the production of hydrogen peroxide, though the authors admit that the theory is weak with regard to iron cycling (i.e. reducing FeIII to the FeII required in Fenton's reagent).

Goodell and co-workers (Goodell, Jellison, Liu, Daniel, Paszczynski, Fekete, Krishnamurthy, Jun and Xu, 1997b; Goodell and Jellison, 1997) also consider the formation of a pH gradient as an important factor in decay. Oxalate chelated FeIII complexes diffuse into the wall where FeIII is transferred to phenolic chelators produced by the fungus (a variety of phenolic compounds capable of chelating iron have been isolated from some brown rot fungi, notably *G. trabeum*). It is proposed that these phenolics then reduce FeIII to FeII (i.e. at a safe distance from hyphae), and could also be involved in the production of hydrogen peroxide from molecular oxygen. The authors are unable to explain the mechanism by which these compounds are then reduced to their original state.

In addition to these, Enoki *et al.* (1992) and Hirano *et al.* (1995) isolated low molecular weight iron containing substances from the brown rot fungi *G. trabeum* and *T. palustris* respectively, which they claim are capable of diffusing into the cell wall and producing the hydroxyl radical through reduction of hydrogen peroxide by complexed FeII. They also suggest that this substance catalyses the reduction of molecular oxygen to hydrogen peroxide by an electron donor such as NADH.

Although Fenton's chemistry has received the greatest attention with regard to brown rot decay other work has considered more closely the involvement of oxalic acid. Several workers have attributed rapid depolymerisation of cellulose and hemicelluloses in initial stages of decay to the low pH reached as a result of oxalic acid production. It is therefore proposed that the low molecular weight degradation agent is the hydronium ion (Shimada, Akamatsu, Ohta and Takahashi, 1991; Green, Larsen, Winandy and Highley, 1991). The removal of hemicelluloses through acid hydrolysis could then open up the pore structure within the wood cell wall to allow access of enzymes (Green *et al.*, 1991). Oxalic acid has also been attributed with a role in softwood bordered pit degradation by sequestering calcium from calcium-pectate complexes in the torus, increasing the susceptibility of pectins to enzymatic degradation (Green, Tschernitz, Kuster and Highley, 1995).

Flournoy, Kirk, and Highley (1991) made an estimate of the size of the degradative agent of the brown rot *Poria (Oligoporus) placenta* (Fr.) Cke. by measuring the volume and width changes in the cell wall pores of decayed sweetgum. They found an increase in pore sizes in the range of 12 to 38Å in wood decayed to 35% weight loss, but with no increase in accessibility above this. They therefore estimated the degradative agent involved to be 12 to 38Å in diameter, possibly 12 to 20Å. These results seem to refute the claim that initial decay by brown rot fungi widen the cell wall voids sufficiently to allow entry of enzymes. Srebotnik and Messner (1991) measured the ability of two proteins of different molecular weights to enter the cell wall of pine decayed by brown rot (*Fomitopsis pinicola* (Swartz: Fr.) Karst.). Even at weight losses of 70% the cell wall was inaccessible to the larger of the two proteins (molecular weight 45000Da; corresponding to the weight of some known wood decay enzymes) and only partially accessible to the smaller (molecular weight 16500Da).

Considerable variation in lignin degrading capability among brown rot fungi has been found, though in general attack is limited to modification rather than any significant depolymerisation (Eriksson *et al.*, 1990). The modifications are largely oxidative, and include demethylation, an increase in the phenolic hydroxyl content (due at least in part to demethylation) and an increase in carbonyl and carboxyl content (Kirk, 1975; Jin, Nicholas and Kirk, 1990a; Jin, Schultz and Nicholas, 1990b). Flournoy (1994) states that, given the correct conditions and an appropriate chelator, such modifications of lignin could be due to the action the Fenton system.

Jin *et al.* (1990a, b) also found evidence of both limited depolymerisation reactions and partial polymerisation of the degradation products. Such depolymerisation and repolymerisation could theoretically lead to a modification of the distribution of lignin as observed by Highley *et al.* (1985) for wood decayed by the brown rot fungus *P. placenta*. Goodell *et al.* (1997b) suggest that without the intervention of a true lignin degrading system, the hydroxyl radical could repolymerise lignin derivatives.

Shimada *et al.* (1997) proposed that in a Fenton's system lignin could first scavenge hydroxyl radicals, after which the resultant lignin derived radicals may oxidise adjacent carbohydrates.

2.3.3 Soft rot mechanisms of decay

The mechanisms of soft rot decay have not received as much attention as those of white and brown rot decay. As mentioned above, soft rot fungi are largely degraders of polysaccharides, with a limited ability to degrade lignin.

Breakdown of polysaccharides is thought to be due to hydrolytic and oxidative enzymes similar to those described for white rot decay. Keilich, Bailey and Liese (1970) and Bailey, Keilich and Husemann (1972) studied enzymes isolated from *Chaetomium*

globosum Kunze and detected endoglucanase, xylanase, mannanase, amylase, dextranase and β-glucosidase, but not exoglucanase. This indicates a similar enzyme complement for polysaccharide breakdown as many brown rot fungi, as discussed above. Exoglucanases have, however, been detected in a number of fungi known to cause soft rot decay (see Eaton and Hale, 1993).

Nilsson (1974) tested 33 fungi found to cause soft rot of birch (*Betula verrucosa* Ehrh.) for cellulases, xylanases and mannanases. He found that not all the soft rot fungi tested possessed each of these enzymes, the fungi for which one or more of these enzyme types were not detected producing only type 1 decay. Nilsson suggested that the negative results were most likely due to imperfect test methods and not an incapability of the fungus to produce these enzymes. He further suggested that the release of enzymes by cavity forming soft rot fungi may be governed by the physical structure of the wood cell wall (i.e. the production of certain enzymes is only induced when the hypha is growing parallel to the cellulose microfibrils in the wood cell wall).

Hale and Eaton (1985b) proposed that a major component at the hyphal tip of the cell wall penetration hyphae is an exoglucanase (with a lack of endoglucanase at this point), while cavity widening is largely due to the release of endoglucanases.

The endoglucanase isolated from *C. globosum* by Bailey *et al.* (1972) was found to have a molecular weight of 30000Da. As mentioned above, this is considered too large to diffuse into the undecayed wood cell wall. Hale and Eaton (1985a) detected a "halo" of degraded material around the edges of soft rot penetration hyphae and proposed a pre-cellulolytic degradation system, possibly involving non-enzymatic decay by a Fenton's type system.

Soft rot fungi are generally considered to degrade lignin to a greater extent than brown rot fungi, but not as effectively as white rot fungi. Levi and Preston (1965)

attributed 40% of Klason lignin weight loss in beech soft rotted by *C. globosum* to be due to demethoxylation. They also found an increase in the alkali solubility of residual lignin, concluding that significant modification of lignin is caused by soft rot fungi.

Haider and Trojanowski (1975) tested the ability of various soft rot fungi to degrade ^{14}C labelled lignin model compounds and dehydropolymers of coniferyl alcohol. They found these fungi able to convert the propyl side chains, methoxyl groups and aromatic rings to carbon dioxide. Assaying for ligninolytic enzymes, Haider and Trojanowski (1975) could not detect laccase in the soft rot fungi, and found lower peroxidase activity than that found in white rot fungi.

Butcher and Nilsson (1982) found that soft rot fungi were less able to decay wood species with a high lignin content and found species containing guaiacyl lignin to be more resistant to soft rot than those containing syringyl - guaiacyl lignin (i.e. similar to white rot as described earlier). Nilsson, Daniel, Kirk and Obst (1989) found that soft rot preferentially attacked the syringyl units in lignin. They proposed that soft rot fungi possess similar but less electropositive peroxidases to the lignin peroxidases in white rot fungi, and that these peroxidases are more able to oxidise syringyl units than guaiacyl units.

2.3.4 Moisture content required for decay

Attempts have been made by workers to establish the minimum and optimum wood moisture contents for decay, though there are difficulties involved in measuring these quantities as moisture content will vary during decay. Moisture content figures will increase as the dry mass of wood substance is reduced, and metabolic water will be produced through the breakdown of wood polymers (theoretically 56% of the weight of cellulose will end up as water after complete decomposition; see appendix 3). Capacity

in the wood for holding water will also increase as decay proceeds. In addition to these, fungi may transport water into the wood from another source to increase moisture content to aid decay. Pure culture decay tests such as the soil block test used in this study use a reservoir of wet soil or agar along with the wood blocks being tested which can be used as a source of water by the decay fungus.

Griffin (1977) used water potential rather than moisture content as a measure of water in wood. Water potential is defined as the free energy of water in a system relative to that of a reference pool of pure water (Rayner and Boddy, 1988). It is argued that such a measure better reflects the availability of the water in wood for use by decay fungi rather than moisture content calculated on a dry wood basis. Water potential is affected by several physical factors including solutes in the water (osmotic potential) and the radii of pores the water occupies in a solid matrix material (matric potential). In the case of wood it is matric potential that is considered the dominant factor. Griffin (1977) differentiates between the water potential at which various fungi can grow and that at which wood decay is possible. In the case of wood decaying basidiomycetes he calculates both of these to be approximately -4MPa, which is consistent with a wood moisture content of 30%.

Viitanen (1997) tested the ability of the brown rot fungus *C. puteana* to decay Scots pine (*Pinus sylvestris* L.) and Norway spruce (*P. abies*) in fixed relative humidity and temperature conditions over saturated salt solutions. The water in the wood at the equilibrium moisture content was therefore the only source available to the fungi. From data recorded Viitanen predicted that the threshold relative humidity for decay was 93-94% (20°C, one year exposure). This is equivalent to a sound wood moisture content in these species of 22-23% and a water potential between -7.7 and -10MPa. This is in line with the "general rule" that wood should be kept at a moisture content below 20% to

prevent decay (Scheffer, 1973). It is notable that the above work suggests that decay is possible, if limited, at moisture contents below fibre saturation point. Theoretically there is no free water in the wood as relative humidities are too low for condensation to occur within the cell lumina. Also, as FSP has not been reached then presumably transient cell wall pores are not at their widest.

The above work refers to the minimum moisture requirements for decay. There is general agreement that optimum conditions for decay by basidiomycetes are at a moisture content above 30% (i.e. above FSP), though both minimum and optimum conditions will vary from fungus to fungus and will also depend on the substrate and on other conditions. Highley and Scheffer (1970) found white rot fungi required higher moisture contents than brown rot fungi to achieve maximum weight loss in laboratory tests. Scheffer (1973) states that the optimum moisture content range for most wood decay fungi is between 40% and 80% or more. At higher moisture contents more of the cell lumen becomes filled with water. This water may impose a physical barrier to growth, and limits gaseous exchange and the supply of oxygen to the fungus (Rayner and Boddy, 1988).

2.4 Chemical modification of wood

For many years attempts have been made to improve the properties of wood by chemical modification. The wood properties that have been given the greatest consideration in this respect are dimensional stability and decay resistance. A wide variety of chemicals have been used to react with hydroxyl groups in wood, including anhydrides, isocyanates, epoxides, aldehydes, acid chlorides, carboxylic acids and others. These modification reactions and the properties of the modified wood have been reviewed by Rowell (1983) and Kumar (1994). The modifying chemicals attracting the

greatest attention, and those that are of importance to this study, are anhydrides and isocyanates.

Anhydrides used in wood modification have included both linear and cyclic anhydrides. The most common modifying chemical used has been the linear acetic anhydride, though studies have also involved the modification of wood with longer chain linear anhydrides such as propionic, butyric, valeric, hexanoic and heptanoic anhydrides (Goldstein, Jeroski, Lund, Nielson and Weaver, 1961; Hill and Jones, 1996). Cyclic anhydrides used to modify wood have included succinic, maleic, phthalic and heptadecenyl succinic anhydride (Matsuda, 1987; Risi and Arseneau, 1958; Codd, Banks, Cornfield and Williams, 1992).

Isocyanates used to modify wood have included mono- and difunctional isocyanates, the latter causing cross-linking reactions within the wood. Wood modification studies involving isocyanates have included the use of methyl, ethyl, n-propyl, allyl, n-butyl, hexyl and phenyl isocyanates (Rowell and Ellis, 1979; Kalnins, 1982; Ellis and Rowell, 1984; Cardias and Hale, 1990) and 1,6-diisocyanatohexane, tolylene-2,4-diisocyanate and isophorone diisocyanate (Ellis and Rowell, 1984; Cardias and Hale, 1990; Martins, 1992).

The extent of chemical modification is usually measured as the percent weight increase of the wood due to modification. This is commonly termed the weight percent gain (WPG):

$$\text{Weight gain (\%)} = \frac{\text{Dry weight after modification} - \text{Initial dry weight}}{\text{Initial dry weight}} \times 100$$

The product of reaction between the wood hydroxyls and anhydrides or isocyanates is discussed further in chapter 3.

2.4.1 Dimensional stability of chemically modified wood

The present study is concerned with the decay resistance imparted to wood by chemical modification. However, to try and understand the mechanisms of this decay resistance, studies on the changes in the physical characteristics of wood when it is modified, specifically the wood – water relationship, were required (chapters 4 and 5). The majority of studies exploring the wood – water relationship in modified wood have involved the study of the dimensional stability of wood when immersed in water. The commonly used measure of dimensional stability is the anti-swell efficiency (ASE). This is a measure of the percent reduction in swelling between oven dry and water saturated dimensions when compared to that of unmodified wood, and is calculated as follows:

$$\text{ASE (\%)} = 100 \times \frac{\text{Percent swell of unmodified wood - Percent swell of modified wood}}{\text{Percent swell of unmodified wood}}$$

An ASE of 100% therefore means that the modified wood is completely dimensionally stable, and its dimensions do not change when it is soaked in water.

The ASE of acetic anhydride modified wood has been measured by several authors. Acetic anhydride modification to WPG's of 20-25% was found to impart an ASE of 70% (see Rowell, 1983). Hill and Jones (1996) found that the ASE of wood modified with a variety of linear anhydrides was governed by the WPG of the modifying chemical, regardless of the chain length of the anhydride involved. They achieved ASE's approaching 90% for wood modified with linear anhydrides to 30% WPG or higher. They attributed ASE purely to the bulking of the wood, and not to blocking of hydrophilic hydroxyl groups.

Risi and Arseneau (1958) measured ASE's of 100% for phthalic anhydride modified wood (60% WPG), though the ASE value and weight dropped with subsequent

water soaks. The permanence of this chemical inside the wood was brought into question.

The ASE values of various isocyanates have also been measured. These include ASE's of 60% for methyl isocyanate modification to 25% WPG (Rowell and Ellis, 1979) and 68% for n-butyl isocyanate modification to 32% WPG (Ellis and Rowell, 1984). Ellis and Rowell (1984) found the ASE's of several other mono- and difunctional isocyanates to be lower than 50%. However, they found significant differences in ASE and WPG were achieved using different reaction times, temperatures and solvent systems, so comparisons are difficult.

2.4.2 Water sorption characteristics of chemically modified wood

Another method used to assess wood – water relations in modified wood has been through studies of the sorption characteristics. There have been several studies of sorption that have involved wood modified with anhydrides or isocyanates.

Stamm and Tarkow (1947) measured the e.m.c. of unreacted and vapour phase acetylated (30% acetyl content) Sitka spruce (*Picea sitchensis* (Bong.) Carr.) at various humidities and found a 67% reduction in water sorbed at 95% RH.

Spalt (1958) used the Hailwood – Horrobin model to fit isotherms to sorption data for several wood species and to White spruce (*Picea glauca* (Moench) Voss) vapour phase acetylated to a 32% acetyl content. He found that the effect of acetylation to this level was to reduce e.m.c. at saturation by 63%. Separating isotherms into the two components, he measured a 67% reduction in surface sorption (monomolecular sorption) at saturation, and a 62 % reduction in capillary condensed water (here Spalt is referring to polymolecular sorption and not to the capillary condensation in the existing, non transient, capillaries as defined by Stamm, 1964). Spalt (1958) calculated that at

saturation, 76% of the reduction in total water sorbed was due to cell wall bulking (as opposed to reduction in sorption sites).

Risi and Arseneau (1957) studied the adsorption of Balsam fir (*Abies balsamea* (L) Mill.) vapour phase acetylated to a range of retentions and found that increasing levels of modification lead to greater reductions in e.m.c. This effect was attributed to a combination of wall bulking and loss of hydrophilic hydroxyl groups. They measured swelling at each relative humidity and found a linear relationship between moisture content and swelling for each WPG. This relationship held even at low relative humidities (and e.m.c.'s) and they concluded that dimensional stability of acetylated wood depended on a combined effect of bulking and loss of sorption sites. The linear relationship between swelling and moisture content was not the same for different levels of modification. As acetyl content increased, the gradient of swelling (%) against moisture content decreased, i.e. at higher levels of reaction, swelling was lower for the same quantity of water in the cell wall (regardless of relative humidity).

Risi and Arseneau (1958) also measured adsorption of phthalic anhydride modified Balsam fir. They found that increasing phthalic anhydride reaction reduced e.m.c. and swelling over the whole range of relative humidities (approx. 15% to 85%). However, at high humidities the difference between the e.m.c.'s for wood modified to between 19 and 32% weight gain (the lowest and highest WPG's tested) appeared only slight, while swelling was significantly reduced as weight gain increased. The improved dimensional stability was attributed to bulking, though this bulking does not appear to have significantly reduced e.m.c.'s at high humidities.

Popper and Bariska (1972) measured the sorption properties of acetic anhydride and phthalic anhydride modified White fir (*Abies alba* Mill.), though weight gains were not reported. Fitting isotherms using the BET and Hailwood - Horrobin models, they

found that reaction with acetic anhydride to significantly reduced monomolecular adsorption as the hydrophilic hydroxyl groups were replaced. This was not found to be the case for phthalic anhydride modified wood, which gave monomolecular adsorption isotherms very similar to untreated wood. They attributed this to the hydrophilic acid hydroxyl introduced during reaction with phthalic anhydride. Reduction in sorption due to phthalic anhydride modification was found to be almost solely due to bulking, causing a reduction in polymolecular sorption. This is not in agreement with Risi and Arseneau (1958), whose data suggests that reductions in water sorbed occurred at the low as well as at high humidities.

Rowell and Rowell (1989) reacted various lignocellulosics, including hardwoods, softwoods, grasses and water plants, with acetic anhydride and measured sorption properties. Plotting the percent reduction in e.m.c. (at 65% RH, 27°C) caused by acetylation against acetyl content they determined a straight line relationship upon which all lignocellulosics lay. Since these lignocellulosics varied significantly in their lignin, cellulose and hemicellulose content, Rowell and Rowell concluded that reduction in hygroscopicity was controlled by a common factor, and was not affected by variations in chemical composition.

In a comprehensive study, Martins (1992) measured the adsorption and desorption characteristics of Corsican pine and Beech (*Fagus sylvatica* L.) with mono- and difunctional isocyanates, including n-butyl isocyanate. Isotherms were fitted using the Hailwood - Horrobin model. In all cases Martins found that increasing WPG caused reduction in sorption. He attributed this to:

1. The increasing number of hydroxyl groups blocked (having a particular effect on monomolecular sorption).

2. Bulking (affecting polymolecular sorption).

3. Cross-linking restricting swelling (in the case of difunctional isocyanates).

The latter of these only affected polymolecular adsorption at high relative humidities. On saturation, however, blocks reacted with difunctional isocyanates were found to swell fully. Martins concluded that cross-linking does not prevent swelling but hinders it, causing lower adsorption at humidities up to saturation. Measuring swelling at various relative humidities, Martins found similar results to those of Risi and Arseneau (1957) for acetylated wood. Swelling and e.m.c. formed a linear relationship for all the isocyanates used (including difunctional isocyanates), with lower swelling per unit increase in moisture content for the wood reacted to higher weight gains.

To test the effect of the size of modifying chemical on sorption, Martins (1992) compared water sorption properties of wood modified with butyl and octadecyl isocyanates. He found adsorption isotherms to be almost identical for the two chemicals at equivalent WPG's. Monomolecular adsorption were very similar despite the significantly lower number of hydroxyls reacted by octadecyl isocyanate. Martins attributed this to site shielding by the large hydrophobic chain introduced into the wood cell wall, causing hydroxyls to become inaccessible for monomolecular adsorption. Monomolecular desorption isotherms were found to differ slightly, with the octadecyl isocyanate modified wood sorbing more water at the monomolecular layer. It was concluded that at saturation the octadecyl chain may move slightly away from the sorption sites within the cell wall, making some extra sites available for sorption.

2.4.3 Decay resistance of chemically modified wood

The majority of work studying the decay resistance of modified wood has again involved acetic anhydride modification. Goldstein *et al.* (1961) acetylated Ponderosa

pine (*Pinus ponderosa* Dougl.) using acetic anhydride in xylene. They tested this against six basidiomycete fungi, five brown rot and one white rot, and found a WPG of 18% to be sufficient for resistance to decay. Peterson and Thomas (1978) acetylated Loblolly pine (*Pinus taeda* L.) to WPG's between 10 and 26% and Green ash (*Fraxinus americana* L.) and Yellow poplar (*Liriodendron tulipifera* L.) to WPG's between 10 and 21%, again using acetic anhydride in xylene. These were tested against the brown rot fungus *G. trabeum* and the white rot fungus *Coriolus versicolor* (L.: Fr.) Quel. (= *T. versicolor*). They found that the white rot was generally easier to control than the brown rot, the lowest level of acetylation (approx. 7%) being nearly as effective as higher ones in reducing weight losses due to white rot. However, ash still lost 6% of its weight to white rot at the highest WPG (20%). In all cases, acetylation to 7-11% significantly reduced decay, though 17-20% was required to protect the wood (with the exception of white rot in ash).

Takahashi, Imamura and Tanahashi (1989) studied the effect of the level of acetylation (using undiluted acetic anhydride) on the decay resistance of Japanese cedar (*Cryptomeria japonica* D. Don), Japanese red pine (*Pinus densiflora* Sieb. *et* Zucc.), Albizzia (*Albizzia falcata* Back.) and Japanese beech (*Fagus crenata* Blume). They exposed these to the brown rot fungi *T. palustris*, *Serpula lacrymans* (Wulfen: Fr.) Schroeter, the white rot fungus *C. versicolor* and to soft rot fungi in unsterile soil. Weight loss to *T. palustris* in all wood species reached almost zero at 20% WPG. For *C. versicolor*, 6% WPG was sufficient to protect softwood, while hardwoods required 12-15% WPG. In the cases of *S. lacrymans* and the soft rot fungi, the effect of acetylation was found to be intermediate between that for *T. palustris* and *C. versicolor* (approx. 15-16% WPG required to protect wood). Takahashi *et al.* (1989) also exposed acetylated Japanese cedar to a solution containing Fenton's reagent to see if acetylation protected

wood against the proposed oxidative, non-enzymatic, decay mechanism of brown rot fungi (see section 2.3.2). Acetylation suppressed weight loss due to the action of Fenton's reagent, and a decline in activity similar to that of *T. palustris* was evident up to approximately 15%, after which increasing acetylation appeared not to further improve the resistance of the wood against Fenton's reagent.

Beckers, Militz and Stevens (1994) measured the threshold levels of protection for acetylation of Scots pine (undiluted acetic anhydride) to a variety of wood decay fungi. They found WPG's of 18% (against *C. puteana* and *G. trabeum*), over 20% (*P. placenta*) and 12% (*C. versicolor*) were required to prevent decay in pure culture test, and 11% was required to protect wood in an unsterile soil soft rot test (12 weeks exposure). After exposing Scots pine acetylated to 10.7% WPG for 32 weeks in conditions suitable for soft rot, Beckers, Militz and Stevens (1995) found no strength loss to occur.

In a piece of work partly based on the present study, Suttie, Hill, Jones and Orsler (1997) modified wood with various linear anhydrides (acetic, propionic, butyric and hexanoic) and succinic anhydride all in pyridine. They exposed these to brown rot (*G. trabeum*, *C. puteana*, *P. placenta*) and white rot (*C. versicolor*) fungi, and in unsterile soil (in a soft rot test). They found that chain length of linear anhydrides made little difference to the decay resistance of wood, and that protection was mainly dependent on WPG. They did suggest that if anything, the shorter anhydrides (acetic and propionic) imparted slightly higher decay resistance, though the range of WPG's tested was small, and this observation was only tentatively made. They found that succinic anhydride did not protect wood from decay.

Dunningham and Parker (1992) modified Radiata pine (*Pinus radiata* D. Don) with succinic anhydride (in methyl isobutyl ketone) and exposed this to pure cultures of *C. puteana* and *C. versicolor*. They found that although weight loss was reduced,

indicating part protection, succinic anhydride modification to 28% WPG was unable to fully protect wood from decay by *C. puteana*. However, they found that wood modified to 15% WPG (the lowest WPG tested) was completely resistant to decay by *C. versicolor*.

Codd (1997) reacted Scots pine with succinic anhydride and an alkenyl succinic anhydride (16-18 carbon alkenyl chain, referred to by Codd *et al.* (1992) as heptadecenyl succinic anhydride), both in dimethylformamide, and exposed these to pure cultures of *C. puteana* and *G. trabeum*. Succinic anhydride modification reduced decay, but complete protection was not achieved even at WPG's in excess of 35%. Water leaching prior to test reduced the efficacy of this modifying chemical. Alkenyl succinic anhydride modification protected wood at WPG's of just over 30%.

Ellis and Rowell (1984) reacted southern yellow pine (*Pinus* spp.) with various isocyanates using a variety of solvent or catalyst systems, and tested these against *G. trabeum*. Decay was greatly reduced by WPG's in excess of 10% of ethyl, n-propyl and n-butyl isocyanates. The best performance was achieved by wood modified with n-butyl isocyanate to 18% WPG (the lowest WPG tested for this isocyanate) and above, which lost just 2% of its weight in the test (unmodified control weight loss was 39%). In contrast to the work of Suttie *et al.* (1997) using linear anhydrides, the results of Ellis and Rowell (1984) suggest that any difference in efficacy of isocyanates is one of improving performance with increasing chain length (up to butyl). However, comparison between the isocyanates is difficult due to the differing reaction conditions used and the low range of weight gains.

Isophorone diisocyanate was unable to completely protect wood even at weight gains of up to 38% (Ellis and Rowell, 1984).

Cardias and Hale (1990) reacted Corsican pine with n-butyl and hexyl isocyanate and with 1,6-diisocyanatohexane in an acetone/pyridine mixture and exposed these to

pure cultures of brown rot (*C. puteana*, *G trabeum*) and white rot (*C. versicolor*, *Pycnoporus sanguineus* (L.: Fr.) Murr.) fungi. For butyl isocyanate they calculated threshold protection levels (WPG) against these fungi to be 15.5%, 10.0%, 9.6% and 12.2% respectively. The threshold values for diisocyanatohexane were very similar. Hexyl isocyanate modified wood was only tested against *C. puteana*, and required 18% WPG for complete protection. In this case, the longer (hexyl) chain isocyanate appeared less effective at protecting wood at a given WPG. Cardias (1992) found modification with butyl isocyanate to 10% weight gain to be sufficient to protect wood against soft rot decay.

2.4.4 Mechanisms of decay resistance of chemically modified wood

As mentioned in chapter 1, the mechanism by which chemical modification protects wood from decay is not fully understood. The theories cited are generally those originally put forward by Stamm and Baechler (1960). Testing acetylated Sitka spruce, Stamm and Baechler proposed that decay was prevented because the FSP was reduced to below 20% (the minimum moisture content requirement for decay fungi; see section 2.3.4). At this level they claimed that there was insufficient moisture available within the cell walls, even in the presence of free water in the cell lumina, to support decay. They also proposed that decay resistance could be explained by the fact that hydroxyl groups, considered susceptible to attack by decay organisms, were removed during reaction. They estimated that wood acetylated to a WPG of 30% would have no available hydroxyl groups remaining.

Stamm and Baechler (1960) found that wood was protected from decay when treated with 2.5% WPG of formaldehyde (cross-linking wood polymers) or with up to 42% WPG of a phenol formaldehyde resin (wood cell wall bulking without reaction to

wood polymers). They concluded that these treatments were not effective in removing the large numbers of hydroxyl groups as was the case with acetylation, and were probably effective in protecting wood against decay by preventing fungal enzymes entering the wood cell wall. Although fungal enzymes have since been found not to penetrate even unmodified wood, and that non-enzymatic degradative agents have been proposed as being responsible for initiating wood decay, this does not invalidate this blocking theory as a potential mechanism.

Peterson and Thomas (1978) stated that the mechanism of protection of acetylation was not biocidal, as cultures of decay fungi could be grown from sections of infected, undecayed, acetylated wood. They attributed the greater efficacy of acetylation against white rot fungi to the inhibition of ligninolytic enzymes caused by the lack of carbohydrate breakdown. They did not expect the substitution of lignin hydroxyls to effect decay resistance to any great extent because of the relative infrequency of hydroxyl groups on the lignin macromolecule. Peterson and Thomas also suggested that in the case of white rot, when enough water is available in the cell wall, erosion of the cell wall should be uninhibited. They therefore concluded that the hydroxyl substitution that occurs when wood is acetylated is far more important than its effect on cell wall equilibrium moisture content. More recently, theories of white rot decay have developed which involve the penetration of the sound wood cell wall by low molecular weight degradative agents (see section 2.3.1), so the effect of cell wall water may not be so easily dismissed.

Rowell and co-workers also questioned the importance of lignin substitution in the protection of wood from decay. They studied the distribution of reacted chemical amongst wood polymers after the wood had been modified with methyl isocyanate (Rowell, 1980) and acetic anhydride (Rowell, 1982a; Rowell, Simonson, Hess, Plackett,

Cronshaw and Dunningham, 1994). In both cases it was found that lignin was modified more quickly than holocellulose with high degrees of substitution being achieved in lignin at relatively low weight gains. The weight gain at which protection against brown rot fungi was achieved coincided with levels at which holocellulose substitution was increasing, and was above levels at which lignin had already become significantly modified. They concluded that lignin substitution did not contribute significantly to the overall protection of wood from decay, and that degree of substitution in the holocellulose fraction seemed to be the most important factor in the protection mechanism. Rowell (1982b) stated further that the substitution of the lignin component in wood was a waste of chemical in the modification process. As with the theory of Peterson and Thomas (1978), the theory of Rowell and co-workers emphasises the importance of hydroxyl substitution over that of the suppression of cell wall moisture content.

Takahashi *et al.* (1989) suggested that the preferential substitution of lignin during the acetylation of wood could be the cause of the greater resistance of acetylated wood to white rot decay when compared to brown rot decay.

Cardias (1992) questioned the theories of Rowell and co-workers, stating that results obtained in her work on isocyanate modified wood suggested that mechanisms other than holocellulose substitution were important. Finding differences in the ability of *C. puteana* and *G. trabeum* to decay isocyanate modified wood, she proposed that this might be due to different types or sizes of cellulases in the degradative systems of these fungi, governing their cell wall penetration and subsequent decay of the wood. However, she concluded that the extent of protection against decay imparted by modification depends upon the extent of the reduction of the absorbed water in the modified wood, and by the physiological capacity of the decay fungi to tolerate water stress. She argued that

C. puteana was able to transport water and increase he moisture content of modified wood more effectively than *G. trabeum*, accounting for the greater tolerance of *C. puteana* to chemical modification.

Rowell (1993) has argued that although brown rot decay cannot proceed when the hemicellulose component is protected, it is the moisture content of the wood cell wall that is the critical factor in protecting wood against decay fungi. He suggested that protection is due to water not being able to get to the glycosidic bonds in wood, and that these bonds in their 'dry state' cannot be broken by fungal action (Rowell, 1996; cited by Suttie, 1997).

Chapter 3. Chemical modification of wood

3.1 Introduction

This chapter describes the procedures and results of the chemical modification reactions conducted prior to fungal testing. Results presented include the weight gain in samples due to the addition of modifying chemical, the degree of substitution of reactive groups in the wood, and the swelling of wood caused by modification. Reaction was confirmed by solvent extraction of ground modified wood samples followed by chemical analysis of this extracted material. Resistance to water leaching was also assessed.

Procedure and results of CCA treatment of wood samples, used as a control throughout fungal testing experiments, are also included.

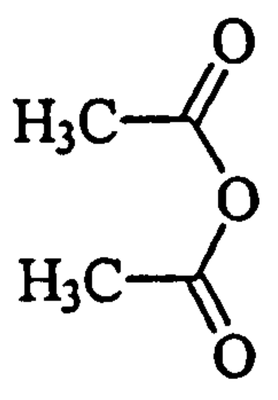
3.2 Materials

3.2.1 Modifying chemicals

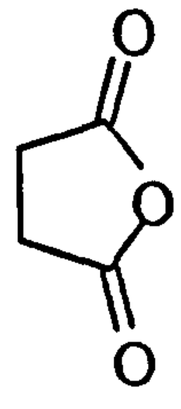
Five modifying chemicals were used in this study. The structures of each of these are given in figure 3.1. They include four anhydrides and one isocyanate. All are chemicals that have been used previously in chemical modification reactions by other workers (see chapter 2), although not all have been tested against decay fungi.

The structures of the four chosen anhydrides differ significantly. They were chosen so that a study could be made on the effect that various structures of modifying chemical have on the performance of chemically modified wood against decay fungi.

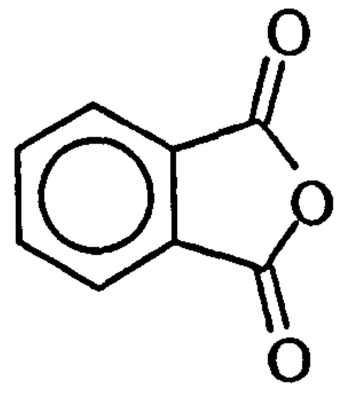
Of these four, the most commonly used and reported is acetic anhydride. This was the only linear anhydride used, and yields acetic acid as a by-product of its reaction with wood. The reaction between wood and acetic anhydride most commonly ascribed to



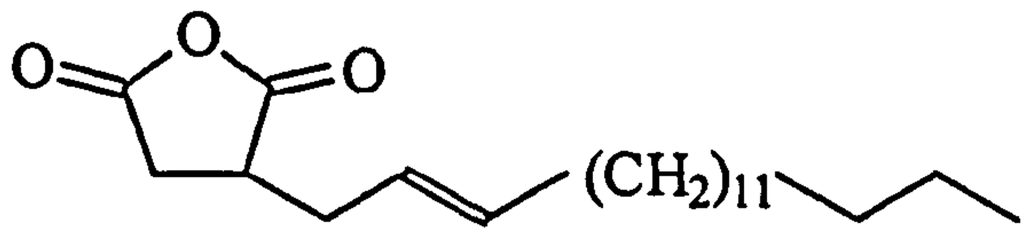
Acetic anhydride



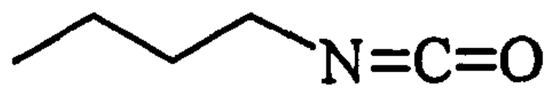
Succinic anhydride



Phthalic anhydride

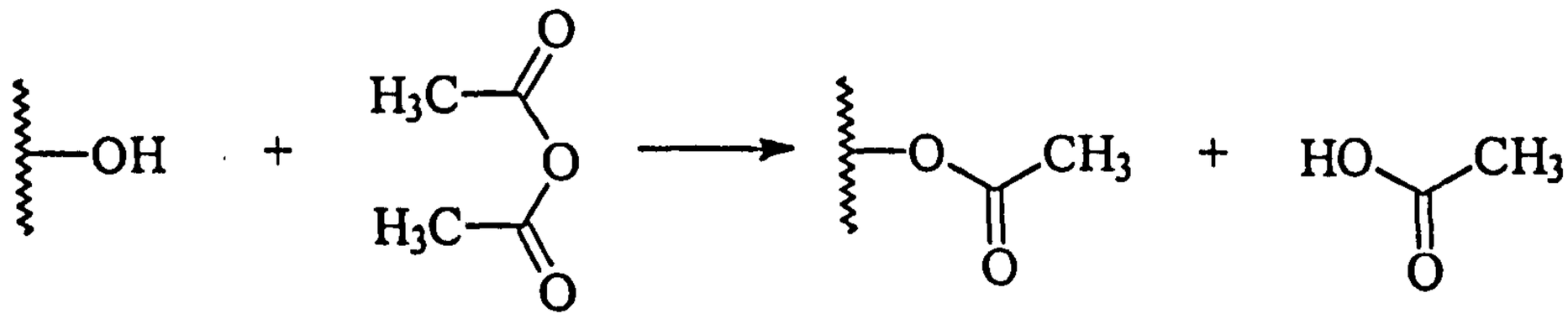


Alkenyl succinic anhydride

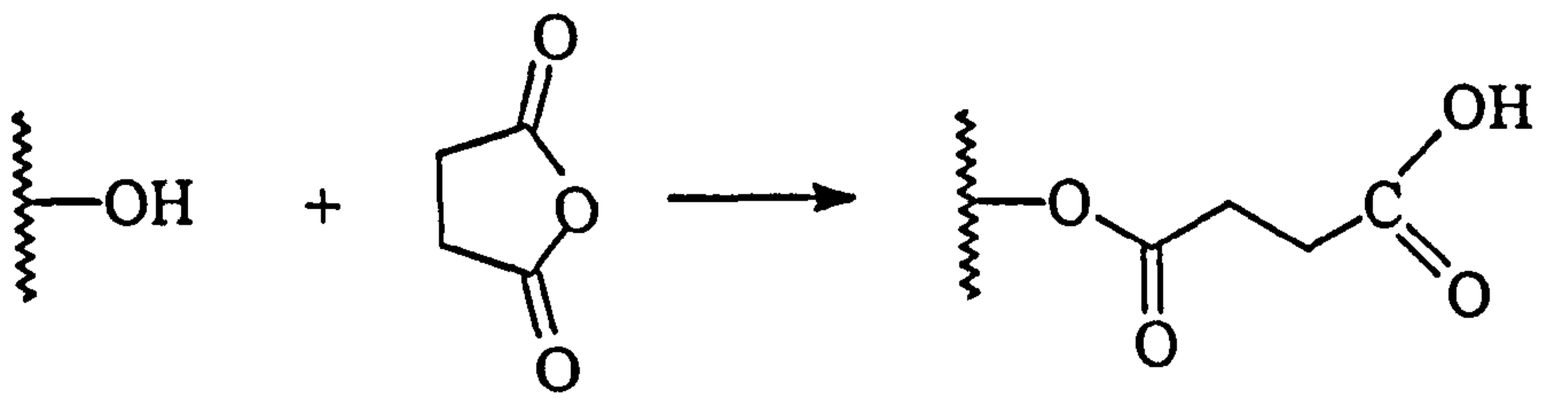


Butyl isocyanate

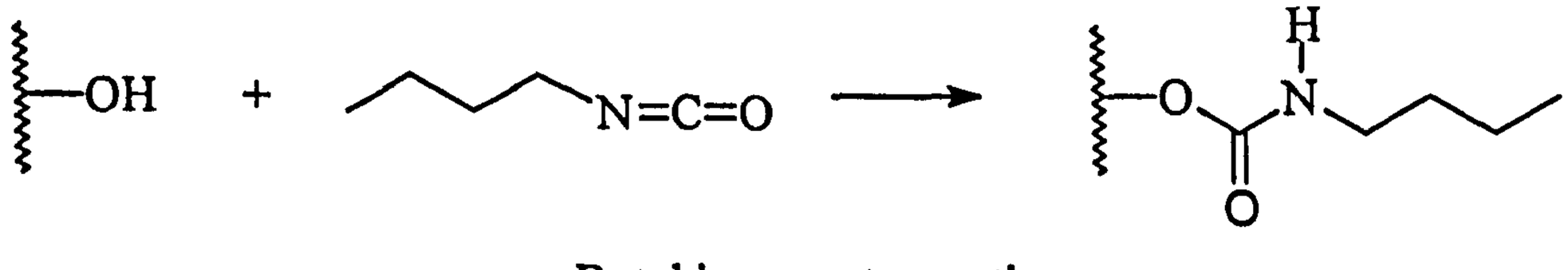
Figure 3.1 Structure of modifying chemicals



a. Acetic anhydride reaction



b. Succinic anhydride reaction



c. Butyl isocyanate reaction

Figure 3.2 Wood modification reactions

by workers involves the formation of an acetyl group as depicted in figure 3.2a. However, Pizzi, Boonstra and co-workers (Pizzi, Stephanou, Boonstra and Pendlebury, 1994; Boonstra, Pizzi, Tekely and Pendlebury, 1996, 1997) have questioned this. They suggest that in addition to the acetylation reaction shown in figure 3.2a, substitution of the aromatic ring in lignin and cross-linking reactions occur. In the present study the traditionally acknowledged reaction (figure 3.2a) was assumed.

The other three anhydrides were all cyclic, which do not yield a by-product but leave a free carboxyl group attached to the wood. These were succinic, phthalic and alkenyl succinic anhydride. Alkenyl succinic anhydride was the largest of the modifying chemicals used in the study. This consists of a succinic anhydride ring substituted with an alkenyl chain. In this study the alkenyl chain was 16 to 18 carbon units in length, and was identical to that used previously by Codd (1997) and Codd *et al.* (1992). It is used commercially as a paper size.

Figure 3.2b shows a diagram of the expected reaction for succinic anhydride. Phthalic and alkenyl succinic anhydride react in a similar way to succinic anhydride, forming an ester and a carboxyl group. In work involving the reaction of succinic, maleic and phthalic anhydride with wood, Matsuda (1987) found that reaction with succinic anhydride involved a small proportion of cross-linking reactions. Although reaction was largely as in figure 3.2b, part of the reaction had involved both ends of the opened succinic anhydride ring forming ester linkages with wood hydroxyls (with no free carboxyl). Due to the relatively small proportion of these, reaction in this study has been assumed to result in monoester reactions (as in figure 3.2b) and the possibility of cross-linking is not further considered.

The isocyanate used was n-butyl isocyanate. This reacts with wood to form a carbamate ester (urethane) as shown in figure 3.2c. The original intention was to include

this as a control for comparison with other work previously undertaken at Bangor such as that by Cardias (1992) on fungal resistance and by Martins (1992) on sorption isotherms. However, the efficacy of this chemical in protecting wood from decay in the present study resulted in it being an extremely useful comparison to the anhydrides, particularly acetic and alkenyl succinic anhydride.

3.2.2 Solvent

For comparison between modifying chemicals it was necessary to use the same reaction system, and therefore the same solvent.

Much work on acetic anhydride modification has involved the use of the undiluted anhydride or the use of xylene as solvent (a non-swelling solvent considered by Goldstein *et al.* (1961) to be the ideal solvent for acetic anhydride modification of wood). Other modifying chemicals used in this study could not be used undiluted (succinic and phthalic anhydrides are solids at room temperature) and/or could not be expected to swell the wood cell wall to the same extent as acetic anhydride. To ensure each modifying chemical had the opportunity of accessing and reacting with wood cell wall polymers a swelling solvent was required. Pyridine was chosen for the following reasons:

1. Each of the modifying chemicals used in this study was found to be highly soluble in or miscible with pyridine.
2. Pyridine swells wood to a greater extent than water (approximately 25% greater: Stamm and Tarkow, 1947; Risi and Arseneau, 1957), allowing even non-swelling modifying chemicals access to cell wall polymers.
3. Pyridine is an organic tertiary amine, considered by Rowell (1983) to be ideal for the catalysis of wood modification reactions.

4. Of several organic solvents tested, Ashton (1974) found pyridine the easiest to remove after impregnation into pine.

The disadvantage of using pyridine in these experiments was its toxicity. Extreme caution was exercised when handling this solvent.

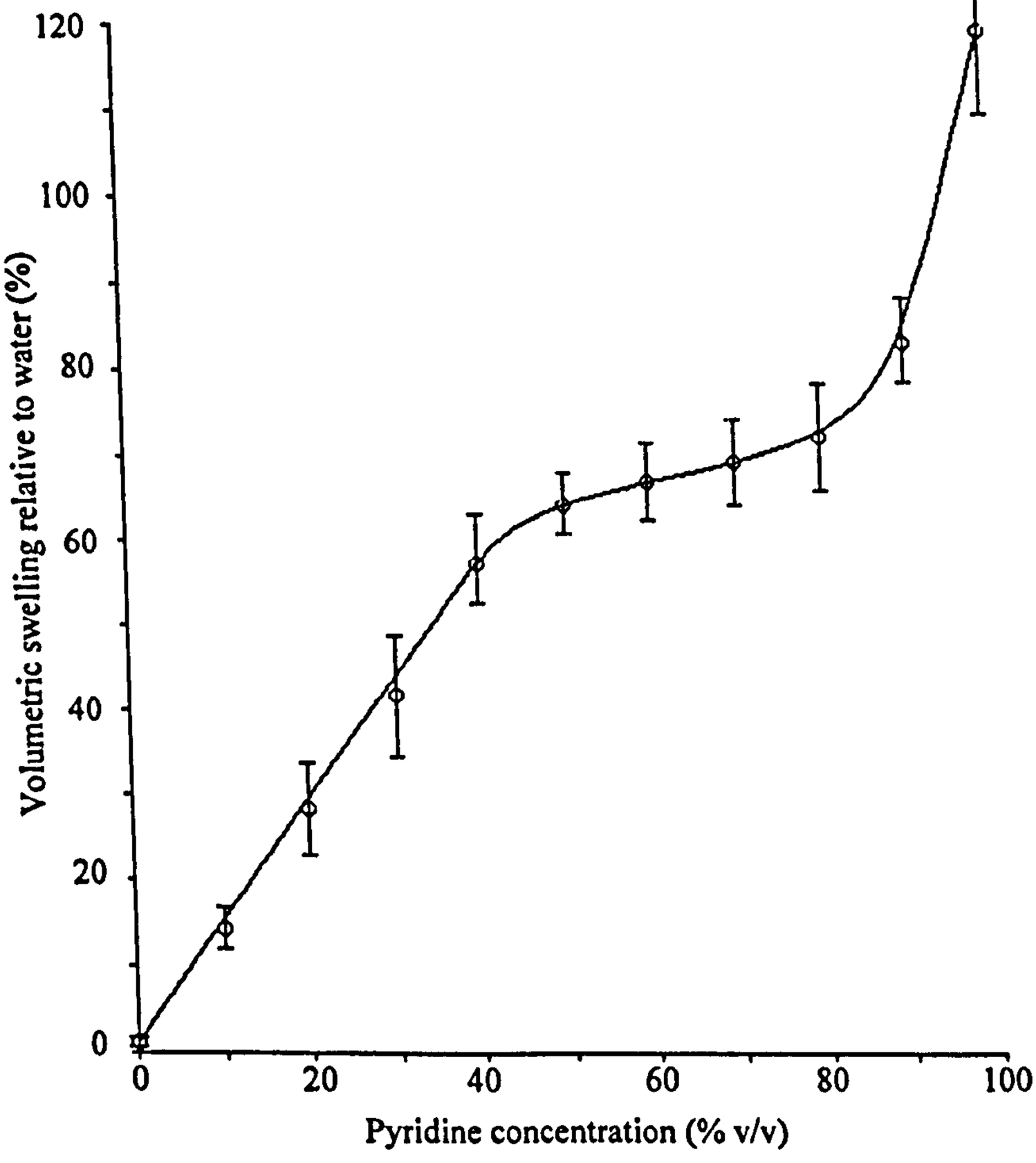


Figure 3.3 Graph of volumetric swelling relative to water with solutions of pyridine in toluene of varying concentration (Source: West 1988)
Mean values (of 4 replicates). Error bars = +/- standard deviation

With regard to the swelling capacity of pyridine (point 2 above) it should be noted that the formation of solution with modifying chemicals would have affected the ability of pyridine to swell the wood. Wood swelling tests by West (1988) in varying ratios of

pyridine to toluene showed the relationship of volumetric swelling to concentration of pyridine to give a sigmoid shape graph (figure 3.3).

Due to the requirement for modification reactions to take place in the absence of water, pyridine was kept as dry as possible by storing it over potassium hydroxide pellets.

3.2.3 Wood

The wood used in these experiments was Corsican pine sapwood obtained from North Wales forests. Pine sapwood was chosen because of its good permeability, essential in wood modification reactions. Corsican pine was selected over the more commonly used Scots pine for ease of cutting large quantities of sapwood. The wider sapwood band in Corsican pine meant that cutting heartwood free samples, particularly for fungal cellar and field trial stakes, was made much easier and waste was minimised.

Kiln dried boards were cut to samples of three sizes depending on the fungal test design:

Pure culture test blocks	10mm (longitudinal) x 20mm (radial) x 20mm (tangential)
Soft rot test stakes	100mm (longitudinal) x 5mm x 15mm
Fungal cellar / field trial test stakes	200mm (longitudinal) x 9mm x 19mm

3.3 Reaction procedure

The purpose of the reaction procedure was to modify wood samples to a range of weight gains which could then be tested for resistance to fungal decay. It was important to ensure that the distribution of the reaction throughout each sample was as even as possible. Unlike traditional biocidal preservatives for which an envelope type protection may be sufficient (i.e. only the outer portion of the wood is treated), modified wood requires the whole cross section to be protected. This is because there is no toxic barrier

to prevent decay fungi growing through an outer treated section and decaying unmodified inner portions of the wood sample.

The procedure used for modifying samples is listed below:

Sample preparation (section 3.3.1)	- Oven drying of wood samples
Impregnation (section 3.3.2)	- Vacuum impregnation of samples with reaction solution
Reaction (section 3.3.3)	- Heating wood samples in excess reaction solution - Quenching reaction in cold acetone
Clean-up (section 3.3.4)	- Reflux in excess acetone - Soxhlet extraction in acetone - Oven drying of modified samples

3.3.1 Sample preparation

In many previous wood modification studies wood was extracted prior to reaction. This was done to remove extractives, which might otherwise be reacted in place of wood cell wall polymers or be extracted from the wood during the reaction procedure so affecting weight gain calculations. Where measures of weight gain are required to be as precise as possible such as in studies of reaction kinetics, prior extraction is essential. However, in studies of fungal resistance such as those referred to in chapter 2, samples have not always been extracted, particularly where possible commercial processes are under consideration. Although this study does not use sample sizes or procedures that have direct implications for commercial use, pre-modification extraction was not undertaken. Any benefits arising from extraction were considered to be outweighed by the extra time and cost involved in extracting the large numbers of samples used.

Samples were oven dried at 105°C for 18 hours to remove all moisture from the wood. Water reacts with anhydrides and isocyanates, and needs to be removed prior to impregnation and reaction so all modifying chemical is available for reaction with wood polymers. Reacting wood with undiluted acetic anhydride, Beckers and Militz (1994) found that wood moisture contents even as high as 15% had little effect on subsequent weight gain achieved. Using undiluted anhydride gives a much greater excess of modifying chemical to reactive sites in wood than the solutions used in this study (see 3.3.3). It is likely that reactions with modifying chemicals as solutions in pyridine would have been much more sensitive to the presence of moisture in wood.

3.3.2 Impregnation

A great deal of work on wood modification has been carried out on fibre or small wood blocks, particularly ones cut short in the longitudinal direction (e.g. 5mm). This has meant that diffusion of reagent into the centre of the block has been quick, and because of this reactions have been started simply by adding dry blocks to ready heated reagent. With larger samples such diffusion cannot be relied upon for adequate reaction at the centre of a sample within reasonable time periods. In these cases vacuum impregnation of the modifying chemical prior to reaction is essential.

In this study wood samples were weighed down in a vacuum desiccator and a vacuum of approximately 700mmHg (approx. 0.08 bar pressure) was drawn for 30 minutes. The reaction solution was then added to immerse the samples, after which the vacuum was released, forcing solution into the samples.

Rowell *et al.* (1994) demonstrated that diffusion effects could also occur across a cell wall. They reacted wood with chloroacetic anhydride and used energy dispersive x-ray analysis of chlorine content across two wood cell walls (between adjacent lumina)

and found that at reaction to low weight gains (using short reaction times) distribution of reaction was uneven, with higher levels being closer to the lumen surface. In the present study samples were allowed to soak overnight in the reaction solution after vacuum impregnation to ensure that the cell wall was fully swollen and modifying chemical was within the cell wall prior to reaction temperature being reached.

Despite this procedure it was not expected that the limiting effects of diffusion could be completely avoided. As reaction occurs and modifying chemical is used up, diffusion of further modifying chemical will take place from the excess solution in which samples are heated, causing higher weight gains on the outside of the block. A similar effect is likely to take place on the microscopic level. As the modifying chemical within the cell wall is used up, diffusion of chemical from the cell lumen occurs. This may affect the distribution of modification across the cell wall, and has been found to have a significant effect in the latewood of modified softwood samples. Because of the small cell lumen and thick cell wall of a latewood tracheid, there will consequently be a low ratio of modifying chemical (in solution in the lumen) to reaction sites in the wall when compared to an earlywood tracheid (large lumen, thin cell wall). This can lead to lower weight gains in the latewood in the core of a sample (where diffusion of modifying chemical from the excess solution outside the sample is low) than in the earlywood. This effect has been thought to be responsible for pockets of decay in interior latewood in otherwise sound modified wood samples. These observations have been made in butylene oxide (Nilsson and Rowell, 1982) and isocyanate (Cardias, 1992) modified pine.

3.3.3 Reaction

To test the durability of modified wood, a range of weight gains was required for each modification type. The most straightforward ways in which this can be done are by

changing the reaction time or the concentration of modifying chemical in the solvent. In this study the latter was chosen. The main benefits of this method are:

1. All samples are subjected to the same conditions for the same amount of time. If time was used to govern weight gain, then low weight gains may have required reaction to be over a significantly shorter time period. This may not have been sufficient to allow heat to diffuse to the centre of larger samples and react the centre of the sample adequately.
2. Excessive quantities of unreacted modifying chemical are not left inside samples after reaction to low weight gains, making clean-up easier.

Trials of different combinations of reaction solution concentrations and reaction times for the various sample sizes were conducted in order to find the optimum solution strengths and reaction times required for a good spread of weight gains. These are given in tables 3.1 and 3.2. Also in table 3.2 are the numbers of each sample size per reaction (i.e. for each concentration of each reagent).

Table 3.1 Concentrations of modifying chemical in pyridine

Modifying chemical	Molecular weight	Range of molar concentrations in pyridine
Acetic anhydride	102	0.5 : 1.0 : 1.5 : 2.0
Succinic anhydride	100	0.25 : 0.5 : 1.0 : 1.5
Phthalic anhydride	148	0.25 : 0.5 : 1.0 : 1.5
Alkenyl succinic anhydride	337	0.25 : 0.5 : 1.0 : 1.5
Butyl isocyanate	99	0.25 : 0.5 : 1.0 : 1.5

Table 3.2 Reaction times

Test sample	Sample size (mm)	Number per reaction	Reaction time (hours at 115°C)
Pure culture	20 x 20 x 10	80	1
Soft rot	100 x 5 x 15	25	1
Fungal cellar	200 x 9 x 19	45	3

After impregnation and overnight soaking the reaction vessel containing blocks in excess solution was brought up to reflux temperature (approx. 115°C) and kept at this temperature for the time shown in table 3.2. At the end of this time period, reaction solution was drained away and the samples were immersed in cold acetone to stop the reaction.

A problem with varying concentration as a means of achieving a range of weight gains is that it is likely to have changed the swelling capacity of the reaction solution, as described earlier. Table 3.3 gives the quantity of pyridine (volume/volume measure) in the highest and lowest concentration solutions used for each modifying chemical. If the modifying chemicals themselves did not contribute to swelling during the reaction procedure, then the effect on swelling during modification of increasing molar concentration of each modifying chemical can be estimated from table 3.3 and figure 3.3. This could potentially have a significant effect on distribution of modification within the wood cell wall and the fungal resistance, particularly if the reaction solution was unable to swell the cell wall to the same extent as water (reaction sites would remain unavailable during the reaction procedure, but may then be exposed to potential decay in fungal tests). A measure of swell during reaction was made to check this (see section 3.4.4).

Table 3.3 Maximum and minimum pyridine content of reaction solutions used

Modifying chemical	Highest concentration of pyridine used (approx.)	Lowest concentration of pyridine used (approx.)
Acetic anhydride	95%	81%
Succinic anhydride	98%	91%
Phthalic anhydride	97%	85%
Alkenyl succinic anhydride	92%	50%
Butyl isocyanate	97%	83%

3.3.4 Clean-up

After reaction it was necessary to remove solvent, unreacted modifying chemical and any reaction by-product from the wood samples. A 90 minute reflux was conducted in the acetone used to quench the reaction. This was followed by a Soxhlet extraction in acetone for the following times:

Fungal cellar and soft rot stakes	overnight
Pure culture test blocks	7 hours

After extraction, samples were oven dried at 105°C for 18 hours and weighed. The weight gain due to reaction was then calculated.

3.3.5 Confirmation of the extent of reaction

An attempt was made to confirm the extent of reaction. This was done for two reasons:

1. Despite the extensive clean-up procedure there was a possibility that some unreacted chemical was still trapped inside the wood. It was necessary to find out if this was the case and establish the quantity of modifying chemical reacted with the wood cell wall polymers.
2. As described earlier, the lack of an extraction step prior to reaction could have caused errors in the calculation of weight gain.

As the method of analysis was a destructive procedure, only a small number of blocks were tested. A sample of 6 of the pure culture blocks was taken from each reaction set. These were ground in a hammer mill to pass a 40 mesh. The ground wood from each of the 6 blocks within a set was mixed together and oven dried. Oven dry weight was recorded. This ground wood was then Soxhlet extracted in acetone for four

hours, redried and reweighed. The weight loss due to extraction was calculated. Samples of this extracted ground wood were then analysed for quantity of reacted chemical.

Anhydrides were analysed using a method similar to that described by Elias (1994) involving the hydrolysis of the ester linkage between modifying chemical and wood. Approximately 0.3 grams of the extracted ground wood sample (oven dry) was weighed (4 decimal places) into a 250ml conical flask. To this was added 25ml of 0.1M KOH and 50ml deionised water. Flasks were then autoclaved for one hour at 110°C (approx. 1.4 bar pressure) to hydrolyse ester linkages. Flasks were allowed to cool to 25°C in a water bath before titrating against 0.1M HCl using bromothymol blue as an indicator. From this the quantity of carboxyl groups in the hydrolysed sample could be calculated. By subtracting the quantity found in unmodified wood (by use of control samples) and taking into account the fact that three of the four hydrolysed anhydrides had two carboxyl groups per molecule, an alternative measure of weight gain was obtained.

Butyl isocyanate modified wood could not be analysed in the same way as anhydride modified wood. Elemental analysis was undertaken by the Chemistry Analytical Unit at the University of Wales, Bangor, on the ground butyl isocyanate modified samples using a Carlo-urba 1108 CHN to establish nitrogen content. The weight gain due to butyl isocyanate modification in a sample was determined from the increase in nitrogen content of modified wood above that of unmodified wood.

3.3.6 Controls

Unmodified controls used throughout this study were of two types. Firstly, a set of each block/stake size was prepared as discussed in section 3.2.3, oven dried and weighed, and put aside for testing. These are known throughout this study as untreated controls. A second set was put through the reaction procedure as a solvent control, with

pyridine only (no modifying chemical) in the vessel at the reaction step. These are referred to as the pyridine controls. In addition to these, water controls were prepared during the CCA treatments, as discussed in section 3.5.

3.4 Results

3.4.1 Weight gain

As stated in chapter 2, the usual method of measuring the extent to which wood has been chemically modified is by calculating the percent weight gain caused by modification (the weight percent gain or WPG). This is calculated as follows:

$$\text{Weight gain (\%)} = \frac{\text{Dry weight after modification} - \text{Initial dry weight}}{\text{Initial dry weight}} \times 100$$

The mean weight gains achieved in this study (along with standard deviations) for the three sample sizes used are given in tables 3.4 to 3.6.

A good range of weight gains was achieved in most cases. The exception was the soft rot stakes, in which lower weight gains than expected were achieved. This was despite achieving higher weight gains in trial reactions. It is not known why weight gains were lower for these samples though it is possible that the pyridine used for these experiments had been allowed to get wet, which may then have suppressed weight gain.

It can be seen from the tables that the wood samples subjected to the reaction procedure in pyridine only (no modifying chemical) actually lost weight. This was due to removal of extractives from the wood. Weight loss was lower for the larger stakes (table 3.4) than for the other samples. Larger samples would have been more difficult to extract than the smaller samples due to the slow relative rate of diffusion of extractives. As discussed earlier, this extraction has implications to the true weight gain achieved during

Table 3.4 Mean weight gains in fungal cellar test stakes

Modifying chemical	Molar conc.	Mean WPG	Standard deviation	Amended mean WPG
Pyridine control	0	-0.3	1.5	0
Acetic anhydride	0.5	5.3	1.1	5.6
	1.0	7.9	0.5	8.2
	1.5	14.4	1.4	14.7
	2.0	17.6	1.6	18.0
Succinic anhydride	0.25	7.4	1.2	7.7
	0.5	11.8	1.7	12.1
	1.0	23.9	2.4	24.3
	1.5	31.2	3.2	31.6
Phthalic anhydride	0.25	6.8	1.2	7.1
	0.5	15.6	1.8	15.9
	1.0	32.8	3.2	33.2
	1.5	47.1	4.2	47.5
Alkenyl succinic anhydride	0.25	5.8	1.5	6.1
	0.5	10.8	1.6	11.1
	1.0	26.1	2.7	26.5
	1.5	36.1	3.7	36.5
Butyl isocyanate	0.25	2.1	0.4	2.4
	0.5	5.6	0.6	5.9
	1.0	15.1	1.6	15.4
	1.5	17.6	2.0	18.0

modification. If extraction occurred in the presence of pyridine then it is likely to have occurred in the presence of pyridine plus modifying chemical. Tables 3.4 to 3.6 contain a column headed 'Amended mean WPG'. This is a calculation of the weight gain due to modification based on the assumption that a level of extraction equivalent to that occurring in the pyridine control reaction occurred in all modification reactions.

3.4.2 Confirmation of the extent of reaction

The results of the extraction and chemical analysis of ground modified wood are given in table 3.7.

Table 3.5 Mean weight gains in soft rot test stakes

Modifying chemical	Molar conc.	Mean WPG	Standard deviation	Amended mean WPG
Pyridine control	0	-1.9	0.4	0
Acetic anhydride	0.5	5.2	0.8	7.2
	1.0	8.6	1.3	10.7
	1.5	12.1	0.7	14.3
	2.0	14	1.2	16.2
Succinic anhydride	0.25	6.7	1.1	8.8
	0.5	12.3	1.7	14.5
	1.0	22.4	2.7	24.8
	1.5	29.6	3.2	32.1
Phthalic anhydride	0.25	9.8	1.3	11.9
	0.5	15.9	1.7	18.1
	1.0	28	1.9	30.5
	1.5	35.3	3.1	37.9
Alkenyl succinic anhydride	0.25	2.8	1.0	4.8
	0.5	7.7	2.0	9.8
	1.0	12.1	4.4	14.3
	1.5	22.5	6.4	24.9
Butyl isocyanate	0.25	0.4	0.6	2.3
	0.5	2.5	0.6	4.5
	1.0	7.3	1.3	9.4
	1.5	12.4	0.9	14.6

Table 3.7 contains a number of measures of weight gain. The first column refers to the actual weight gain of the 6 selected blocks calculated from their aggregate dry weights before and after modification. The next two columns refer to the acetone extraction procedure. These are the weight loss due to extraction and the recalculated weight gain on the basis of this extraction. A weight loss of 0% was found on extracting the pyridine control samples. It was therefore assumed that weight loss from modified samples was due to extraction of modified chemical (either unreacted or reacted and subsequently removed from the cell wall during extraction) or reaction by-product not removed during the earlier clean-up procedure.

Table 3.6 Mean weight gains in pure culture test blocks

Modifying chemical	Molar conc.	Mean WPG	Standard deviation	Amended mean WPG
Pyridine control	0	-2.2	0.7	0
Acetic anhydride	0.5	5.9	0.8	8.3
	1.0	12.4	1.8	14.9
	1.5	17.0	1.0	19.6
	2.0	20.3	0.9	23.0
Succinic anhydride	0.25	5.3	0.7	7.7
	0.5	16.2	1.2	18.8
	1.0	30.2	2.0	33.1
	1.5	38.2	3.3	41.3
Phthalic anhydride	0.25	7.4	0.9	9.8
	0.5	16.5	1.2	19.1
	1.0	31.6	2.2	34.6
	1.5	39.1	3.3	42.2
Alkenyl succinic anhydride	0.25	4.4	0.8	6.7
	0.5	10.2	1.2	12.7
	1.0	23.4	1.5	26.2
	1.5	31.8	2.3	34.8
Butyl isocyanate	0.25	2.8	0.7	5.1
	0.5	9.0	0.7	11.5
	1.0	16.3	1.2	18.9
	1.5	21.8	1.0	24.5

At this point it should be noted that the oven dry weight prior to extraction (recorded for the purpose of calculating weight loss due to the extraction procedure) was taken after the blocks had been ground and oven dried, as stated above. If there was any residual pyridine, acetic acid or other volatile substance trapped in the blocks, it may have been removed during grinding and oven drying, i.e. prior to the extraction procedure. Any resultant weight loss from such an effect would be unrecorded. Any such weight loss has been assumed to be negligible here.

A further amendment was made in the penultimate column by taking into account the removal of extractives during reaction, as described earlier for tables 3.4 to 3.6.

Table 3.7 Comparison of weight gain measurements for modified wood

Modification	WPG after reaction	Weight loss due to extraction (%)	WPG after extraction	WPG amended for pyridine extraction	WPG from analysis
Pyridine control	-2.2	0	-2.2	0	0
Acetic anhydride	5.6	0.4	5.2	7.6	7.8
	12.2	0.6	11.5	14.0	13.8
	17.2	0.6	16.5	19.1	18.3
	20.4	1.0	19.2	21.9	20.4
Succinic anhydride	5.9	0.2	5.6	8.0	7.6
	15.2	1.4	13.6	16.2	14.9
	29.5	2.5	26.2	29.0	29.8
	39.6	3.3	35.0	38.0	35.0
Phthalic anhydride	7.3	0.6	6.7	9.0	8.0
	16.3	1.6	14.4	16.9	15.3
	30.7	3.2	26.5	29.3	26.3
	38.5	4.4	32.4	35.4	30.4
Alkenyl succinic anhydride	3.9	0.5	3.4	5.6	3.7
	10.8	0.9	9.8	12.3	7.3
	22.6	1.4	20.8	23.5	12.1
	31.3	1.6	29.2	32.1	14.0
Butyl isocyanate	3.0	0	3.0	5.3	4.8
	9.1	0.1	9.0	11.5	13.5
	15.8	0.2	15.6	18.2	20.2
	22.1	0.2	21.9	24.6	25.2

Finally, the weight gain calculated from chemical and elemental analysis is included.

Weight losses due to extraction make an interesting comparison. Butyl isocyanate figures are very low, indicating that the original clean-up procedure in acetone after reaction was successful in removing unbonded chemical. Extraction weight losses are higher for anhydride modified wood, particularly succinic and phthalic anhydride modified wood, which in some cases had relatively high weight losses. The differences in the weight losses listed in table 3.7 for the extraction of ground wood are not necessarily accounted for by differences in the amount of unbonded chemical not

removed by the initial clean-up procedure. Hot acetone was previously found to be a good solvent for the modifying chemicals used, and since the initial clean-up and the ground wood extraction both involved Soxhlet extraction in acetone, no difference in solubility was expected between the procedures. If the clean-up had not been efficient at removing unreacted chemical from the centre of the blocks (subsequently exposed by grinding) then a similar weight loss would be expected for all blocks, including butyl isocyanate. Additionally there seems to be no reason why phthalic anhydride (the modifying chemical subject to the greatest extraction) should be trapped to a greater extent than both the smaller butyl isocyanate and the larger alkenyl succinic anhydride. The explanation of the different weight losses after extraction of ground modified wood may not lie in the efficacy of the clean-up but in the ease of removal of reacted chemical; reacted chemical may have been removed from wood polymers during the grinding and extracting stage. Wood samples were not oven dry when put in the hammer mill, and may have been subject to hydrolysis of modifying chemical as grinding proceeded and the wood material heated up. Rowell and Ellis (1979) report that the urethane bond resulting from isocyanate reaction is stable to hydrolysis. The susceptibility of succinic and phthalic anhydrides to hydrolysis has been reported by Matsuda (1987). This could therefore account for the differing results in table 3.7.

Titration and elemental analysis are subject to error and are by no means ideal ways of calculating weight gain due to modification. They do however provide an interesting comparison with the mass change measures in table 3.7. The most obvious difference in weight gain recorded by these methods was for alkenyl succinic anhydride. Titration did not prove a good method for analysing the weight gain for this modifying chemical. This could be due to the hydrophobicity of the long alkenyl chain making the hydrolysis step less effective.

In a comparison of the various weight gain measures, weight gains measured by titration for the other three anhydrides are relatively high at low weight gains and relatively low at higher weight gains. This again may be a reflection of inadequacies in the titration method, particularly as the number of ester groups in the wood increased.

Weight gains calculated for butyl isocyanate modified wood using elemental analysis were relatively high compared with other weight gain measures. This lends weight to the argument that true weight gains were in the region of that corrected for the pyridine extraction. Due to the vastly different procedures nothing can be concluded from a comparison between the results for elemental analysis and those for titration, and the way in which they differ from the mass change measures.

Throughout the rest of the study the weight gain measure used was that originally measured from oven dry weights before and after modification (i.e. without amendment). It should be remembered when analysing results that the actual quantity of modifying chemical reacted with wood cell wall polymers may have differed from the weight gain figure used, and will probably have been slightly higher.

3.4.3 Substitution

An alternative measure of the extent of reaction is the substitution level. This measures the number of moles of hydroxyl substituted (or the number of moles of modifying chemical that has reacted with the wood). By measuring the extent of hydroxyl substitutions rather than the weight increase, a better measure of the reactivity of each modifying chemical with the wood is obtained.

Substitution level (mMoles per gram of wood) is calculated as follows:

$$\text{Substitution (mMoles/g)} = \frac{\text{Dry weight after modification} - \text{Initial dry weight}}{\text{Initial dry weight} \times \text{Molecular weight of adduct}} \times 1000$$

The molecular weight of the adduct is equal to the molecular weight of the modifying chemical in all cases except acetic anhydride. During wood modification using acetic anhydride, part of the molecule is removed as by-product (acetic acid). In this case the molecular weight of the adduct is 42. The equation assumes that one mole of wood hydroxyl groups is reacted for every mole of modifying chemical (i.e. no cross-linking or polymerisation reactions).

The substitution level as a function of the molar concentration of reaction solution for each modifying chemical is depicted in figure 3.4.

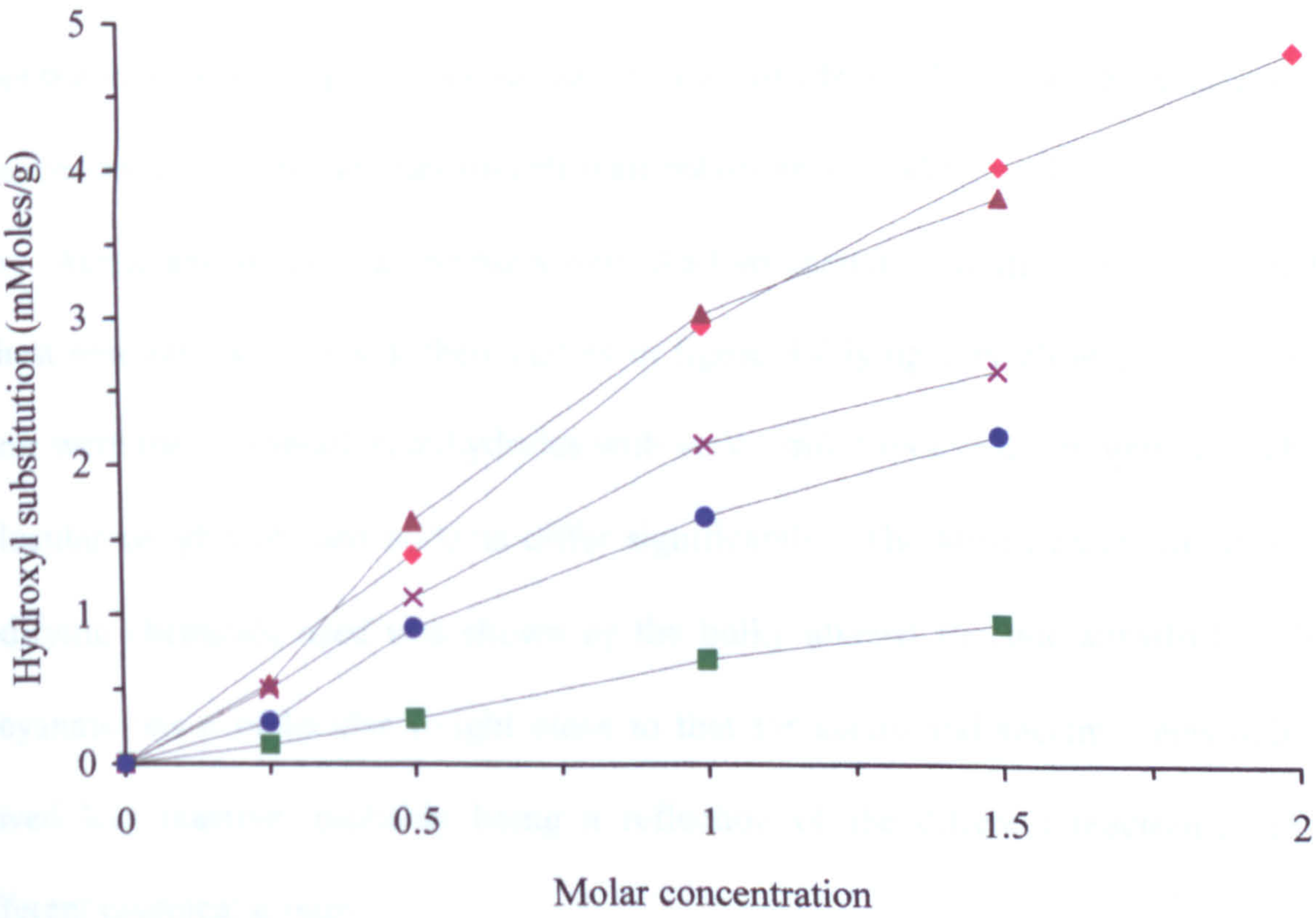


Figure 3.4 The substitution level of wood modified with varying molar concentrations of different modifying chemicals

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲); Phthalic anhydride (×); Butyl isocyanate (●)

In figure 3.4 lines have been drawn to the origin (any other value at zero molar concentration would be invalid as a molecular weight of adduct is required in the above equation). As before, this figure is affected by the question as to whether weight gains (and therefore substitution levels) should be higher to account for extraction by pyridine. The deformation of curves at low weight gains is a direct effect of this as errors in substitution calculation from not accounting for extraction will have a relatively greater effect at lower than at higher weight gains. The value of this figure as a comparison between different modifying chemicals is not lost.

The reactivity of the anhydrides used tends to reflect the molecular weight, the lower the molecular weight the more reactive the anhydride. This could be a function of the accessibility of reactive sites on cell wall polymers to modifying chemicals of varying sizes. Acetic and succinic anhydrides were the two chemicals in this study showing the highest reactivity with wood, their curves in figure 3.4 lying very close to one another. These were the two smallest anhydrides with very similar molecular weights, though the molecular weights of their adducts differ significantly. The lowest reactivity of all the modifying chemicals used was shown by the bulky alkenyl succinic anhydride. Butyl isocyanate has a molecular weight close to that for acetic and succinic anhydride but proved less reactive, probably being a reflection of the different reactivities of the different chemical groups.

3.4.4 Volume increase due to modification

Of the eighty pure culture blocks (20mm x 20mm x 10mm) reacted, a random set of 15 were chosen and their dimensions measured (to an accuracy of +/- 0.01mm) before and after modification. From these measurements block volume and volume increase due to modification were calculated. Measurements were also made immediately after the

clean-up stage (prior to oven drying) to give an indication of swell during reaction. This was done in a fume cupboard as samples were still saturated with acetone. Measuring at this stage does not give a true reading of swell during reaction because acetone has different swelling properties to pyridine (and to the reaction solutions), though the unpleasant nature and toxicity of pyridine led to the decision to make these measurements once this solvent had been removed. Acetone swells wood to a much lesser extent than pyridine. Stamm (1964) calculated their swelling of wood relative to water (i.e. water = 100) to be 63 for acetone and 118 for pyridine. In this work it was thought that the clean-up procedure would have resulted in some shrinkage of the wood due to the replacement of pyridine with acetone, though that a solvent exchange mechanism may have occurred in which the pyridine swollen state was at least partly preserved.

The results are given in table 3.8 and figures 3.5 and 3.6. Table 3.8 lists the mean swell during the reaction procedure (post clean-up) and after oven drying. Volumetric swell due to reaction (i.e. measured after oven drying) is depicted in figure 3.5 which gives the mean values for each reaction set, while in figure 3.6 all points are plotted, giving a better indication of the spread of the data.

The mean volumetric swell of untreated wood due to water saturation was found to be 18% (see chapter 4). In comparison with the volumetric swell during reaction listed in table 3.8, the pyridine control samples were found to swell by 16.8%. Pyridine was expected to swell wood to a greater extent than water, and the result in table 3.8 is almost certainly due to the replacement of pyridine with acetone before measurement, causing a certain amount of shrinkage. It should be noted that these samples did not shrink down to a size expected for the swelling of wood by acetone alone, so it appears that the swollen state was partially preserved as suggested above (though the quantity of residual pyridine in the block at this point is not known). Despite any shrinkage that may occur, mean

Table 3.8 Mean and (standard deviation) volumetric swell during and after chemical modification reaction

Modifying Chemical	WPG	Volumetric swell (%)			
		During reaction		After reaction	
		Mean	s.d.	Mean	s.d.
Pyridine control	-2.5	16.8	2.9	1.9	1.1
Acetic anhydride	5.7	19.6	2.7	6.1	1.1
	13.5	22.0	3.0	9.8	1.8
	17.1	21.6	1.7	11.0	1.5
	20.4	23.1	2.6	12.6	1.4
Succinic anhydride	5.4	19.5	2.3	5.3	1.5
	15.7	21.5	1.8	7.4	1.2
	30.4	34.0	5.6	14.2	1.9
	37.0	43.7	5.6	19.5	2.3
Phthalic anhydride	6.9	20.2	1.8	5.6	1.0
	16.3	23.3	2.3	9.8	1.6
	31.9	27.3	1.9	16.4	2.3
	38.6	36.2	4.7	19.3	2.4
Alkenyl succinic anhydride	3.9	18.5	2.2	4.8	1.0
	10.4	21.0	2.1	8.0	1.0
	23.5	24.8	3.2	14.5	1.8
	30.7	23.5	2.9	16.0	1.8
Butyl isocyanate	2.7	19.4	2.7	5.0	1.4
	8.9	20.2	3.3	7.9	1.4
	15.8	22.4	2.5	11.5	1.5
	22.0	21.7	2.3	13.4	1.3

volumetric swell during the reaction procedure for the reacted samples was always in excess of the 18% measured for water soak, dispelling the worry that swelling due to the reaction solution (and the penetration of modifying chemical throughout the wood cell wall) may be significantly reduced at higher molar concentrations. In many cases an increase in swell during reaction was recorded with increasing molar concentration, indicating that the addition of modifying chemical caused increased swelling in these conditions. In most cases this was only slight, with the exceptions being high molar concentrations (high WPG samples) of succinic and phthalic anhydrides. In the case of succinic anhydride modified wood, the very large increase in dimensions resulted in

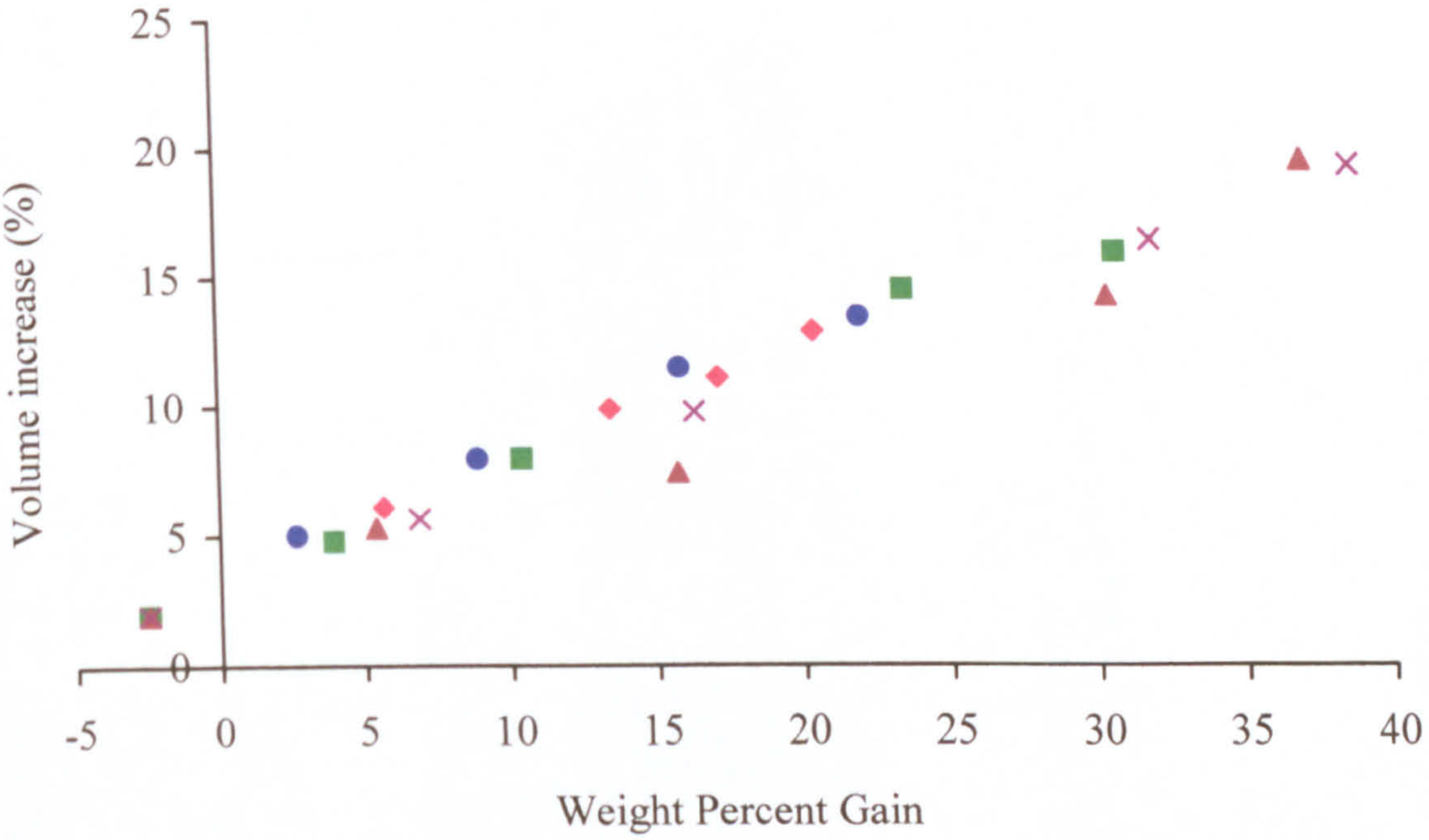


Figure 3.5 Mean volume increase in wood blocks due to chemical modification

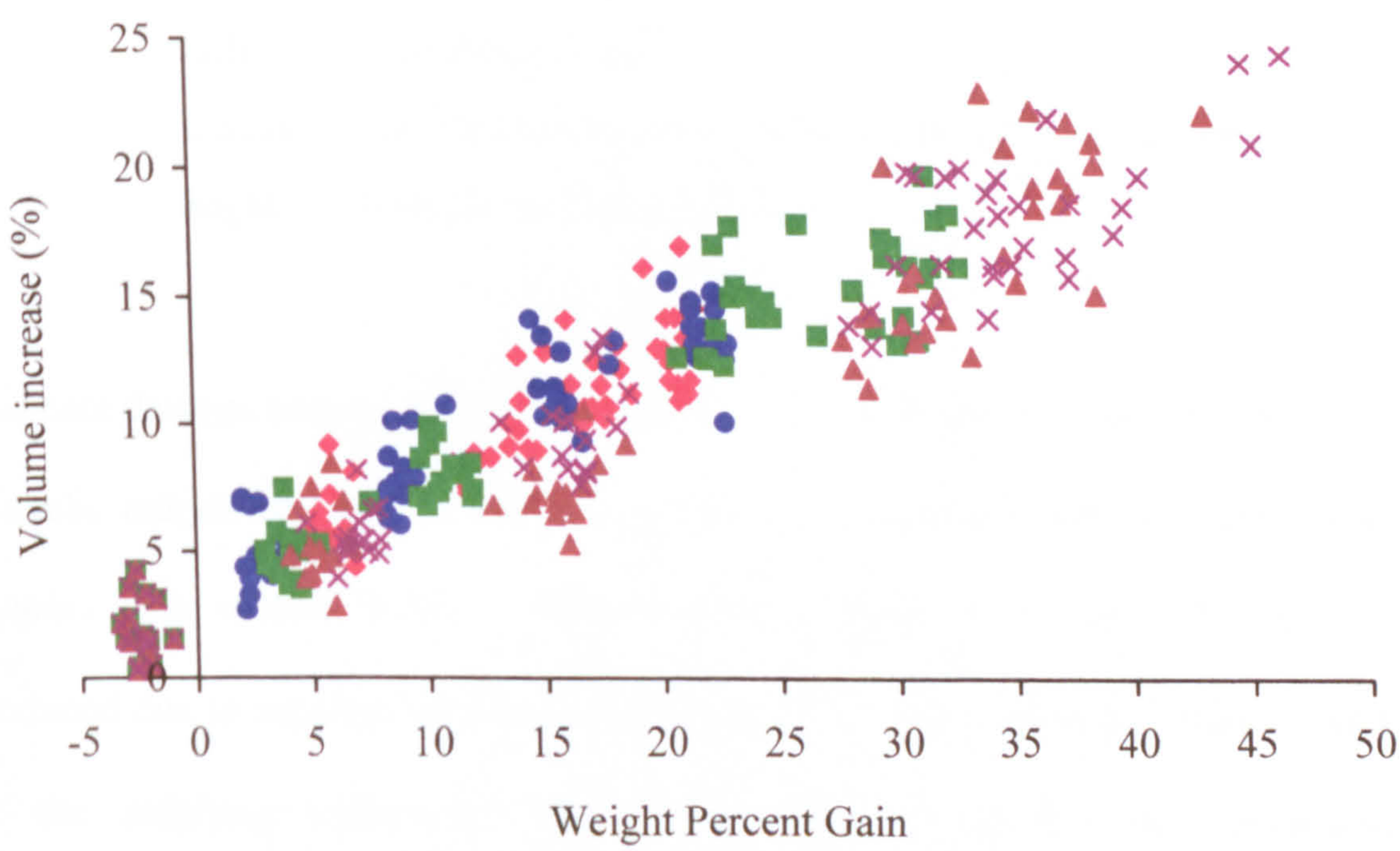


Figure 3.6 Volume increase in wood blocks due to chemical modification

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲); Phthalic anhydride (×); Butyl isocyanate (●)

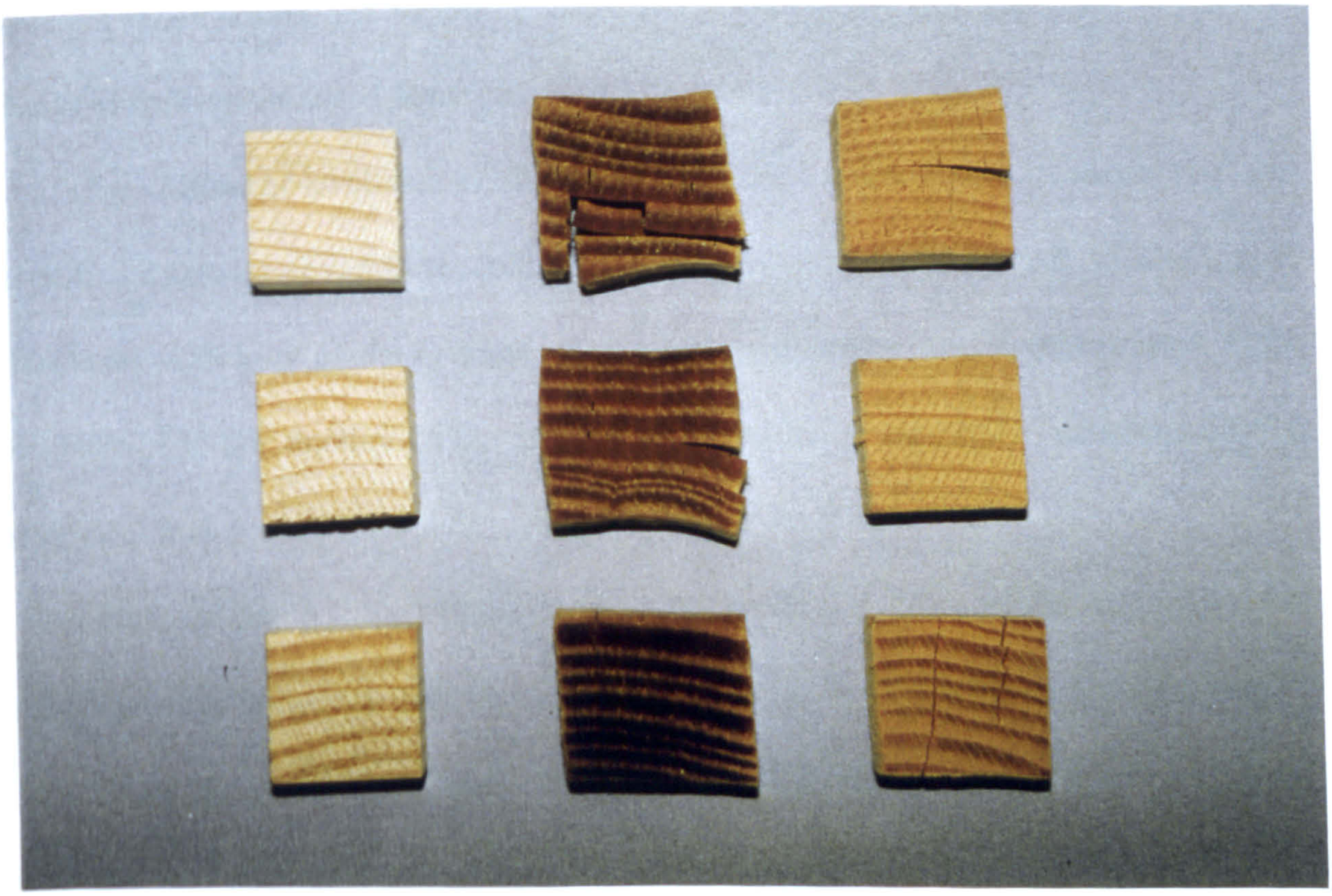


Plate 3.1 Volumetric swell of wood samples (original size 10mm x 20mm x 20mm) due to chemical modification with a 1.5 molar solution of succinic anhydride in pyridine.

Left: Unmodified blocks

Centre: Modified blocks prior to final oven dry (acetone saturated)

Right: Oven dry modified blocks

significant damage occurring in many of the blocks, as shown in plate 3.1. In the case of the cyclic anhydrides, carboxylic acid groups are introduced onto the wood cell wall polymers (see section 3.2.1). Nakano (1993) found that carboxylic acid groups introduced due to succinic anhydride modification ionised at pH's between 5 and 8, and that the resulting carboxylate groups repelled one another and increased the volumetric swell of modified wood. In the base pyridine solution this effect could have contributed to excessive swell, particularly where a large number of carboxyl groups are introduced (high WPG's of succinic and phthalic anhydride). In the case of succinic

anhydride modification, the repelling forces and resultant swell might have become so great as to damage the wood structure.

For volumetric swell due to modification, figure 3.6 shows that although there was a significant amount of variation within blocks of a particular modification (due in part to differences in density of the original wood blocks), different modifications show close relationships to one another. This trend is easier to see in figure 3.5, which shows the mean values.

From figure 3.5 the relationships between WPG and swelling for acetic and alkenyl succinic anhydride and for butyl isocyanate appear linear up to a weight gain of 15 to 20%. Above this level there is evidence of a tail off in the relationship for butyl isocyanate and alkenyl succinic anhydride modified wood, with lower swelling for an equivalent increase in WPG. This kind of relationship is in agreement with that reported by Stamm and Tarkow (1947) for acetic anhydride. Phthalic anhydride does not display such a tail off, and although swelling was slightly lower at equivalent WPG's the linear relationship of swelling with weight gain appears to continue to WPG's in excess of 40%.

Succinic anhydride modification exhibits no such linear relationship; a sigmoid shape relationship occurred with a higher ratio of swelling to weight gain at the lowest and highest WPG's. This relationship is more in line with that found by Hill and Jones (1998) for linear anhydrides (including acetic anhydride).

One of the most striking features of figures 3.5 and 3.6 (and table 3.8) is the swelling evident in pyridine control blocks (averaging approximately 2%), which actually lost weight. Two possible reasons for this are that solvent might have remained in the blocks after an inefficient clean-up stage (despite the recorded weight loss) or that a solvent exchange mechanism occurred from the swollen state in pyridine via acetone before the block was dried, leaving blocks in a slightly swollen state. However, on

remeasuring the blocks after leaching (procedure described in section 3.7) this swelling was found not to have been reversed. It therefore seems that swelling in these blocks may have been due to a relaxation of stresses in the wood developed during initial kiln drying (or even in the growing tree) or to damage of the wood structure during the reaction procedure. Since the perceived curves or lines for each relationship between swelling and weight gain for each modifying chemical appear to pass through this point (positive swelling at negative WPG) it seems that all blocks were subject to this effect.

The establishment of a definite relationship between swelling and weight gain would require more reactions for each chemical than the four used here. The purpose here was to compare the cell wall swelling properties of each modifying chemical as a factor which might affect biological resistance.

3.4.5 Visual appearance

Colour or brightness changes were evident in all samples, most evident being darkening of wood reacted to higher loadings of the three cyclic anhydrides. Of the greatest relevance to this work was block damage occurring during reaction with succinic anhydride. After succinic anhydride reaction to high weight gains, wood splinters were commonly found in the reaction vessel upon removal of the samples. Fungal cellar and soft rot stakes were prone to splitting along the grain, while pure culture blocks were often broken into two or more pieces after reaction (see plate 3.1). Breakage tended to occur along the latewood - earlywood interface. Breakage and splintering in this way seems to suggest that damage occurred in the lignin or non-crystalline polysaccharide part of the wood cell wall matrix.

3.5 CCA treatment

For each test of resistance to decay fungi CCA treated samples were included as a control. CCA was prepared to type 2 specification using laboratory grade reagents or better (British Standard BS4072, 1974). A sample of treated wood was later analysed for salt balance (using x-ray fluorescence: Oxford Instruments QX XRF wave dispersive instrument) at Hickson Timber Products Ltd, Castleford, and found to comply with the specification. Nominal British Standard composition and the actual composition of treated wood from analysis are given in table 3.9.

Table 3.9 Nominal and actual composition of CCA

Ingredient	**Nominal composition (CCA type 2)**	**Actual composition**
Copper (as $CuSO_4.5H_2O$)	35.0%	35.0%
Dichromate (as $Na_2Cr_2O_7.2H_2O$)	45.0%	42.4%
Arsenic (as $As_2O_5.2H_2O$)	20.0%	22.6%

3.5.1 CCA treatment of fungal cellar and soft rot test stakes

Fungal cellar and soft rot test stakes were treated using a full-cell vacuum and pressure impregnation process. This involved an initial vacuum of approximately 650 mmHg (approx. 0.15 bar) in the treatment cylinder for 30 minutes, after which the cylinder was flooded with preservative. A pressure of 12 bar was then applied for one hour. Uptake and retention of preservative were measured for each individual stake from the difference in weights before and after impregnation. Stakes were stored in a sealed container for 2 weeks in order for fixation to take place. A small beaker of xylene was also placed in the container as a fumigant to prevent mould growth.

Solution strengths used and mean (and standard deviation) retentions are given in table 3.10.

Table 3.10 Mean CCA retentions of fungal cellar and soft rot test stakes

Solution strength (%)	Hydrated salt retention (kg/m^3)			
	Fungal cellar stakes (45 no. 9mm x 19mm x 200mm)		Soft rot stakes (25 no. 5mm x 15mm x 100mm)	
	Mean	Standard deviation	Mean	Standard deviation
0 (water control)	0	0	0	0
0.13	0.8	0.03	0.9	0.04
0.25	1.6	0.06	1.8	0.11
0.5	3.2	0.11	3.5	0.19
1.0	6.4	0.23	7.0	0.40

3.5.2 CCA treatment of pure culture test blocks

Pure culture test blocks were treated using a single vacuum impregnation process. Blocks were placed and weighed down in a vacuum desiccator and a vacuum of approximately 700mmHg (approx. 0.08 bar pressure) was drawn for 30 minutes, after which preservative was introduced into the vessel to immerse the stakes and the vacuum released. The blocks were allowed to stand in the preservative solution for a further 30 minutes before being drained and weighed. They were then stored for fixation as described in 3.5.1 above. Solution strengths used and mean (and standard deviation) retentions are given in table 3.11.

Table 3.11 Mean CCA retentions of pure culture test blocks

Solution strength (%)	Hydrated salt retention (kg/m^3) (80 blocks 20mm x 20mm x 10mm)	
	Mean	Standard deviation
0 (water control)	0	0
0.07	0.5	0.02
0.14	1.0	0.04
0.28	2.0	0.09
0.56	4.0	0.18

3.6 Leaching

Although chemically modified samples were exposed in fungal tests unleached, a subset of 4 of the pure culture test blocks from each of the reactions was set aside for leaching in accordance with European Standard EN84 (1989). This involved the vacuum impregnation of modified wood blocks with deionised water followed by a 14 day soak with regular water changes. Oven dry weight before and after leaching was recorded and weight loss was calculated. Results are given in table 3.12 (chemically modified wood) and table 3.13 (CCA treated wood).

Table 3.12 Leaching weight losses for chemically modified wood blocks

Modifying chemical	Mean WPG	Weight loss (%)	
		Mean	Standard deviation
Untreated control	0	2.1	0.26
Pyridine control	-2.8	2.2	0.07
Acetic anhydride	4.5	1.0	0.28
	14.2	0.6	0.13
	17.5	0.6	0.21
	19.4	0.6	0.05
Succinic anhydride	5.1	1.3	0.08
	15.2	2.3	0.28
	30.4	3.5	0.34
	35.9	4.2	0.27
Phthalic anhydride	6.9	2.6	0.26
	16.4	4.0	0.44
	34.3	6.5	0.24
	35.4	7.1	0.16
Alkenyl succinic anhydride	4.1	0.3	0.10
	8.7	0.4	0.18
	25.6	0.4	0.12
	31.7	0.3	0.31
Butyl isocyanate	2.3	0.6	0.18
	8.4	0.4	0.08
	16.3	0.5	0.09
	21.1	0.7	0.10

Table 3.13 Leaching weight losses for CCA treated wood blocks

Hydrated salt retention kg/m^3	Weight loss (%)	
	Mean	Standard deviation
0	2.2	0.18
0.5	1.7	0.12
1.0	1.8	0.26
2.0	1.9	0.09
4.0	1.9	0.17

The pyridine and untreated control samples listed in table 3.12 and the water control (0kg/m^3) samples in table 3.13 were all subject to very similar weight losses. This was despite the fact that the pyridine control samples had already undergone a solvent extraction process (in the form of refluxing in pyridine and then acetone and a Soxhlet extraction in acetone during clean-up) and had already lost nearly 3% of their original weight. It seems that certain water extractable substances remained in the blocks at the end of the reaction procedure only to be removed during leaching.

CCA treated blocks and blocks modified with acetic and alkenyl succinic anhydride and with butyl isocyanate all lost marginally less weight than their corresponding control blocks, indicating that these treatments were not susceptible to leaching in these conditions. Succinic and phthalic anhydrides were subject to increasing weight loss due to leaching with increasing weight gain of modifying chemical. This is a similar result to that obtained from the acetone extraction of ground modified wood in section 3.4.2. As mentioned in section 3.4.2 this cannot solely be explained by the removal of unreacted chemical, and it seems that succinic and phthalic anhydride are both susceptible to hydrolysis and leaching. This is in accordance with the results of Matsuda (1987) who found wood modified with succinic, phthalic and maleic anhydrides to be susceptible to hydrolysis of the ester bond at high humidities.

The reason why succinic and phthalic anhydride modification are more susceptible to hydrolysis than acetic anhydride may be due to the free carboxyl group formed on modification. The rigidity of the phthalic group could then be responsible for the higher susceptibility to hydrolysis of wood modified with phthalic anhydride by holding the carboxyl group in relatively close proximity to the ester. However, if wood modified with succinic and phthalic anhydride (and maleic anhydride, according to Matsuda, 1987) is susceptible to hydrolysis, then it might reasonably be expected that this would also be the case for alkenyl succinic anhydride. In the latter case the hydrophobic chain could protect the ester bond from water, or even restrict the mobility of the free carboxyl (if proximity of this group to the ester is indeed a requirement of hydrolysis). Alternatively, hydrolysis may still occur at a rate equivalent to that for succinic anhydride modified wood, but the insolubility of alkenyl succinic anhydride (or its diacid) in water results in it remaining unleached from the wood. In this case, hydrolysis of the ester would not be detected purely by measuring weight loss in the leaching test. Whether the alkenyl succinic anhydride remained bonded to the wood in this study is not known and was not further tested.

3.7 Summary of points raised in chapter 3

- A range of weight gains of modification were achieved by varying the concentration of modifying chemical in pyridine.
- The weight gain (WPG) of modification was calculated on the basis of oven dry weight before and after the reaction procedure. The lack of pre-modification extraction meant that some weight loss was likely to have occurred during reaction which was not accounted for in the WPG calculation. Consequently genuine WPG's may have been slightly higher than those measured.

- Extraction of ground modified wood with acetone confirmed that reaction to wood polymers had occurred. Weight loss due to this extraction procedure was higher for anhydride (particularly succinic and phthalic anhydride) modified wood than for butyl isocyanate modified wood. This effect may be due to the varying ease with which reacted modifying chemicals can be removed from the wood polymers rather than the extraction of unreacted chemical not adequately removed in the original clean-up procedure.
- For anhydrides, reactivity with wood polymers (based on the level of substitution in reacted wood) appears to increase with decreasing molecular weight. This may be due to the accessibility of wood cell wall hydroxyl groups to smaller modifying chemicals. Butyl isocyanate was less reactive than anhydrides of equivalent molecular weight (and less reactive in this study than all but alkenyl succinic anhydride).
- A measure of swell during reaction indicated that during all modification reactions, wood samples were swollen to a greater extent than water saturated untreated wood. Theoretically, all hydroxyls available to degradative agents released by decay fungi (when wood is water swollen) were available for reaction. The only restriction to the substitution of any given hydroxyl was therefore its accessibility to a given modifying chemical.
- Volumetric swell due to modification was calculated. Very similar relationships of volumetric swell against WPG were recorded for the different modifying chemicals within the range of weight gains achieved. This was particularly the case for acetic anhydride, alkenyl succinic anhydride and butyl isocyanate modified wood.
- Modification of wood with succinic anhydride to high WPG's caused quite severe damage to samples, commonly causing splitting and even the breaking of samples into

two or more pieces. This was thought to occur in the lignin or non-crystalline polysaccharide portions of the wood matrix, and to be caused by carboxylate groups repelling one another during the reaction procedure, causing the wood to swell excessively and ultimately to break apart.

- When subject to water leaching, acetic anhydride, alkenyl succinic anhydride and butyl isocyanate modified wood and CCA treated wood lost less weight than their corresponding controls, and so appeared resistant to leaching. Phthalic and succinic anhydride modified wood lost more weight than other samples. This weight loss increased as WPG increased. In these two cases, modifying chemical appeared to be susceptible to hydrolysis and leaching. It was thought possible that the ester bond formed on modification with alkenyl succinic anhydride was also hydrolysed, with the modifying chemical remaining unleached.

Chapter 4. Water adsorption and swelling of chemically modified wood

4.1 Introduction

The wood - water relationship is one of the most important physical properties of wood, particularly when considering dimensional stability and decay resistance (the importance of moisture content in wood decay was discussed in chapters 1 and 2). Chemical modification changes the properties of wood due to the reaction of hydrophilic chemical groups within the wood and by bulking the wood cell wall. This chapter describes an experiment to measure the effect of these changes on the attraction of the modified wood for water and the capacity of the cell wall to hold water. This involved exposing modified wood to increasing relative humidities and calculating adsorption isotherms from measured equilibrium moisture contents.

Several models for fitting sorption isotherms to empirical data have been proposed. One such model is that proposed by Hailwood and Horrobin (1946), as described in section 2.2.2. Due to the close fit found when the Hailwood – Horrobin (single hydrate) model has been applied to e.m.c. data for wood (Spalt, 1958; Simpson, 1980) and for chemically modified wood (Martins, 1992), this model was also used in the present study.

A further advantage of the Hailwood – Horrobin model is the ease with which the total water sorbed can be attributed to monomolecular and polymolecular sorption (Martins, 1992). This is potentially of a great deal of interest when assessing and comparing wood reacted with a variety of modifying chemicals.

In this study, hysteresis was not measured as this was not considered important to the durability of modified wood. Only e.m.c.'s in adsorption were measured and the adsorption isotherms for the various modified woods calculated.

In addition to the sorption study, the swelling of modified wood was measured by recording the change in dimensions on soaking in water. This was measured because it was thought that the ability of water to swell the modified wood cell wall might influence the accessibility of wall polymers to fungal degradative agents.

4.2 Experimental method

4.2.1 Modified wood

Samples were selected from the pure culture test blocks (10mm x 20mm x 20mm) modified as described in chapter 3. Two blocks from each reaction set were selected and cut in half to form four samples measuring approximately 5mm (longitudinal) x 20mm x 20mm. This was done so as to improve diffusion of water vapour to the centre of the sample and speed up the process of reaching e.m.c. when exposed to a particular relative humidity. Each half of the block was assumed to have the same WPG. Though cutting blocks like this theoretically exposes sorption sites not available to reaction, such an increase would be insignificant compared to the number available to water sorption throughout the sample.

All four of these samples were oven dried and oven dry weights recorded.

4.2.2 Control of relative humidity

The method for controlling relative humidity was identical to that used by Martins (1992). Test samples were kept above saturated solutions of selected salts in containers stored in a controlled temperature room set at 20°C (variation +/- 1°C). Six salts were chosen and these are listed in table 4.1 along with the relative humidity of the atmosphere above each saturated solution at 20°C (according to Kaye and Laby, 1973).

Table 4.1 Saturated salt solutions used and their resultant relative humidities at 20°C

Salt	Relative Humidity (%)
Potassium nitrate	93
Sodium chloride	76
Sodium dichromate	55
Potassium carbonate	44
Potassium acetate	23
Lithium chloride	12

Data published by Kaye and Laby (1973) show the equilibrium relative humidity above saturated solutions of these salts to be insensitive to any variation in temperature expected in the controlled temperature room (a variation around 20°C of +/- 5°C causing a maximum variation of +/- 1% RH).

Excess salt was present within each solution to ensure saturation was maintained. The solution and air in the container were agitated by bubbling air through the solution.

An attempt was made to measure relative humidity within the containers by using a hygrometer and a wet and dry bulb psychrometer. Neither of these proved accurate, and both gave inconsistent readings. The psychrometer was not suitable for use in this experiment due to the lack of sufficient air flow required to evaporate water at the wet bulb. The relative humidities listed in table 4.1 were therefore assumed to be correct. Thin wood wafers were used in each container to monitor any variation in humidity above a particular salt solution. These wafers were expected to equilibrate much faster than the test samples and so give an indication of any unexpected variation. No such variation was detected.

The lack of an accurate measure of relative humidity for verification in this test was unfortunate, but this does not affect the comparisons made between modified (and unmodified) woods exposed in identical conditions.

4.2.3 Sample conditioning

The oven dry wood samples were placed in the container with the lowest relative humidity. They were left to equilibrate for 3 weeks and then weighed once a week until it became obvious that no significant weight change had occurred since the last weight was recorded (and e.m.c. had been attained). When all blocks had reached e.m.c. they were removed to the container with the next lowest relative humidity (blocks were exposed to saturated solutions in order of increasing humidity so adsorption isotherms could be fitted). Equilibrium moisture content was reached within 6 weeks for all but the two highest humidities, which required longer exposure times.

Moisture contents at each relative humidity were calculated as follows:

$$\text{m.c. (\%)} = 100 \times \frac{\text{wet weight (modified sample)} - \text{dry weight (modified sample)}}{\text{original (unmodified) dry weight of sample}}$$

The original (unmodified) weight of the sample was calculated from the dry weight of the modified wood and the WPG (the actual original weight of the sample was unknown as the sample had been cut after modification, as described in section 4.2.1).

The above equation gives a calculation for moisture content on an original wood weight basis. This was done to give a more valid measure of change in wood hygroscopicity as it is unaffected by weight changes due to modification. What is being measured is the ratio of water present to actual wood polymer material.

4.2.4 Swelling test

Swelling was not tested at each relative humidity as it was thought that the relationship between swelling and moisture content was of minor importance to this work. However, a measure of the volumetric swell of modified wood at saturation was required to be able to further assess the properties of the modified cell wall.

Samples tested were the adsorption test samples (after e.m.c. at the highest RH had been measured) and selected modified blocks (pre-modification size 10mm x 20mm x 20mm) which had been measured before and after modification as described in section 3.4.4. These latter samples were included so measures of aggregate swelling (swelling due to modification plus swelling due to water soaking) could be made and compared.

Oven dry weights and dimensions (+/- 0.01mm) of all samples were recorded. A vacuum (approx. 700mmHg, 0.08 bar) was applied to the samples for 30 minutes, after which they were immersed in deionised water and the vacuum released. Samples were left to soak for 48 hours before measurement. After wet measurement the samples were oven dried and dry dimensions were remeasured. Studies of the dimensional stability of chemically modified wood have found that results from the first water soak cycle can differ significantly from those in subsequent cycles (Rowell and Ellis, 1978, Hill and Jones, 1996). In an attempt to avoid any such anomalies in this experiment, the process was repeated and a second set of wet and dry dimensions was taken. After each soak, oven dry weights were also recorded so that any loss of chemical due to leaching was detected.

Swelling was calculated in two ways:

1. As swelling due to the water soak

$$\text{Swelling (\%)} = \frac{\text{Volume of wet modified wood - Volume of dry modified wood}}{\text{Volume of dry modified wood}}$$

2. As the aggregate swelling due to modification plus water soak

$$\text{Swelling (\%)} = \frac{\text{Volume of wet modified wood - Volume of dry unmodified wood}}{\text{Volume of dry unmodified wood}}$$

Swelling due to water soak was calculated on the basis of the volume of dry modified wood. If it is assumed that the cell lumen size is constant, this therefore gives a measure of the capacity of the modified cell wall to swell. If the extent to which wood

can swell affects the size of pores in the wall, which in turn affects the accessibility of wood polymers to degradative agents released by decay fungi, then any reduction in the capacity of wood to swell could be a cause of decay resistance in wood.

4.3 Isotherm fitting

As described in section 2.2.2, the Hailwood – Horrobin model divides total moisture sorbed into its monomolecular and polymolecular components. The equation for the Hailwood – Horrobin model is as follows:

$$m = mh + md = \frac{Mo\,Kh\,Kd\,h}{(1 + Kh\,Kd\,h)} + \frac{Mo\,Kd\,h}{(1 - Kd\,h)} \qquad (4.1)$$

where:

h is the vapour pressure (h = RH/100)

m is the wood moisture content in equilibrium with h

mh is the moisture content relating to the hydrate water (monomolecular sorption)

md is the moisture content relating to the dissolved water (polymolecular sorption)

In addition to these are the three constants calculated from isotherm fitting:

Mo is the moisture content corresponding to complete polymer hydration

Kh is the equilibrium constant between the hydrate and the dissolved water

Kd is the equilibrium constant between dissolved water and water vapour

The assumptions and derivation of the Hailwood – Horrobin model are not discussed here, but are fully explained and discussed in the original paper (Hailwood and Horrobin, 1946) and by Skaar (1988).

On the right hand side of equation 4.1 the first term corresponds to mh and the second to md. This allows isotherms for these components to be plotted separately once constants have been calculated.

For ease of fitting, the equation 4.1 is transformed to the quadratic equation:

$$h/m = A + B\,h - C\,h^2 \qquad (4.2)$$

As h/m and h can both be obtained experimentally, a curve can be fitted to experimental data and values of A, B and C obtained. The constants in equation 4.1 are then calculated as follows:

$$Kd = \frac{-B + \sqrt{(B^2 + 4AC)}}{2A} \qquad (4.3)$$

$$Kh = \frac{1}{1 - Kd\,(B/C)} \qquad (4.4)$$

$$Mo = \frac{100}{A\,Kd\,(Kh + 1)} \qquad (4.5)$$

Once values for these constants are obtained for each modified wood, values of m, mh and md can be calculated for values of h between 0 and 1, and the isotherm can be plotted.

In this study the results obtained (section 4.2.3) were plotted in the form of h/m against h for each set of 4 samples (i.e. each WPG of each modifying chemical). As 6 relative humidities were used, this resulted in 24 points being plotted for each set. A curve of the form shown in equation 4.2 was fitted through each set of 24 points (least squares method). From these curves, values for A, B and C and the R^2 value were obtained.

4.4 Results and discussion - sorption isotherms

4.4.1 Isotherm fitting

The mean and standard deviation moisture contents (of 4 blocks) at each relative humidity are given in table 4.2. A curve of the form of equation 4.2 was fitted to these

Table 4.2 Mean (and standard deviation) values for experimentally derived e.m.c.'s, and the coefficients of determination (R^2) from curve fitting

Modifying chemical	WPG	E.M.C.'s at increasing relative humidities						R^2
		12% RH	23% RH	44% RH	55% RH	76% RH	93% RH	
Untreated control	0	2.64 (.06)	4.21 (.09)	7.06 (.08)	8.38 (.11)	12.27 (.07)	18.88 (.23)	0.973
Pyridine control	-2.6	2.49 (.12)	4.03 (.08)	6.85 (.13)	8.13 (.15)	11.99 (.09)	18.92 (.1)	0.947
Acetic anhydride	5.4	2.10 (.13)	3.43 (.11)	5.79 (.12)	6.98 (.1)	10.43 (.1)	15.68 (.3)	0.939
	11.7	1.78 (.06)	2.93 (.07)	5.03 (.17)	6.08 (.18)	9.26 (.27)	14.29 (.11)	0.940
	16.2	1.41 (.08)	2.29 (.1)	3.94 (.1)	4.78 (.06)	7.32 (.04)	11.21 (.26)	0.934
	20.4	1.18 (.06)	2.03 (.05)	3.59 (.1)	4.38 (.06)	6.76 (.09)	10.39 (.33)	0.932
Succinic anhydride	6.5	2.23 (.06)	3.76 (.05)	6.50 (.07)	7.80 (.04)	11.55 (.09)	17.53 (.34)	0.965
	16.8	2.10 (.09)	3.50 (.13)	6.10 (.17)	7.44 (.16)	11.25 (.18)	17.11 (.36)	0.932
	29.4	2.25 (.1)	3.65 (.14)	6.32 (.21)	7.79 (.23)	11.97 (.31)	17.84 (.81)	0.905
	38.6	2.18 (.05)	3.57 (.05)	6.33 (.05)	7.91 (.05)	12.26 (.27)	17.86 (.49)	0.958
Phthalic anhydride	6.5	2.17 (.11)	3.50 (.15)	6.33 (.21)	7.61 (.17)	11.46 (.31)	17.92 (.33)	0.896
	17.5	1.89 (.17)	3.11 (.15)	5.58 (.15)	6.90 (.15)	10.59 (.17)	17.28 (.87)	0.886
	32.9	1.97 (.08)	3.19 (.12)	5.53 (.2)	6.92 (.2)	10.96 (.2)	18.32 (1.44)	0.937
	45.1	1.95 (.1)	3.20 (.02)	5.50 (.08)	7.00 (.12)	11.38 (.12)	19.40 (1.04)	0.972
Alkenyl succinic anhydride	4.5	1.95 (.11)	3.36 (.09)	5.91 (.16)	7.07 (.15)	10.70 (.13)	16.44 (.26)	0.906
	12.0	1.73 (.05)	2.95 (.06)	5.24 (.09)	6.36 (.11)	9.81 (.16)	15.12 (.3)	0.960
	22.4	1.52 (.03)	2.68 (.03)	4.69 (.07)	5.71 (.04)	8.81 (.06)	13.35 (.19)	0.973
	31.8	1.52 (.09)	2.59 (.08)	4.61 (.15)	5.60 (.14)	8.55 (.07)	12.72 (.15)	0.901
Butyl isocyanate	2.1	2.39 (.09)	3.81 (.12)	6.59 (.09)	8.39 (.33)	12.29 (.34)	19.46 (.96)	0.908
	8.2	1.85 (.11)	3.15 (.12)	5.60 (.18)	6.76 (.12)	10.37 (.12)	16.30 (.01)	0.963
	15.6	1.65 (.24)	2.82 (.23)	4.99 (.32)	6.10 (.3)	9.33 (.27)	14.33 (.26)	0.926
	22.9	1.42 (.22)	2.53 (.21)	4.55 (.3)	5.55 (.25)	8.48 (.22)	12.68 (.24)	0.867

data points as described in section 4.3. R^2 (the coefficient of determination) for each of these curves is also given in table 4.2. R^2 is a statistical measure of the proportion of variation that can be explained by the regression line (e.g. for untreated controls the regression line accounted for 97% of variation). The R^2 values in this test ranged from 0.867 to 0.973, indicating good fit to the experimental results. Values for the constants Kh, Kd and Mo were calculated using the formula established from curve fitting and the equations 4.2, 4.4 and 4.5. These values are listed in appendix 1.

4.4.2 Isotherms for chemically modified wood

Figures 4.1 to 4.5 show the isotherm families for each modification type. Mean values of the experimentally derived e.m.c.'s at each humidity (from table 4.2) are also plotted. The isotherms for monomolecular and polymolecular adsorption are plotted in figures 4.6 to 4.15.

Figure 4.1 shows the isotherms for the acetic anhydride modified wood and the controls. The isotherms for the untreated control and for the pyridine control are very close, as would be expected (in figures 4.2 to 4.5 only the pyridine control is shown). Figures 4.6 and 4.7 show corresponding monomolecular and polymolecular isotherms for the control and the acetic anhydride modified wood. The pyridine control (with weight loss due to extraction of 2.6%) displays lower monomolecular adsorption and slightly higher polymolecular adsorption. These results are similar to those of Wangaard and Granados (1967). Wangaard and Granados measured the sorption isotherms of extracted and unextracted woods and found that extractives in wood cause a slight increase in monomolecular sorption, but a significant decrease in polymolecular (and total) sorption. They attributed the increase in monomolecular sorption to extractives being slightly more hygroscopic (per unit weight) than wood cell wall material, therefore leading to greater

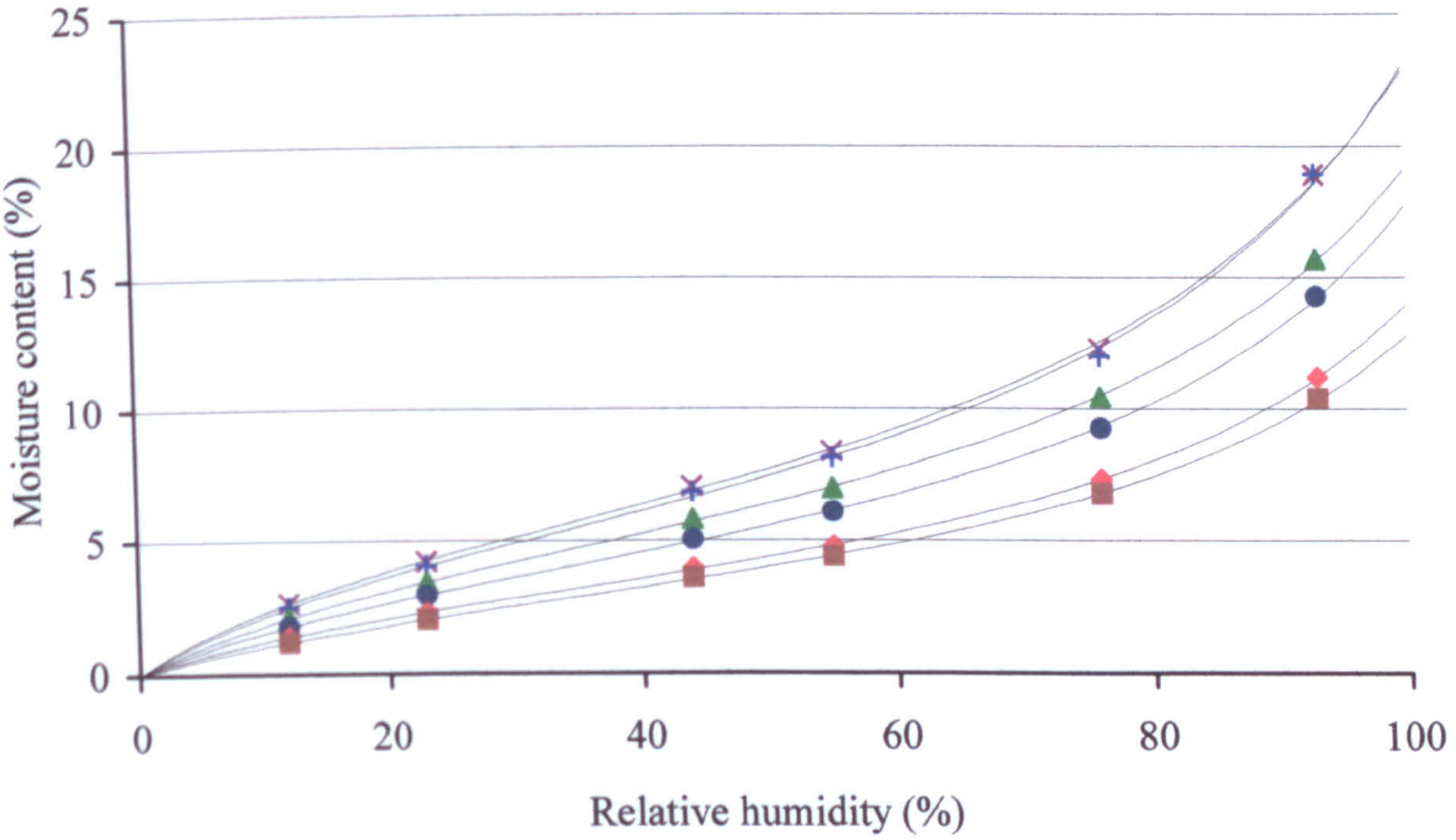


Figure 4.1 Adsorption isotherms for acetic anhydride modified wood

Pyridine control (+); 5.4 WPG (▲); 11.7 WPG (●); 16.2 WPG (◆); 20.4 WPG (■); Untreated control (×)

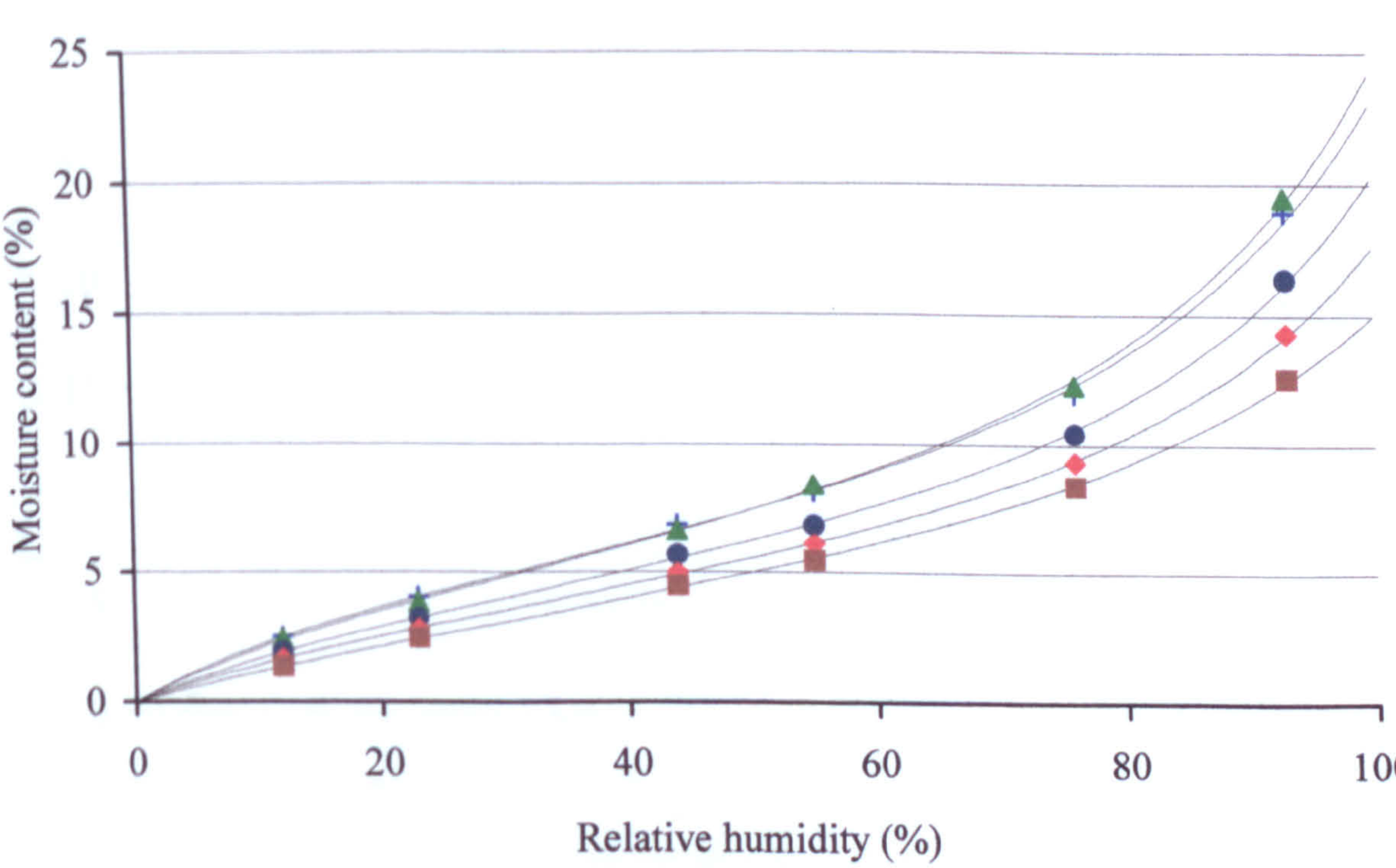


Figure 4.2 Adsorption isotherms for butyl isocyanate modified wood

Pyridine control (+); 2.1 WPG (▲); 8.2 WPG (●); 15.6 WPG (◆); 22.9 WPG (■)

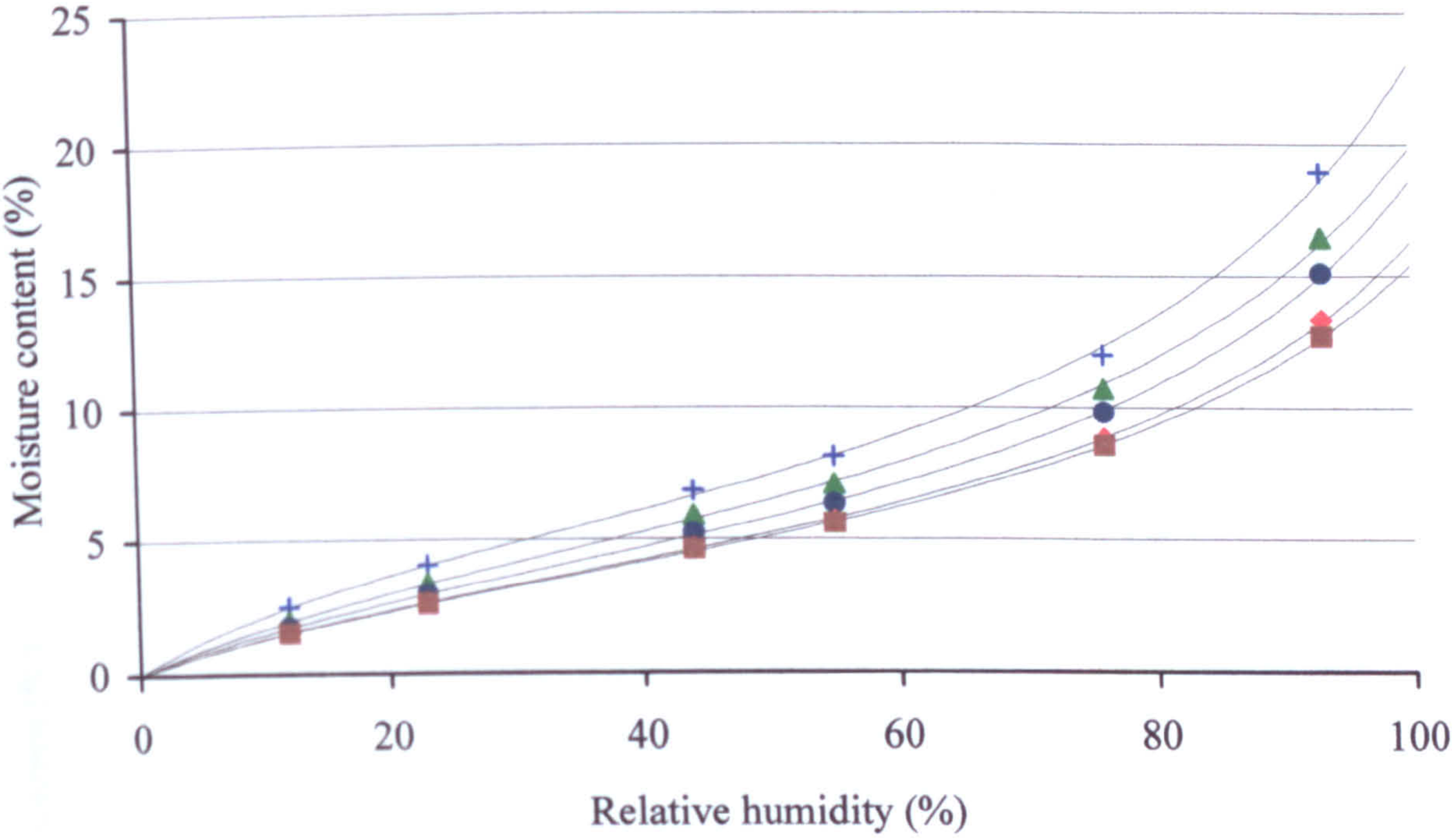


Figure 4.3 Adsorption isotherms for alkenyl succinic anhydride modified wood

Pyridine control (+); 4.5 WPG (▲); 12.0 WPG (●); 22.4 WPG (◆); 31.8 WPG (■)

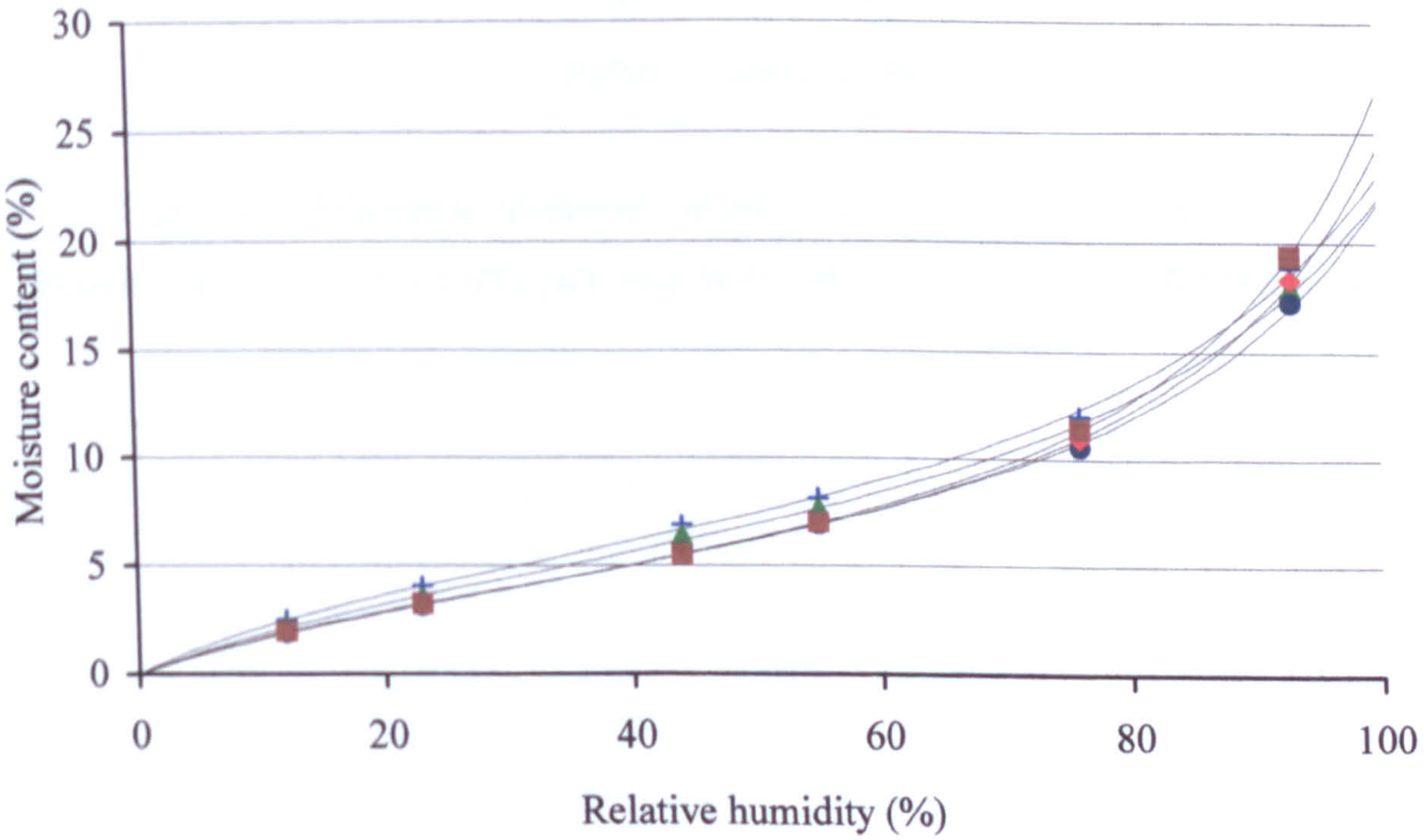


Figure 4.4 Adsorption isotherms for phthalic anhydride modified wood

Pyridine control (+); 6.5 WPG (▲); 17.5 WPG (●); 32.9 WPG (◆); 45.1 WPG (■)

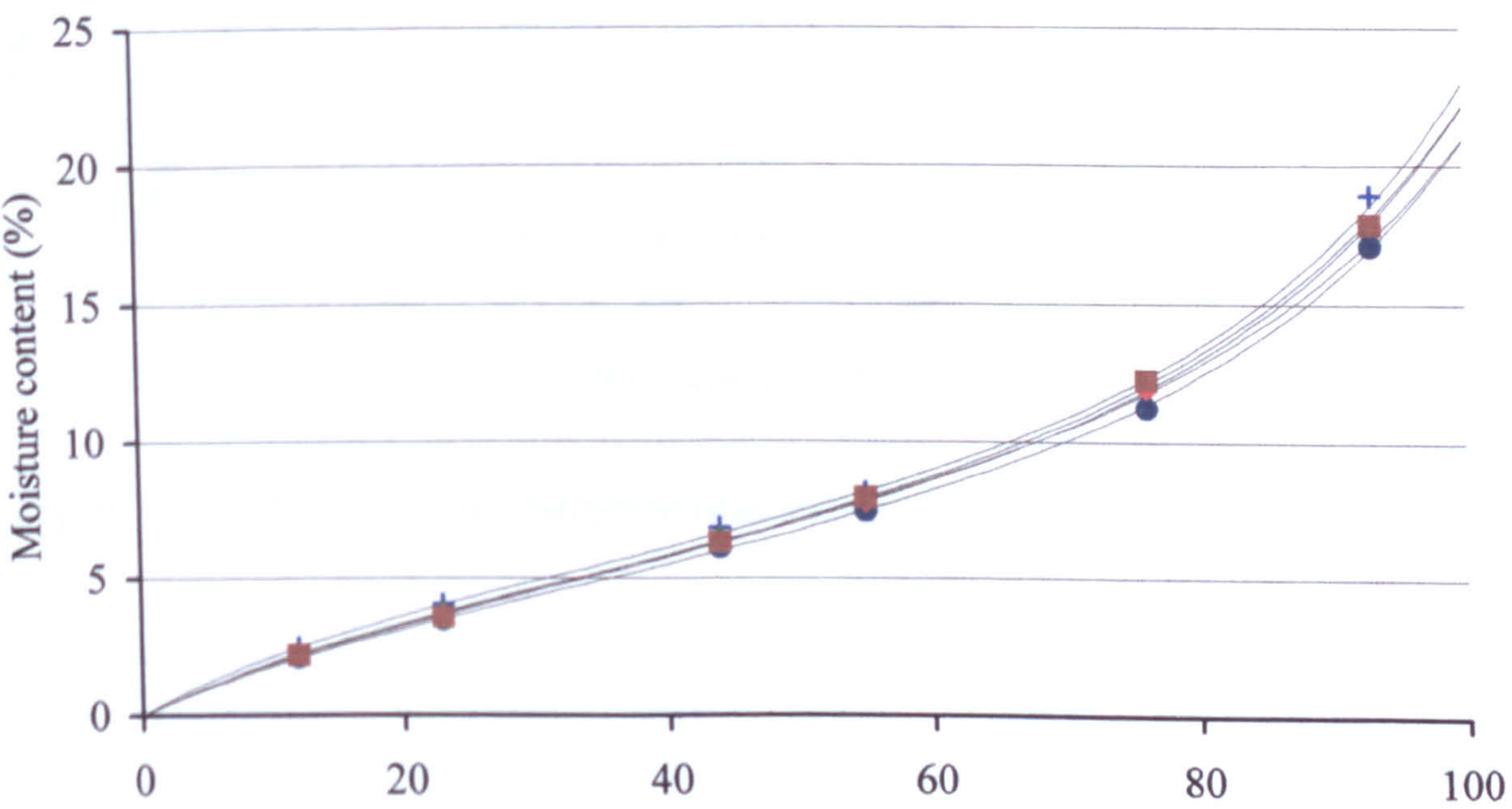


Figure 4.5 Adsorption isotherms for succinic anhydride modified wood

Pyridine control (+); 6.5 WPG (▲); 16.8 WPG (●); 29.4 WPG (◆); 38.6 WPG (■)

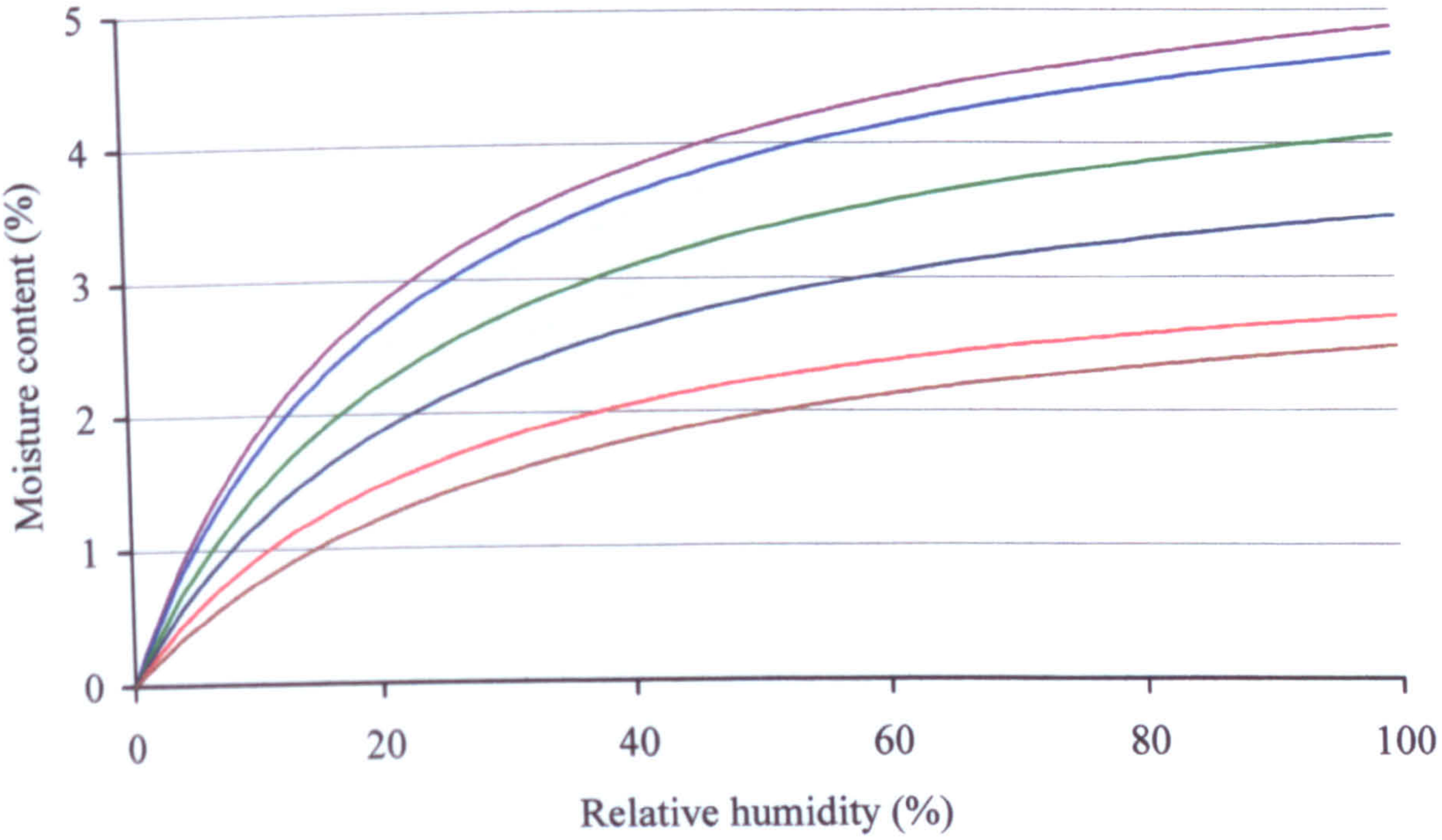


Figure 4.6 Monomolecular adsorption isotherms for acetic anhydride modified wood

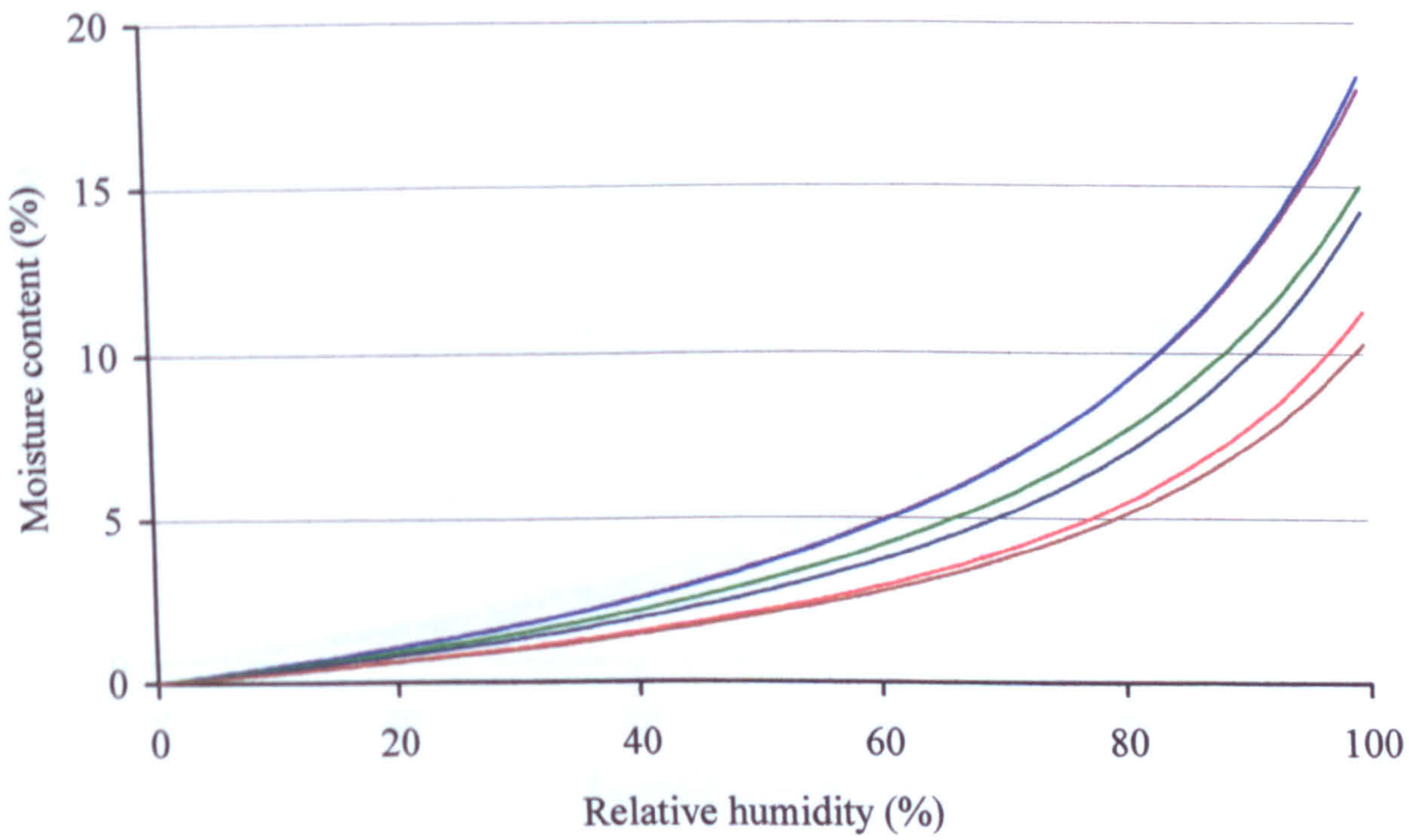


Figure 4.7 Polymolecular adsorption isotherms for acetic anhydride modified wood

Pyridine control (—); 5.4 WPG (—); 11.7 WPG (—); 16.2 WPG (—); 20.4 WPG (—); Untreated control (—)

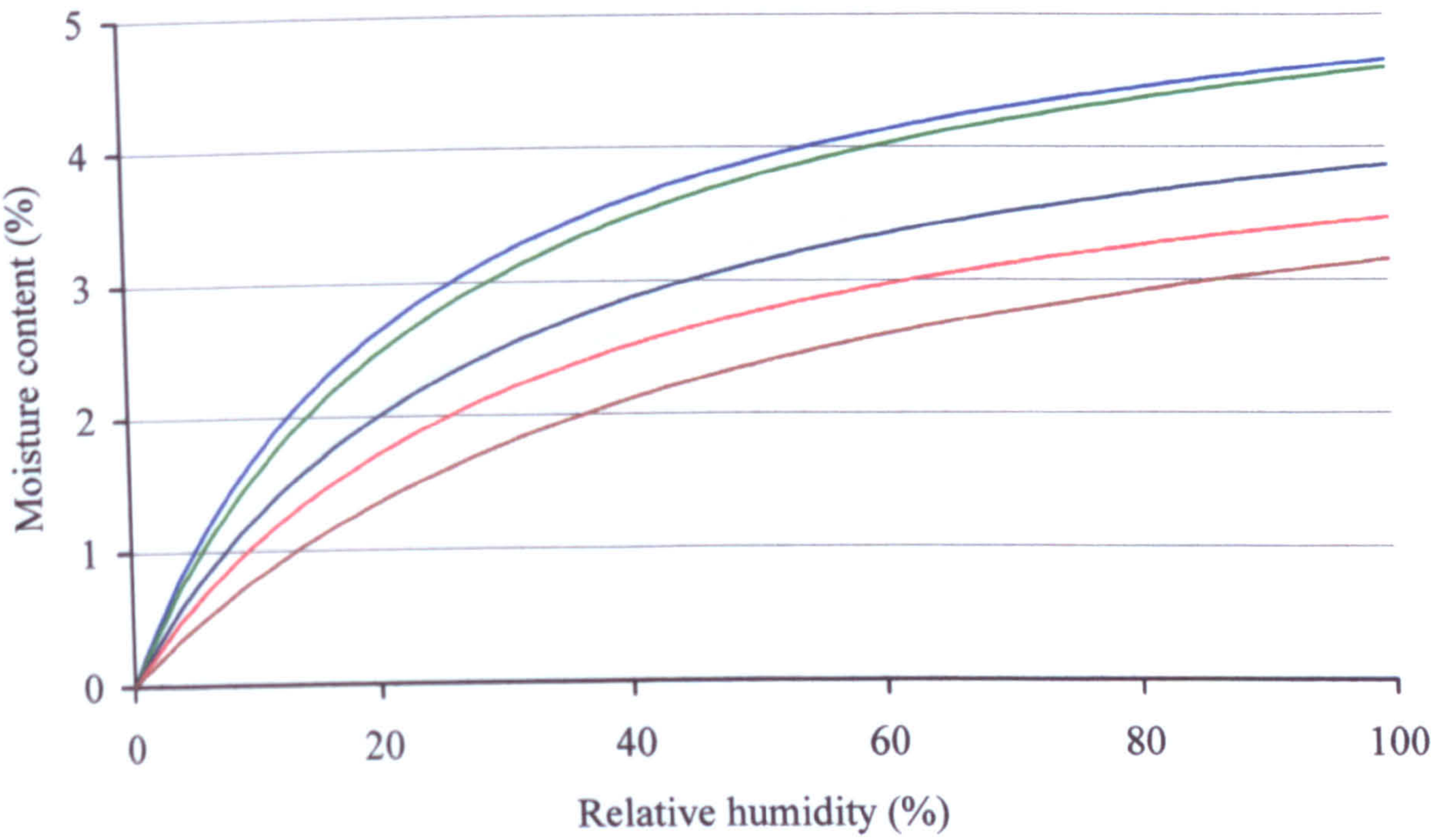


Figure 4.8 Monomolecular adsorption isotherms for butyl isocyanate modified wood

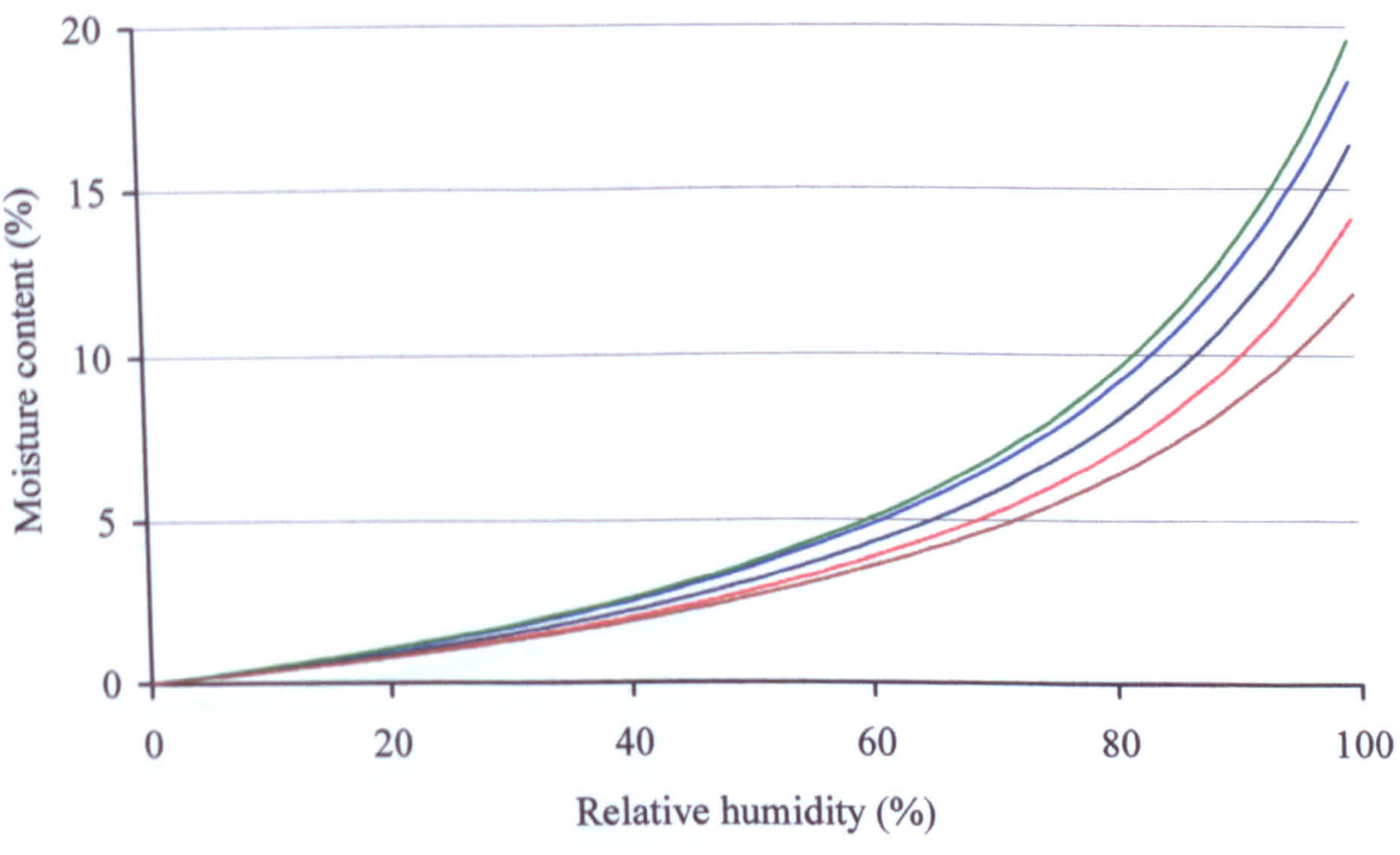


Figure 4.9 Polymolecular adsorption isotherms for butyl isocyanate modified wood

Pyridine control (—); 2.1 WPG (—); 8.2 WPG (—); 15.6 WPG (—); 22.9 WPG (—)

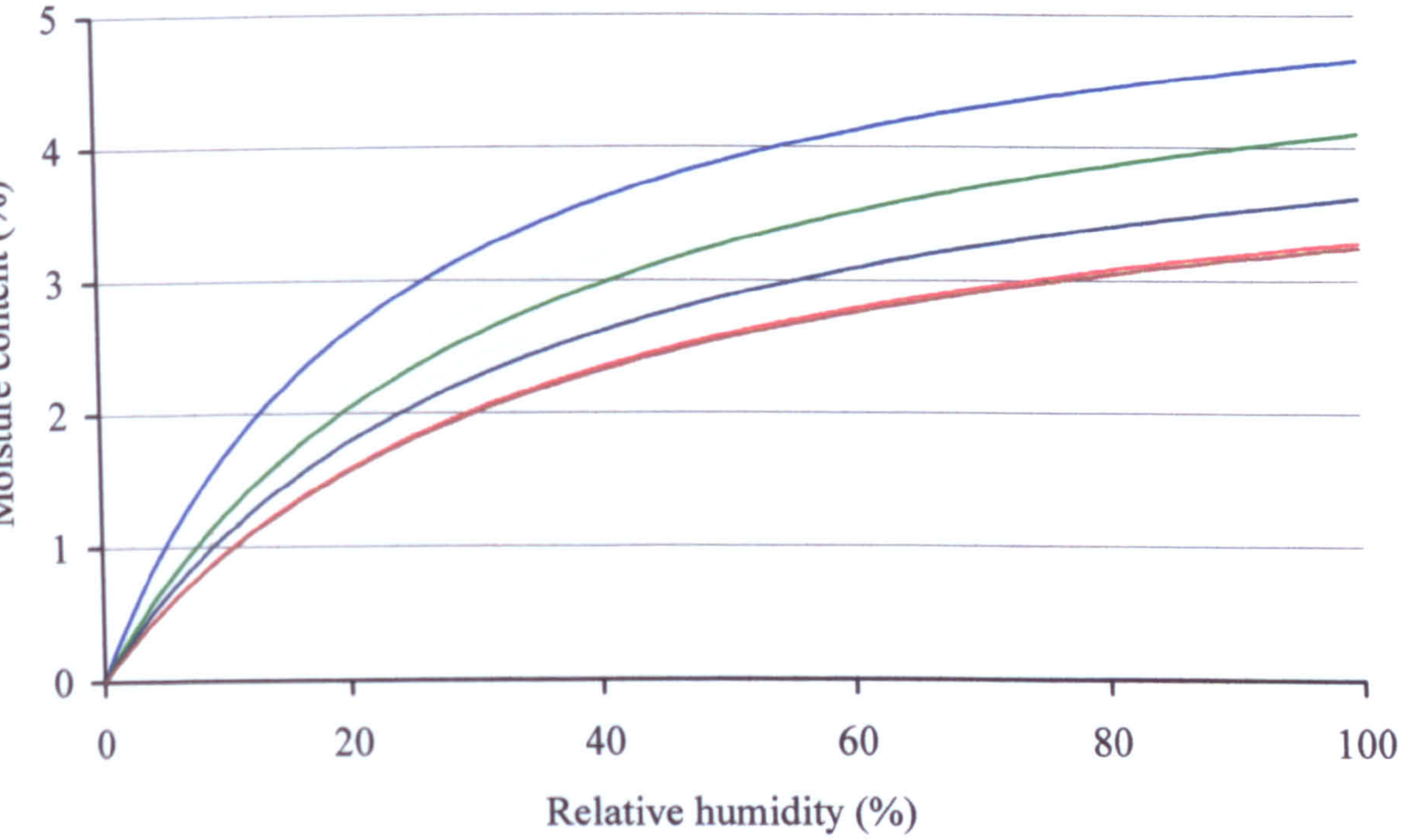


Figure 4.10 Monomolecular adsorption isotherms for alkenyl succinic anhydride modified wood

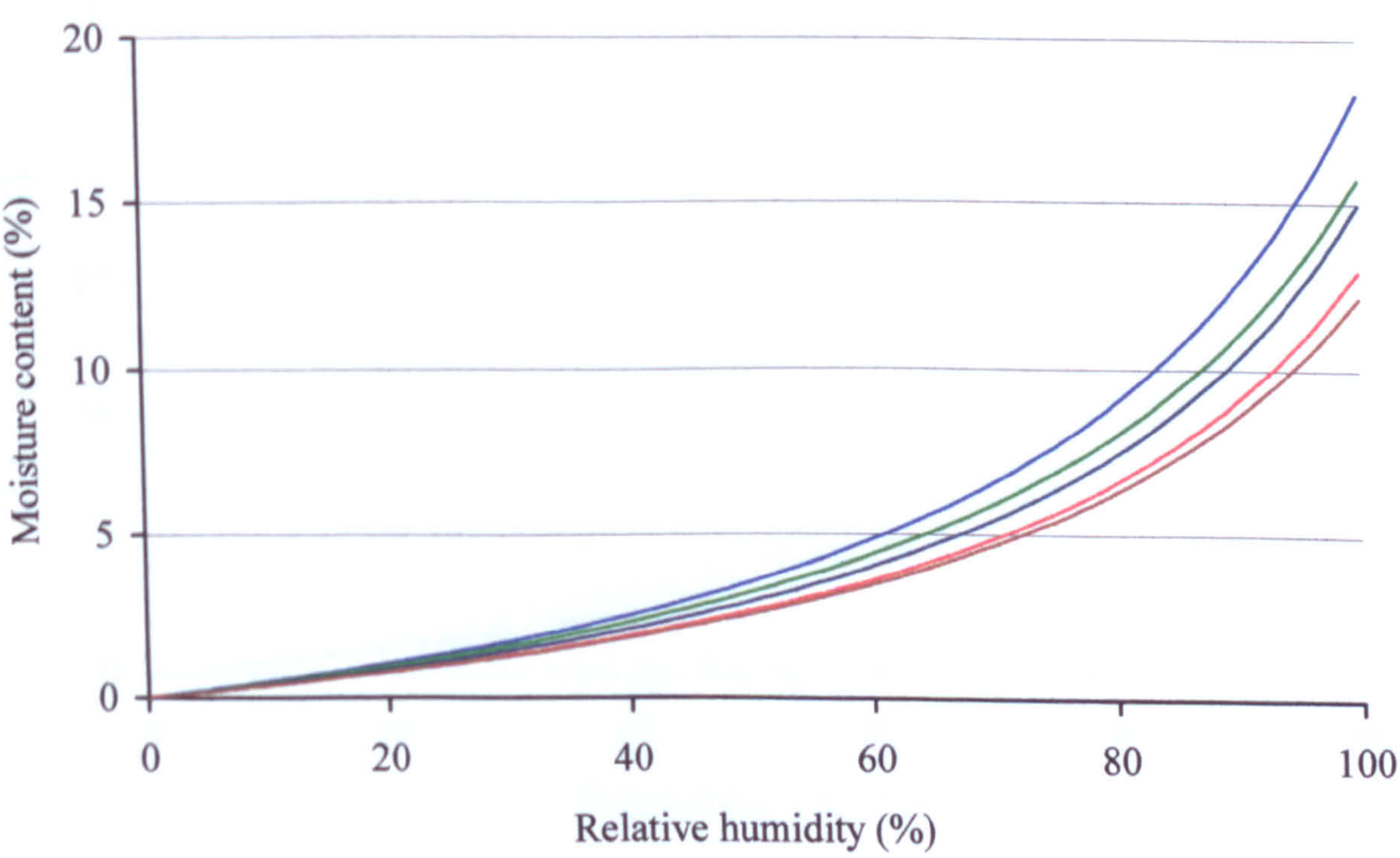


Figure 4.11 Polymolecular adsorption isotherms for alkenyl succinic anhydride modified wood

Pyridine control (—); 4.5 WPG (—); 12.0 WPG (—); 22.4 WPG (—); 31.8 WPG (—)

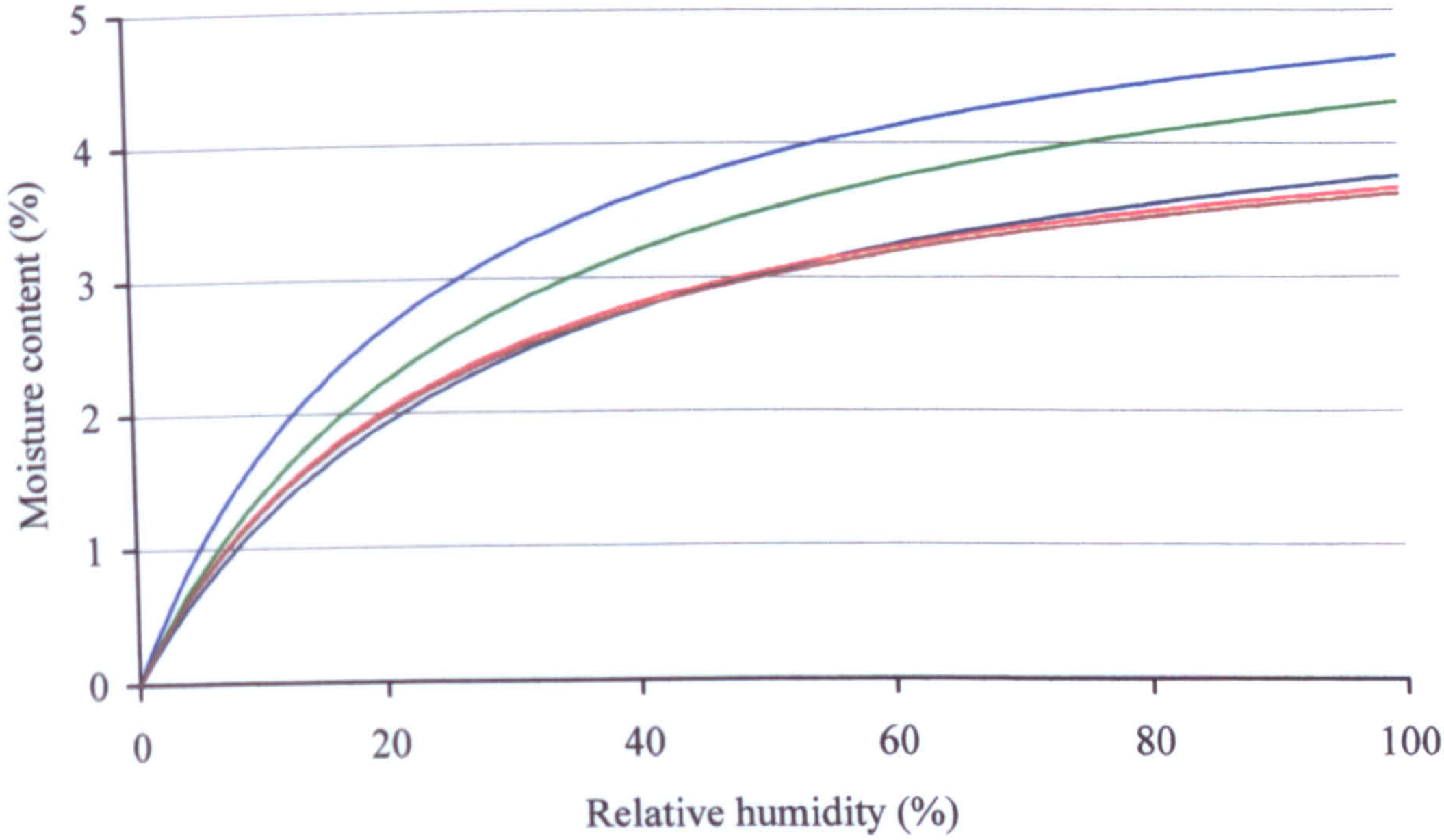


Figure 4.12 Monomolecular adsorption isotherms for phthalic anhydride modified wood

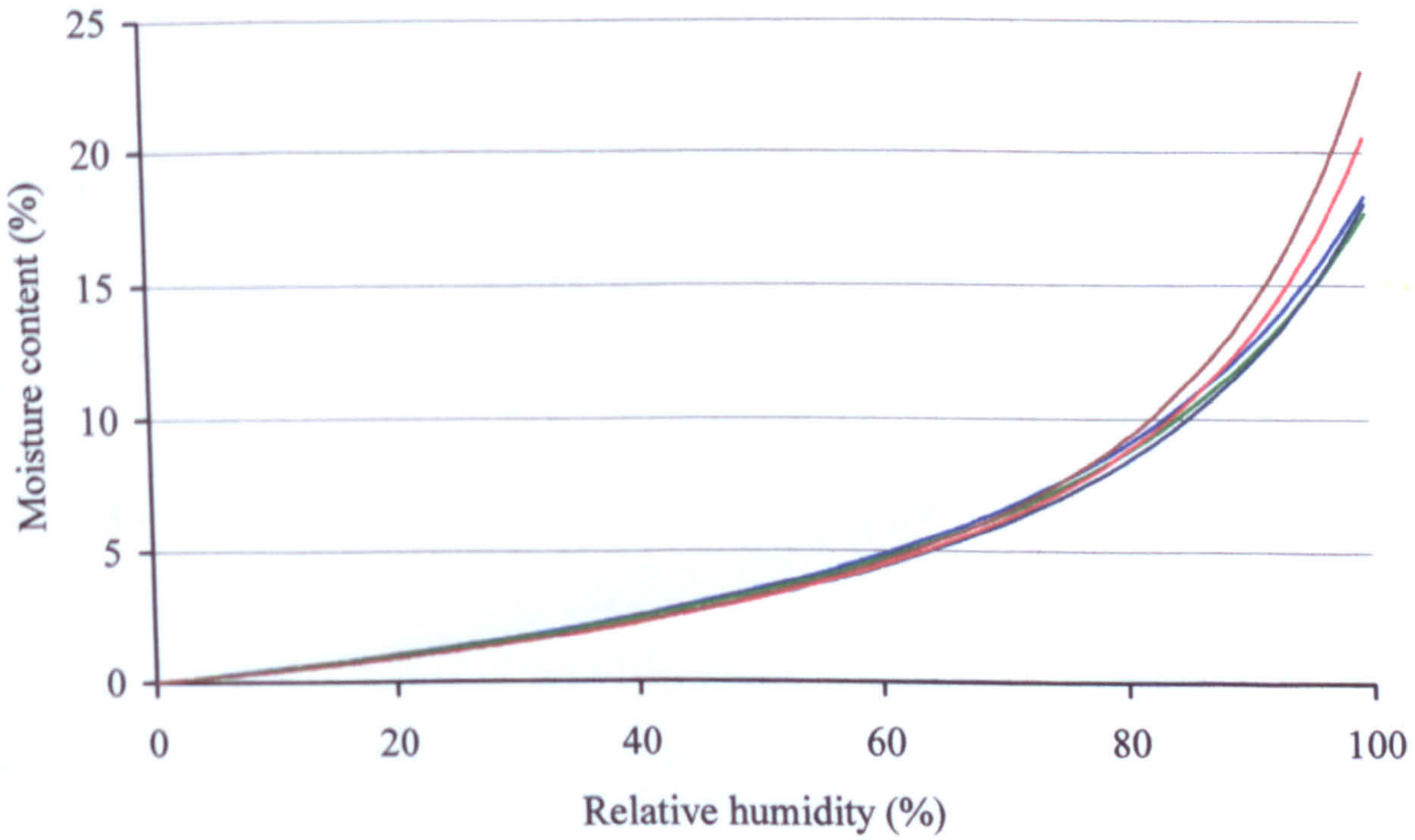


Figure 4.13 Polymolecular adsorption isotherms for phthalic anhydride modified wood

Pyridine control (—); 6.5 WPG (—); 17.5 WPG (—); 32.9 WPG (—); 45.1 WPG (—)

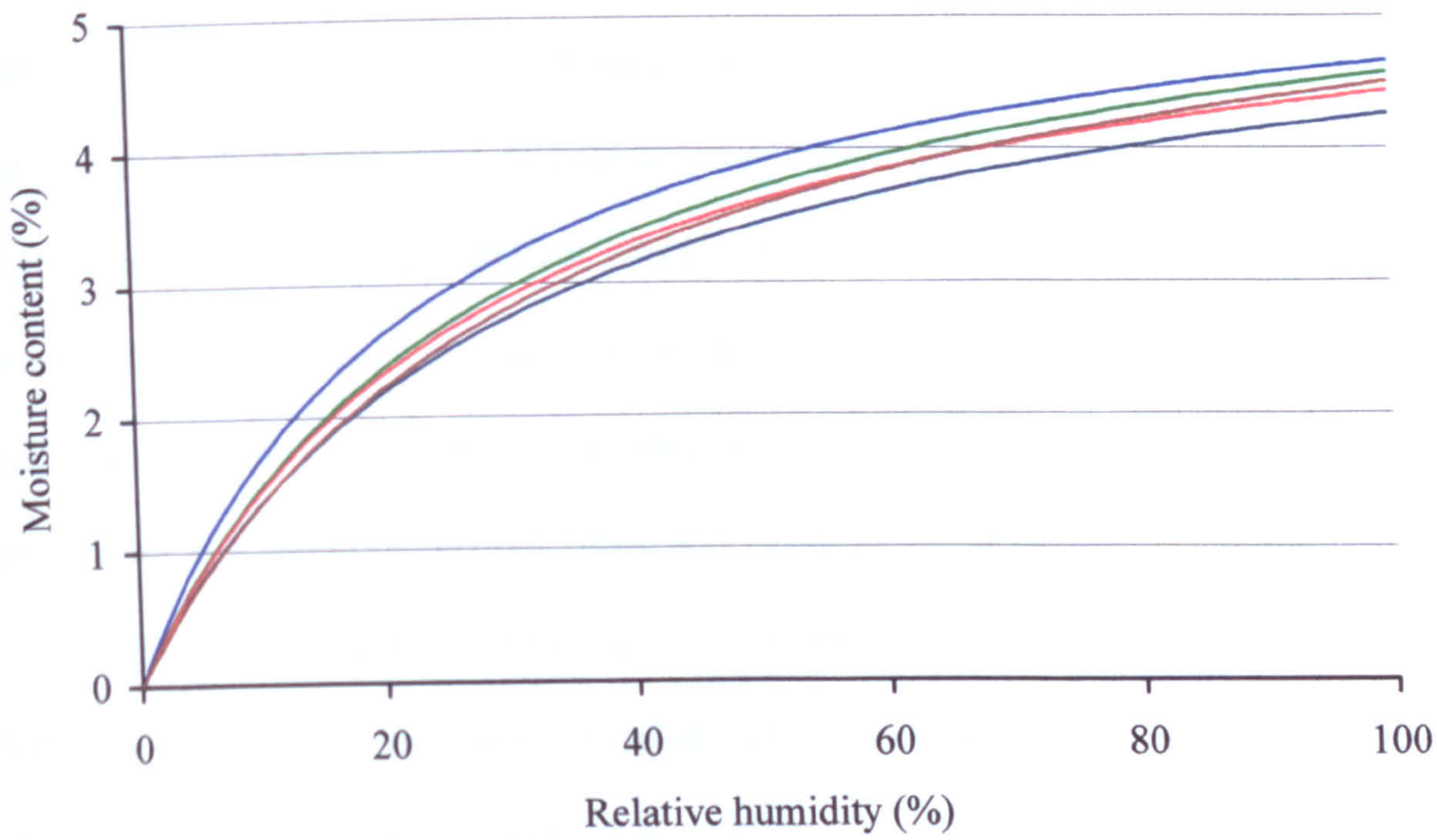


Figure 4.14 Monomolecular adsorption isotherms for succinic anhydride modified wood

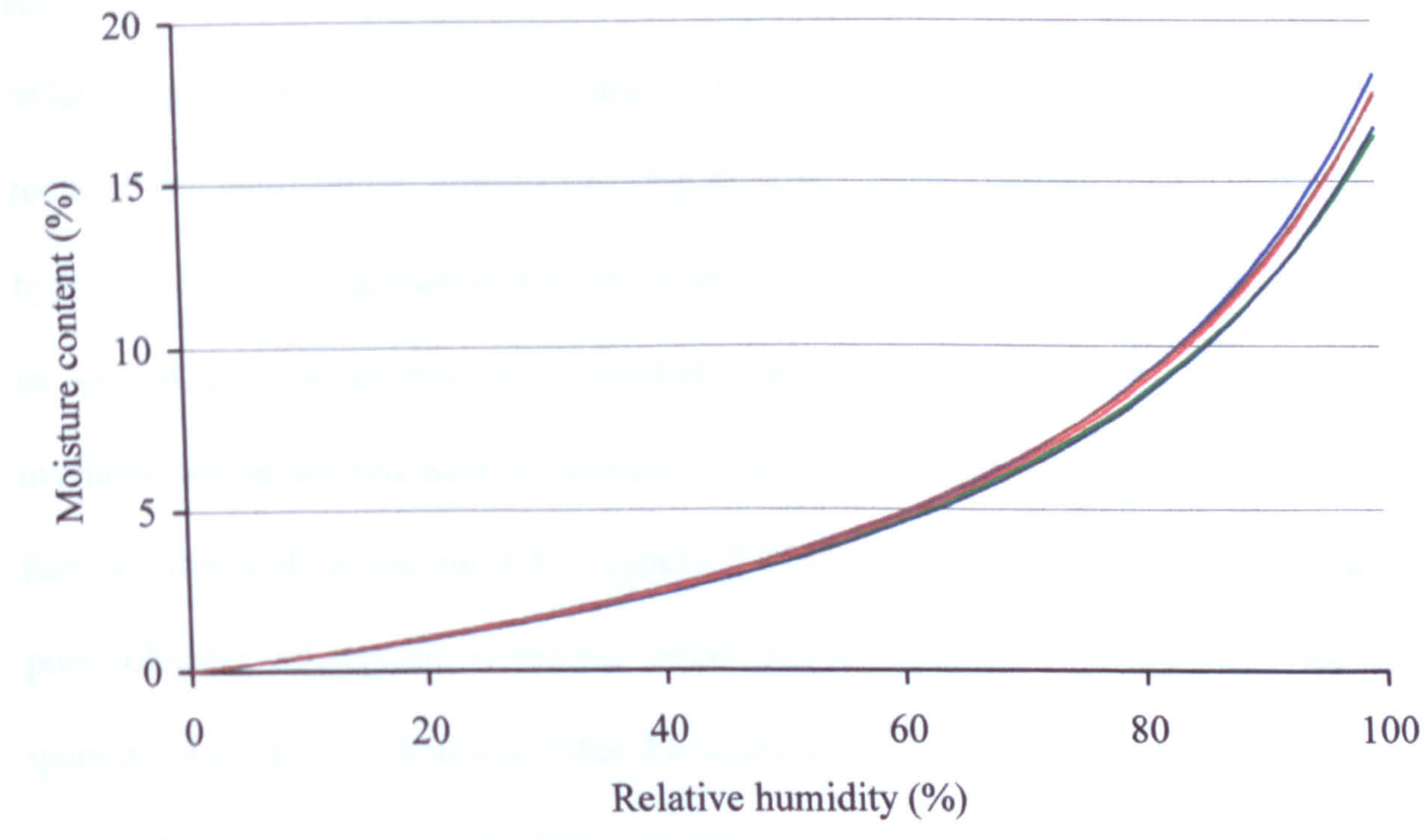


Figure 4.15 Polymolecular adsorption isotherms for succinic anhydride modified wood

Pyridine control (—); 6.5 WPG (—); 16.8 WPG (—); 29.4 WPG (—); 38.6 WPG (—)

surface sorption. The decrease in polymolecular sorption they attributed to extractives being within the wood cell wall, bulking the wall, and excluding polymolecular water. In the present study the difference in polymolecular sorption between the controls was only slight, and may even be an artefact of calculating the e.m.c. on the basis of original weight (this calculation was made for the pyridine control samples as well as for the modified samples). In this case the extractives in the sapwood may be largely located in cell lumina, and therefore may not affect polymolecular sorption.

Figure 4.1 indicates an obvious trend of reducing hygroscopicity with increasing WPG for acetic anhydride modified wood. This also holds for the monomolecular and polymolecular isotherms in figures 4.6 and 4.7. Similar trends were found for butyl isocyanate and alkenyl succinic anhydride modified wood (figures 4.2 and 4.3). The result for 2.1% WPG of butyl isocyanate is somewhat anomalous as this appears not to reduce hygroscopicity. Butyl isocyanate modification of wood to 2.1% WPG slightly reduced monomolecular adsorption (figure 4.8). This would be expected as the hydrophilic hydroxyl groups within the wood cell wall are reacted. However, an increase in polymolecular adsorption was recorded (figure 4.9), indicating that more space was available within the cell wall for dissolved water than in the controls. These effects are further discussed in section 4.5. Higher WPG's behave as expected, with total and polymolecular adsorption isotherms being lower as WPG increases (an increasing quantity of modifying chemical bulks the wood cell wall).

Alkenyl succinic anhydride modified wood behaved more in line with the expected trend of increasing WPG leading to reduced e.m.c.'s. Of interest in figure 4.3 is the only slight difference in the isotherms for wood modified to 22.4% and to 31.8% WPG. This appears to indicate that after a certain level of modification was reached only small reductions in hygroscopicity were achieved. There was a slight reduction in

polymolecular adsorption at 31.8% WPG, indicating that some bulking occurred (figure 4.11), though this was a much smaller reduction than might be expected from the quantity of chemical being introduced. The fact that no reduction in monomolecular adsorption was evident (figure 4.10) leads to the conclusion that additional reaction above 22.4% occurred in areas of the cell wall already heavily modified at 22.4%, and at hydroxyl sites that would otherwise be shielded from water by the long alkenyl chain of nearby reacted modifying chemical. The reason why these hydroxyl groups are reacted, and not those available for monomolecular sorption at 22.4% WPG, may be that the large alkenyl succinic anhydride molecule cannot reach and react with the less accessible hydroxyls within the wood cell wall structure.

Trends for phthalic and succinic anhydride modified wood (figures 4.4 and 4.5) are not obvious, with isotherms clustering together and in some instances crossing one another.

Phthalic anhydride modification reduces monomolecular adsorption up to WPG's of 17.5%, after which there was no significant change (figure 4.12). In figure 4.13 the polymolecular adsorption curves show no such reduction, with significant increases in polymolecular adsorption at WPG's of 32.9% and 45.1%. This causes the adsorption isotherms in figure 4.4 to cross over one another, with high levels of modification causing lower monomolecular but higher polymolecular adsorption. It appears that phthalic anhydride modification creates significantly more space in the wood cell wall but not a large number of new sorption sites. Isotherms drawn for phthalic anhydride modified wood by Risi and Arseneau (1958) show that at low relative humidities the e.m.c.'s were lower for wood modified to increasing WPG's, but as relative humidity reached the maximum measured (over 80%) the differences became smaller as the isotherms for the wood modified to higher weight gains became steeper. This seems to indicate the same

effect found in the present study, in which monomolecular adsorption was reduced while polymolecular adsorption increased. Again, this is further discussed in the context of the results from the swelling test (section 4.5).

Initial increases in WPG of succinic anhydride modified wood caused decreases in monomolecular and polymolecular adsorption as shown in figures 4.14 and 4.15, though for WPG's of 29.4% and above, isotherms begin to indicate increasing hygroscopicity (though at all weight gains, modified wood was less hygroscopic than unmodified wood). At WPG's of 29.4% and above, monomolecular adsorption started to increase, having reached a minimum at 16.8% WPG.

It should be noted that in the cases of each of the three cyclic anhydrides (succinic, phthalic and alkenyl succinic anhydrides) a hydrophilic carboxyl group is created each time a hydroxyl group is substituted (this assumes no cross-linking reactions occur as discussed in section 3.2.1). Any reduction in monomolecular adsorption was therefore presumably due to the shielding of adjacent hydrophilic sites (either on wood polymers or reacted modifying chemical) by the modifying chemical. The effect of such shielding would be expected to be much greater for alkenyl succinic anhydride (due to the long alkenyl group introduced for each hydroxyl reacted) than for the other two cyclic anhydrides.

4.4.3 Fibre saturation point

In this study the maximum capacity of the modified cell wall to hold water is of primary interest, as this will arguably have the greatest influence on the ability of fungi to decay wood. As stated earlier, the e.m.c. of wood at 100% RH as indicated by the sorption isotherm is sometimes used as a measure of FSP. Using this method to measure FSP is not ideal. Stamm (1964) argues that since adsorption curves rise sharply above a

relative humidity of 90%, extrapolation above this level cannot be made with any confidence. A better measure of FSP would probably be gained from the desorption isotherm as measurement in desorption involves drying down from the fully swollen state. Hysteresis effects tend to mean that FSP measurements from desorption are generally higher than those measured in adsorption. Such a method, however, is still open to error. Stamm (1964) and Skaar (1988) discuss several methods of measuring FSP and their advantages and disadvantages.

Using the method of extrapolation from the adsorption isotherm, untreated (unextracted) Corsican pine was found to have a FSP of 22.8%. Usually FSP would be expected to be 25-30% (Skaar, 1988). Spalt (1958) measured FSP from adsorption isotherms for various softwoods and found results similar to the present study, e.g. 22.8% (Redwood; *Sequoia sempervirens* Endl.), 24.6% (Ponderosa pine; *Pinus ponderosa* Dougl.) and 23.9% (Western white pine; *Pinus monticola* Dougl.). In most cases Spalt found FSP measured from desorption to be 3 to 5% higher than the adsorption measure (i.e. much closer to the expected measure).

The e.m.c. values at 100% RH measured here from the extrapolation of the adsorption isotherms, though not accurate measures of FSP, serve as an indication of relative values of FSP (Note: FSP values calculated here are done so on the basis of original wood weight). These values are plotted in figures 4.16 to 4.18 for total, polymolecular and monomolecular adsorption respectively.

The behaviour for total adsorption at 100% RH very much reflects that for polymolecular adsorption. This is as expected due to the much larger proportion of polymolecular sorption compared to monomolecular adsorption at high relative humidities, and is in line with findings of Spalt (1958). In the present study

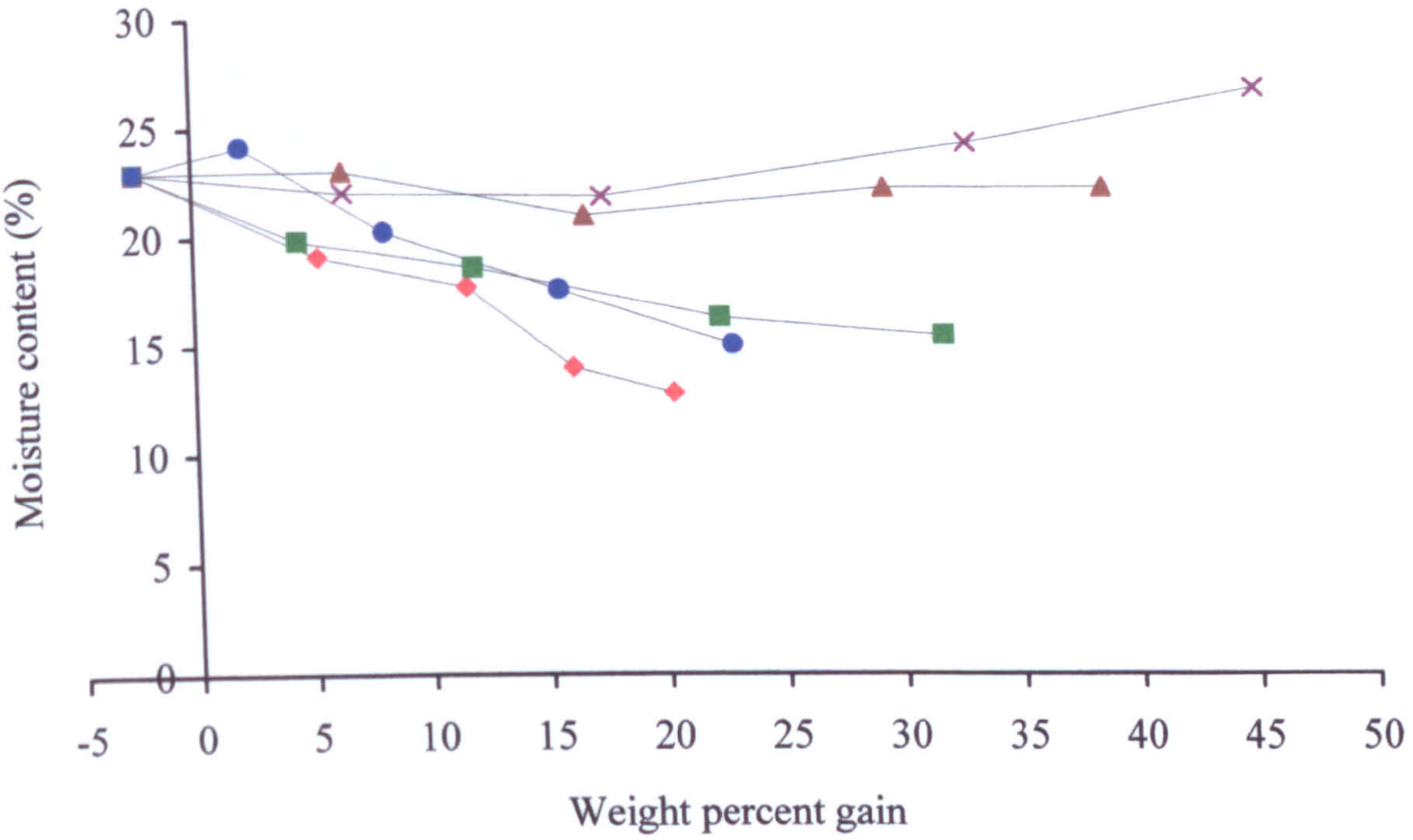


Figure 4.16 Total water adsorbed at 100% relative humidity (from isotherms)

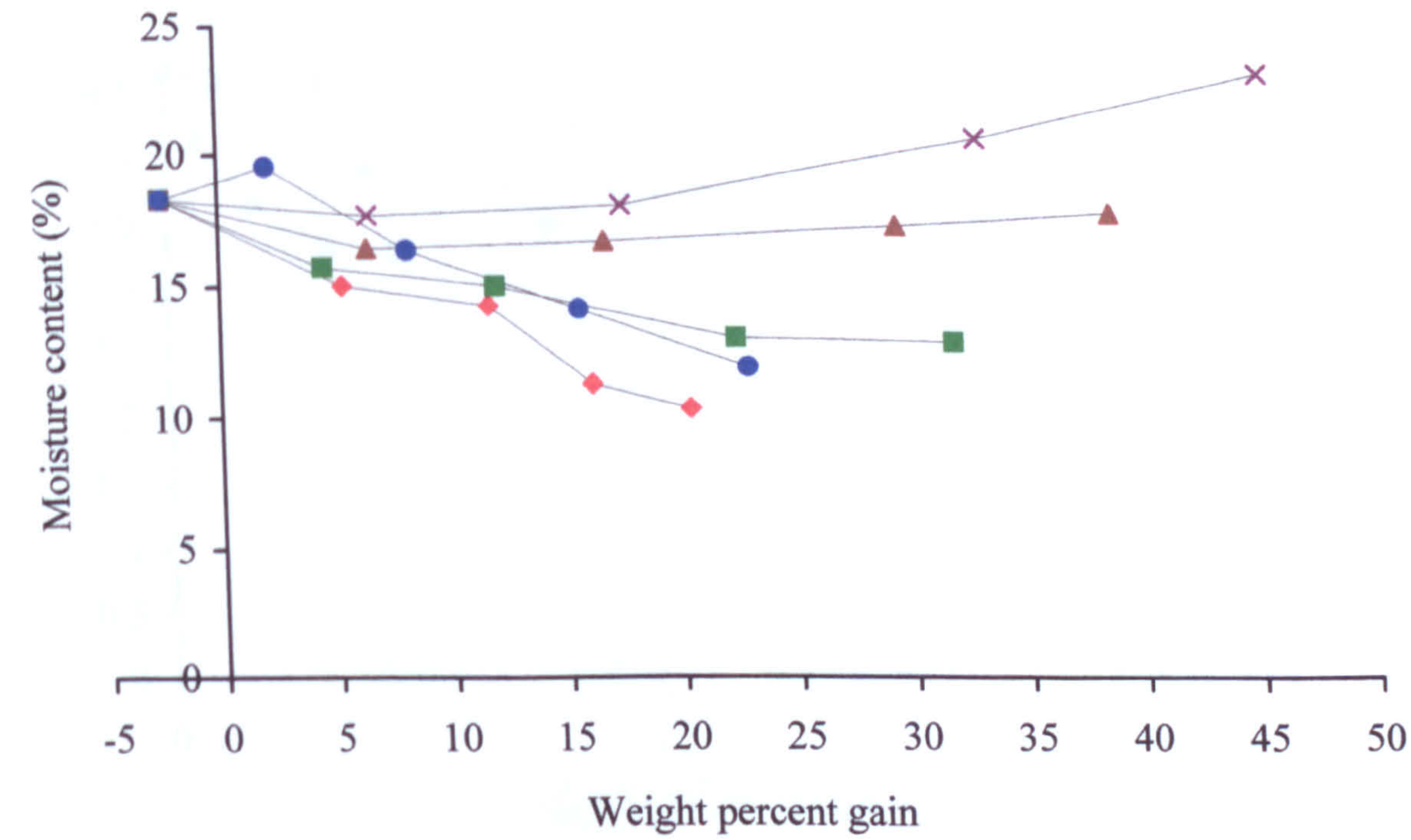


Figure 4.17 Polymolecular adsorption at 100% relative humidity

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲); Phthalic anhydride (×); Butyl isocyanate (●)

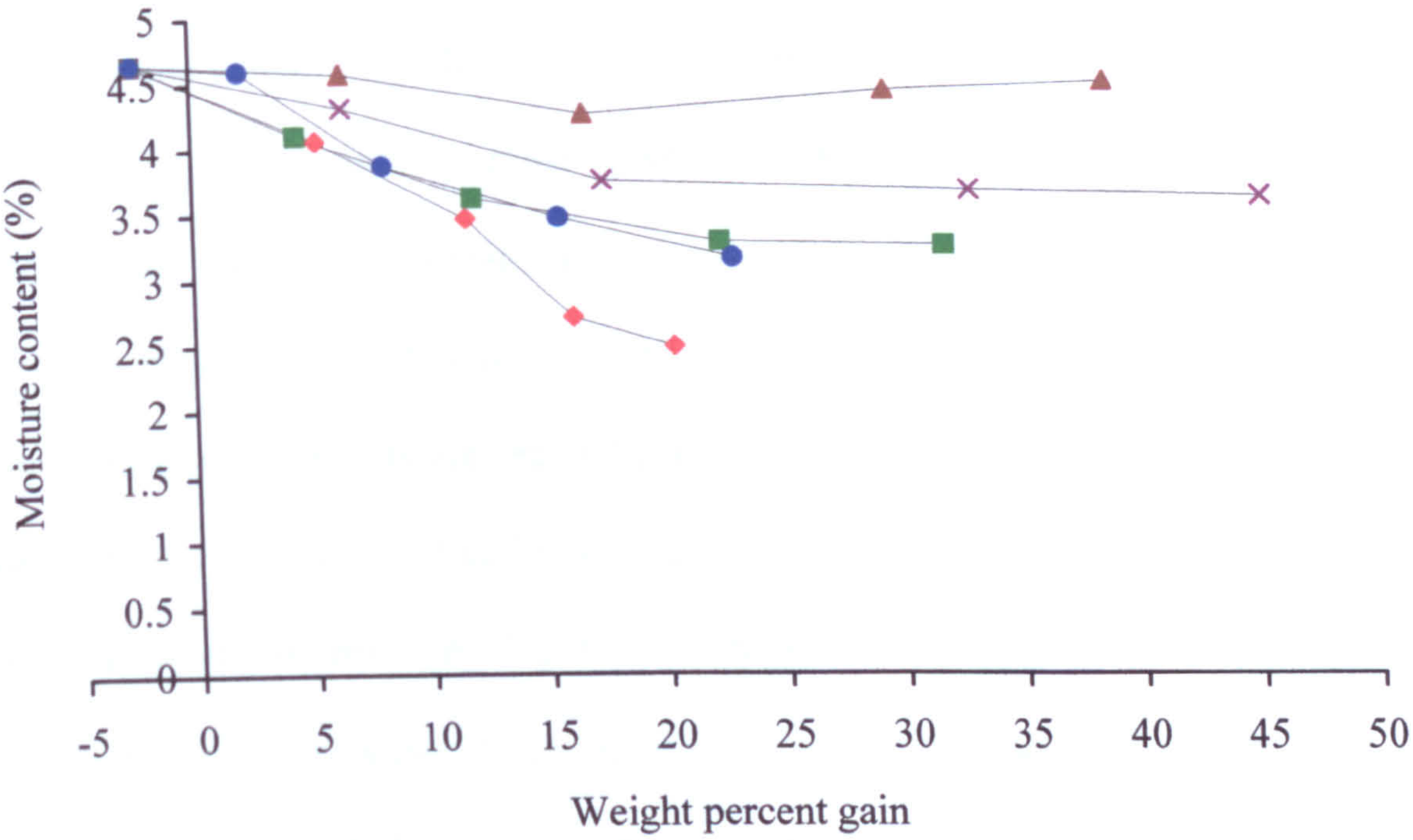


Figure 4.18 Monomolecular adsorption at 100% relative humidity

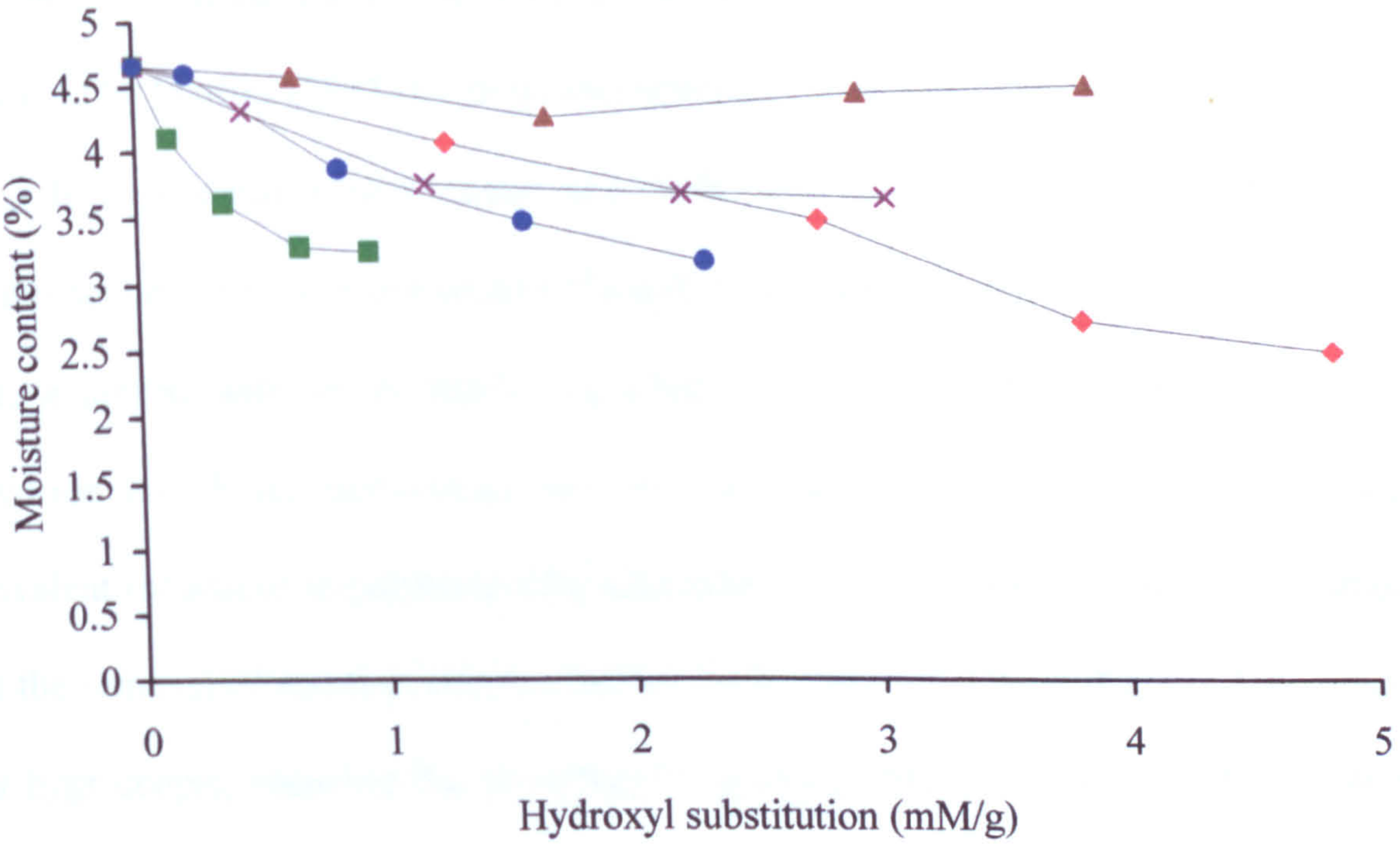


Figure 4.19 Monomolecular adsorption at 100% relative humidity as a function of hydroxyl substitution

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲); Phthalic anhydride (×); Butyl isocyanate (●)

monomolecular adsorption of different modified woods (at 100% RH, figure 4.18) also showed a similar pattern (with the exception of phthalic anhydride modified wood).

Acetic anhydride was the most effective chemical at reducing adsorption in both monomolecular and polymolecular forms. At the monomolecular level it is unsurprising that acetic anhydride modification causes greatest reductions in adsorption, since a greater number of hydroxyls are reacted per unit weight. Any shielding of sites by the longer hydrophobic chains of butyl isocyanate and alkenyl succinic anhydride was not as effective as actual site reaction. Figure 4.19 shows a plot of monomolecular adsorption (at 100% RH) against hydroxyl substitution due to modification. It can be seen that alkenyl succinic anhydride was much more effective at reducing monomolecular adsorption per hydroxyl reacted, followed by butyl isocyanate. This shows that the hydrophobic chains of these chemicals did shield hydroxyl sites. This result is similar to that found by Martins (1992) for butyl and octadecyl isocyanates (see section 2.4.1).

It is interesting that acetic anhydride also caused the largest reduction in polymolecular adsorption per weight of modifying chemical. Despite the higher weight gains achieved with larger modifying chemicals (note particularly alkenyl succinic anhydride and butyl isocyanate) and the corresponding bulking of the cell wall, equivalent reductions in polymolecular adsorption were not attained. This could indicate that the removal of sorption sites caused the wood to swell less as the cell wall became less hygroscopic, reducing the potential for polymolecular adsorption in line with the reduction in monomolecular adsorption. This explanation does not concur with the findings of Martins (1992) for isocyanate modified wood, who concluded that polymolecular sorption was not affected by a reduction in monomolecular sorption.

4.5 Results and discussion - swelling

4.5.1 Swelling of modified wood due to water saturation

Percent swelling (at saturation) of modified wood as a function of WPG is depicted in figure 4.20. Swelling in this figure has been calculated on the basis of modified volume, which in all cases was greater than reacted volume as described in section 3.4.4. It could be argued that calculation of swelling on the basis of modified volume (which increased as WPG increased) would cause the measurement of percent swelling to decrease even if the same amount of water were to enter the cell wall and cause the same increase in volume. Upon recalculating these figures on the basis of original (unmodified) volumes (i.e. replacing the denominator in equation 1, section 4.2.4, with 'Volume of dry unmodified wood'), this effect was found to be relatively small, and not to affect the relative positions of the various data points.

All the modifying chemicals were effective to some extent in reducing swelling. At higher levels of modification, phthalic and succinic anhydride were the least effective modifying chemicals at reducing volumetric swell. Butyl isocyanate and alkenyl succinic anhydride reduced swelling to a greater extent than acetic anhydride at low weight gains, though further reductions in volumetric swell became smaller as WPG increased, and the curves in figure 4.20 level off. No such level off occurred for acetic anhydride modified wood, and at WPG's over 17% this appears to be the most effective modifying chemical at reducing swell.

On closer inspection, the results for volumetric swell in figure 4.20 do not seem to correlate with those in figure 4.16 for moisture content at FSP. Wood modification with phthalic anhydride and succinic anhydrides both caused reduction in swelling despite insignificant changes or even increases in calculated adsorption at 100% RH (compare

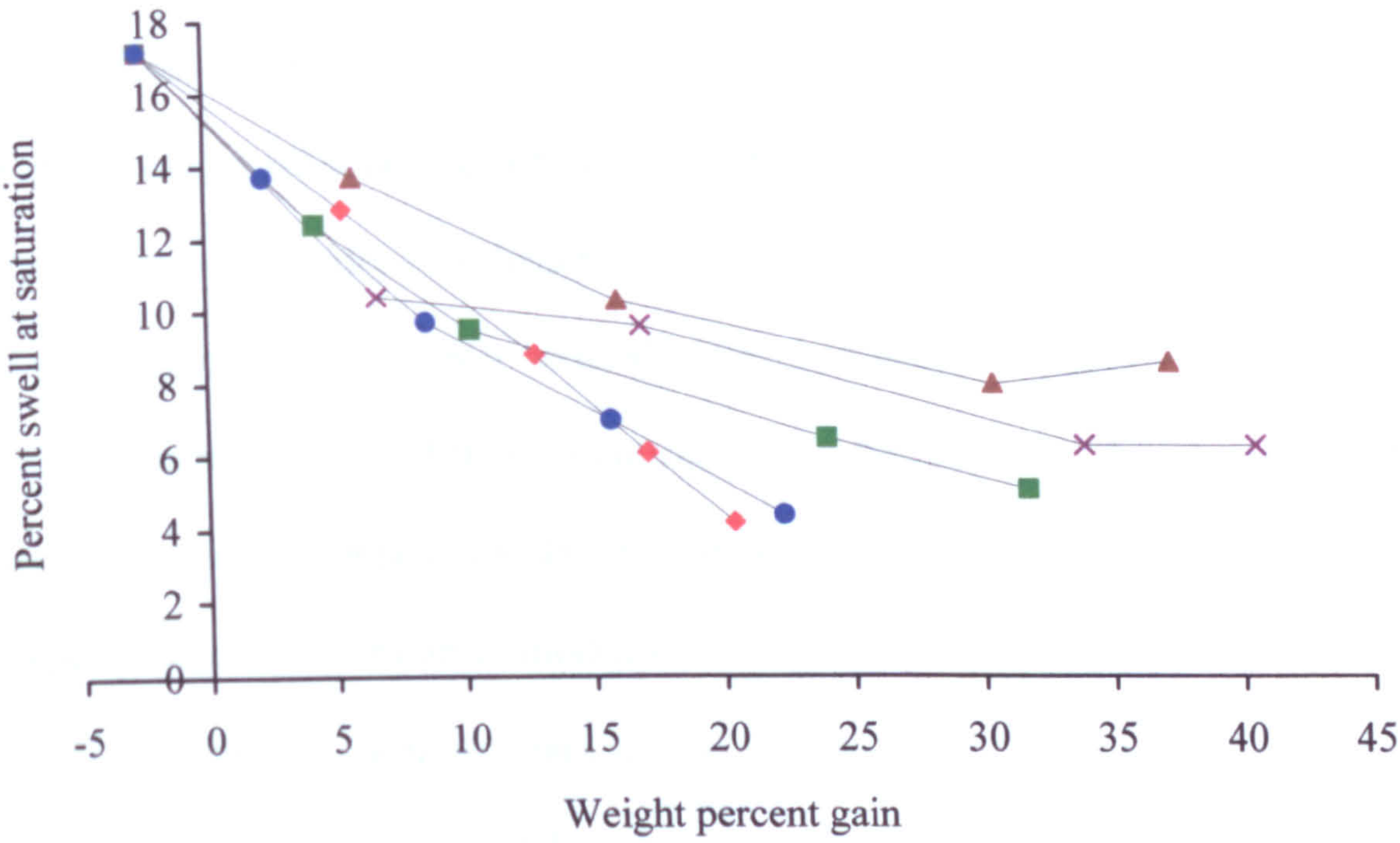


Figure 4.20 Percent volumetric swell of water saturated chemically modified wood

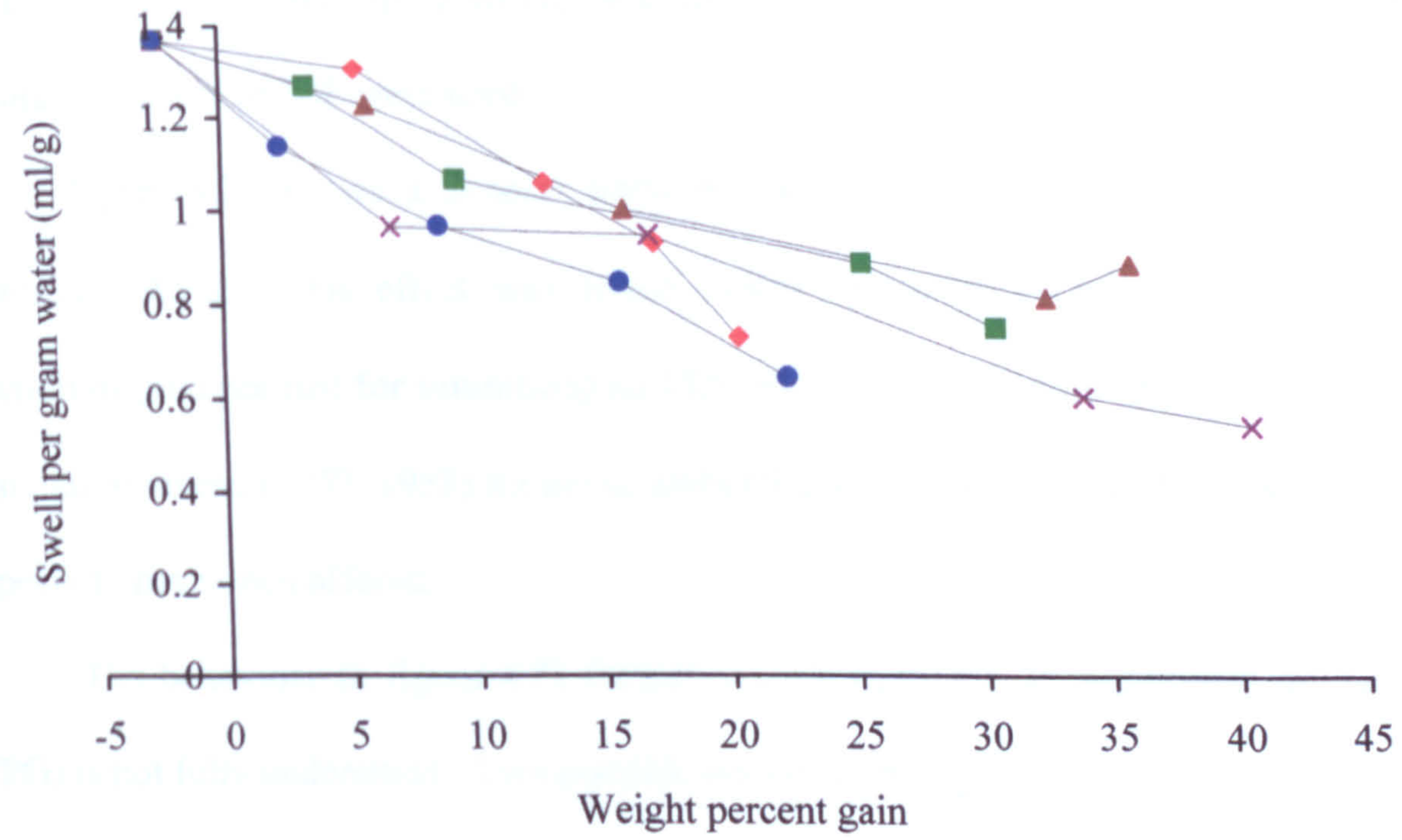


Figure 4.21 Swell per gram of water (at FSP) of chemically modified wood

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);
Phthalic anhydride (×); Butyl isocyanate (●)

figures 4.20 and 4.16). Additionally, the behaviour of the other three modifying chemicals seems to differ, with acetic anhydride modified wood appearing to swell more for a given weight of water adsorbed than butyl isocyanate (and in some instances, alkenyl succinic anhydride). To better illustrate this, the volumetric swell of modified wood per gram of water (at FSP) was calculated and is plotted against WPG in figure 4.21. This also removes any difficulties in comparing figures 4.16 and 4.20, in which the former deals with a percentage calculated on an original wood weight basis, and the latter with a percentage calculated on an modified wood volume basis.

The measure of swell per gram for the pyridine control (0% weight gain) in this figure is very high, at approximately 1.4ml/g. This is because of the low calculated moisture content at FSP (from extrapolation of the adsorption isotherm as discussed above); all points in the figure would be expected to fall lower on the y-axis if a more accurate measure of FSP were used.

Figure 4.21 shows a general trend of reduction in swell per unit water with increasing WPG. This effect was found across the range of e.m.c.'s and relative humidities (and not just for saturation) by Martins (1992) for various isocyanates and by Risi and Arseneau (1957, 1958) for acetic and phthalic anhydrides, though no explanation appears to have been offered.

The behaviour in figure 4.21 (reduced swelling power of water with increasing WPG) is not fully understood. Two possible explanations follow:

1. The difference in swell per gram of water is due to bulk wood effects, i.e. changes in the size of the cell lumina.
2. The cell lumina are constant in size, and the apparent change in swelling capacity of cell wall water is due to a void volume introduced into the wall with the modifying chemical.

The first of these two relies on modification either causing an increase in swelling (due to saturation) into the cell lumina or a reduction in the way in which the lumen size increases due to water swelling, both of which could cause the effect seen in figure 4.21. Cell lumina in some wood species have been found to enlarge or shrink when wood is saturated and swells, though even where this occurs it is often only by a small amount, and negligible compared to the swelling of the cell wall (Stamm, 1964; Siau, 1996). Although any change in lumen size in dry or saturated modified wood was not measured, it was assumed that no such change occurred. Any change in dimension due to water swell was considered to be solely due to the swelling of the wood cell wall.

The second explanation involves the theory that when wood is modified, the swell due to this modification is greater than might be expected by only taking the density of the modifying chemical into account. Nakano (1988; cited by Hill and Jones, 1998) suggested that the volume increase in wood amounted to the sum of the volume occupied by the modifying chemical (Vi) plus a void volume (also known as free volume, Vf) created in the cell wall polymeric network. If the explanation for reducing swell per gram of water can be explained on the lines of this free volume model, then the water within the modified cell wall will consist of water occupying Vf plus water causing the wall to swell.

In a recent study of wood modification with linear anhydrides (including acetic anhydride) Hill and Jones (1998) found that this void volume increased in proportion of the volumetric swell (due to modification) as the size of modifying chemical increased. Values for the proportion of Vf in volumetric swell were calculated by Hill and Jones for different size anhydrides. These values for the proportion of Vf for acetic anhydride and butyric anhydride modified wood (33% and 42% respectively) were applied to the

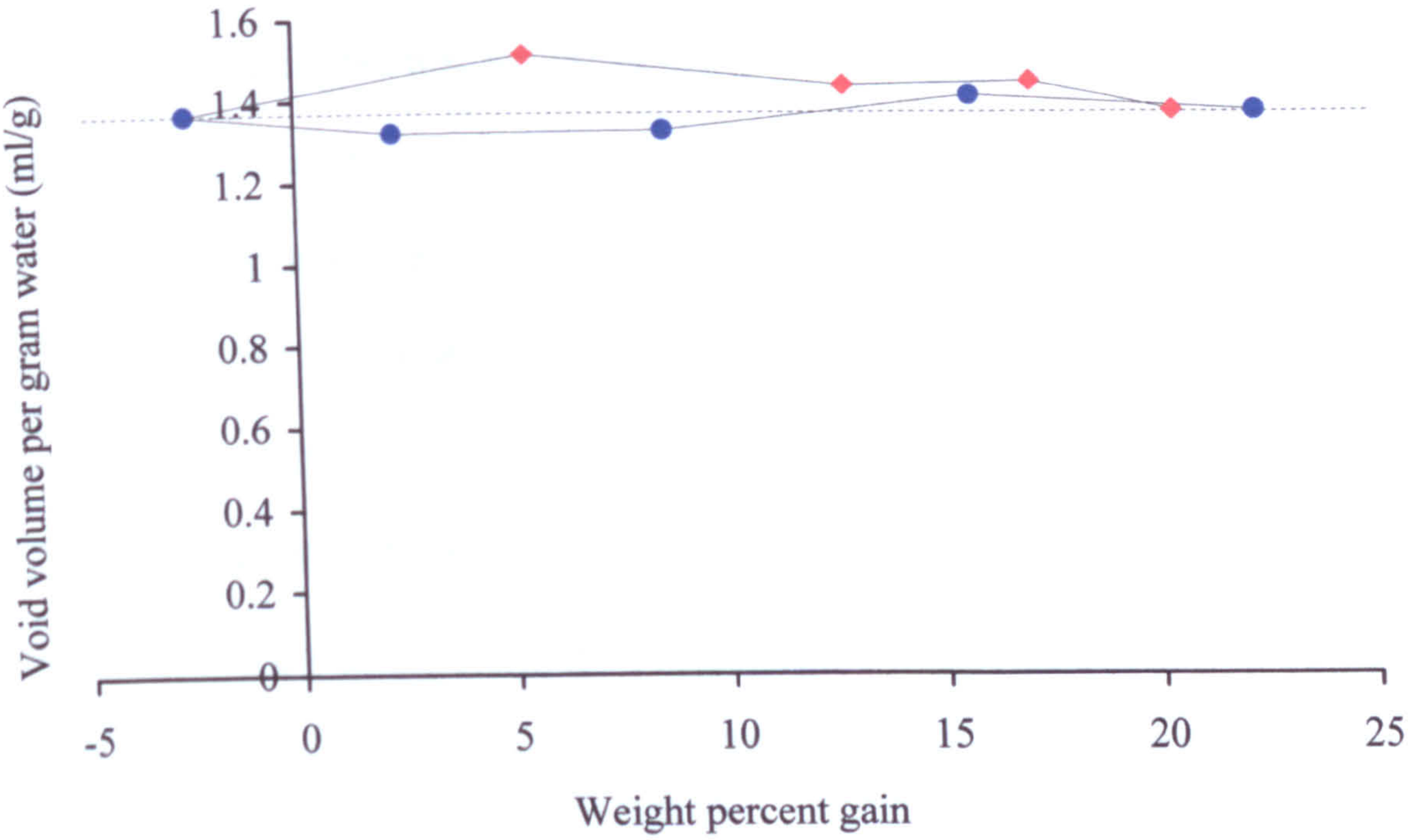


Figure 4.22 Cell wall void volume per gram of water (at FSP) of chemically modified wood

Acetic anhydride (◆); Butyl isocyanate (●)

swelling data in the present study for acetic anhydride and butyl isocyanate (the butyric anhydride value was applied to butyl isocyanate modified wood due to the similar size of adduct, though it is conceded that there are obvious chemical differences between the two). Assuming that Vf is filled with water without causing volumetric swell, this was added to the value for volumetric swell to give a figure for the apparent cell wall void volume occupied by water. This apparent cell wall void volume per gram of water was then plotted against WPG in the same way that swell per gram was plotted in figure 4.21. These recalculated data are given in figure 4.22. These recalculated data points for both chemicals were found to lie reasonably close to the horizontal, having similar measures of void volume per gram of water as the control (i.e. close to 1.4ml/g in this case). The free volume model therefore seems to offer a reasonable explanation of the differences in swelling recorded for wood modified to varying WPG's of different modifying chemicals.

This model is not without its problems when applied to data in this study. Hill and Jones (1998) calculated that for any of the modifications tested, the proportion of Vf in volumetric swell (due to modification) was higher at the lowest WPG's. This seems to fit the data for butyl isocyanate modified wood (as the void volume per gram of water is below the horizontal at low WPG's), though acetic anhydride modified wood does not follow this pattern. Further, if Vf was significantly larger for butyl isocyanate than acetic anhydride, as suggested by this calculation, then a greater volumetric swell due to modification to a given WPG would be expected for butyl isocyanate. The results presented in section 3.4.4 show that within the experimental constraints of this study, both chemicals appear to have caused wood to swell by the same volume.

There is no conclusive evidence here to suggest whether the change of swelling properties (i.e. differing swell per gram of cell wall water) is due to bulk wood effects (i.e. change in lumen size) or cell wall effects (i.e. introduction of free volume, Vf). Theoretically this could have a bearing on the decay resistance of chemically modified wood. As described in section 4.2.4, the swelling capacity of the cell wall could have an effect on the accessibility of the cell wall polymers to degradative agents released by decay fungi. If the only change is in lumen size, and swelling is proportional to the cell wall moisture content regardless of modification type or of WPG, then the effect of swelling becomes inextricable from the effect of cell wall moisture content. This is further discussed in chapter 9.

4.5.2 Aggregate volumetric swelling of modified wood

The percent volumetric swell of wood due to the effects of modification plus water saturation (aggregate swell) was calculated for comparison with saturated untreated wood. These results are given in table 4.3, alongside the 'dry' measures for

Table 4.3 Mean (and standard deviation) percent volumetric swelling of dry and water saturated modified wood calculated on the basis of pre-modification volume

Modifying Chemical	WPG	Volumetric swell (%)		Weight loss (%)
		Dry	Water saturated	
Untreated control	0	0	18.0 (1.6)	2.5
Pyridine control	-2.6	2.2 (1.5)	18.7 (2.8)	1.8
Acetic anhydride	5.4	8.7 (0.6)	21.8 (1.8)	1.0
	12.8	10.6 (1.6)	20.5 (2.7)	0.7
	17.1	11.6 (1.3)	18.4 (2.0)	0.6
	20.4	12.2 (0.4)	17.2 (1.3)	0.5
Succinic anhydride	5.8	6.1 (1.8)	20.4 (2.5)	2.5
	15.9	7.5 (1.5)	20.0 (1.5)	4.1
	32.5	12.9 (0.7)	23.9 (0.2)	5.5
	35.9	19.7 (2.0)	32.4 (2.9)	6.8
Phthalic anhydride	5.9	5.8 (1.0)	18.4 (1.4)	4.8
	15.3	9.7 (1.6)	20.2 (2.1)	8.6
	34.5	16.2 (0.6)	22.9 (0.9)	11.8
	40.5	18.5 (1.5)	26.2 (2.4)	14.8
Alkenyl succinic anhydride	3.4	5.6 (0.5)	18.9 (0.6)	0.8
	9.3	9.4 (2.1)	19.5 (2.6)	0.4
	25.3	16.4 (1.9)	23.1 (2.2)	0.2
	30.5	17.6 (3.5)	23.1 (3.7)	0.1
Butyl isocyanate	2.3	5.6 (2.1)	20.1 (3.0)	1.3
	8.6	8.9 (2.5)	19.3 (3.4)	0.7
	15.7	13.2 (1.8)	21.1 (2.5)	1.3
	22.3	14.3 (1.7)	19.7 (2.7)	0.5

volumetric swell due to modification, as calculated in section 3.4.4. The results listed are the mean values of 3 to 6 replicate samples. Standard deviations in some cases are quite large, reflecting a high spread of data in a single set of samples. While actual quantities listed should be regarded with some caution (a higher number of replicates would ideally be required), results are intended to be indicative.

The final column in table 4.3 shows the mean weight loss (due to leaching) occurring in samples over the two water soak stages.

Acetic anhydride and butyl isocyanate both cause an increase in aggregate swell at very low WPG's, but this does not continue with further addition of modifying chemical.

This may be due to low level of reaction causing a relaxation of the mechanical forces within the wood cell wall that resist volumetric swell, or even damaging the wood cell wall. Some evidence for this is that wood modified to a weight gain of 2.1% butyl isocyanate did not reduce monomolecular sorption in line with the reductions at higher weight gains (figure 4.8), possibly due to the exposure of new sorption sites. This effect and the finding of Hill and Jones (1998) that volumetric swell caused by low WPG's had a higher proportion attributable to Vf than higher WPG's help account for the increased sorption recorded for 2.1% butyl isocyanate modification (figure 4.2).

While there is no discernible pattern of increase or decrease in aggregate swell for butyl isocyanate modified wood (table 4.3), modification of wood with acetic anhydride reduces aggregate swell (after the initial increase described in the previous paragraph) to levels ultimately below that of untreated wood. This may be indicative of a reduction in saturated volumetric swell being caused by the decrease in sorption sites in the cell wall, i.e. a reduction in monomolecular adsorption leading to a reduction in polymolecular adsorption (and hence aggregate volumetric swell) as suggested in section 4.4.3. Even if this was the case, bulking seems to have been the predominant factor in the dimensional stabilisation of modified wood.

Modification of wood with the three cyclic anhydrides tended to cause increased aggregate swell with increasing WPG. In table 4.3 this is particularly evident for high WPG's of succinic and phthalic anhydride, where it appears that the natural forces restricting swelling in the wood cell wall have been overcome. In chapter 3 it was proposed that damage in succinic anhydride modified wood was caused by ionised carboxyl groups (introduced during modification) repelling one another and causing the wood to swell excessively, and ultimately break apart. It is also possible that these effects caused the extra swell recorded in table 4.3 for each of the cyclic anhydrides. Nakano

(1993) found that swelling due to ionisation of these groups in wood occurred significantly as pH increased above 5, so this could easily have occurred in the water soak. The excessive aggregate swell in succinic anhydride modified wood (35.9% WPG) was almost certainly due to cell wall damage. The increase in volumetric swell per gram of water at this WPG (in figure 4.21) also points to this. For phthalic anhydride modified wood, the increase in aggregate swell between the highest WPG's (34.5% and 45.1%) was greater than the increase due to modification only (table 4.3), which may be indicative of the beginnings of cell wall damage (damage to and weakening of the cell wall would account for a reduction in the mechanical resistance of the wall to swell).

Alkenyl succinic anhydride differs significantly in its sorption and swell characteristics from the other two cyclic anhydrides (as seen in figure 4.16 and 4.20). This is due in part to the difference in the number of carboxyl groups introduced onto the wood at a given WPG. Succinic anhydride introduces more than 3 times the number of carboxyl groups as alkenyl succinic anhydride at the same WPG. Bearing this in mind, it seems surprising that the difference in total swell between wood modified with the three cyclic anhydrides is not greater. This could be due to the high weight losses (due to leaching) of wood modified with succinic and phthalic anhydride, causing the measured values of volumetric swell to be lower than they otherwise would be at the listed WPG's. It is interesting to note that the weight losses for succinic and phthalic anhydride modified wood proved to be particularly high when compared with the 14 day leaching trial results (section 3.6). The higher than expected weight losses in the swelling trial are thought to be due to the oven dry and resoak procedure (as described in section 4.2.4). The oven drying (105°C) of water saturated modified samples may have created ideal conditions for the hydrolysis of susceptible ester linkages in succinic and phthalic anhydride modified

wood. When resoaked, any hydrolysed modifying chemical would have then leached out of the sample, resulting in the high weight losses recorded.

4.6 Summary of points raised in chapter 4

- On the basis of the coefficients of determination (R^2) the Hailwood - Horrobin adsorption isotherm gave a good fit to experimental data for unmodified and modified wood in this study. The e.m.c. value at 100% RH given by the fitted adsorption isotherm was taken as a measure of FSP, though this method gave lower values for FSP than would be expected from other measures.
- The differences between the adsorption isotherms for the untreated wood and the pyridine control samples was attributed to the presence of extractives in the former.
- Acetic anhydride proved the most effective modifying chemical at reducing water sorption at a given WPG. Succinic and phthalic anhydride proved largely ineffective, and even caused increasing levels of sorption at higher WPG's.
- Acetic anhydride modification proved the most effective at reducing monomolecular adsorption. This indicates that the actual reaction of hydroxyl groups is more effective at reducing access to sorption sites than site blocking. The latter was proved to be a mechanism of reduction of monomolecular sorption by butyl isocyanate and alkenyl succinic anhydride modified wood.
- Acetic anhydride also proved the most effective modifying chemical at reducing polymolecular adsorption, even being more effective than higher WPG's of other modifying chemicals and the corresponding increase in the bulking of the cell wall reacted with these chemicals. This is thought to have been due to the reduction in the number of sorption sites leading to a reduction in the potential for polymolecular

sorption. Some additional evidence for this was found when measuring the total swell (swell due to modification plus water saturation) of modified wood.

- Succinic and phthalic anhydride were the worst of the modifying chemicals at reducing volumetric swell. At low weight gains, butyl isocyanate and alkenyl succinic anhydride modified wood reduced swelling to a greater extent than acetic anhydride, though as the effectiveness of these two chemicals dropped with increasing WPG's, acetic anhydride became the most effective dimensional stabiliser (at WPG's greater than 17%).
- Swell per gram of water dropped with increased WPG for all chemicals. A possible explanation of this was that a void volume (Vf) was introduced into the wood cell wall during modification. This was found to provide a reasonable explanation for the lack of correlation between moisture content and swelling for different WPG's of different modifying chemicals.
- The total volumetric swell of the cyclic anhydride modified wood to levels above that of the water swollen untreated wood was thought to be due to the repelling effects of ionised carboxyl groups. The excess volumetric swell at high WPG's of succinic and phthalic anhydride modified wood was attributed to damage of the wood cell wall (leading to a reduction in the mechanical resistance of the cell wall to resist swell).
- The sorption behaviour of modified wood was accounted for by the different values of Vf for the different modifying chemicals and by the differing ability of the chemicals to increase or decrease aggregate volumetric swell (i.e. the volumetric swell due to the effect of modification plus the effect of water saturation).

Chapter 5. Cell wall pore accessibility of chemically modified wood

5.1 Introduction

The previous chapter studied the reduction in equilibrium moisture content caused by increasing weight gains of modifying chemical. This was attributed mainly to the bulking action of chemical in the wall displacing water that would otherwise be adsorbed in the cell wall as bound water. In addition, the extent of swell of modified wood due to water saturation was studied. Decay mechanisms have been proposed for brown and white rot fungi in which degradative agents diffuse into the wood cell wall and decay wood polymers at a distance from the fungal hyphae (see section 2.3). It was thought possible that the bulking of the cell wall by modifying chemical, along with reduced swell, could be causing a restriction in pore size. If pore size was restricted to the extent that degradative agents could no longer enter the cell wall, then decay would presumably be prevented.

To assess whether this is a likely mechanism of protection of wood by chemical modification, a measurement of the accessibility of these pores to probes of various sizes was attempted. This is known as the solute exclusion technique This was developed by Stone and Scallon (1968a, b) and used for decayed wood by Flournoy *et al.* (1991, 1993) as discussed in section 2.2.3.

5.2 Materials and method

5.2.1 Modified wood

Samples were selected from the pure culture test blocks (10mm x 20mm x 20mm) reacted as described in chapter 3. Time restrictions meant that only two weight gains of

each modifying chemical (highest and third highest) and the pyridine control were tested. Four blocks were selected from each relevant reaction set and these were cut in half to form samples measuring approximately 5mm (longitudinal) x 20mm x 20mm, as in chapter 4. This was done to speed up the diffusion of the probes used for the solvent exclusion technique into the centre of each sample. Again due to limited time, only half of each block (i.e. four samples total) were tested for each weight gain of each sample. Cutting the blocks in this way (i.e. after modification) may affect the results of this experiment as pathways direct to the middle portions of the cell wall may be opened that were inaccessible to modifying chemical or had received lower modification due to diffusion effects from the cell lumina.

Samples were oven dried and oven dry weight was recorded.

5.2.2 Determination of pore size

Samples were vacuum impregnated with a solution of 0.05% sodium azide and soaked for 3 months to ensure complete saturation. Sodium azide was used as a preservative to prevent bacterial attack of the wood (or probes) during the initial soaking or the experiment. Before incubation with probe solutions, the soaking solution was changed in an attempt to remove any material that had leached from the blocks during the initial soak so that this would not contaminate the solutions used in the experiment.

The probes selected were identical to those used by Flournoy *et al.* (1991) and are listed, along with their molecular weights and diameters, in table 5.1.

Of the six (non-water) probes, three were sugars and three (the three largest probes) were dextrans.

Stone and Scallon (1968b) list three properties of probes necessary for the applicability of the solute exclusion technique:

Table 5.1 Molecular weights and diameters of probes

Probe	Molecular weight	Probe diameter (Å)*
Water	18	4
Glucose	180	8
Maltose	342	10
Raffinose	504	12
Fluka AG	6000	38
Pharmacia T10	11200	51
Polysciences 15-20K	17500	61

* Probe diameters from sources cited by Flournoy *et al.* (1991)

1. Probes act as spheres of a particular diameter. For linear dextrans of the type used in this study, this has been found to be the case (Grotte, 1956, cited by Stone and Scallon, 1968b).
2. The range of molecular diameters for a probe (governed by the molecular weight range for a given dextran) should be small compared to the range of pore sizes within the porous body being examined. Stone and Scallon (1968b) concluded that the dextrans used (similar to those used here) satisfied this condition because the wood and pulp fibres they measured had a comparatively wide pore distribution. Although it was expected that chemical modification would reduce pore size (rather than increase it as in the case of pulping), the relative distributions of the probes and the modified wood pores was not considered further in the present study. The dextrans used in this study were not expected to be able to penetrate the modified wood cell wall to any significant extent. Flournoy *et al.* (1991) found that the same three dextrans were unable to penetrate unmodified undecayed wood. The three probes expected to penetrate wood were all sugars, having no molecular weight variation within a sample.
3. Probe molecules should not be adsorbed onto the surface of the wood, as this would cause a reduction in the concentration of the solution and an apparent increase in

accessibility. Stone and Scallon (1968b) found adsorption to be negligible for wood and pulp fibre, and in this study it was assumed also to be negligible for modified wood samples.

Stock solutions (3% concentration) of each probe were prepared in aqueous 0.05% sodium azide solution.

Wet modified wood samples were dabbed dry and weighed in a sample tubes to 4 decimal places. Probe solution was then added (approx. 7ml per sample) and the weight (4 d.p.) recorded. Sample tubes were then sealed and incubated in a control temperature room (20°C) for one week. Preliminary tests had shown this time to be sufficient for the penetration of the sample by the probes and equilibrium concentration of the solution to be reached. After this period the solution was decanted from the sample and set aside for concentration measurement. Wood samples were then soaked in aqueous 0.05% sodium azide (approx. 100ml of solution per sample) for one week to remove the probe from the wood. To ensure probe was fully removed, the soaking solution was changed after two and five days. The procedure was then repeated for the next probe.

The quantity of water in each modified wood sample was calculated by subtracting oven dry weight from the saturated weight of the sample. The quantity of this water accessible to each probe was calculated from the change in concentration of the probe solution after incubation with the wood block. This was determined using a Knauer differential refractometer in a controlled temperature room (20°C) with the undiluted 3% stock solution of the given probe in the control cell. A comparison of the differential refractometer reading with readings on a calibration curve, prepared by adding known quantities of water (0.05% sodium azide) to the stock solution, gave the quantity of water in the wood sample which had diluted the stock solution during incubation.

The quantity of water in the sample which had diluted the probe solution during incubation, as measured above, was the accessible water in the sample to that given probe. This was divided by the original (unmodified) weight of the wood block to give a measure of the volume accessible water per gram of wood (ml/g). By presenting this measure as volume accessible it has been assumed that the specific gravity of water in the sample (and in the wood cell wall) is 1. The original (pre-modification) weight of the sample was calculated on the basis of modified weight and WPG as in chapter 4. The unmodified weight was used in preference to modified wood weight because it was decided that this would give a more comparable of the accessible water in a given volume of swollen cell wall material.

In the calculation of accessible volume in the cell wall to probes of a given size a further assumption has been made. This is that the concentration of the solute in the accessible pores is equal to that in the solution surrounding the sample. This assumption has been criticised by Alince (1991) as discussed in section 2.2.3. Since each probe would have been subject to the same effects for each modification type, as presumably would degradative agents diffusing into the wall from fungal hyphae, the measurement of the apparent accessible volume was still considered valuable.

5.3 Results and discussion

Table 5.2 gives the mean accessible pore volumes available to each probe in water swollen modified wood. It was noticed that there was significant variation between wood samples in a given set. This was attributed to variation in density of the wood samples (prior to modification). To overcome problems of wood density variation, results have been presented in table 5.3 on the basis of the volume of accessible water in the cell wall, and not that in the whole wood sample. To do this it was assumed that the three largest

Table 5.2 Mean (and standard deviation) pore volumes of chemically modified wood accessible to probes of various sizes

Modifying chemical	W.P.G.	Accessibility (ml/g) to probes of increasing diameter						
		4Å	8Å	10Å	12Å	38Å	51Å	61Å
Pyridine control	-2.6	1.70 (.11)	1.57 (.08)	1.52 (.09)	1.51 (.10)	1.33 (.11)	1.33 (.10)	1.30 (.12)
Acetic anhydride	12.8	1.32 (.07)	1.19 (.07)	1.19 (.07)	1.18 (.08)	1.10 (.05)	1.09 (.06)	1.06 (.05)
	20.7	1.39 (.12)	1.28 (.12)	1.28 (.02)	1.28 (.12)	1.23 (.13)	1.23 (.13)	1.20 (.15)
Succinic anhydride	15.9	1.35 (.06)	1.24 (.04)	1.19 (.04)	1.14 (.04)	1.01 (.03)	1.02 (.05)	1.02 (.05)
	35.2	1.36 (.04)	1.17 (.05)	1.08 (.04)	1.09 (.05)	0.98 (.05)	0.93 (.06)	0.95 (.06)
Phthalic anhydride	16.5	1.41 (.04)	1.18 (.03)	1.19 (.05)	1.23 (.05)	0.98 (.06)	1.01 (.06)	1.00 (.06)
	38.7	1.36 (.06)	0.88 (.07)	1.04 (.07)	1.03 (.04)	0.87 (.05)	0.84 (.04)	0.88 (.05)
Alkenyl succinic anhydride	9.9	1.41 (.06)	1.29 (.05)	1.24 (.05)	1.26 (.06)	1.16 (.06)	1.17 (.06)	1.10 (.02)
	29.3	1.22 (.04)	1.07 (.03)	1.02 (.02)	1.11 (.03)	1.03 (.04)	1.06 (.05)	0.95 (.02)
Butyl isocyanate	8.7	1.41 (.07)	1.26 (.08)	1.26 (.08)	1.26 (.08)	1.17 (.06)	1.18 (.07)	1.14 (.07)
	21.6	1.35 (.09)	1.27 (.08)	1.23 (.09)	1.24 (.10)	1.19 (.11)	1.21 (.09)	1.20 (.11)

probes had not penetrated the wood cell wall (as found by Flournoy *et al.*, 1991). The measured accessible water for these three probes for each sample was averaged to obtain a measure of lumen volume. This was then subtracted from the measured quantity of water accessible to each probe, giving the accessible cell wall water in each sample. The results in table 5.3 are in the form of means and standard deviations. In the majority of cases there has been a significant reduction in the standard deviation when compared to those in figure 5.2. This confirms that a large amount of the variation between samples was due to differences in original wood density (i.e. to difference in lumen volume per gram of wood).

Table 5.3 Mean (and standard deviation) cell wall pore volumes of chemically modified wood accessible to probes of various sizes

Modifying chemical	W.P.G.	Accessibility (ml/g) to probes of increasing diameter						
		4Å	8Å	10Å	12Å	38Å	51Å	61Å
Pyridine control	-2.6	0.38 (.01)	0.25 (.03)	0.20 (.02)	0.19 (.02)	0.01 (.01)	0.01 (.01)	-0.02 (.02)
Acetic anhydride	12.8	0.24 (.02)	0.11 (.02)	0.11 (.02)	0.10 (.03)	0.02 (.00)	0.01 (.01)	-0.02 (.01)
	20.7	0.17 (.03)	0.06 (.02)	0.06 (.04)	0.06 (.04)	0.01 (.02)	0.01 (.01)	-0.02 (.02)
Succinic anhydride	15.9	0.33 (.06)	0.22 (.02)	0.17 (.01)	0.12 (.03)	-0.01 (.02)	0.00 (.01)	0.01 (.02)
	35.2	0.40 (.03)	0.21 (.04)	0.13 (.03)	0.13 (.01)	0.02 (.01)	-0.02 (.01)	0.00 (.01)
Phthalic anhydride	16.5	0.41 (.02)	0.18 (.04)	0.19 (.01)	0.23 (.01)	-0.02 (.05)	0.01 (02)	0.00 (.03)
	38.7	0.49 (.02)	0.01 (.03)	0.18 (.04)	0.17 (.03)	0.01 (.02)	-0.02 (.02)	0.02 (.01)
Alkenyl succinic anhydride	9.9	0.26 (.03)	0.14 (.02)	0.10 (.02)	0.11 (.03)	0.02 (.03)	0.03 (.03)	-0.04 (.05)
	29.3	0.21 (.01)	0.06 (.02)	0.00 (.02)	0.10 (.01)	0.02 (.01)	0.04 (.02)	-0.06 (.02)
Butyl isocyanate	8.7	0.24 (.01)	0.09 (.02)	0.09 (.02)	0.10 (.02)	0.01 (.01)	0.02 (.00)	-0.03 (.02)
	21.6	0.15 (.02)	0.07 (.01)	0.03 (.01)	0.04 (.01)	-0.01 (.01)	0.01 (.01)	0.00 (.01)

The results in table 5.3 are depicted for each modifying chemical in figures 5.1 to 5.5.

5.3.1 Fibre saturation point

The solute exclusion technique has been used to measure the fibre saturation point of wood, as discussed in section 2.2.1. The fibre saturation point in the present study can be calculated from the accessible volume listed for water (4Å diameter) in table 5.3 or the accessible volume at 4Å in figures 5.1 to 5.5. In figures 5.1 to 5.5 the y-axis has been drawn at 4Å to better illustrate this point.

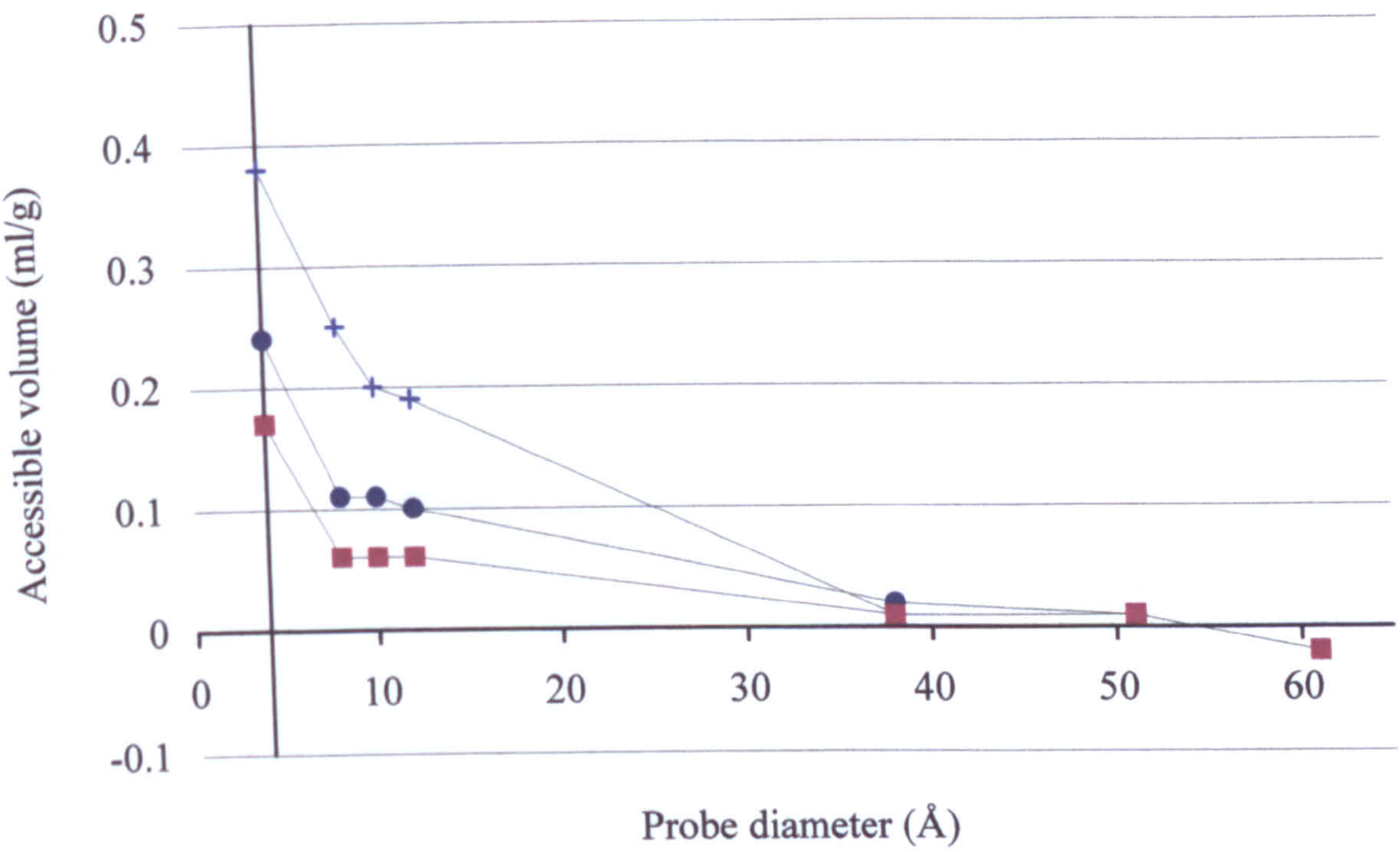


Figure 5.1 Average accessible cell wall water in acetic anhydride modified wood

Pyridine control (+); 12.8 WPG (●); 20.7 WPG (■)

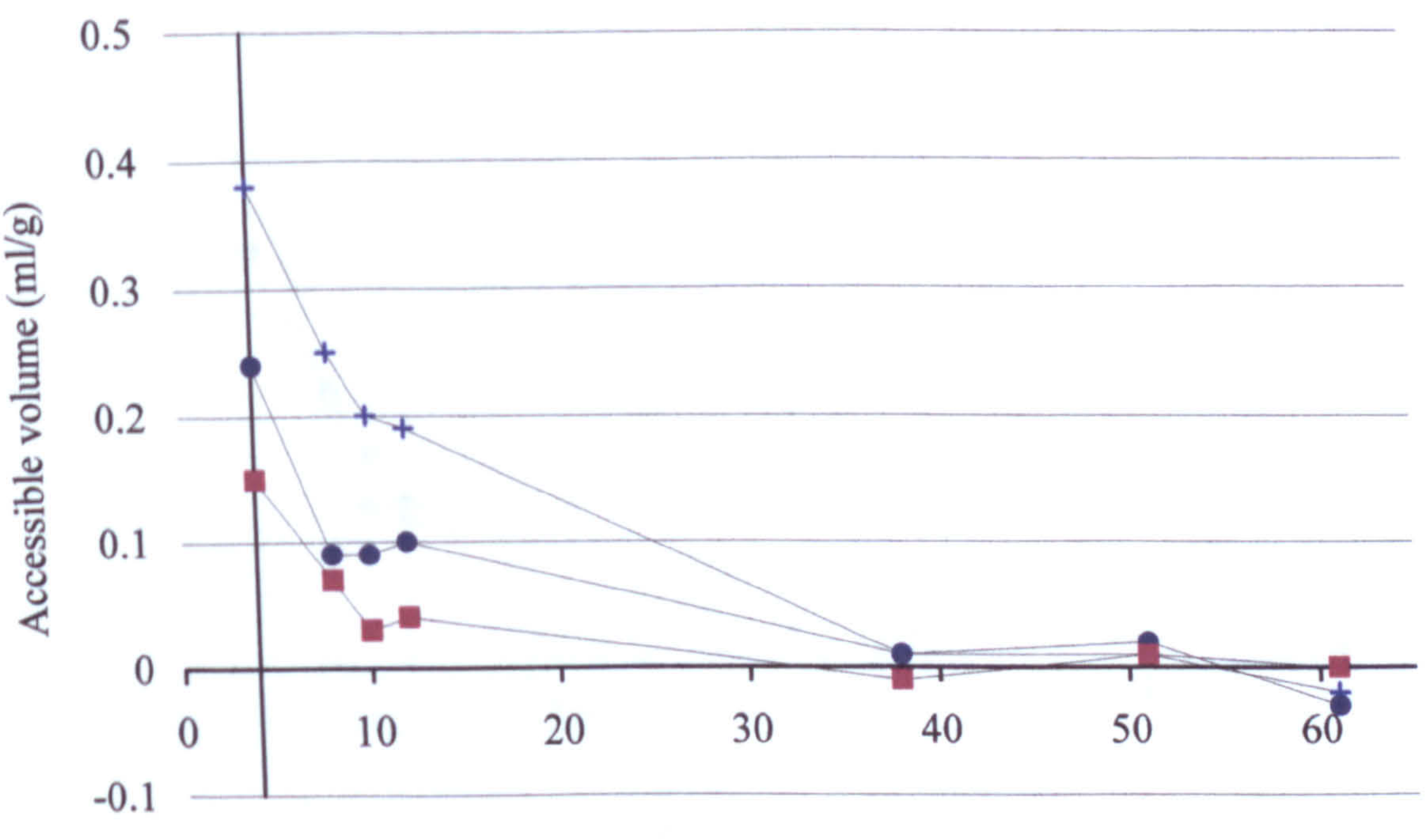


Figure 5.2 Average accessible cell wall water in butyl isocyanate modified wood

Pyridine control (+); 8.7 WPG (●); 21.6 WPG (■)

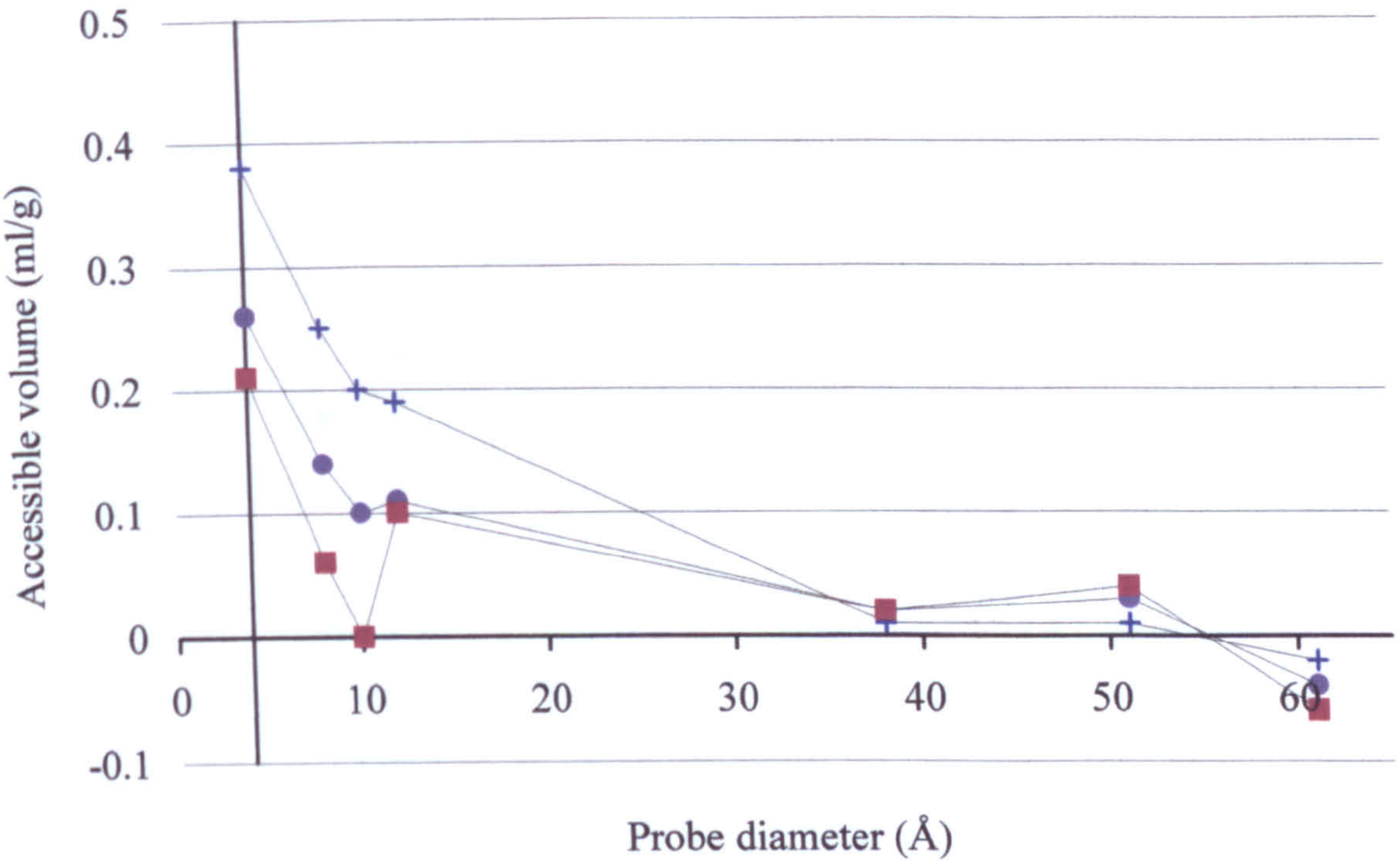


Figure 5.3 Average accessible cell wall water in alkenyl succinic anhydride modified wood

Pyridine control (+); 9.9 WPG (●); 29.3 WPG (■)

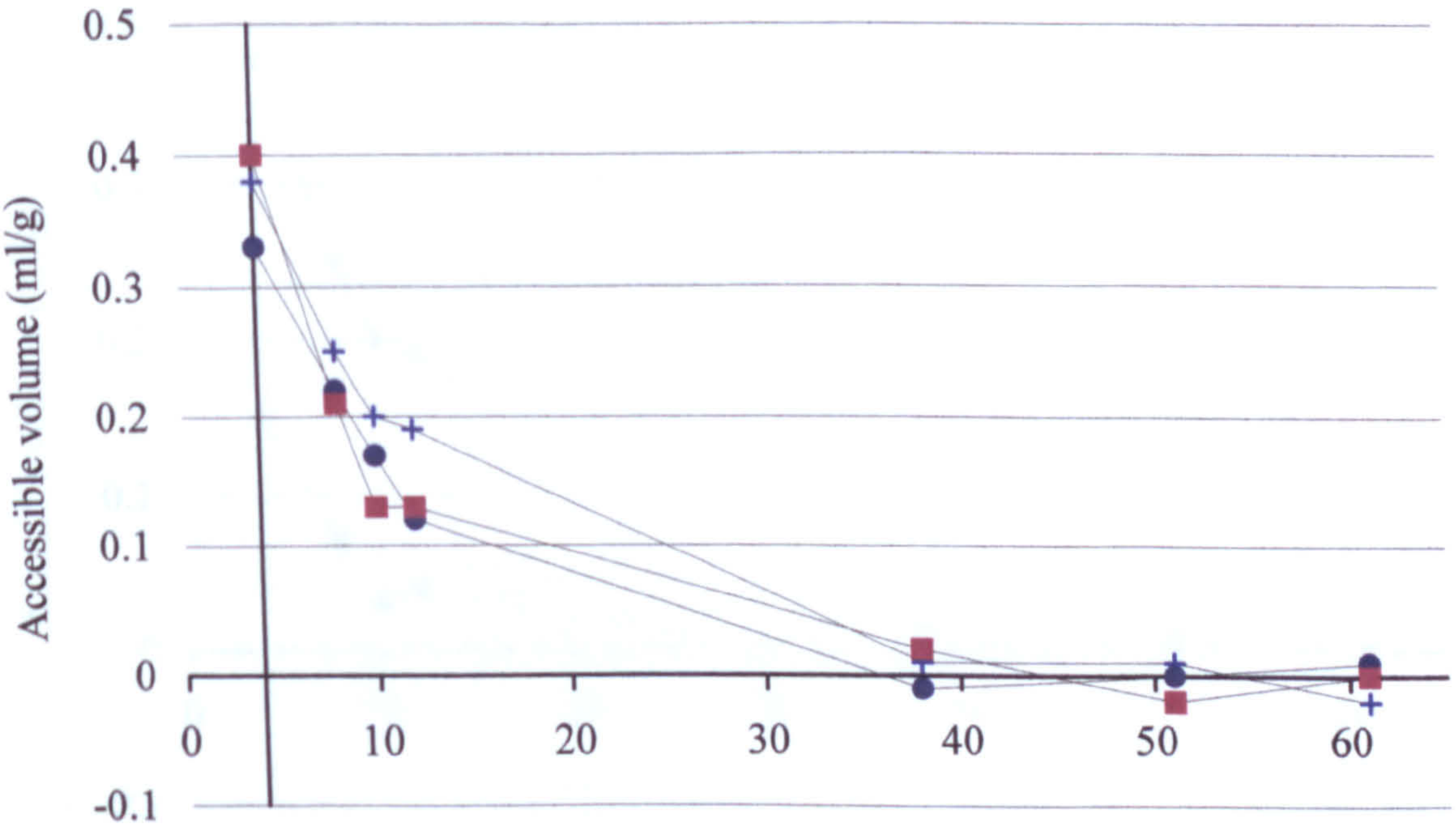


Figure 5.4 Average accessible cell wall water in succinic anhydride modified wood

Pyridine control (+); 15.9 WPG (●); 35.2 WPG (■)

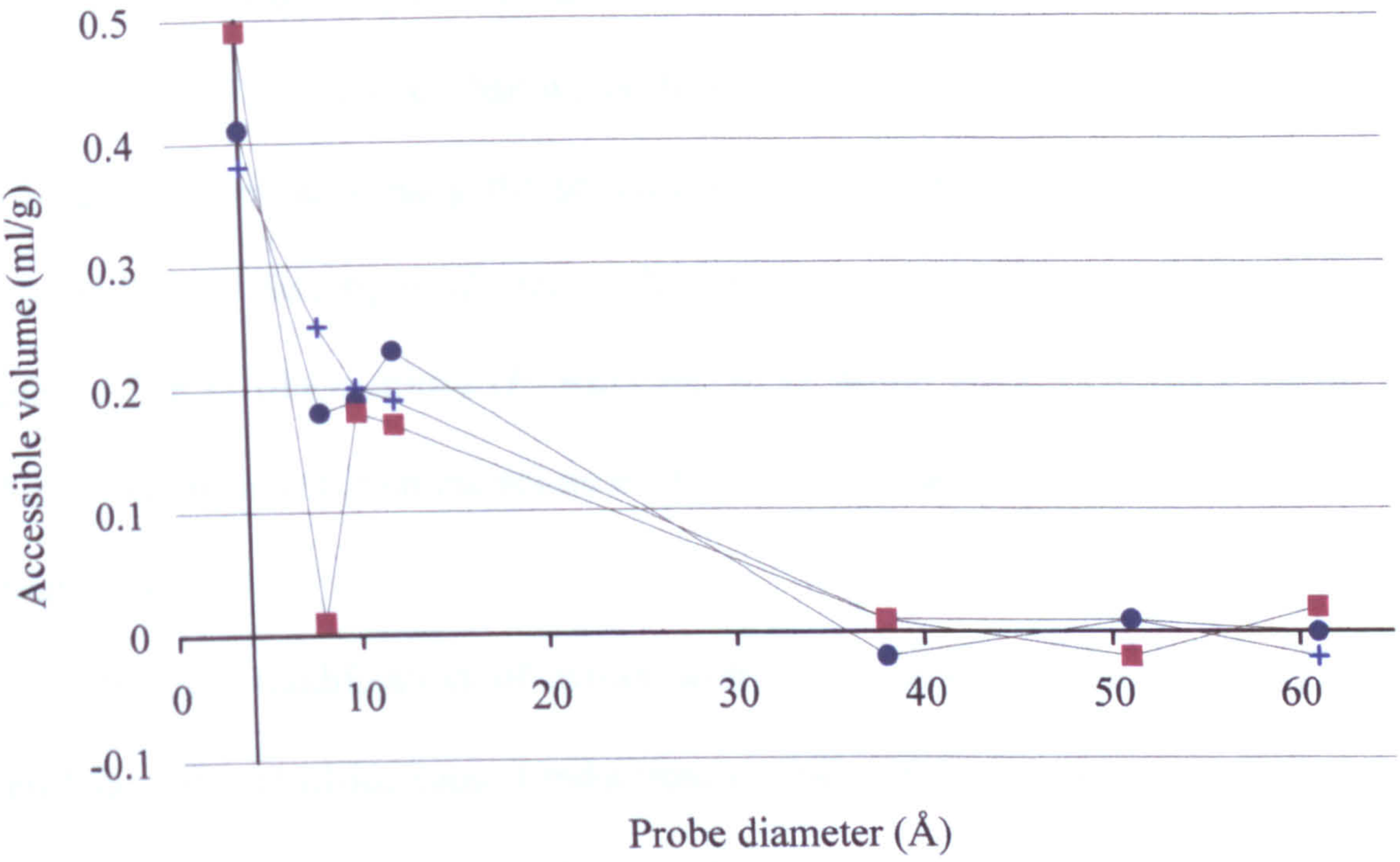


Figure 5.5 Average accessible cell wall water in phthalic anhydride modified wood

Pyridine control (+); 16.5 WPG (●); 38.7 WPG (■)

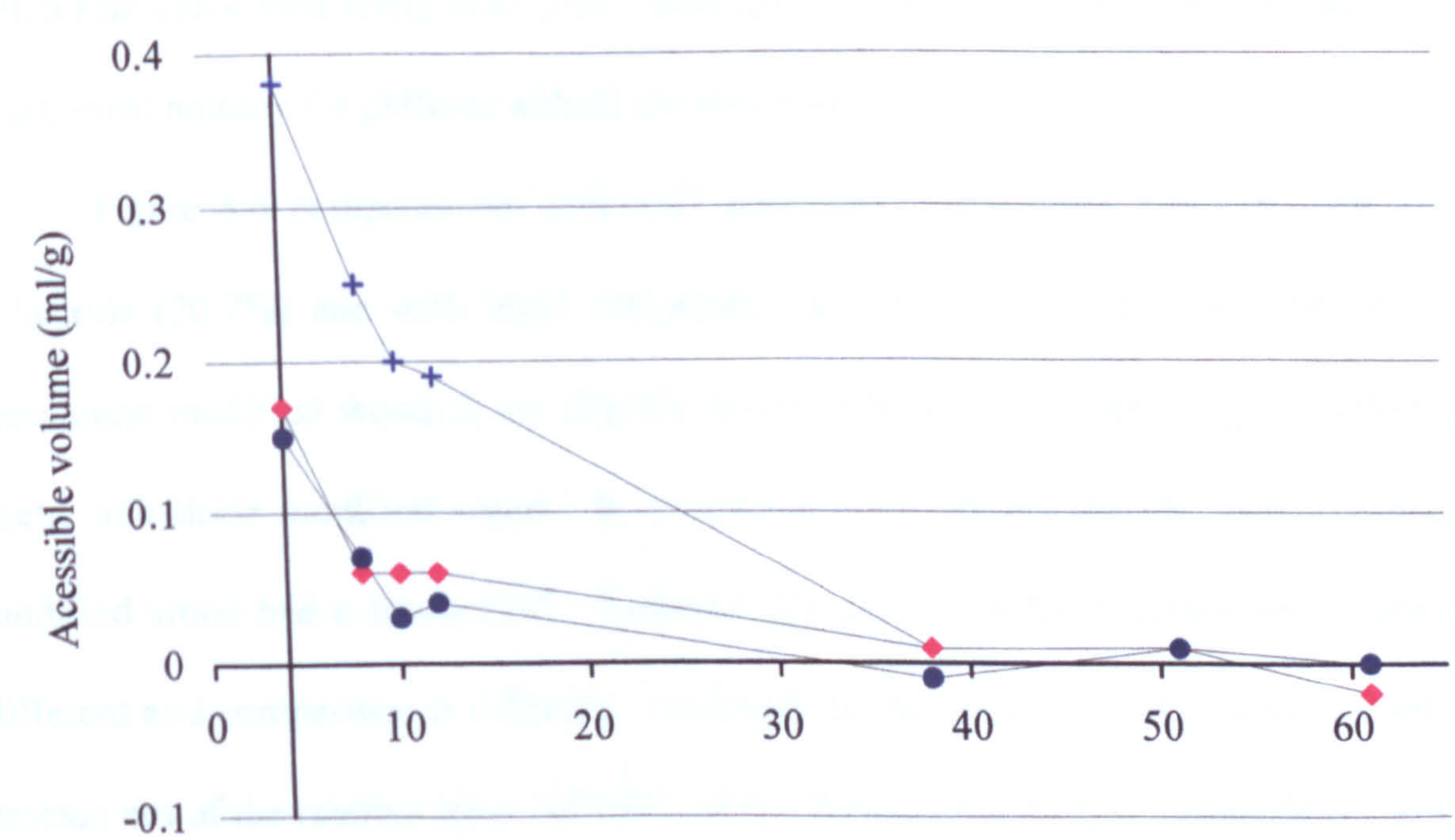


Figure 5.6 Average accessible cell wall water in acetic anhydride and butyl isocyanate modified wood

Pyridine control (+); Acetic anhydride 20.7 WPG (◆); Butyl isocyanate 21.6 WPG (●)

FSP for control samples was calculated to be 38% (0.38ml/g as listed in table 5.3). This measure for FSP is higher than would be expected using other techniques, but agrees with other FSP measures using the solute exclusion technique. Flournoy *et al.* (1991) calculated FSP of Sweetgum (*L. styraciflua*) to be 35% and Stone and Scallon (1967) calculated FSP of Black spruce (*P. mariana*) to be 40%. Possible reasons for the high FSP measures obtained from the solute exclusion technique are given by Siau (1996) (see section 2.2.1).

Chemical modification of wood with acetic anhydride, butyl isocyanate and alkenyl succinic anhydride caused reductions in FSP with increasing WPG (figures 5.1 to 5.3). This was not the case for phthalic and succinic anhydride modified wood (figures 5.4 and 5.5), high modification levels of which both caused an apparent increase in FSP above that of the control samples. This is not surprising given the results in chapter 4 in which FSP calculated using adsorption isotherms was found to increase at higher weight gains, most notably for phthalic anhydride modification.

Figure 5.6 compares the cell wall accessibility for wood modified with acetic anhydride (20.7%) and with butyl isocyanate (21.6%). This shows the FSP for butyl isocyanate modified wood to be slightly lower (albeit at a slightly higher WPG) than acetic anhydride modified wood. In chapter 4 it was found that the acetic anhydride modified wood had a lower FSP. However the two methods of measurement are very different and comparison is difficult. Additionally, to be able to make a more confident assessment of the relative levels of FSP using solute exclusion, the measurement of many more samples would be required.

5.3.2 Pore accessibility

Figures 5.1 and 5.2 show that both acetic anhydride and butyl isocyanate modified wood generally had lower accessibility for each sugar probe, though there seems to be no discernible pattern. For wood modified with acetic anhydride to a 20.7% weight gain, the same accessibility was measured for the three sugar probes. Although this could be due to a change in pore size distribution within the wood cell wall, other results indicate that such a conclusion cannot confidently be drawn. In particular, results for the raffinose probe (12Å diameter) were unpredictable among all the samples tested. In several cases an increase in accessibility was measured above the smaller maltose (and even glucose) probe. This cannot be easily explained, though it is likely to be due to experimental errors.

Figure 5.7 displays data for acetic anhydride and butyl isocyanate modified wood on the basis of inaccessible volume. This was calculated by subtracting the accessible volume to a given probe in a single sample from the total water in the sample. Figure 5.7 shows the averages for each set of samples. Displaying data in this way makes it easier to see changes in the distribution of pore sizes. In figure 5.7 it appears that the water inaccessible to the glucose probe remained constant or even increased at the lower weight gains of these chemicals, but falls below the control at higher weight gains. This could be evidence of a limited amount of cell wall damage at low WPG's causing an increase in the number of small diameter pores (as suggested in chapter 4). Alternatively, initial reaction may be occurring in larger pores (>8Å diameter) causing no change in, or even increasing, the volume of pores below 8Å diameter (as larger pores narrow). Then, at higher weight gains, reaction occurs in pores inaccessible to even glucose, and the total volume of these pores is reduced.

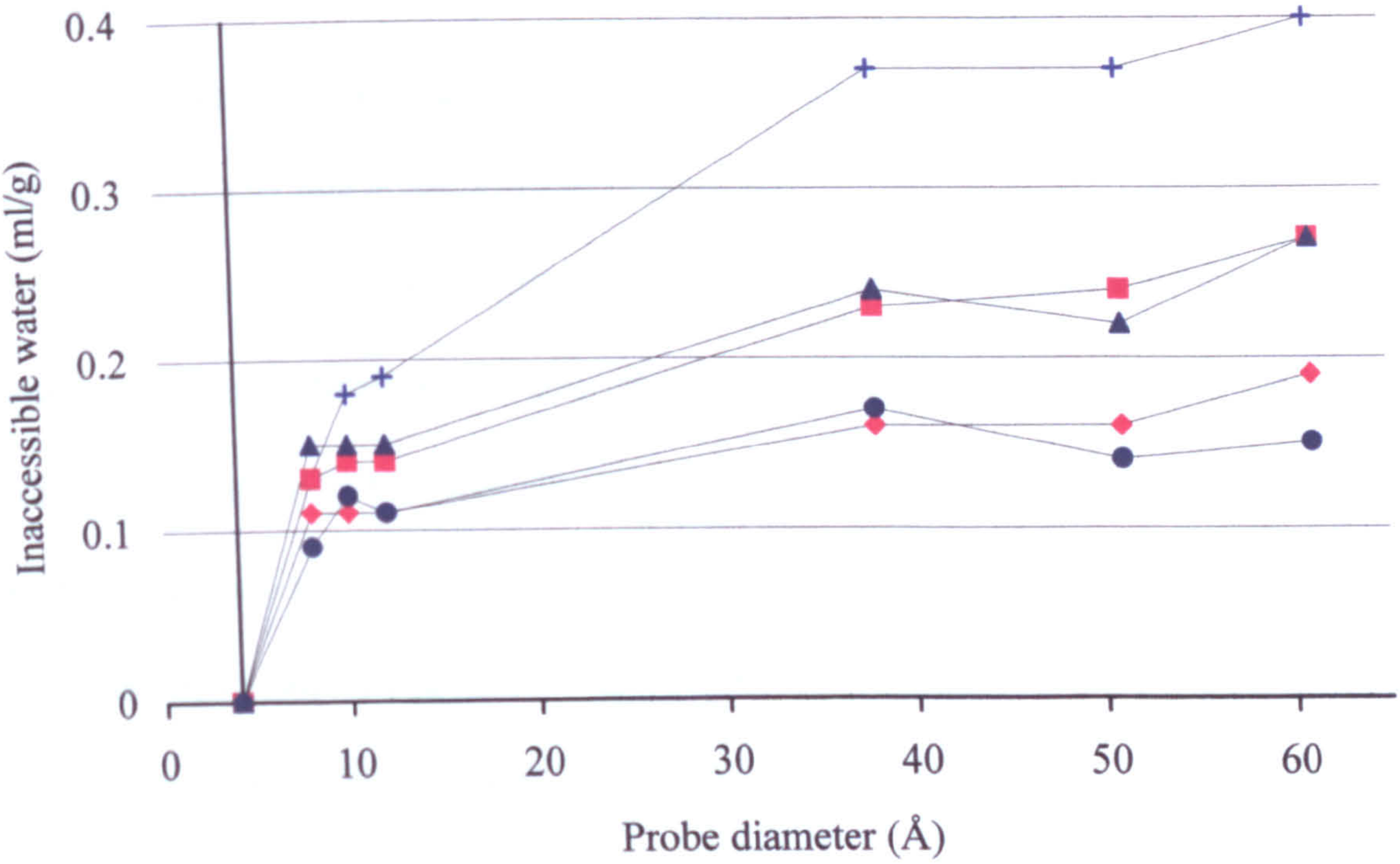


Figure 5.7 Average inaccessible water in acetic anhydride and butyl isocyanate modified wood

Pyridine control (+); Acetic anhydride 12.8 WPG (■), 20.7 WPG (◆);
Butyl isocyanate 8.7 WPG (▲), 21.6 WPG (●)

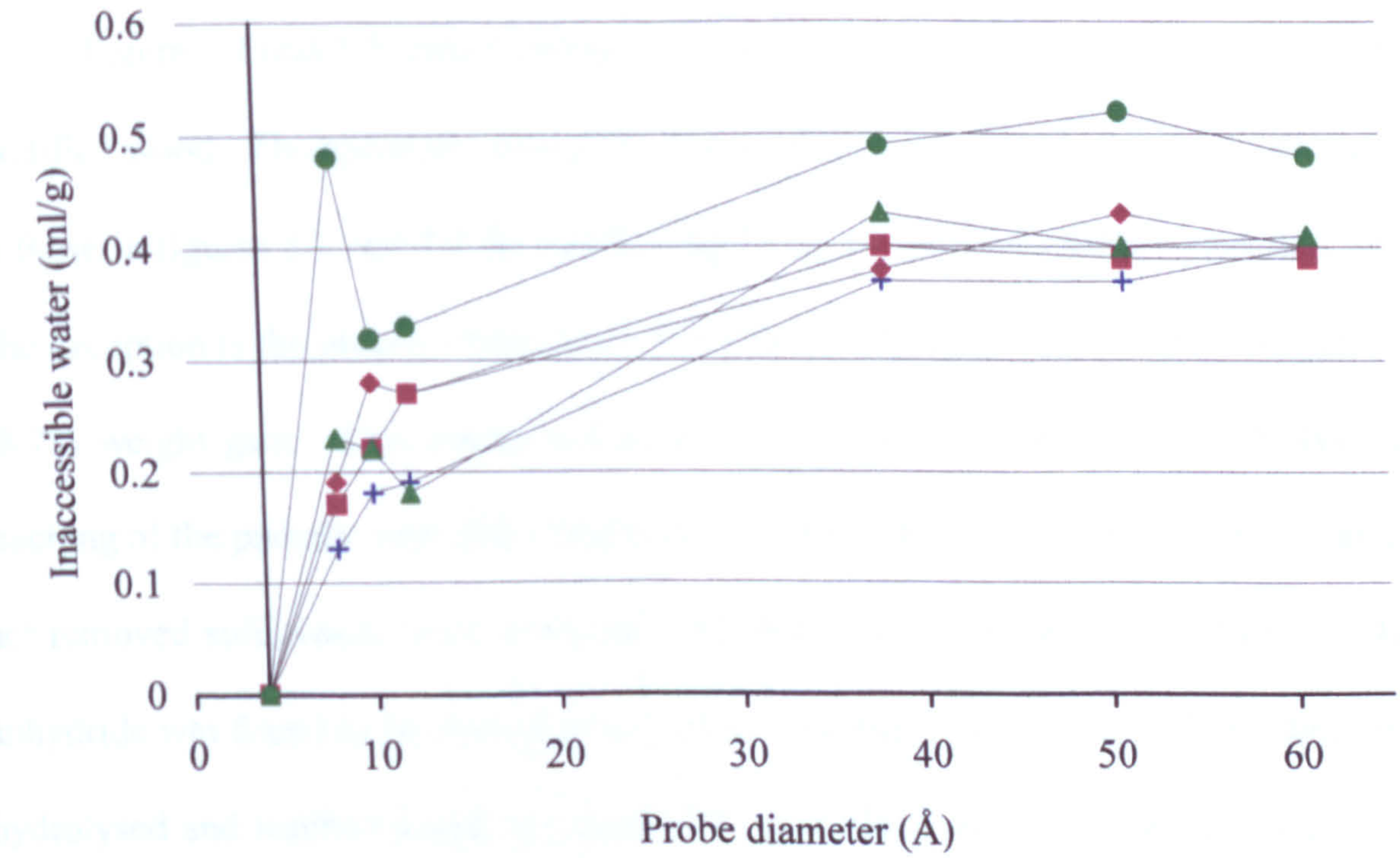


Figure 5.8 Average inaccessible water in succinic and phthalic anhydride modified wood

Pyridine control (+); Succinic anhydride 12.8 WPG (■), 20.7 WPG (◆);
Phthalic anhydride 8.7 WPG (▲), 21.6 WPG (●)

In figures 5.6 and 5.7 slight differences in accessibility between acetic anhydride and butyl isocyanate modified wood are evident, though due to the questionable sensitivity of this test this is not a conclusive result.

Figure 5.3 depicts data for alkenyl succinic anhydride modified wood. The results obtained for this modification proved particularly unpredictable. There was a fairly large variation in accessibility between the three largest probes, and at 29.3% weight gain the measured increase in penetration with increasing probe size between maltose and raffinose is particularly large. From the measurement using the maltose probe it would appear that access to the cell wall is restricted to chemicals of less than 10Å in size, though the raffinose measurement contradicts this completely. This result is not understood and consequently no conclusion can be drawn from this data. Results for alkenyl succinic anhydride modified wood have thus not been presented in the form of inaccessible volume.

Figure 5.4 and 5.5 depict accessibility results for succinic and phthalic anhydride modified wood. The apparent changes in accessibility are relatively small in comparison to those in figures 5.1 and 5.2 for acetic anhydride and butyl isocyanate modified wood. The exception is the glucose measurement for wood modified with phthalic anhydride to 38.7% weight gain. This anomalous result is most likely to be due to hydrolysis and leaching of the phthalic anhydride modification during the initial soaking, which was then not removed sufficiently prior to sample incubation with the glucose solution. Phthalic anhydride was found to be particularly prone to leaching in section 3.6. Extraction of the hydrolysed and leached modifying chemical (most likely in acid form) into the solution would have affected the refractometer reading to give the appearance of inaccessibility to this probe.

The inaccessible water for succinic and phthalic anhydride modified wood is shown in figure 5.8. Phthalic anhydride caused a general increase in inaccessible water, particularly at high weight gains, as the cell wall pore volume apparently increases. This could be due to hydrolysis and leaching of modifying chemical in all probe solutions (resulting in lower accessibility measurements), though an increase in pore volume for this chemical is in line with findings in chapter 4 when measuring adsorption isotherms. Succinic anhydride did not cause any significant increase in measured FSP, as stated above. From figure 5.8 there does appear to be a redistribution of pore size, with a greater amount of total pore volume within the cell wall consisting of smaller pores (increased volume of water in pores of 4 to 8Å in diameter). If this was solely due to narrowing of existing larger pores then it would be expected that total pore volume (and therefore FSP) would be reduced. This is not the case, from which it is concluded that new pores are created (as proposed in chapter 4).

5.3.3 Test sensitivity

As mentioned in the above discussion, several problems were encountered during the experiment and in the analysis of results. Results in most cases indicate a reduction in pore space or a change in the distribution of pore size within the cell wall due to chemical modification. Beyond this, results are viewed with some caution due to the apparent variability in the test and unpredictability of some of the measurements. Though it remains plausible that the restriction of pore size could cause modified wood to become resistant to decay fungi, a definite conclusion cannot be drawn from this experiment.

It is thought that problems have generally stemmed from the sample selection and the number of samples used. To obtain more meaningful results it would have been necessary to cut samples to the appropriate size (≤5mm longitudinal) prior to

modification, and from a single wood strip (matched densities). The analysis of modified wood fibre could also be considered. In addition, a wider range of weight gains, a larger number of replicates and even a wider range of probes would be required in a comprehensive study of this type. Time restrictions meant that this was not possible within the confines of this study.

5.4 Summary of points raised in chapter 5

- Questions were raised regarding the sensitivity of the pore size exclusion experiment. In particular, these concerned the numbers of replicates and weight gains tested, unmatched samples, and sample cutting after modification. These stem from the fact that samples were originally reacted in batches primarily intended for decay testing. Conclusions drawn from this chapter are therefore done so tentatively.
- Fibre saturation point was measured for the pyridine control samples to be 38%. This is high in retention to other measures (e.g. that measured in chapter 4), but in line with the results of other workers using the solute exclusion technique.
- Wood modification with acetic anhydride, alkenyl succinic anhydride and butyl isocyanate caused a reduction in FSP from that measured for the pyridine controls, while modification with succinic and phthalic anhydride caused an increase. This is in keeping with results in chapter 4.
- Only slight differences in accessibility were measured between wood modified to equivalent WPG's of acetic anhydride and butyl isocyanate. No definite conclusion could be drawn from these differences.
- Pore exclusion results for alkenyl succinic anhydride and phthalic anhydride modified wood were unpredictable. In the case of phthalic anhydride modification this was

thought to be due to modifying chemical leaching into the probe solution and distorting the reading given by the differential refractometer. The reasons for the unpredictable results obtained for alkenyl succinic anhydride modified wood are not known.

- Succinic anhydride modification did not cause a decrease in total accessibility (in fact it caused an increase), but it did seem to lead to a redistribution of pore sizes within wood. It was concluded that this was due to cell wall damage, which had caused the creation of new small diameter pores.

- The results for acetic anhydride and butyl isocyanate modified wood indicated either that reaction to low WPG's caused a limited amount of cell wall damage (as suggested in chapter 4) or that initial reaction (to low WPG's) occurred in larger pores, causing them to narrow, with subsequent reaction (to higher WPG's) occurring increasingly in smaller pores.

Chapter 6. Pure culture decay tests against brown and white rot fungi

6.1 Introduction

The aim of this section of the study was to give an indication of the ability of each modifying chemical to protect wood from decay by brown and white rot fungi and to establish, where applicable, the level of modification that completely protected wood against decay (the threshold level of protection).

It has often been stated that the low moisture content in modified wood is the cause of its decay resistance (see section 2.4.4). Part of this study therefore involved the manipulation of test moisture content to examine the ability of modifying chemicals to protect wood in conditions of forced wetting. Control blocks kept in sterile test conditions provided a measure of the equilibrium moisture contents for undecayed modified wood in the test. It was thus hoped that an insight into the importance of moisture content on decay would be gained.

6.2 Materials and methods

6.2.1 Modified wood samples

Corsican pine sapwood blocks measuring 20mm x 20mm x 10mm (radial x tangential x longitudinal) were chemically modified as described in chapter 3. Solvent and untreated controls were also exposed as were CCA treated blocks (treated as described in chapter 3). Prior to exposure, blocks were conditioned at 20°C and 60% RH for 2 weeks, packed in an argon atmosphere and sterilised by gamma irradiation (2.5 Mrad.).

6.2.2 Test fungi

Four decay fungi were used; the brown rot fungi *Coniophora puteana* (Schum.: Fr.) Karst. and *Gloeophyllum trabeum* (Pers.: Fr.) Murr., and the white rot fungi *Trametes versicolor* (L.: Fr.) Pilat and *Pycnoporus sanguineus* (L.: Fr.) Murr. (table 6.1). The first three of these were chosen as they are fungi that are commonly used in wood decay testing, including the testing of chemically modified wood. The latter was chosen as it had provided good white rot attack of pine in a previous study of the durability of chemically modified wood (Cardias, 1992).

Table 6.1 Decay fungi and test conditions

Fungus	**Strain number**	**Decay type**	**Test conditions**
Coniophora puteana	FPRL 11E	Brown rot	high and low m.c. test
Gloeophyllum trabeum	FPRL 108N	Brown rot	high and low m.c. test
Trametes versicolor	CTB 863A	Simultaneous white rot	high m.c. test
Pycnoporus sanguineus	DPF 44	Simultaneous white rot	high m.c. test

6.2.3 Test conditions

Soil block test jars were prepared using 500 ml Beatson squat jars (French, Flint and Orme Co.) containing 200 g of John Innes No. 2 compost wetted up to achieve water holding capacity (WHC = 35%) plus 30%. Three Corsican pine sapwood feeder strips (28 x 34 x 5 mm) were gently pressed into the top of the soil in each jar. Block sizes and soil moisture content were in accordance with AWPA Standard E10 (1991). After autoclaving, active cultures of brown and white rot test fungi (precultured on 4% malt agar) were inoculated into the test jars. These jars were incubated at 27°C and 75% RH for two weeks prior to introducing the test blocks.

Two test blocks and one virulence control block, which had received no treatment, were placed in each test jar on top of the feeder strips.

To examine the effect of different wetting conditions on modified wood, two types of test were performed. In the first test the blocks were overlaid with wetted vermiculite (BS1982: 1990, Part 1). This is referred to as the high moisture content (high m.c.) test. The second test was a conventional soil block test without the vermiculite overlay, and is referred to as the low moisture content (low m.c.) test. Performance against white rot fungi was assessed in the wetter test only. The low moisture content test allowed equilibrium block wetting of unmodified wood to *ca.* 30%, ideal for initiation of decay by brown rot fungi, while the vermiculite allowed initial capillary wetting to *ca.* 75%, providing conditions more suitable for white rot decay. Performance against brown rot fungi was assessed using both test methods to enable a comparative assessment to be made, and also to provide an additional wetting challenge against the moisture reducing effect of some types of chemical modification. These conditions are summarised in table 6.1.

The vermiculite (Micafil) was sieved to remove dust particles and was wetted to WHC + 10% (WHC = 500%). This was then autoclave sterilised and added to the jars to cover the test blocks.

Following 16 weeks incubation in a conditioning room at 27°C and 75% RH, the blocks were removed, weighed, oven dried, and reweighed. Moisture contents and weight losses were calculated. Blocks were then split open and visually and microscopically examined for evidence of fungal decay. This visual assessment was taken into account in the estimation of threshold levels of protection for each modification type against each fungus.

6.3 Results and discussion

Data for weight loss and the percent moisture content at the end of test for modified wood are presented in figures 6.1 to 6.16. Each point represents the mean of eight test blocks. Data from which these figures have been drawn are given in the tables in appendix 2 along with data for CCA treated wood. In appendix 2 WPG figures are the mean values for the whole reaction set. This is so that equivalent weight loss (and moisture content) data can be listed side by side. In figures 6.1 to 6.16 the WPG's are the mean of the eight blocks exposed in each particular test. The only noticeable differences between these and the WPG's listed in appendix 2 are for WPG's in excess of 30%, where the spread of weight gains in a single reaction set could be relatively wide (see chapter 3).

6.3.1 Determination of performance

Following assessment of the test, it was found that high levels of reaction can have a significant bearing on the interpretation of results where weight losses are used as the criteria for assessment. For example, significant non-decay related weight losses were recorded for the sterile control blocks treated with succinic and phthalic anhydrides (figures 6.1 and 6.3). In some cases these non-decay related weight losses were found to increase with WPG at high modification levels. In an attempt to show weight losses solely attributable to decay, sterile control weight losses were subtracted from the fungal weight loss data to give the corrected data plotted in figures 6.5, 6.7, 6.9, 6.11, 6.13 and 6.15. In the case of wood modified with succinic or phthalic anhydride this amounted to substantial corrections to the data. For example, blocks treated with phthalic anhydride to 38% WPG showed a 13% weight loss in the sterile controls of the high moisture content test.

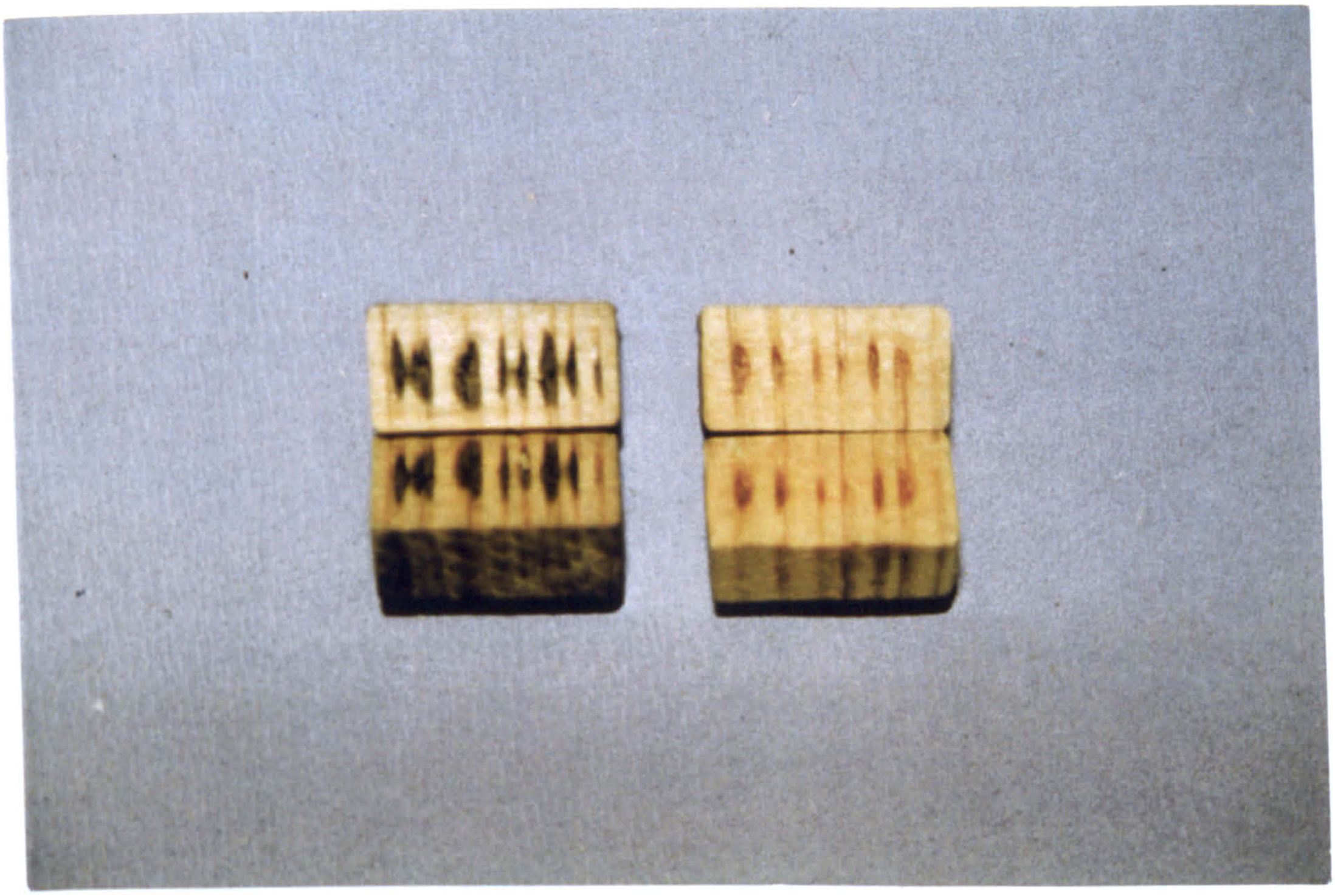


Plate 6.1 Acetic anhydride modified wood blocks decayed by *Gloeophyllum trabeum*. Left; 17% WPG acetic anhydride, 16% weight loss. High moisture content test. Right; 20% WPG acetic anhydride, 6% weight loss. Low moisture content test.

Having corrected the data it is also important to consider that the presence of the test fungi may have increased the amount of non-decay related weight loss by block wetting and/or fungal mediated changes that might increase lability of the compounds.

The results were also affected by inconsistent distribution of modifying chemical within individual blocks. Plate 6.1 shows the interior of *G. trabeum* decayed acetic anhydride modified test blocks. Pockets of brown rot are evident in the latewood regions inside each block; an identical pattern to that found in brown rotted butyl isocyanate modified wood by Cardias and Hale (1990). In the present study, the pattern shown in plate 6.1 was also evident in *C. puteana* decayed modified wood, and in butyl isocyanate and alkenyl succinic anhydride modified wood, but in these latter cases it was not so

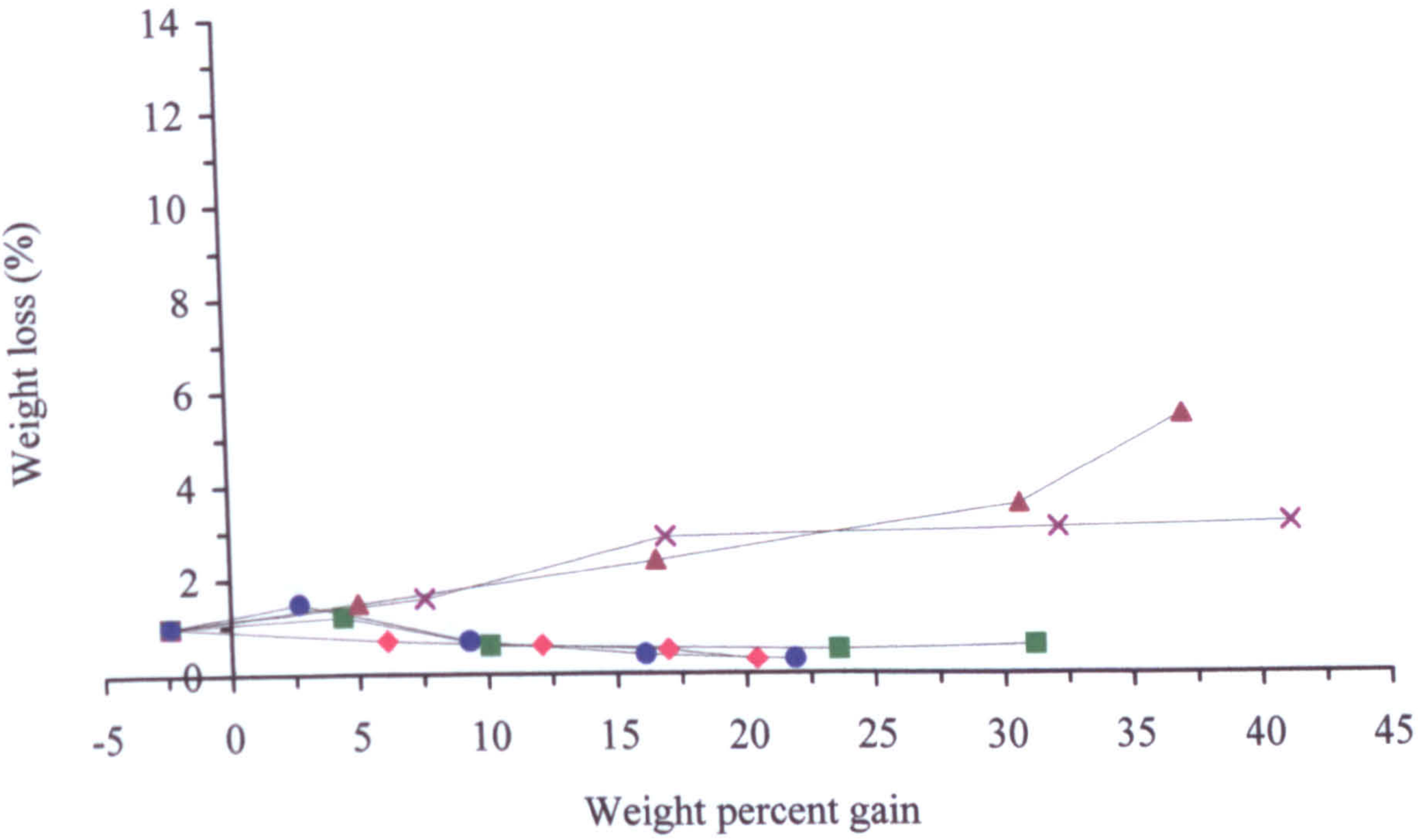


Figure 6.1 Sterile control (low moisture content test). Weight loss (%)

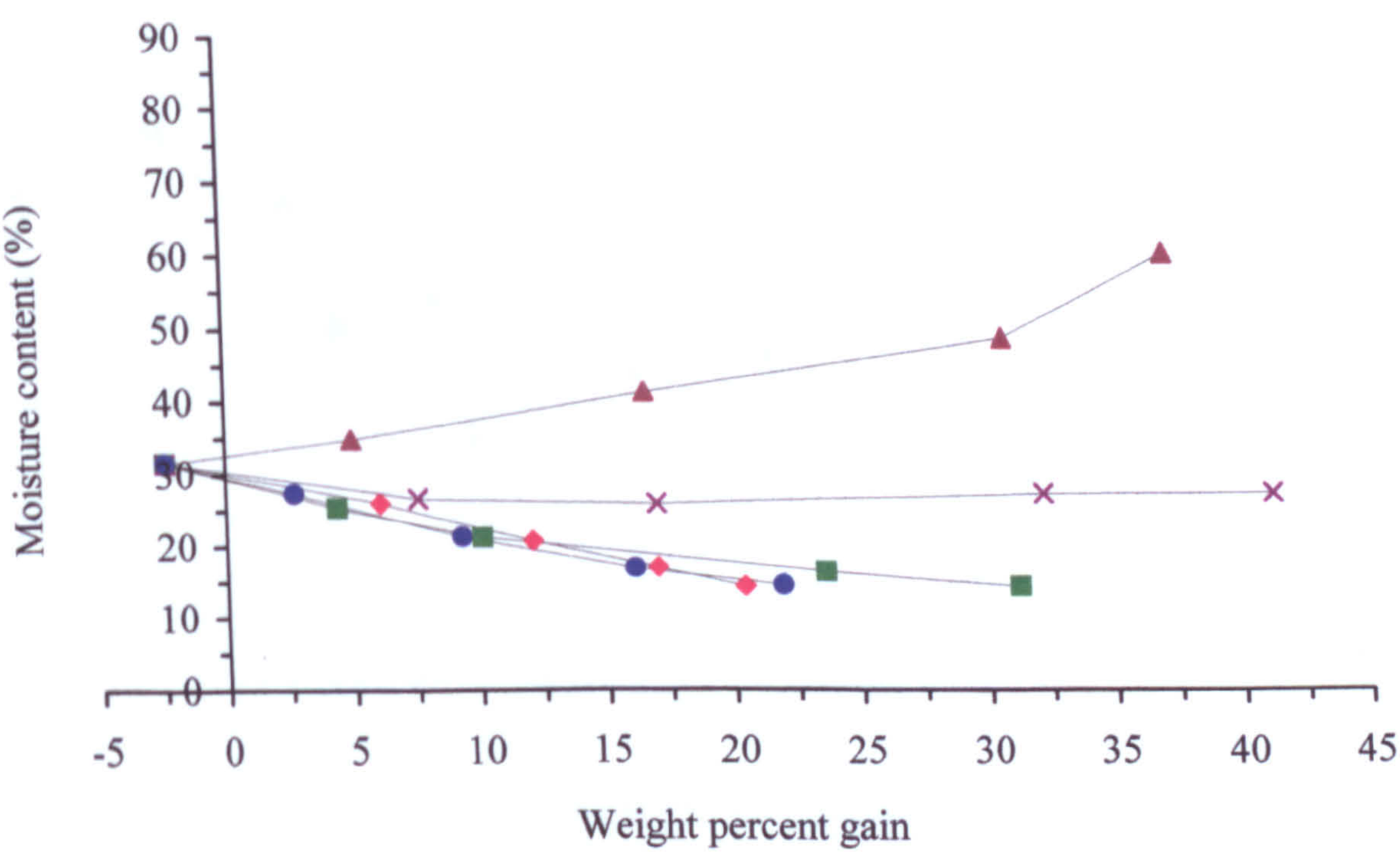


Figure 6.2 Sterile control (low moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲); Phthalic anhydride (×); Butyl isocyanate (●)

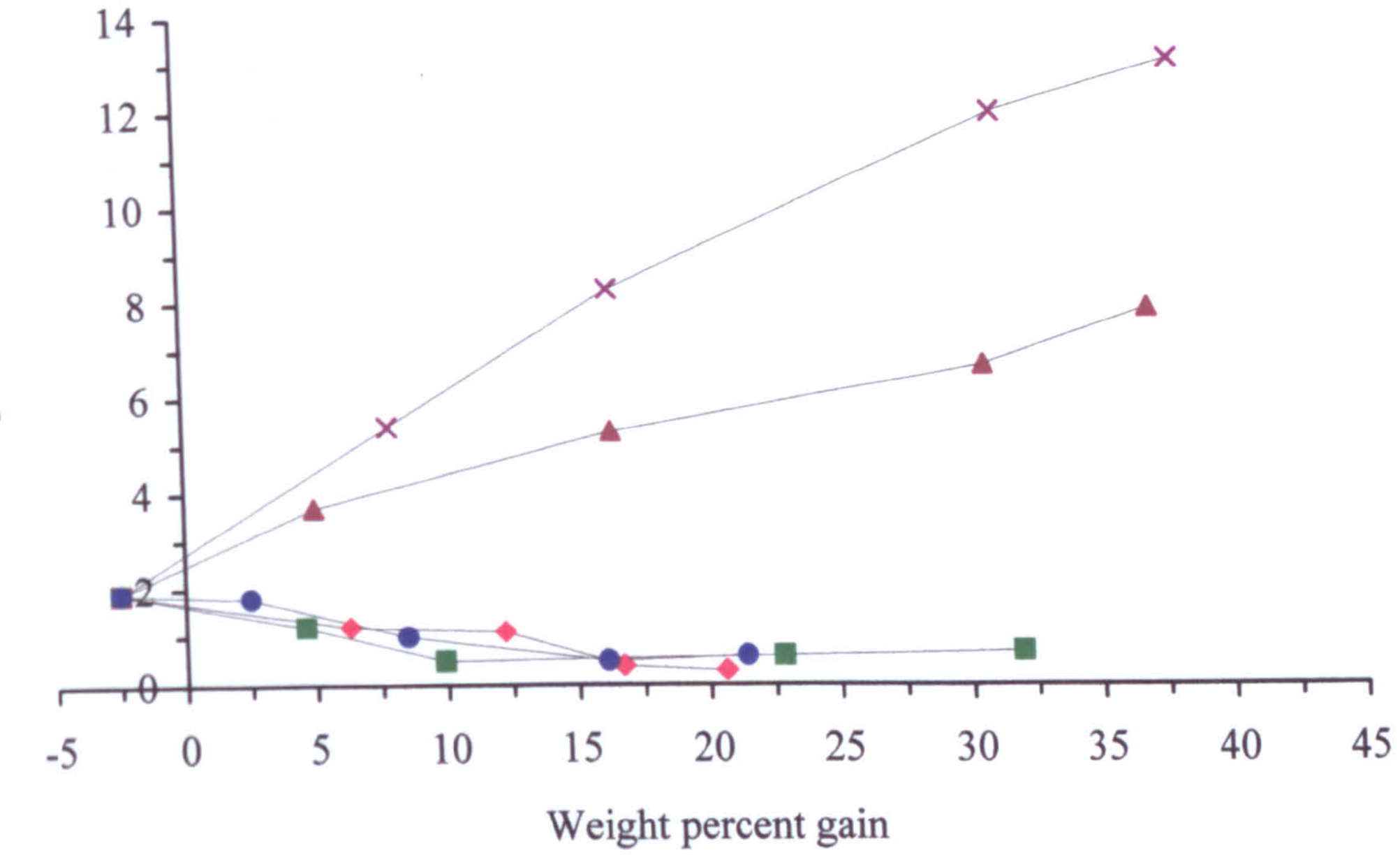


Figure 6.3 Sterile control (high moisture content test). Weight loss (%)

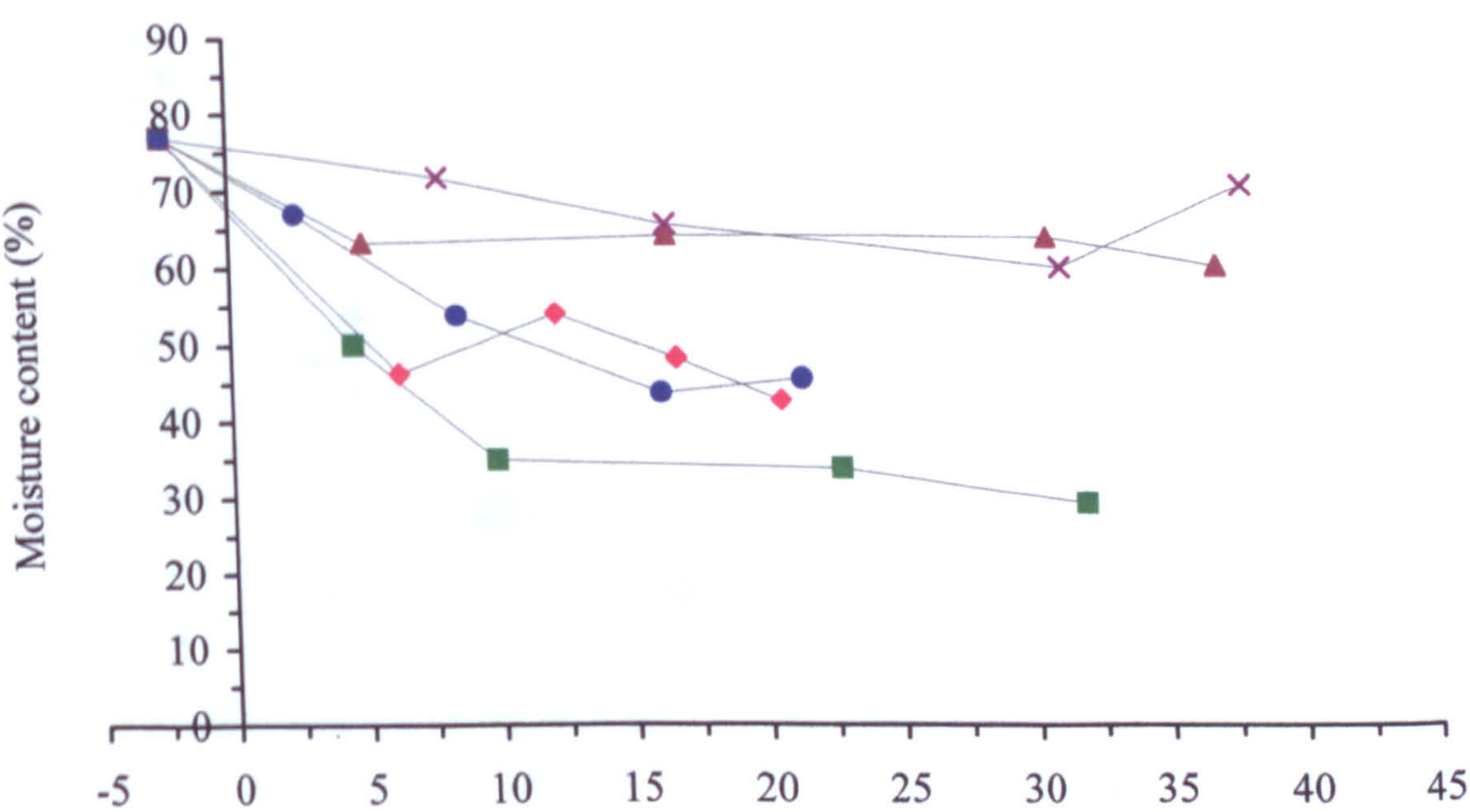


Figure 6.4 Sterile control (high moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲); Phthalic anhydride (×); Butyl isocyanate (●)

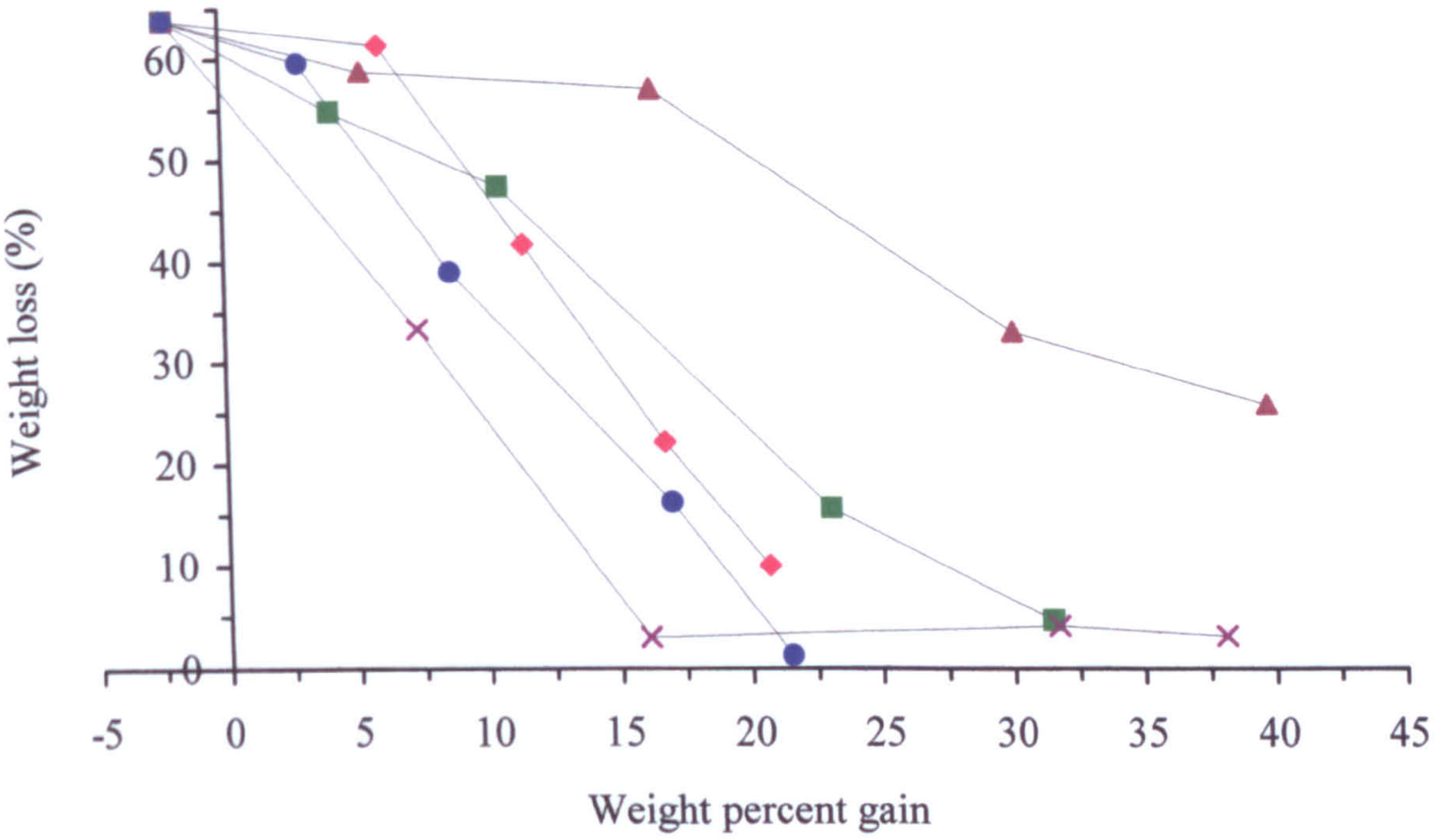


Figure 6.5 *Coniophora puteana* (low moisture content test). Corrected weight loss (%)

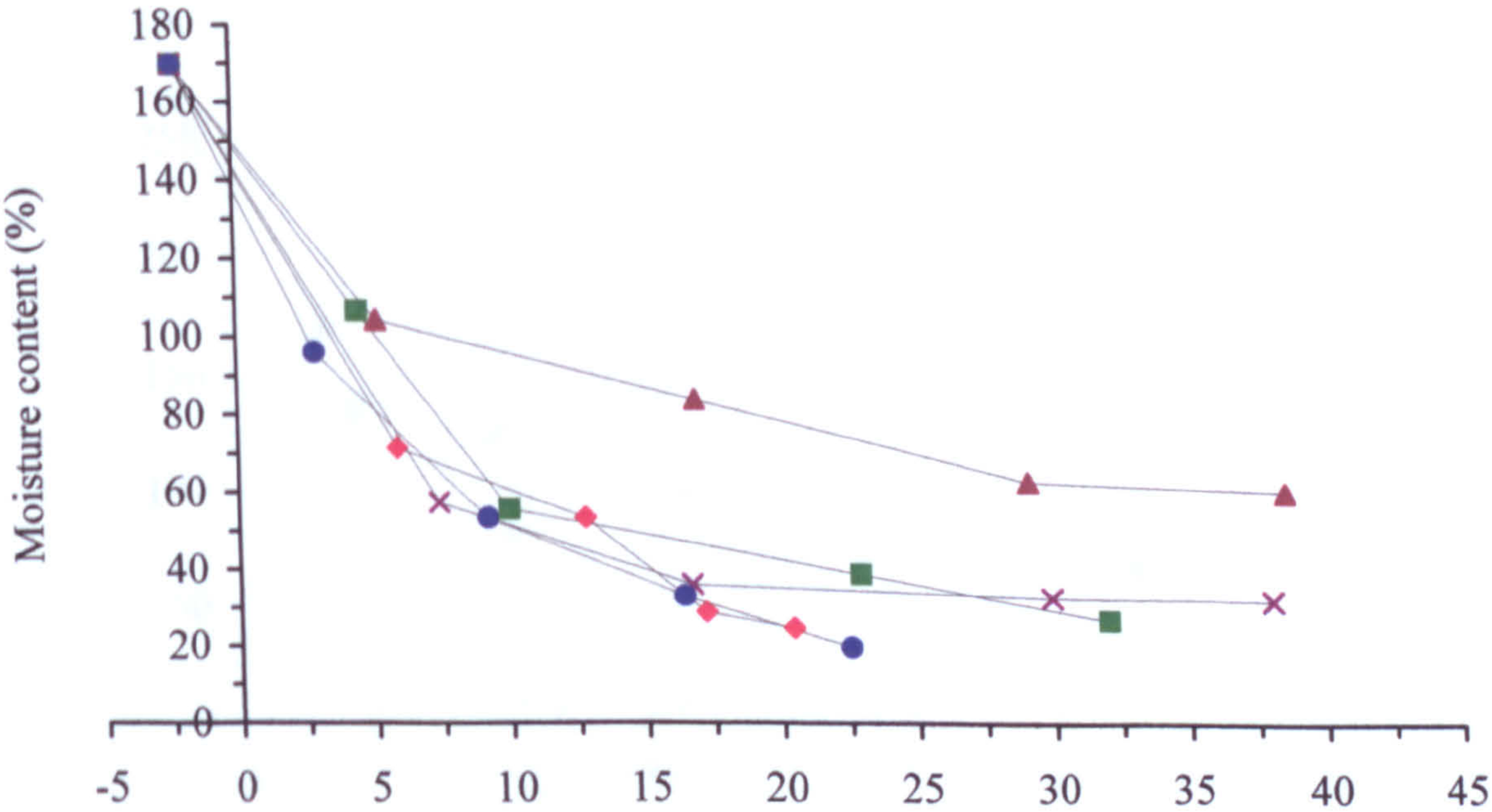


Figure 6.6 *Coniophora puteana* (low moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);
Phthalic anhydride (×); Butyl isocyanate (●)

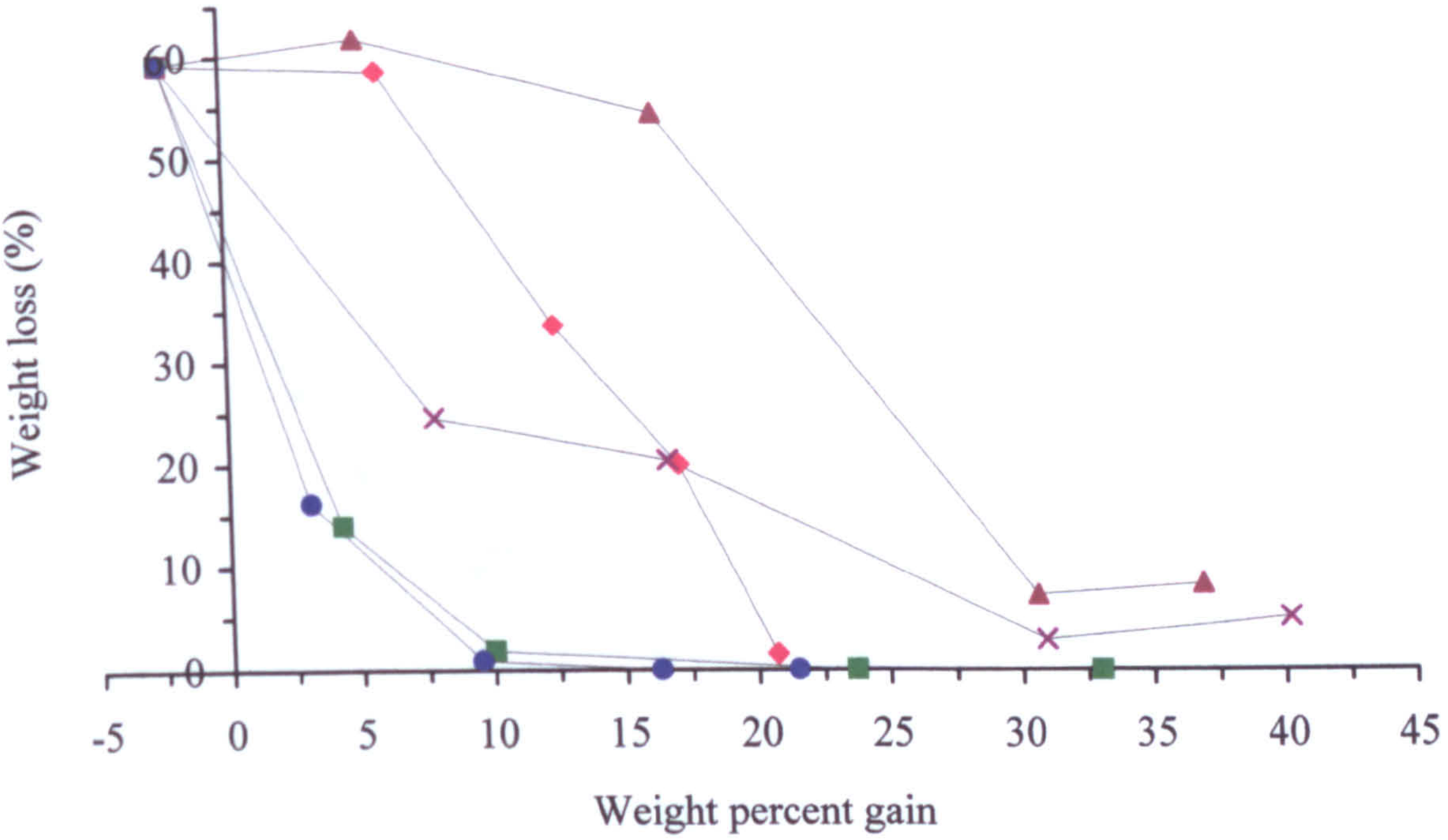


Figure 6.7 *Coniophora puteana* (high moisture content test). Corrected weight loss (%)

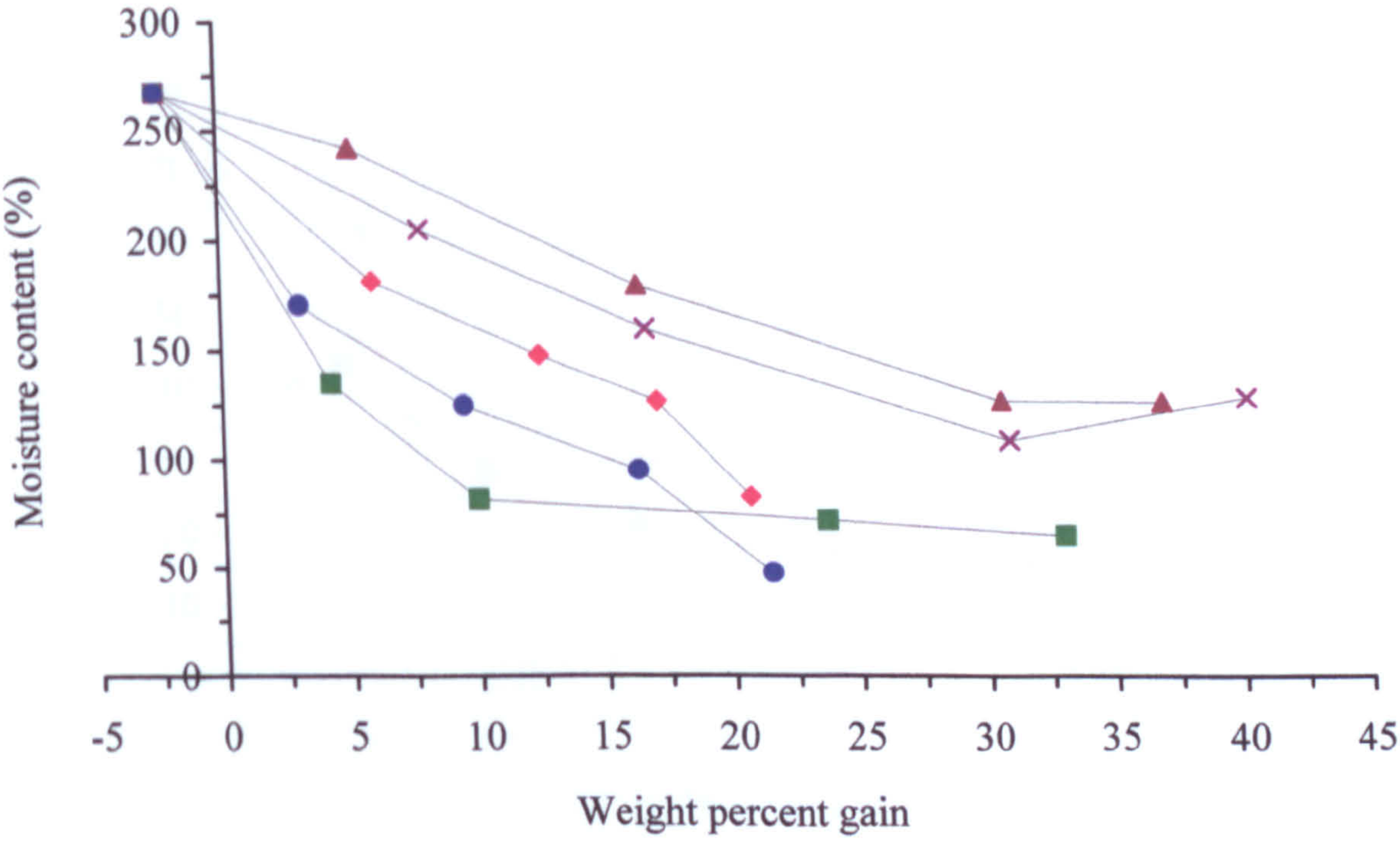


Figure 6.8 *Coniophora puteana* (high moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);
Phthalic anhydride (×); Butyl isocyanate (●)

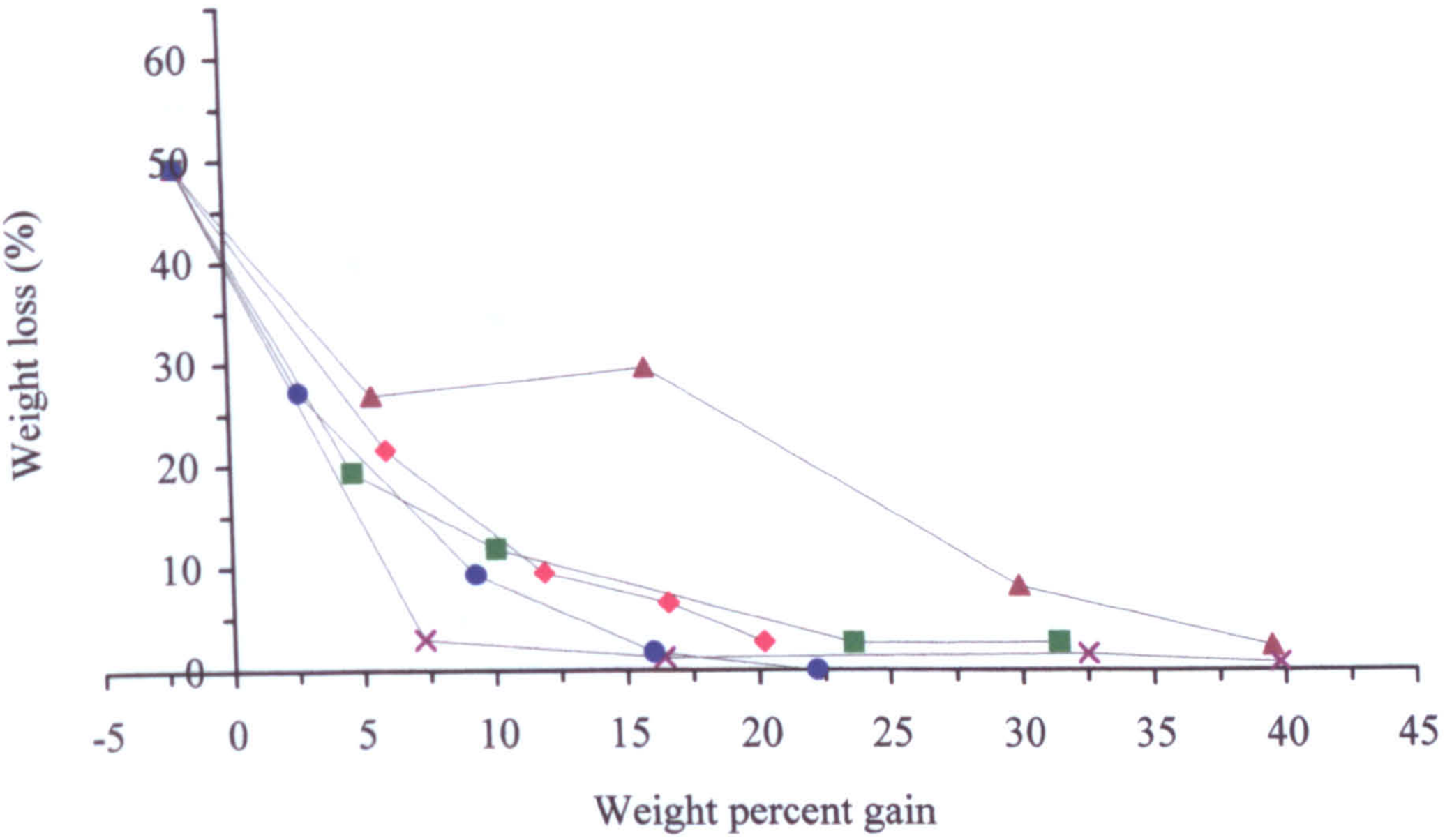


Figure 6.9 *Gloeophyllum trabeum* (low moisture content test). Corrected weight loss (%)

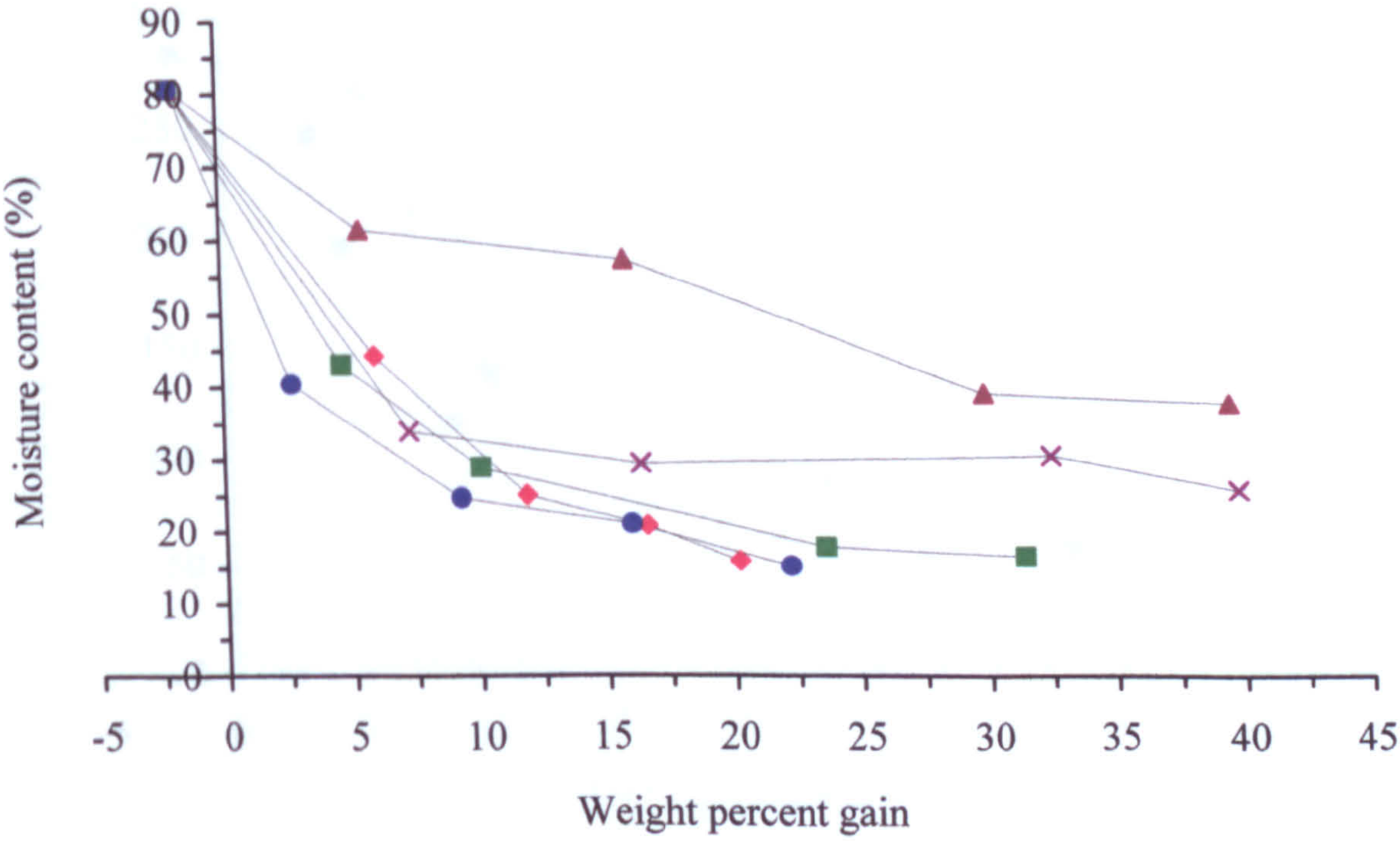


Figure 6.10 *Gloeophyllum trabeum* (low moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);
Phthalic anhydride (×); Butyl isocyanate (●)

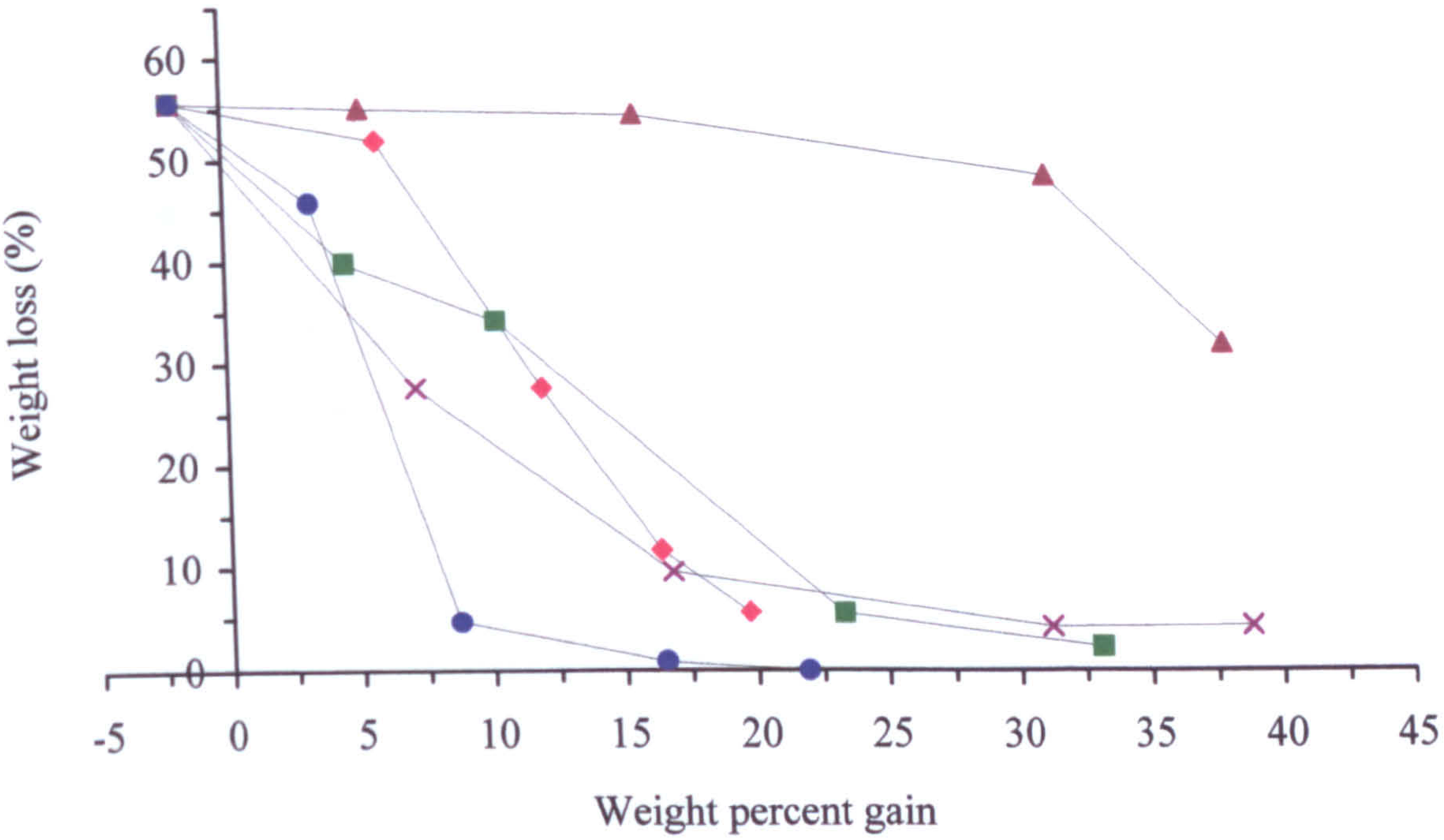


Figure 6.11 *Gloeophyllum trabeum* (high moisture content test). Corrected weight loss (%)

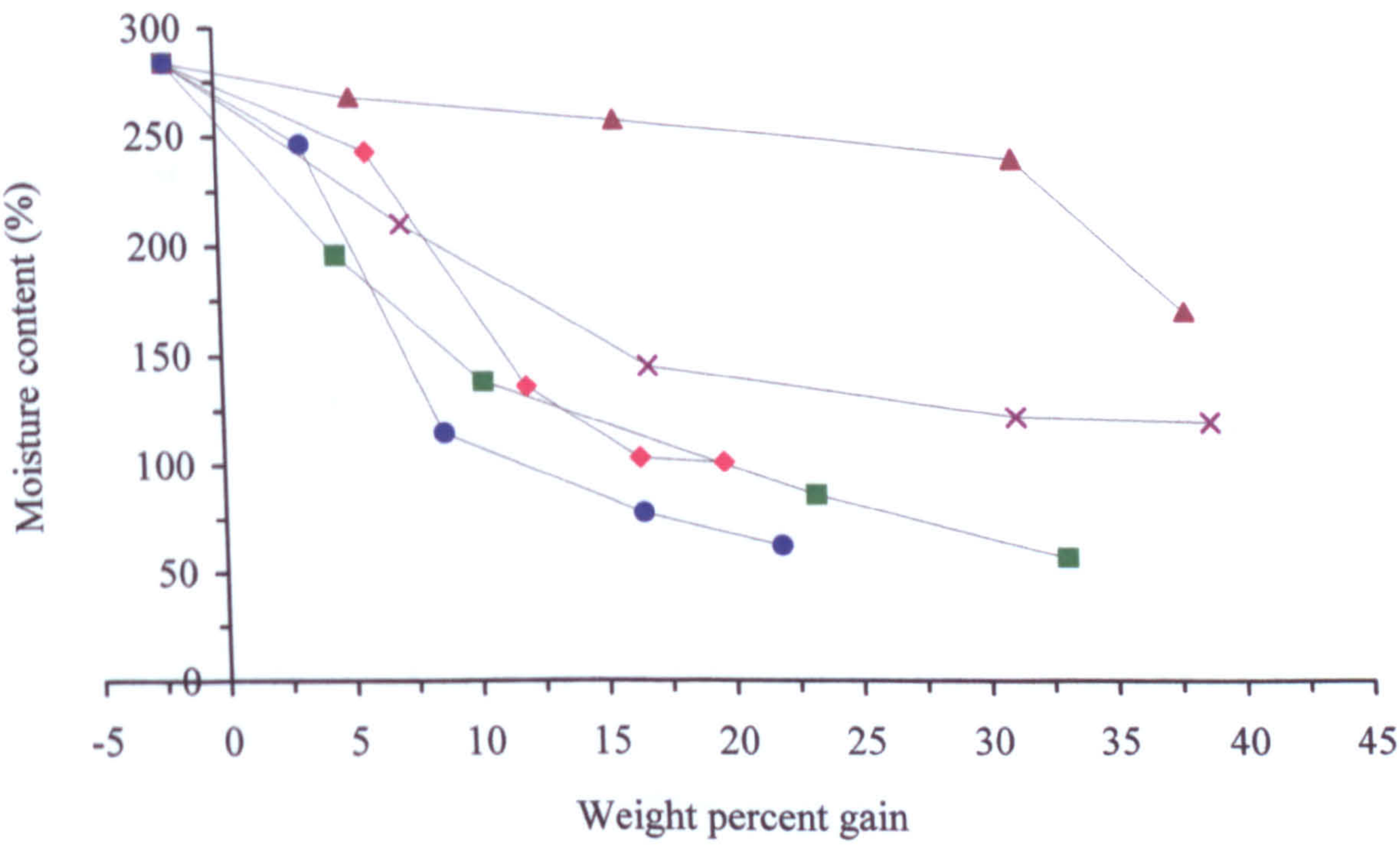


Figure 6.12 *Gloeophyllum trabeum* (high moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);
Phthalic anhydride (×); Butyl isocyanate (●)

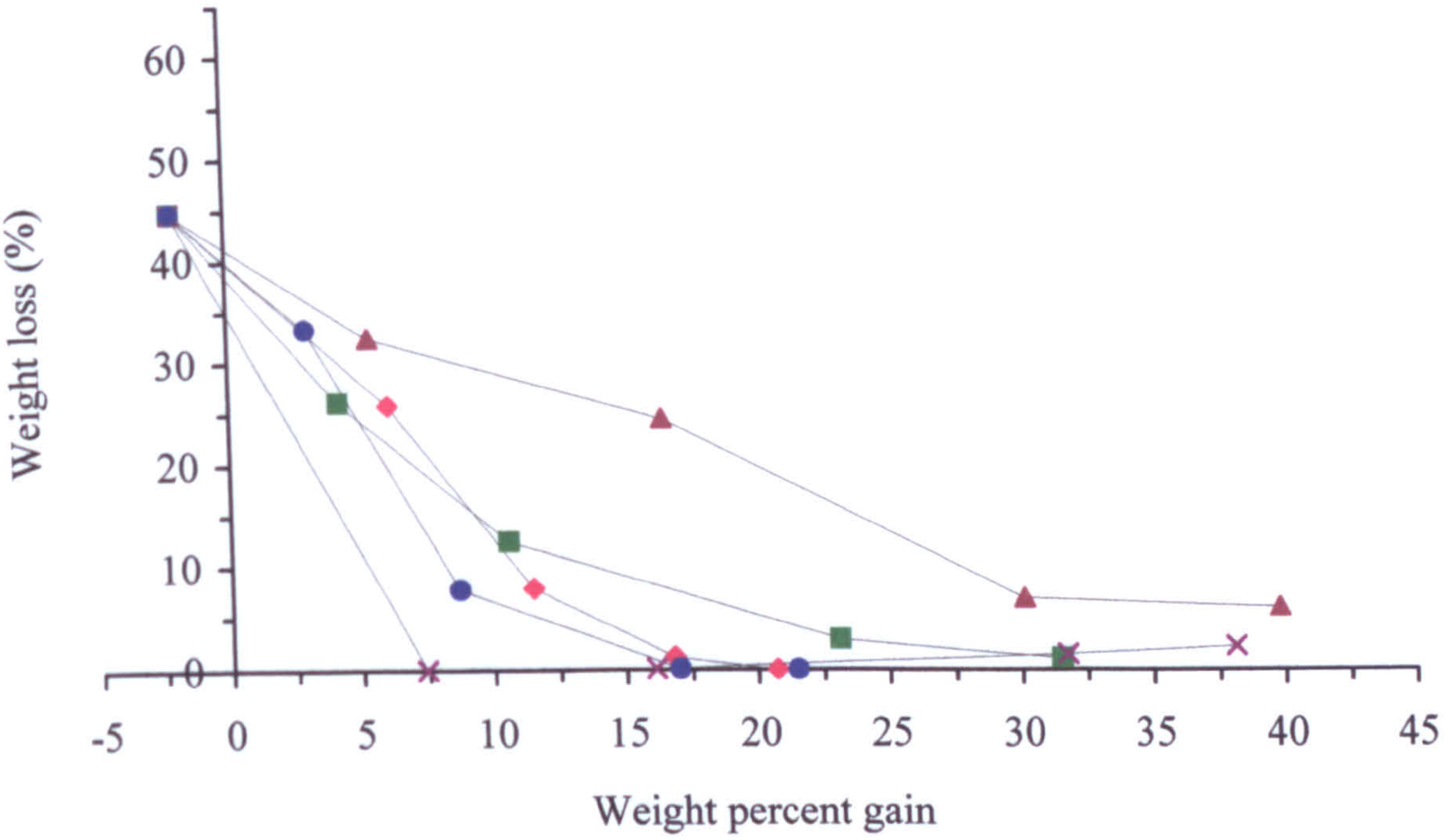


Figure 6.13 *Trametes versicolor* (high moisture content test). Corrected weight loss (%)

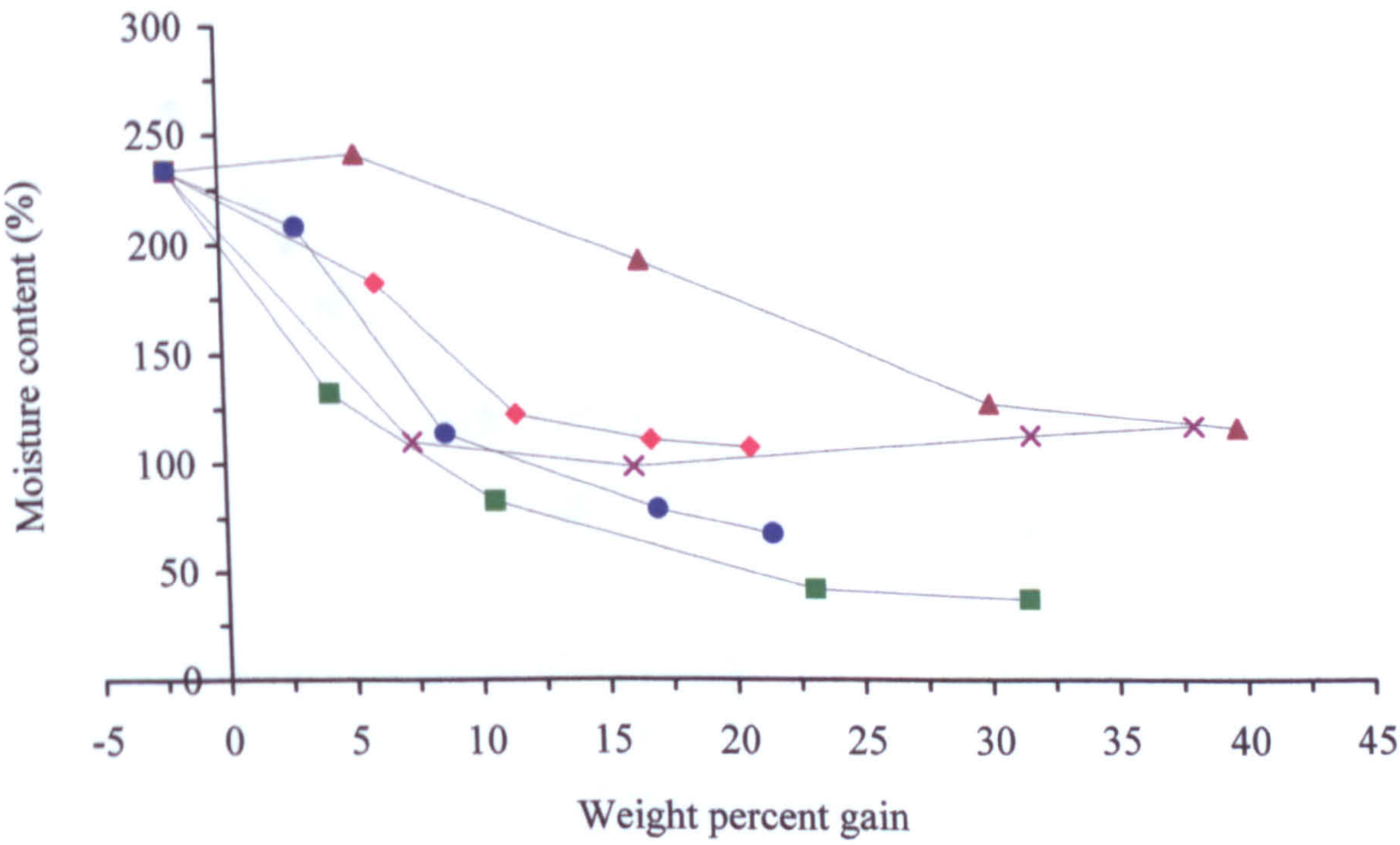


Figure 6.14 *Trametes versicolor* (high moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);
Phthalic anhydride (×); Butyl isocyanate (●)

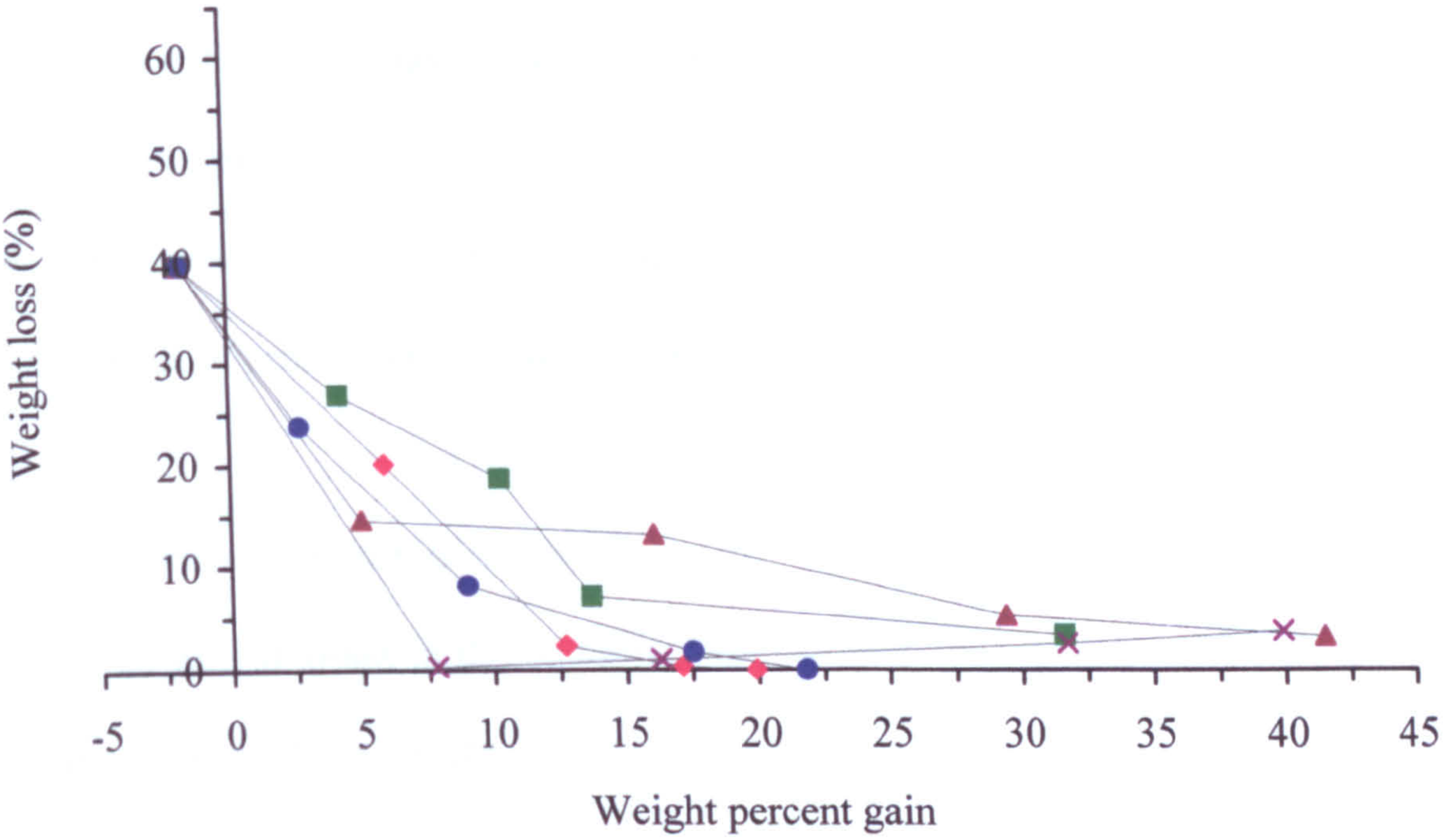


Figure 6.15 *Pycnoporus sanguineus* (high moisture content test). Corrected weight loss (%)

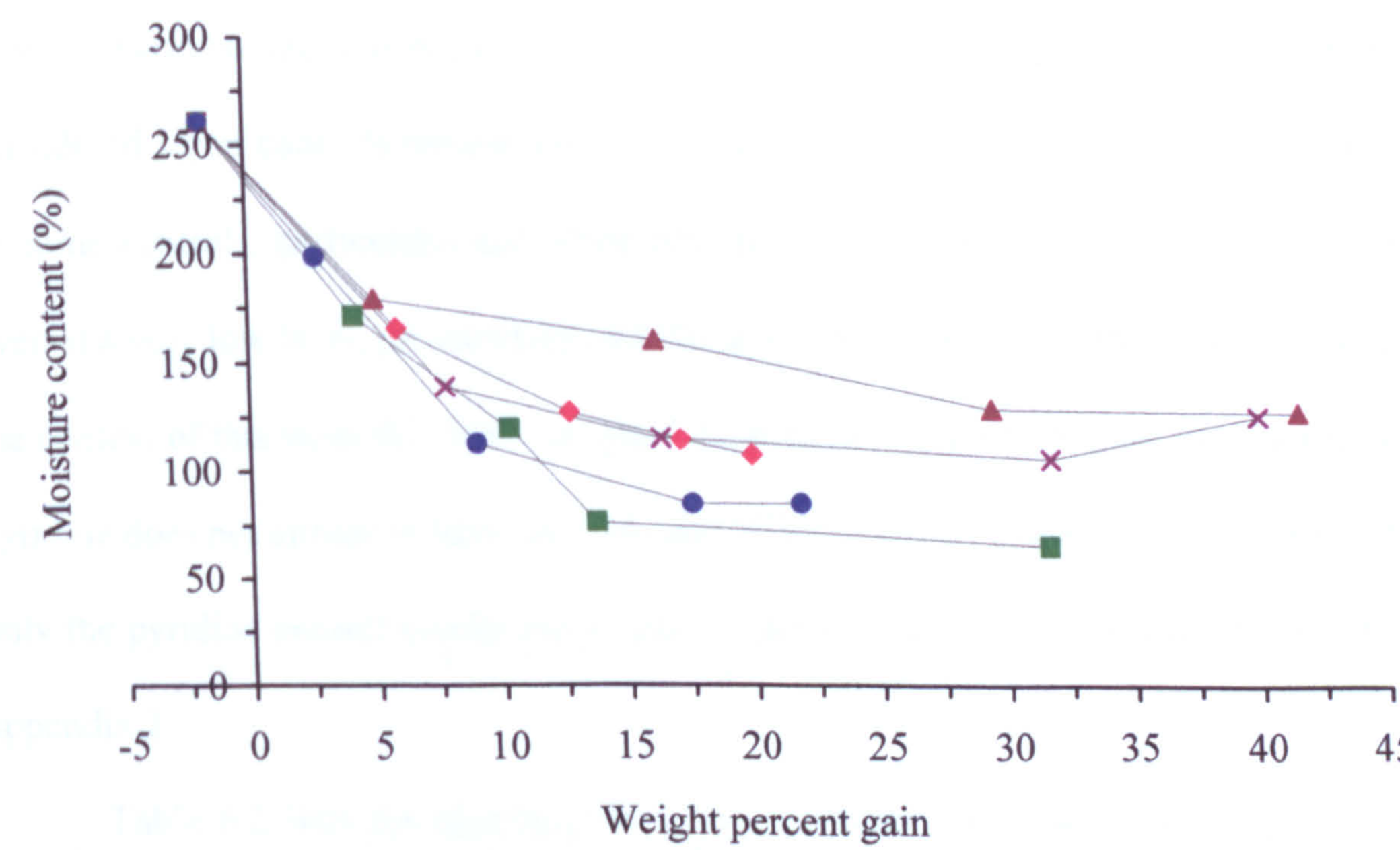


Figure 6.16 *Pycnoporus sanguineus* (high moisture content test). Moisture content (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);
Phthalic anhydride (×); Butyl isocyanate (●)

pronounced as in the acetic anhydride modified wood. Uneven distribution of modification in the wood may be due to a lower ratio of modifying chemical to cell wall material during modification in the latewood (small lumen, thick cell wall), leading to lower localised level of reaction. This has implications when considering the genuine threshold level of protection of a modifying chemical.

6.3.2 Performance against decay fungi

High weight losses in the untreated and pyridine controls were achieved using all four fungi (figures 6.1 to 6.16). Average wood weight losses for pyridine controls of 65%, 58%, 47% and 40% were achieved by *C. puteana, G. trabeum, T. versicolor* and *P. sanguineus* respectively. These are compared with weight losses for the untreated controls of 59%, 59%, 28% and 33%. High standard deviations mean that differences between pyridine and untreated control weight losses against brown rot fungi cannot be considered significant. However, there does seem to be a an increase in weight loss for pyridine controls, particularly for white rot fungi. It is possible that residual pyridine, even at a very low level, provided an additional nitrogen source for decay fungi, though in the context of this work this is not of great importance. Important is the fact that residual pyridine does not appear to have any biocidal affect on decay fungi. In figures 6.1 to 6.16 only the pyridine control results are given. Results for the untreated control are listed in appendix 2.

Table 6.2 lists the threshold levels of protection estimated from figures 6.5 to 6.15. The modifications were able to control the decay by white rot more easily than by brown rot in Corsican pine sapwood. However, the two brown rot fungi gave very similar threshold levels to each other for each modifying chemical, as did the white rot fungi. In the case of the brown rot fungi, exposure to *G. trabeum* tended to result in greater weight

Table 6.2 Estimated threshold levels of protection (WPG)

Modifying chemical	**Brown rot fungi**		**White rot fungi**	
	C. puteana	*G. trabeum*	*T. versicolor*	*P. sanguineus*
Acetic anhydride	**24%***	**23%**	17%	17%
Alkenyl succinic anhydride	**38%***	**38%**	**36%**	**40%**
Succinic anhydride	No threshold	No threshold	No threshold	43%
Phthalic anhydride	35%	35%	<8%	<8%
Butyl isocyanate	**22%***	20%*	17%	20%
CCA	1.5 kg/m^3*	**6 kg/m^3**	0.75 kg/m^3	<0.5 kg/m^3

Where modified wood was exposed to a fungus in both moisture conditions (brown rot fungi), only the higher threshold value (worst case) is given. Values given refer to exposure in the high moisture content test except where marked with an asterisk (*). Those in **bold** indicate an extrapolated threshold level, which was above any level of modification achieved in the test.

losses in the high moisture content test, while exposure to *C. puteana* resulted in greater weight losses in the low moisture content test. Cardias and Hale (1990), working with isocyanate modified wood, and Codd *et al.* (1992), working with alkenyl succinic anhydride, found threshold modification levels for *G. trabeum* to be lower than those for *C. puteana*. It was suggested by Cardias and Hale (1990) that this is due to the ability of *C. puteana* to increase the moisture content of the wood it is attacking. In this study, a higher wood moisture content enabled *G. trabeum* to cause decay at modification levels similar to those decayed by *C. puteana*. However at sub-threshold levels of modification, in worst case test conditions, weight loss tended to be greater for *C. puteana* than *G. trabeum*.

In comparison to threshold data reported by other workers (section 2.4.2) the above figures are slightly high. For acetylation, Goldstein *et al.* (1961) reported a general protection level against all basidiomycete fungi tested (brown and white rot) of 18%. Beckers *et al.* (1994) also found 18% WPG acetylation to protect against *G. trabeum* and *C. puteana*, but found *P. placenta* required over 20%. They found wood acetylated to 12% to be protected against *Coriolus (Trametes) versicolor*.

For alkenyl succinic anhydride, Codd (1997) reported threshold protection against *C. puteana* and *G. trabeum* at just over 30%, but found succinic anhydride unable to protect wood at WPG's achieved. In the present study, succinic anhydride proved more effective in protecting wood against decay by white rot fungi when compared to brown rot fungi (as did all modifications), though not to the extent reported by Dunningham and Parker (1992). They found succinic anhydride modified wood (15% WPG) to be completely resistant to decay by *C. versicolor*.

Finally, Rowell and Ellis (1984) reported that a WPG of 18% (or less) was sufficient for butyl isocyanate modification to protect wood against *G. trabeum*. In the present study, weight losses in butyl isocyanate modified wood decayed by *G. trabeum* were very low at WPG's above 17%. For the same chemical, Cardias and Hale (1990) reported thresholds of 15.5%, 10%, 9.6%, 12.2% against *C. puteana*, *G trabeum*, *C. versicolor* and *P sanguineus* respectively.

Threshold CCA retentions are also given in table 6.2 for comparison. The weight losses in CCA treated wood exposed to brown rot fungi are shown in figure 6.17. As in the case for modified wood, exposure to *G. trabeum* resulted in greater weight losses in the high moisture content test, while exposure to *C. puteana* resulted in greater weight losses in the low moisture content test.

6.3.3 Influence of the structure of the modifying chemical

Figure 6.18 shows weight loss of modified wood in the *C. puteana* low moisture content test plotted against the number of hydroxyl groups reacted by each modifying chemical (millimoles per gram of wood). This differs from the figures presented on the basis of weight percent gain due to the large differences in molecular weights. It is

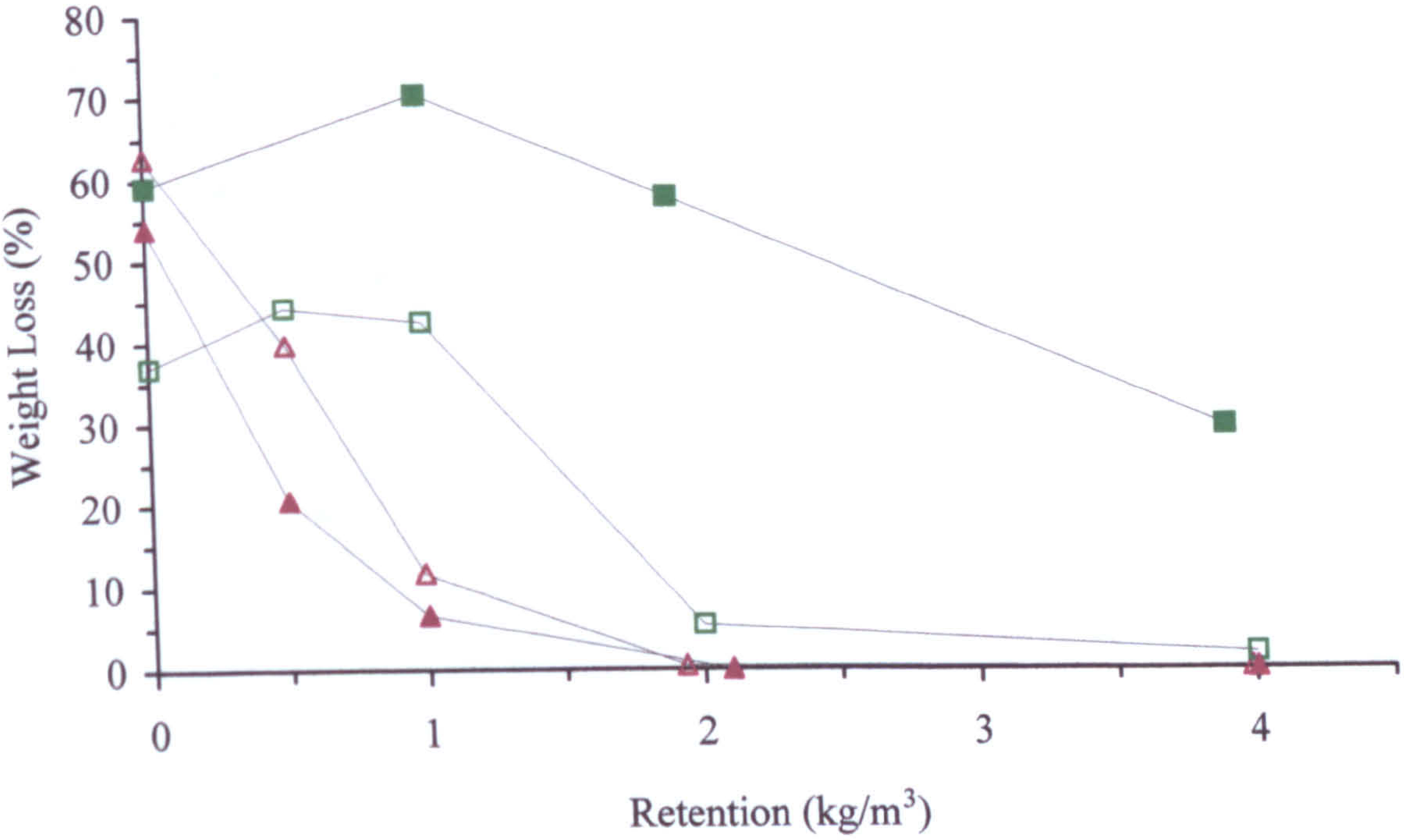


Figure 6.17 Weight losses (%) of CCA treated wood

Gloeophyllum trabeum (low moisture content) (□);

Gloeophyllum trabeum (high moisture content) (■);

Coniophora puteana (low moisture content) (△);

Coniophora puteana (high moisture content) (▲)

obvious from the large variation in performance between modifying chemicals that structural differences are important in the protection of wood.

Figures 6.1 to 6.4 show that the succinic and phthalic anhydride modified wood exposed in sterile jars behaved very differently from the other three modifications. High non-decay related weight losses were apparent for both of these chemicals, coupled with the inability to effectively reduce test moisture contents.

After exposure, phthalic anhydride modified blocks differed visually from other modified blocks in two respects. Firstly white deposits were found on the blocks which were evident even in those blocks exposed in sterile conditions. Fourier transform infrared spectroscopy revealed this to be phthalic acid. This would be the expected end

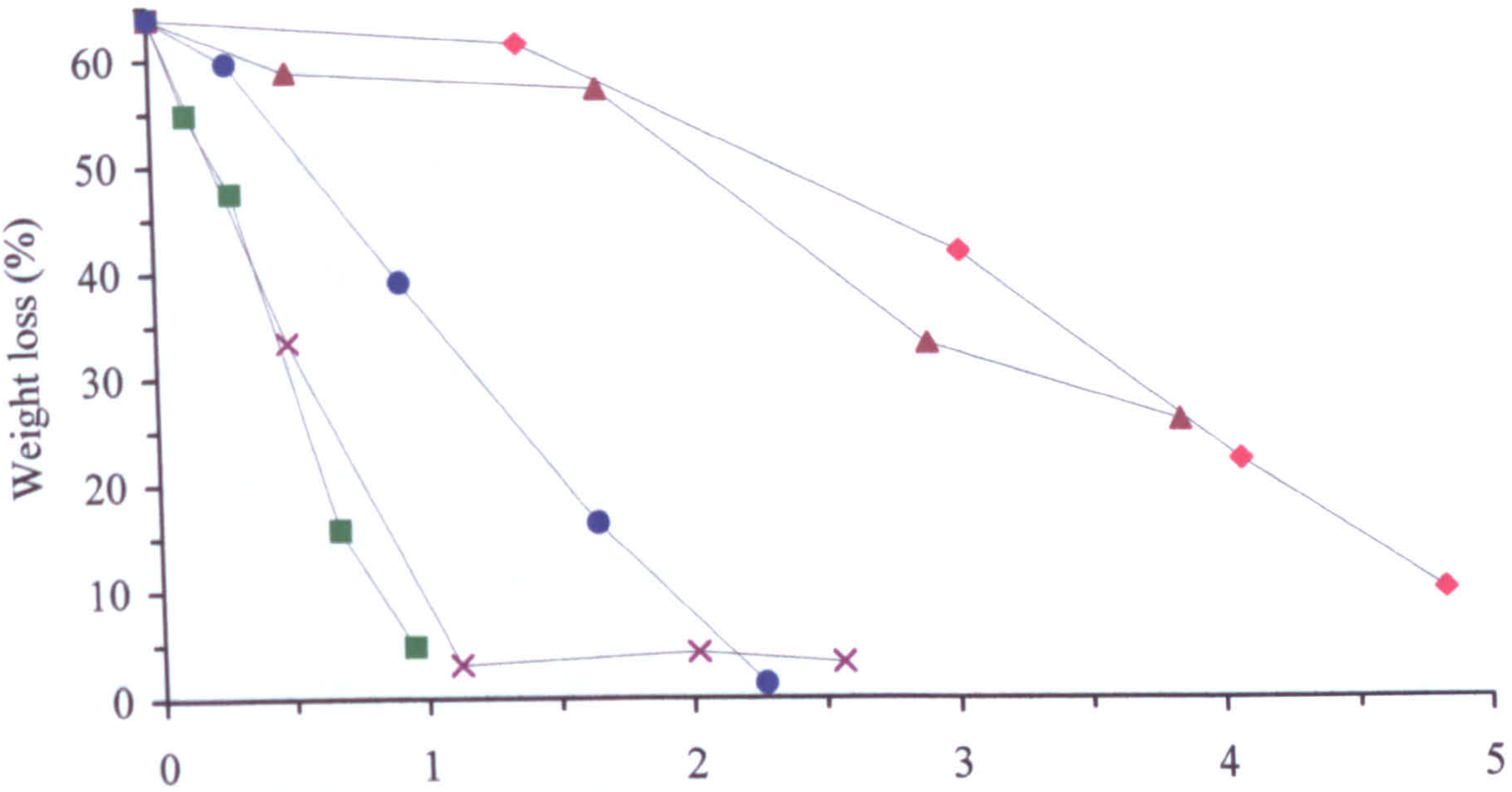


Figure 6.18 Corrected weight loss in blocks exposed to *Coniophora puteana* (low moisture content) test as a function of wood hydroxyl groups reacted

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲); Phthalic anhydride (×); Butyl isocyanate (●)

product if the ester bond formed on reaction of phthalic anhydride with wood were then hydrolysed. Secondly, decay fungi did not appear to grow into wood blocks modified to above threshold levels of phthalic anhydride A similar observation was made for undecayed CCA treated wood. In other modified woods fungal hyphae appeared to enter even those modified blocks that they were unable to decay. It is possible that relatively large quantities of phthalic acid within modified blocks had a biocidal effect on decay fungi, and prevented them from entering and decaying the wood. This would account for the results in figures 6.5, 6.9, 6.13 and 6.15, which show phthalic anhydride to be one of the more effective modifying chemicals in this test. A phthalic anhydride WPG of 39% is equivalent to 195 kg/m3 (the conventional measure of preservative retention in wood). In comparison to CCA retentions it can be seen that this is particularly high. Due to the high

non-decay related weight loss in phthalic anhydride treated blocks, it is unlikely that decay resistance would have been maintained in the longer term. Prolonged exposure may well have left the wood susceptible to decay as the modifying chemical was lost. The results of longer (unsterile) exposure tests are reported in chapter 8.

Succinic anhydride modification in the present test was observed to allow an increase in moisture content (above that of controls) in the low moisture content test (figure 6.2). In chapter 4 it was found that high WPG's of succinic anhydride did not cause a reduction in sorption, and phthalic anhydride modification caused an increase. In this test phthalic anhydride modification caused no increase in moisture content, and the increase in moisture content for succinic anhydride modified wood was significantly above FSP, and so could not be accounted for by sorption effects. It is possible that the carboxyl groups formed during succinic anhydride modification caused an increase in the wettability of the wood cell wall surface (i.e. reduced the contact angle between wood and water), and so promoted the flow of free water into the cell lumina to a greater extent than unmodified wood. Although these carboxyl groups are also formed on modification with alkenyl succinic anhydride and phthalic anhydride, a similar increase in moisture content did not occur. For alkenyl succinic anhydride the large hydrophobic chain would have caused a decrease in wettability. For phthalic anhydride modification it is possible that the positioning of the carboxyl in modified wood (due to the inflexibility of the molecule) was such that wettability was not increased.

Succinic anhydride was largely ineffective in preventing decay by the fungi tested (figure 6.5). This could be due to weakness of the bond that succinic anhydride forms on reaction with the wood, which would account for weight losses in sterile conditions (figures 6.1 and 6.3).

In most cases acetic anhydride, butyl isocyanate and alkenyl succinic anhydride modified woods showed similar relationships between WPG and both weight loss and moisture content. This was most evident in low moisture content test, particularly for the WPG / moisture content relationship (figures 6.2, 6.6 and 6.10). However, despite close threshold levels for butyl isocyanate and acetic anhydride modifications, examination of the figures reveals that at sub-threshold WPG's butyl isocyanate almost invariably protected wood to a greater degree than acetic anhydride. Both these chemicals tended to be more effective in protecting wood than alkenyl succinic anhydride; estimated threshold levels of protection were significantly greater for the latter (38%, *C. puteana*, table 6.2) than for the other two (22% and 24%, *C. puteana*).

This order of efficacy; butyl isocyanate, then acetic anhydride, then alkenyl succinic anhydride, may indicate an optimum chain length for the modifying chemical with regard to wood protection. However, differences in structure (other than size) between the three chemicals mean such a conclusion cannot be drawn in this work. The possibility of an optimum chain length of modification for decay resistance is apparent in other work using isocyanate modification. Ellis and Rowell (1984) found butyl isocyanate modified wood to be more decay resistant than that reacted with the shorter chain ethyl and propyl isocyanates, while Cardias and Hale (1990) found wood modified with butyl isocyanate also to be more effective than wood reacted with the longer chain hexyl isocyanate.

6.3.4 The relationship between moisture content and decay

Assessing the relationship between moisture content and decay in modified wood is not straightforward. Relatively little can be gained from a direct comparison of end of

test moisture content and weight loss as increasing levels of decay lead to increasing moisture content. Reasons for this include:

- A reduction in the dry weight of wood (the denominator in the moisture content calculation.
- The production of water through metabolism of wood polymers. For example, complete metabolism of cellulose would theoretically result in the production of a mass of water equal to approximately 56% of the mass of cellulose (see appendix 3).
- The creation of additional void space in which water can condense or sorb.
- An increasing quantity of fungal hyphae within the wood, which themselves contain a high proportion of water.

However, there is still value in comparing end of test moisture contents between modification types and also in examining the moisture content of blocks after exposure in sterile conditions, which give an indication of moisture available to decay fungi before decay occurs.

Initial observations indicate that suppression of moisture content was not the mechanism by which decay was suppressed. In the high moisture content test all moisture contents reached high enough levels to theoretically support decay (i.e. greater than 20%). In the white rot tests alkenyl succinic anhydride modified blocks lost more weight than butyl isocyanate or acetic anhydride modified blocks despite having lower moisture contents at the end of the test (even before the increase in moisture content caused by decay through fungal metabolic water production and reduction in dry wood weight are taken into account) (see figures 6.13 to 6.16). In the high m.c. *G. trabeum* test alkenyl succinic anhydride modified wood showed greater weight losses than butyl isocyanate or acetic anhydride modified wood despite lower equilibrium moisture

contents for alkenyl succinic anhydride modified wood in the sterile jars (figures 6.4 and 6.11).

Each of the above cases involves the high moisture content test, with resulting end moisture contents well above FSP. However, it is possible that it is the distribution of water in wood that is important to the ability of fungi to decay wood rather than the gross moisture content. More specifically the amount of water in the wood cell walls may be of greater importance than free water in the cell lumina (as suggested by Stamm and Baechler, 1960; see section 2.4.4). In the above cases, alkenyl succinic anhydride modified wood was decayed to a greater extent than acetic anhydride or butyl isocyanate modified wood despite having a lower moisture content. Results from chapter 4 indicate that alkenyl succinic anhydride modified wood adsorbs more cell wall water than acetic anhydride or butyl isocyanate modified wood (at equivalent weight gains of above 15%; see section 4.4.3). The lower moisture content for alkenyl succinic anhydride modified wood in this test could be due to a greater water repellency, resulting in a lower free water content. If the long hydrophobic chain causes a greater wetting angle between water and the modified wood cell wall, this could lead to reduced bulk water flow into the cell lumina, and a lower overall moisture content (despite a higher cell wall moisture content).

In order to assess the effect of the cell wall moisture content on decay resistance, a comparison of weight loss with FSP is required. This is further discussed in chapter 9.

An interesting comparison can be made between the low and high moisture content tests for the brown rot fungi. The only difference between the two tests is the moisture content of the blocks, and from a comparison of the moisture contents in the sterile tests (figures 6.2 and 6.4) it is evident that the extra water will have largely (if not wholly) been in the form of free water within the cell lumina. This free water had a

significant effect on decay. As stated earlier, weight losses in the *C. puteana* test were reduced due to the high moisture content, while those in the *G. trabeum* test increased. It is probable that weight losses increased in the latter case because moisture content was raised into a range considered optimal for decay by that fungus (see section 2.3.4). The availability of water could have caused more rapid growth of the hyphae and hence more rapid decay. In a time limited test such as this one, the rate of decay will help determine the extent of decay at the end of the test.

The reduction in decay in the *C. puteana* test is less straightforward. Moisture content may have increased to a level above optimal for this fungus. Above optimal moisture content, the excess water is thought to restrict decay by imposing a physical barrier to growth or by limiting gaseous exchange (Rayner and Boddy, 1988), though this does not explain why different modifications should affect decay in these conditions to a different extent. Particular is the case of acetic anhydride modified wood, which showed a lesser reduction in weight loss in these conditions than butyl isocyanate and alkenyl succinic anhydride modified wood (compare figures 6.5 and 6.7). When the equilibrium moisture content in the high m.c. (sterile) test is considered (figure 6.4), then it is seen that acetic anhydride modified wood had a higher equilibrium moisture content certainly than alkenyl succinic anhydride modified wood, and in certain cases also butyl isocyanate modified wood. The differing extent to which bulk water appears to be affecting decay of modified wood could be due to different water repellency properties of modifications. If the hydrophobic chains of butyl isocyanate and alkenyl succinic anhydride caused the lumen - cell wall surface to be more water repellent (have a greater contact angle with water) even when at fibre saturation point, then the distribution of free water within the lumen could be affected. This in turn could change the way free water influences the decay process.

6.4 Summary of points raised in chapter 6

- Non-decay related weight losses (weight losses in sterile jars) were particularly high for succinic and phthalic anhydride modified wood. This is in agreement with results from chapters 3 and 4.

- Patterns of uneven decay were evident in brown rotted acetic anhydride modified wood, and to a lesser extent in butyl isocyanate and alkenyl succinic anhydride modified wood. This took the form of pockets of decay in the latewood in the interior of the blocks. It was concluded that this was due to a lower ratio of modifying chemical to cell wall material in the inner latewood during reaction, leading to lower WPG's in this portion of the wood. The uneven distribution could have caused measured threshold protection levels against decay fungi to be higher than if modification had been evenly distributed.

- High weight losses in unmodified controls were achieved for all four fungi in this test. Pyridine control weight losses were, if anything, higher than untreated controls, so it was concluded that the use of this solvent had no adverse effects on the test.

- The threshold levels of protection estimated from weight loss results in this work tended to be higher than those reported by other workers.

- Decay by white rot fungi was more easily controlled than decay by brown rot fungi. The threshold levels of protection for the two white rot fungi were very close to one another, as were the thresholds for the two brown rot fungi.

- *C. puteana* caused higher weight losses in the low m.c. test, while *G. trabeum* caused higher weight losses in the high m.c. test. In worse case conditions, weight losses for *C. puteana* were generally higher than those for *G. trabeum*.

- Phthalic anhydride proved to be one of the more effective modifying chemicals in protecting wood from decay in this test. However, this chemical was highly labile, and it is thought that the large quantities of modifying chemical in the wood had a biocidal effect on the basidiomycete fungi.

- Succinic anhydride was not effective in protecting wood from decay. Interestingly, succinic anhydride modification increased the amount of free water in wood in sterile conditions. This modifying chemical may cause a reduction in the contact angle between the cell wall and water.

- Of the other three modifying chemicals, butyl isocyanate was more effective than acetic anhydride, which was more effective than alkenyl succinic anhydride in protecting wood from decay. This may indicate an optimum chain length for a modifying chemical, though other differences between these three chemicals mean such a conclusion cannot be drawn here.

- The end of test moisture content in decay tests is not a valid measure of the wood - water relationship in modified wood (due to decay effects).

- Even in undecayed modified wood, moisture content in the high m.c. test was always above 30%, and so was always theoretically high enough to support decay. Overall moisture content does not appear to be the governing factor with regard to the mechanism of protection of modified wood from decay. It was suggested that the water within the cell wall may be more important.

- Having said this, results from the high m.c. test for brown rot fungi showed that free water in the lumina can have a significant effect on the extent of end of test weight losses in modified wood (as it can on weight losses in unmodified wood). In the case

of *C. puteana* it was thought that different modifications could have caused different distributions of free water within the cell lumina, which in turn could hinder the decay process.

Chapter 7. Soft rot test

7.1 Introduction

This chapter describes a test in which chemically modified stakes were exposed in unsterile soil in conditions suitable for soft rot. An attempt was made in this test to establish the threshold levels of protection for these chemicals specific to soft rot fungi. CCA treated stakes were tested alongside the modified stakes for comparison.

7.2 Materials and methods

7.2.1 Modified wood samples

Corsican pine sapwood stakes measuring 5mm x 15mm x 100mm (radial x tangential x longitudinal) were chemically modified as described in chapter 3. Pyridine and untreated controls were also exposed, as were CCA treated stakes (treated as described in chapter 3).

7.2.2 Test conditions

Stakes were planted to a depth of 80mm in tanks of John Innes No. 2 compost wetted to 110% of its water holding capacity. Lids were placed over the tanks with gaps being created between lid and tank for ventilation purposes. These tanks were stored in a conditioning room at 27°C and 70% relative humidity for 20 months. Soil moisture content was monitored and sustained throughout the duration of the test.

These conditions were slightly wetter than those stated in European pre-standard ENV807 (1993), and ensured severe wetting conditions for the hydrophobic modified wood. The exposure time was also significantly longer than the 32 weeks specified in

ENV807. Stakes were visually assessed for signs of decay throughout the test, and sets were removed after 13 and 20 months. Weight loss and end of test moisture content for these sets of stakes were calculated after oven drying (105°C) to constant weight. Weight loss and moisture content figures presented are therefore on the whole stake, including the section remaining above the surface of the soil. Light microscopy was used to confirm the presence of soft rot decay (soft rot cavity attack of the S_2 layer was taken as evidence of soft rot).

7.3 Results and discussion

7.3.1 Performance of chemically modified wood in unsterile soil

The relative ability of the different modifications to prevent decay in these conditions is depicted in figure 7.1 (13 months exposure) and figure 7.2 (20 months exposure). Data from which these figures have been drawn are given in the tables in appendix 4 along with data for CCA treated wood. In these figures each point represents mean weight loss and mean WPG data for 10 stakes. In some cases this mean covers stakes which vary significantly in WPG or weight loss so figures 7.1 and 7.2 should only be used for comparative purposes. Figures 7.3, 7.4 and 7.5 show scatter plots of weight loss data of all the individual stakes modified with acetic anhydride, alkenyl succinic anhydride and butyl isocyanate respectively (the three most effective modifications) after 20 months exposure. Figure 7.6 depicts the end of test (20 months) moisture content results for butyl isocyanate modified wood. Figure 7.7 gives weight loss data for CCA treated stakes (20 months). Regression lines/curves have been drawn on these figures to aid the assessment of threshold level of protection.

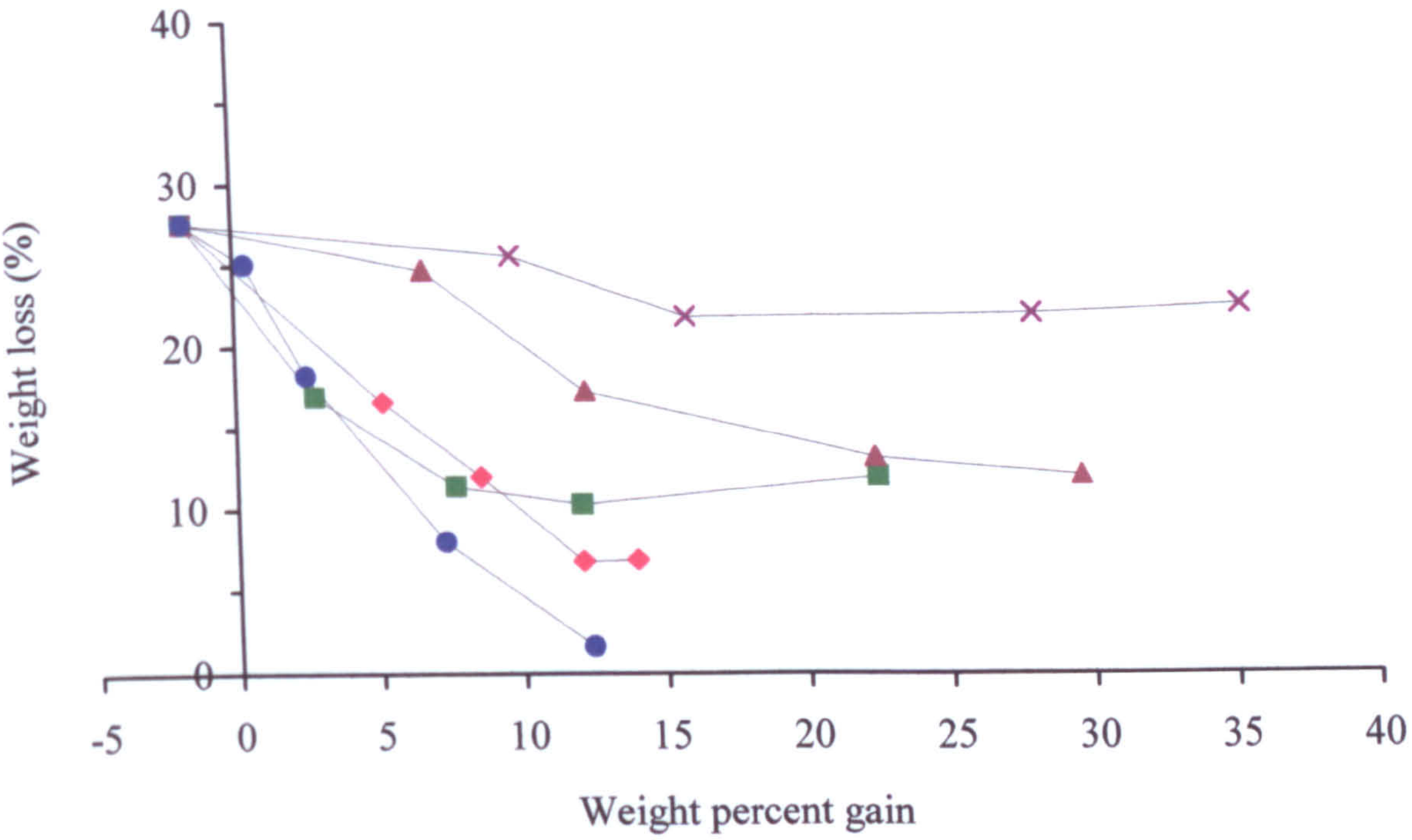


Figure 7.1 Thirteen months exposure in unsterile soil. Weight loss (%)

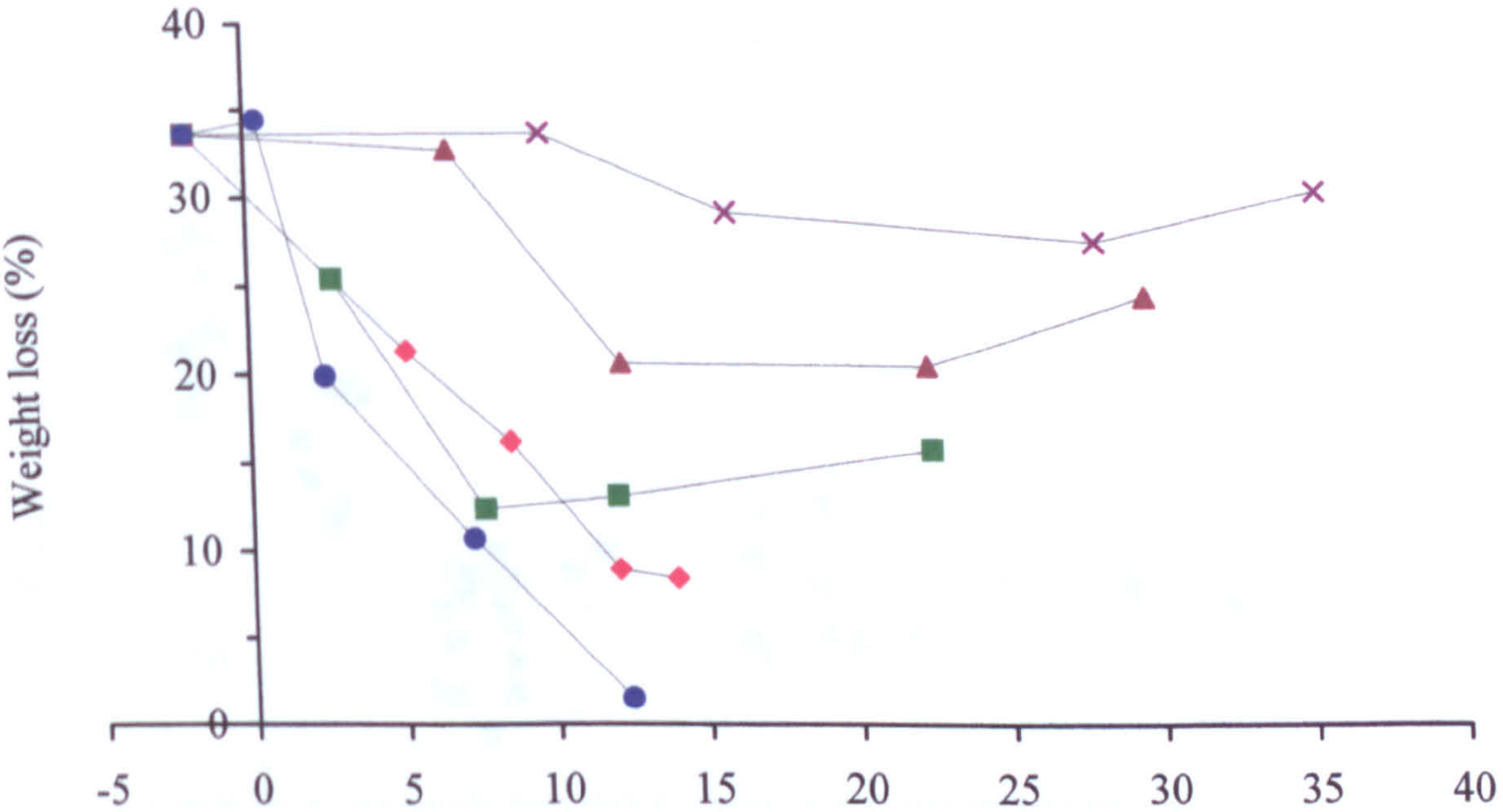


Figure 7.2 Twenty months exposure in unsterile soil. Weight loss (%)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Succinic anhydride (▲);

Phthalic anhydride (×); Butyl isocyanate (●)

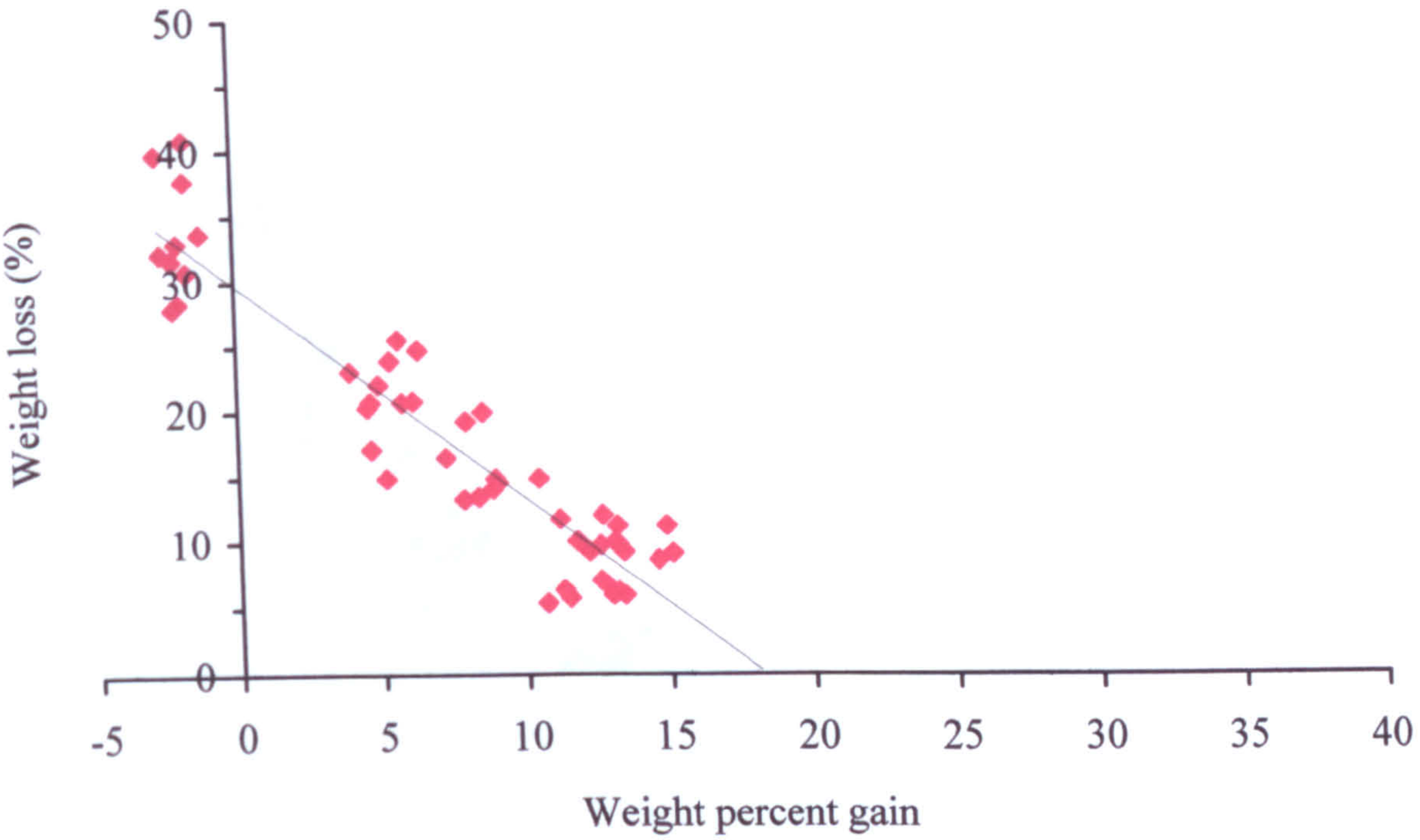


Figure 7.3 Weight loss in acetic anhydride modified wood exposed for 20 months in unsterile soil

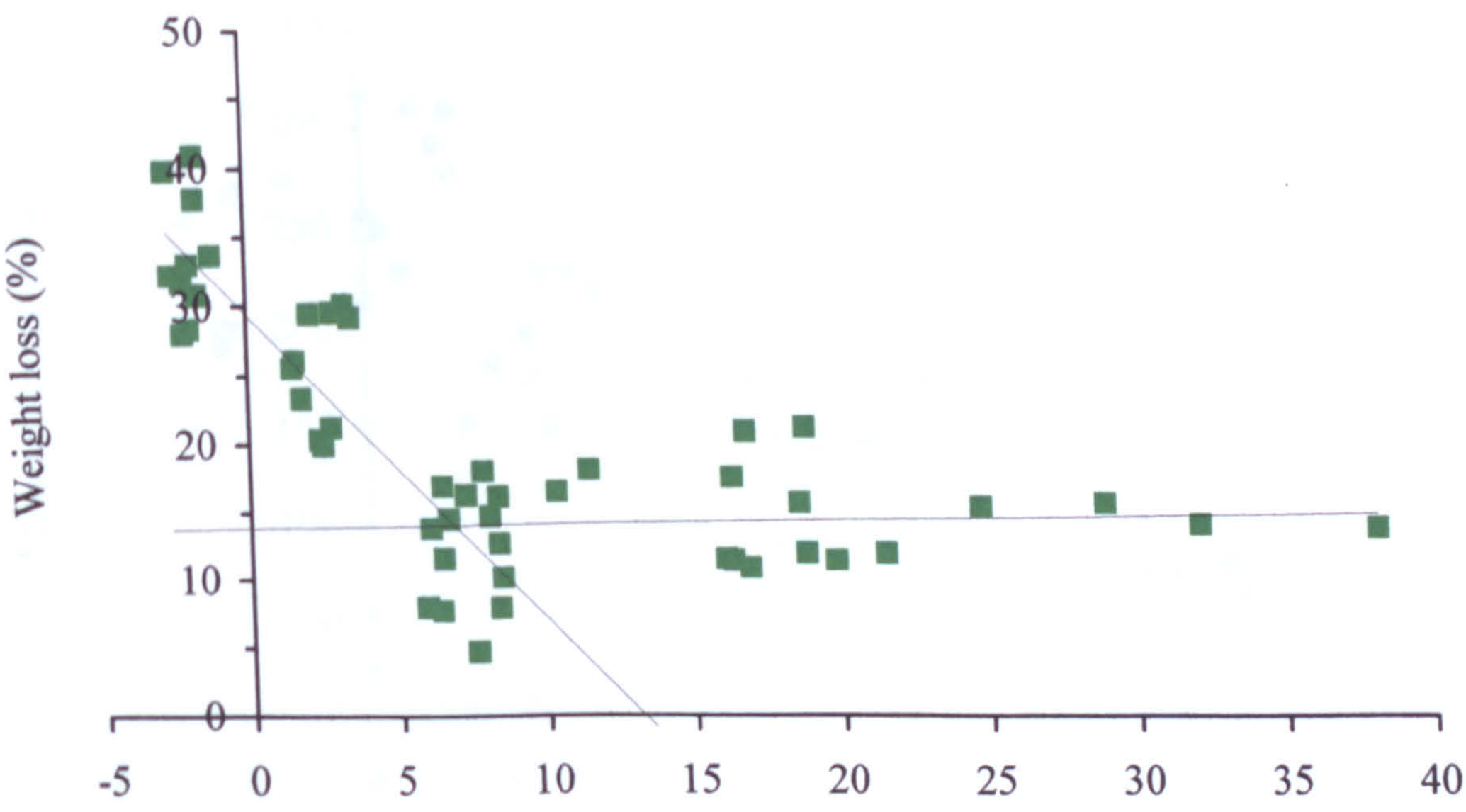


Figure 7.4 Weight loss in alkenyl succinic anhydride modified wood exposed for 20 months in unsterile soil

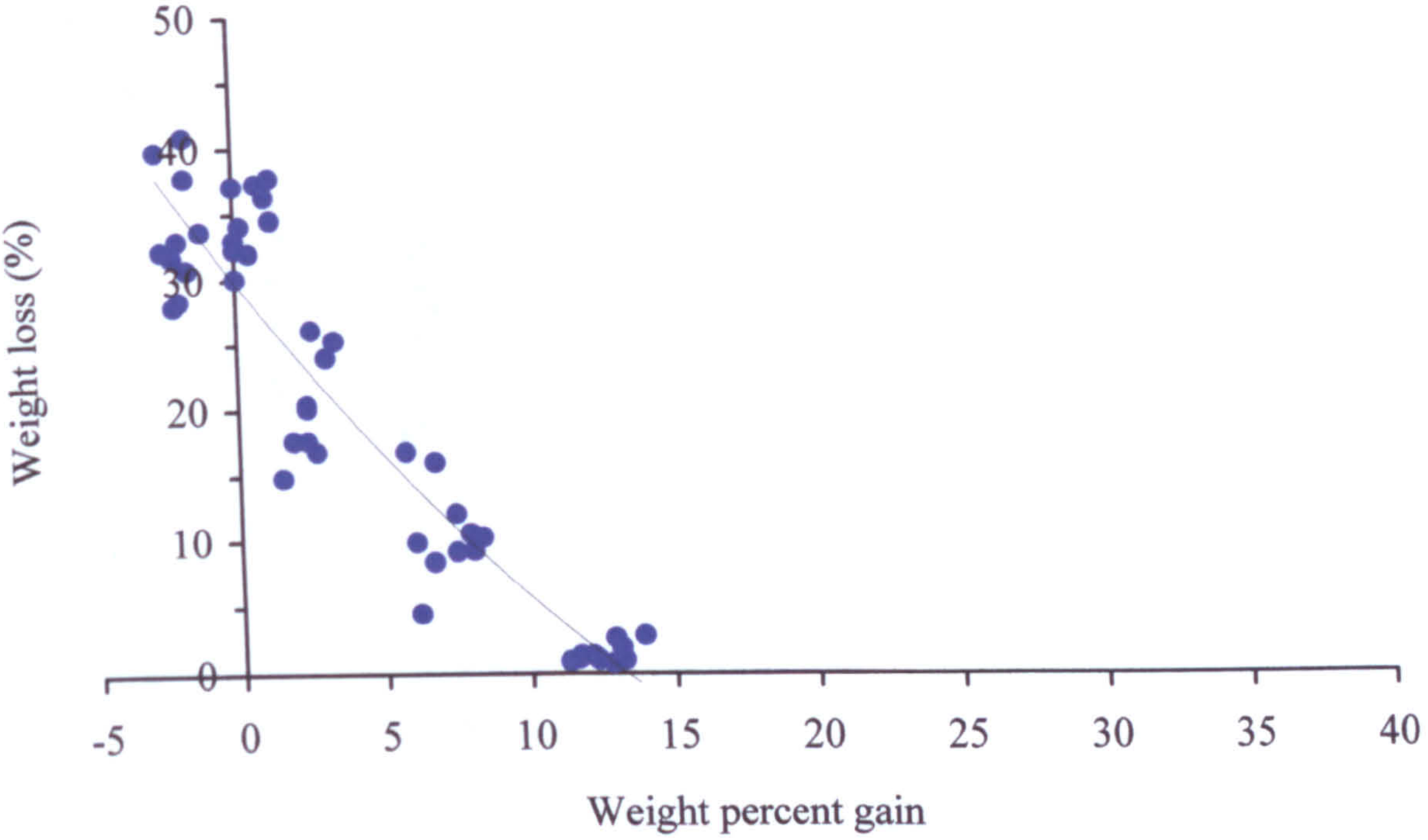


Figure 7.5 Weight loss in butyl isocyanate modified wood exposed for 20 months in unsterile soil

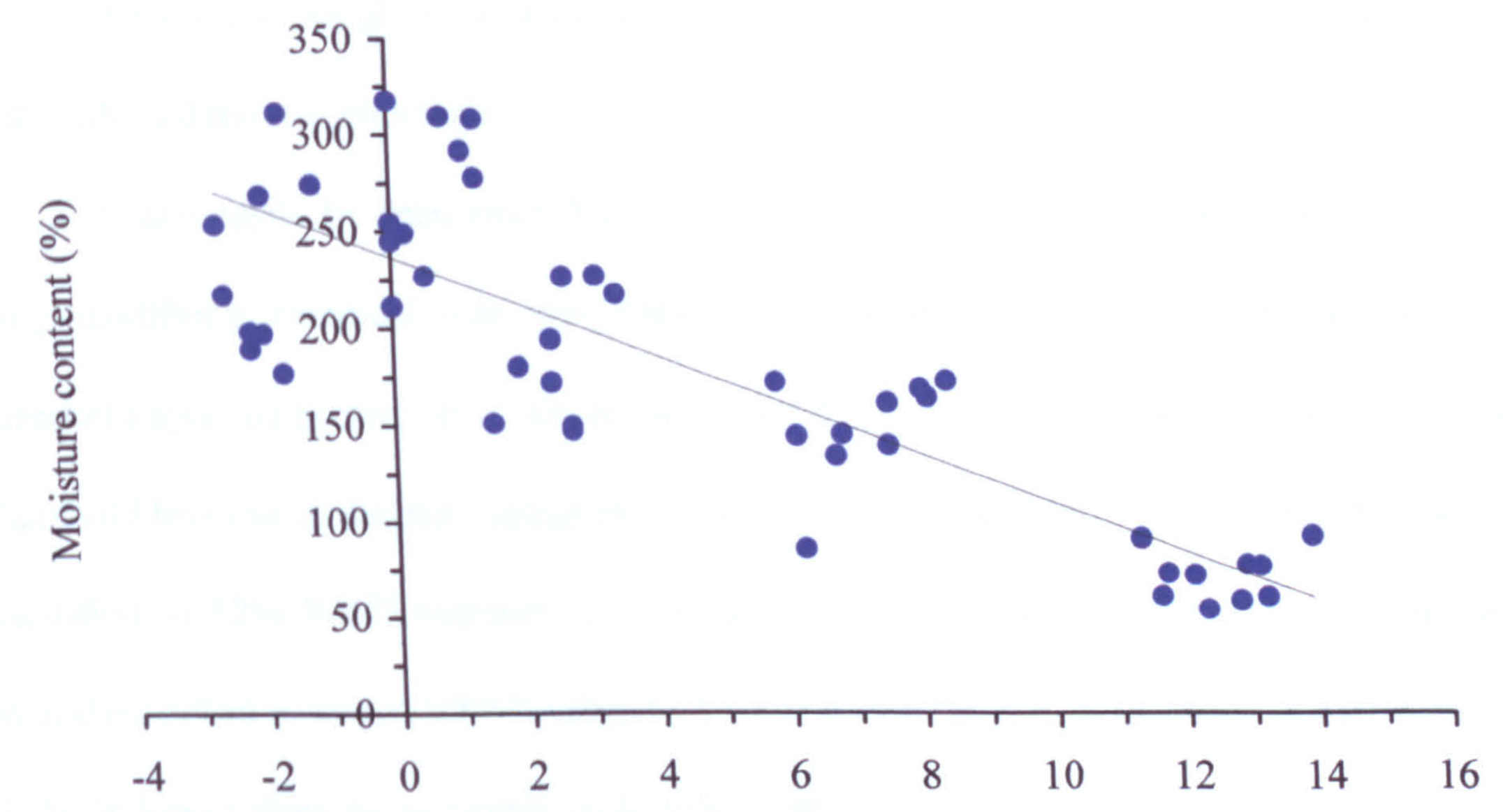


Figure 7.6 End of test moisture content of butyl isocyanate modified wood exposed for 20 months in unsterile soil

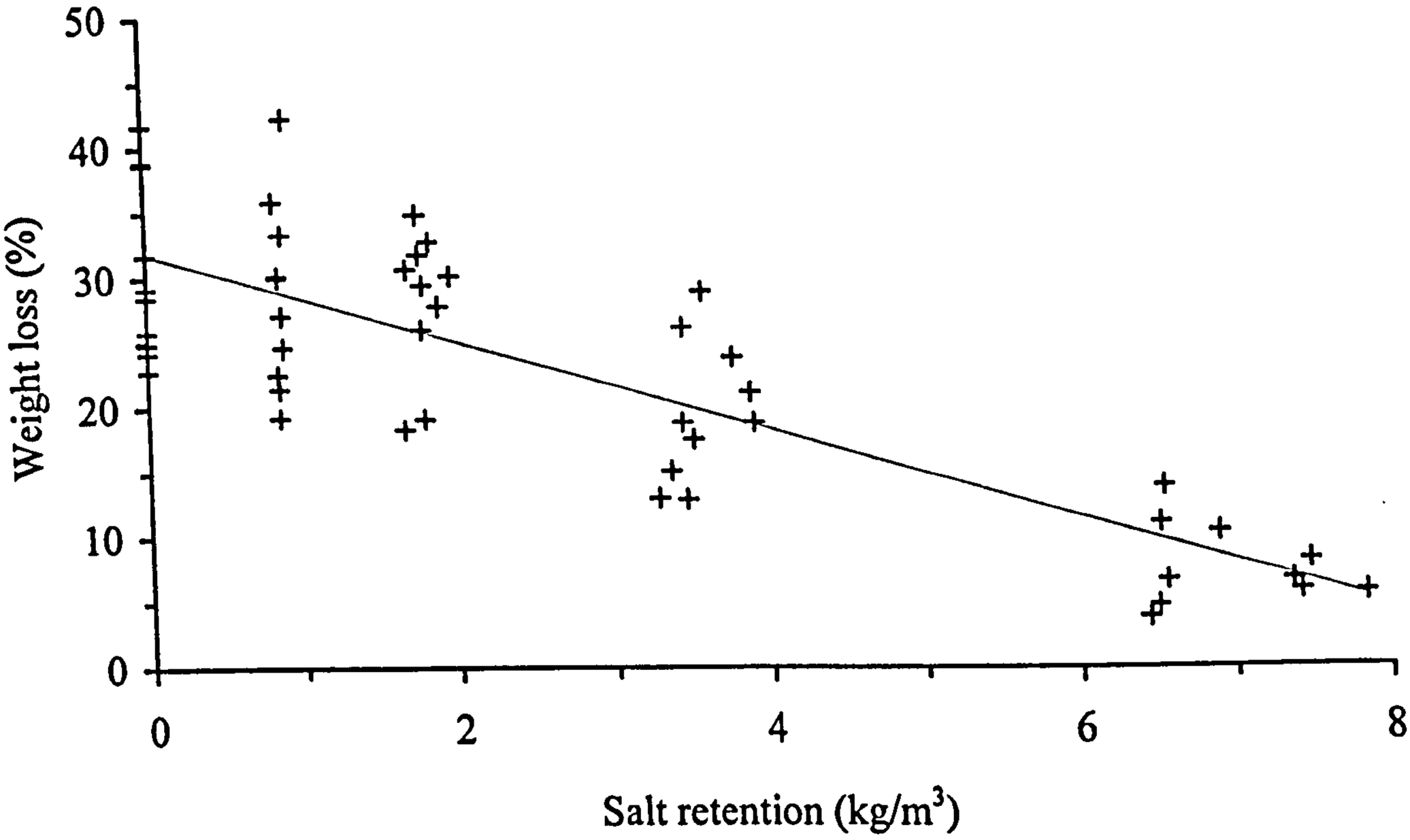


Figure 7.7 Weight loss in CCA treated wood exposed for 20 months in unsterile soil

An average weight loss of 33% was achieved in unreacted control stakes (pyridine only) after 20 months exposure.

It can easily be seen from Figures 7.1, 7.2 and 7.5 that butyl isocyanate was the only modifying chemical that was completely effective in controlling decay, with a threshold level of protection of approximately 12% WPG. This appears to be a genuine threshold level as no further weight loss (above the 1.6% recorded) was apparent in wood modified to 12% WPG between 13 and 20 months (figures 7.1 and 7.2, appendix 4). Wood modified to lower WPG's continued to lose weight over this period. A threshold of 12% is lower than those levels reported in chapter 6 to protect wood from decay by basidiomycete fungi in pure culture (22% *C. puteana*, 17% *T. versicolor*), indicating that soft rot is easier to control using this modifying chemical. However, a comparison such as this is difficult to make as the test methods vary significantly in duration and in type (e.g. sample size, pure culture or unsterile etc.).

Acetic anhydride failed to protect pine from soft rot decay, though disappointingly low WPG's were achieved (maximum 15%) as described in section 3.4.1. From Figure 7.3 it is seen that threshold protection would have been reached at around 18% WPG (assuming a straight line relationship). Takahashi *et al.* (1989) found that acetylation to 15% weight gain (WPG) was sufficient to protect softwood from decay in this test, and proposed that soft rot ranked between white and brown rot fungi in their ability to decay acetylated softwood, where the level of acetylation required to protect against brown rot was greatest and white rot was least. A proposed value of 18% in this test is greater than that found by Takahashi *et al.*(1989), though the test duration in the present study was significantly longer (20 months as opposed to 6 months). Acetic anhydride modification to 18% WPG would still rank between the threshold figures reported in chapter 6 for white rot and brown rot fungi (17% and 23-24% respectively). Without further exposure of stakes at higher weight gains, such conclusions cannot be drawn with any confidence.

Stakes modified with alkenyl succinic anhydride showed increasing resistance to decay in these conditions with increasing WPG up to about 8%. In figure 7.4 a regression line has been drawn for this section of the data, indicating that on this basis a threshold level of protection close to that for butyl isocyanate would be expected. Above 8% no further improvements were evident. This is illustrated by the second, horizontal, regression line drawn through data points with WPG's greater than 8% (Figure 7.4). This particular modifying chemical proved less effective at protecting pine against soft rot than it did against brown and white rot decay in pure culture tests, where thresholds of 36-40% WPG were estimated. No threshold level of protection appears achievable against soft rot fungi in these conditions. It is possible that the bulkier alkenyl succinic anhydride molecule had greater difficulty in penetrating the wood cell wall than other modifying chemicals. Any resultant uneven distribution across the wall may be reflected in a lesser

ability of this chemical to protect against cell wall based soft rot (type 1) decay than against decay by brown or white rot fungi.

Succinic and phthalic anhydrides both proved inadequate at protecting wood, microscopic examination revealing both to be heavily soft rotted at high WPG's. Weight loss in sterile conditions of both of these chemicals was reported in the previous chapter, and loss of modifying chemical may have made modified wood susceptible to decay in this test. During the early part of the exposure period succinic anhydride modified stakes were quickly covered in heavy mould growth (plate 7.1). This growth was much greater than that evident on control stakes. This could indicate that succinate can be metabolised by mould fungi and/or that colonisation by competing fungi was inhibited by succinic anhydride modification. The participation of succinate in the tricarboxylic acid (Krebs) cycle and therefore its potential as an energy source would suggest that the former is a distinct possibility.

Similarly, phthalic anhydride modified stakes were quickly colonised by black staining fungi not apparent in control stakes or stakes modified with other chemicals (plate 7.2). Again this could be due to the ability of certain stain fungi to metabolise phthalate or to competitive inhibition by the modification. In chapter 6 phthalic anhydride modified wood showed good decay resistance against basidiomycete fungi, though this was attributed to possible biocidal properties of this chemical. Microscopy revealed phthalic anhydride modified wood to be soft rotted at all WPG's. It is not known if soft rot fungi were able to decay wood despite phthalic anhydride modification, or whether loss of chemical through leaching or action of the stain fungus enabled subsequent access.

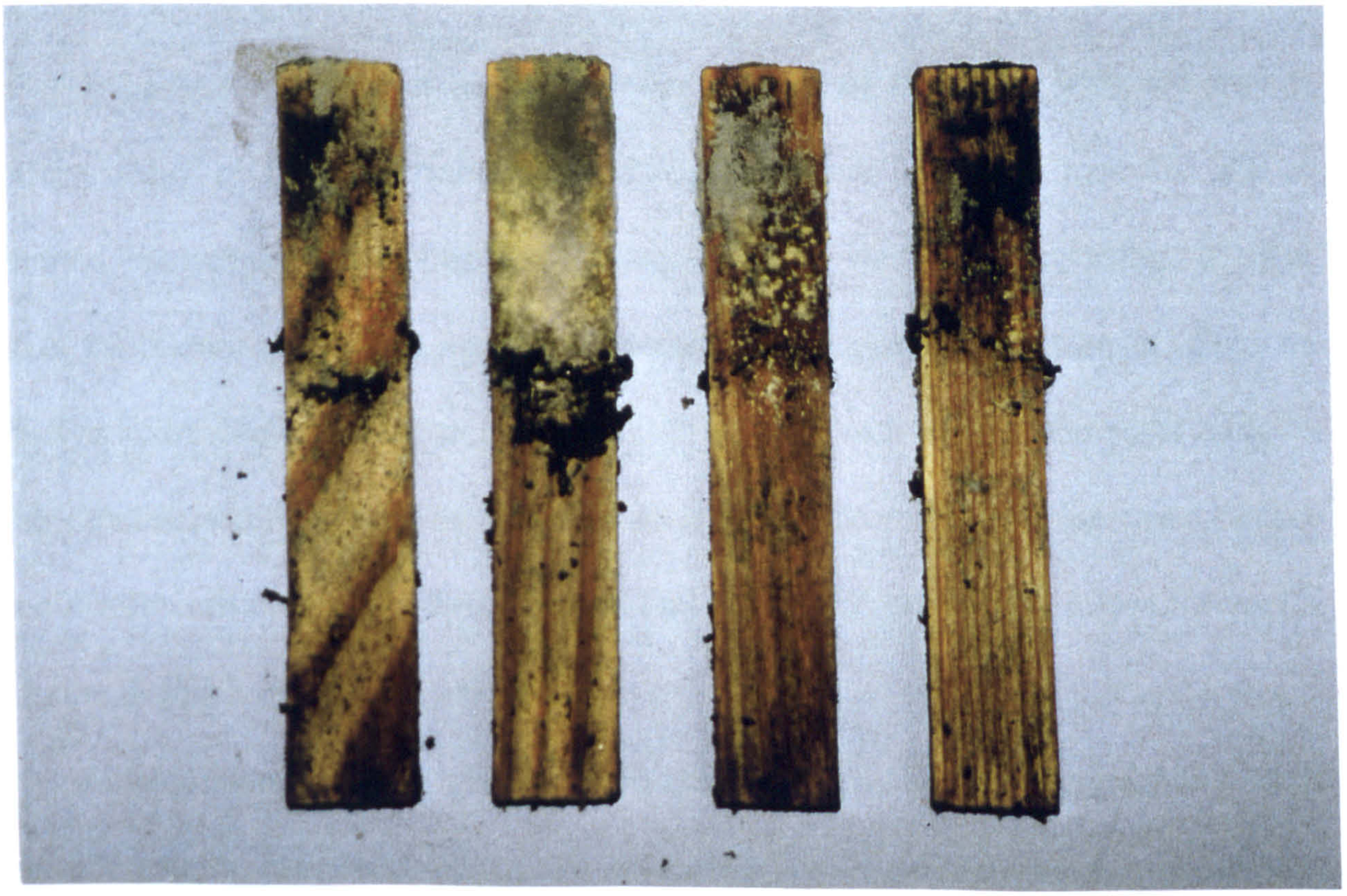


Plate 7.1 Mould growth on succinic anhydride modified stakes.

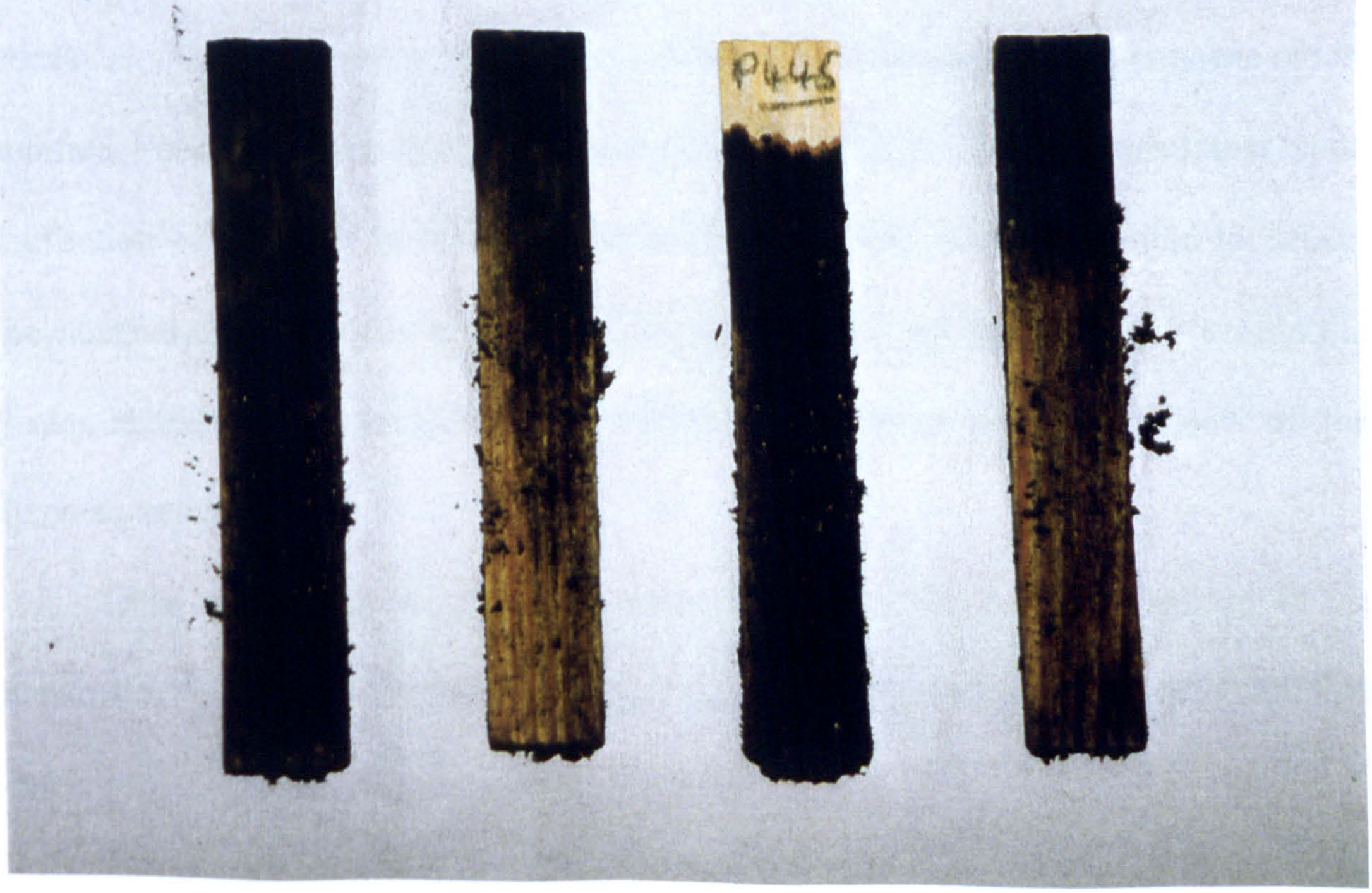


Plate 7.2 Colonisation of phthalic anhydride modified wood by a black staining fungus

7.3.2 Distribution of decay

Even at low levels of acetylation the exterior of the stake remained sound. Surface decay of butyl isocyanate and alkenyl succinic anhydride modified stakes occurred, but was not apparent as WPG increased despite weight loss occurring. In these stakes the interior of the stake was decayed while the outside remained sound. Such an effect is most likely to be due to higher WPG's being achieved at the stake surface. Although the modifying solution was vacuum impregnated into stakes, increased surface modification can occur from diffusion of additional modifying chemical from the excess solution in which stakes were reacted.

Light microscopy of acetic anhydride and butyl isocyanate modified woods revealed specific patterns of decay. At increasing WPG's soft rot was only apparent in latewood cell walls. Tunnelling bacterial attack was found in cell walls at the earlywood - latewood boundary, where no soft rot decay occurred. Earlywood cell walls remained undecayed. A similar pattern was found by Nilsson and Rowell (1982) in butylene oxide modified Ponderosa pine exposed in unsterile soil, and it was concluded that poor distribution of chemical in the latewood resulted in areas more susceptible to decay. They attributed the presence of tunnelling bacteria in areas not attacked by soft rot to the greater ability of these organisms to overcome the decay inhibiting effects of the chemical treatment.

Light microscopy of alkenyl succinic anhydride also revealed soft rot in the latewood only. This effect was identical in stakes reacted to a weight gain of 8% as it was in stakes reacted to a weight gain of over 30%.

In the pure culture study (section 6.3.1) pockets of brown rot were found in the latewood of wood modified with acetic anhydride, butyl isocyanate and alkenyl succinic

anhydride. These were attributed to a lower ratio of modifying chemical to cell wall material during modification in the latewood, leading to lower localised level of reaction.

7.3.3 Moisture content - weight loss relationship

In the one set of stakes which remained undecayed in this study (butyl isocyanate, 12% WPG), an average moisture content at the end of test of 72% was achieved (figure 7.6). This is theoretically sufficient to support decay by soft rot or basidiomycete fungi.

Figure 7.8 shows a plot of moisture content at the end of the test against weight loss for acetic anhydride and butyl isocyanate modified wood, irrespective of weight percent gain. Wood modified with succinic, phthalic and alkenyl succinic anhydrides each gave a very similar moisture content - weight loss relationship, but these have not been plotted for the sake of clarity. Data for soft rotted CCA treated wood, which shows reasonably close agreement with the wood modification treatments. If anything, CCA treated wood is slightly drier at each given weight loss than modified wood.

If the mechanism of wood protection by chemical modification is through suppression of moisture content then the fact that decay of CCA treated wood leads to a similar moisture content - weight loss relationship is difficult to explain. Results from the pure culture test showed that CCA retentions of up to 4 kg/m^3 (hydrated salt retention) did not suppress moisture content (see table A2.9, appendix 2) The explanation of the relationships in figure 7.8 are better described not by increased moisture contents leading to increased weight losses, but by weight loss resulting in increased moisture content (reasons for which were given in chapter 6).

These observations support those made in the previous chapter that overall moisture content of the modified wood does not explain the susceptibility or resistance of the wood to decay. However, overall wood moisture content measured here does not give

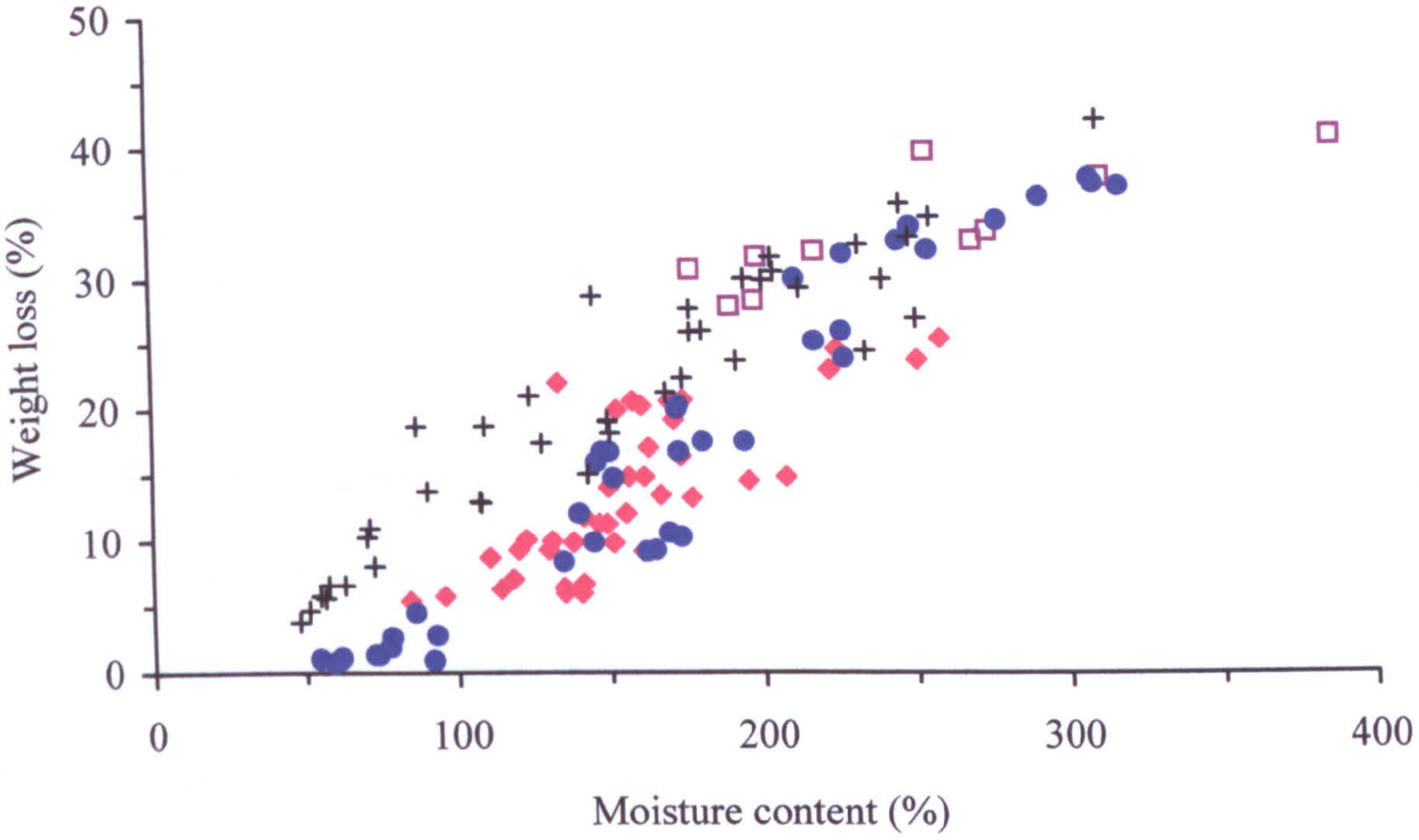


Figure 7.8 Moisture content / weight loss relationship of treated wood after 20 months exposure in unsterile soil

Acetic anhydride (◆); Butyl isocyanate (●); CCA (+); Pyridine control (□)

an indication of the distribution of the water in the cell walls and lumina, and again the quantity of water in the cell walls cannot be discounted as a potential mechanism of the protection mechanism of chemical modification.

7.4 Summary of points raised in chapter 7

- Threshold level of protection of butyl isocyanate modified wood was 12%. This is significantly lower than the thresholds reported against brown and white rot fungi in pure culture (chapter 6). Butyl isocyanate was the only chemical for which threshold protection was achieved, though WPG's tested against soft rot were lower than in other sections of the study.

- It was estimated that acetic anhydride modification would have achieved threshold protection at 18% WPG; similar to, if just above, that for white rot fungi, but below that for brown rot fungi (in pure culture).

- Modification with alkenyl succinic anhydride protected wood up to a WPG of 8%, after which no further improvement in decay resistance was achieved. No threshold protection level appears possible against soft rot using this modifying chemical. Decay of modified samples was confined to the latewood, and it was concluded that the size of the alkenyl succinic anhydride molecule prevented it from penetrating the latewood cell wall sufficiently to protect it from soft rot cavity attack.

- Succinic anhydride afforded little protection to wood in this test. Heavy mould growth on succinic anhydride modified samples was thought to be due to the ability of the mould to utilise succinate hydrolysed from wood polymers; succinic anhydride participates in the tricarboxylic acid cycle. Mould could also have flourished due to the inhibition of competitors in the wood, though this seems unlikely due to the apparent inability of this chemical to protect wood.

- Phthalic anhydride was also unable to protect wood. This could have been due to loss of modifying chemical from the wood or to soft rot fungi being able to decay wood despite modification. Samples were quickly and extensively colonised with black staining fungus. This black stain may have been able to metabolise phthalate or have flourished due to competitive inhibition.

- Acetic anhydride and higher WPG (sub-threshold) butyl isocyanate modified wood were only decayed in latewood on the inside of each sample (the outside remaining sound). Similar to the pure culture test, this was attributed to an uneven distribution of modifying chemical in the wood. Additionally, tunnelling bacterial attack was found

in the earlywood - latewood boundary, these organisms apparently having a greater ability to overcome the decay inhibiting effects of modification.

- Even in undecayed butyl isocyanate modified stakes, moisture content was theoretically high enough to support decay. Factors other than the overall moisture content of the wood are important in the decay of modified wood by soft rot fungi, possibly cell wall moisture content.

- Evidence presented indicated that the end of test moisture content was governed largely by weight loss, and not vice versa (as discussed in chapter 6).

Chapter 8. Fungal cellar and field trial

8.1 Introduction

Further ways in which the durability of chemically modified wood was tested in this study were through the exposure of stakes in a fungal cellar test and in a field trial. The soil moisture content in the fungal cellar was adjusted to be brown rot selective, though increasing moisture content with increasing depth in the soil provided conditions suitable for a range of decay organisms. These tests therefore provided a measure of the performance of wood modifications or preservatives in ground contact conditions, against a variety of wood decay organisms (brown, white and soft rot and bacteria). The wood samples used in these tests were the largest of those modified in this study. This provides an extra test for chemically modified wood as problems associated with the impregnation of sufficient modifying chemical into wood are likely to be greater than in the pure culture or soft rot tests.

In addition to the modified stakes, sets of stakes were exposed which had been impregnated with modifying chemical but not brought to reaction temperature. The aim of this procedure was to give a measure of any efficacy of modification chemicals not attached to the wood polymers. Restrictions in time and space meant that impregnated unreacted wood was only tested in this part of the study, and only for a relatively short time period.

8.2 Materials and methods

8.2.1 Modified wood samples

Corsican pine sapwood stakes measuring 9mm x 19mm x 200mm were chemically modified or treated with CCA as described in chapter 3. Pyridine and untreated controls were also included in the experiment.

Stakes impregnated with modifying chemical but not reacted were treated using acetone as carrier solvent rather than pyridine. Acetone was chosen as it has a lower boiling point than pyridine (56°C acetone, 115°C pyridine) and so could be removed easily in a vacuum oven without increasing temperature to a level where significant reaction between the wood and the modifying chemical would occur.

The method for impregnating stakes was as follows:

1. Vacuum impregnation of solution into oven dry stakes (procedure as in chapter 3).
2. Overnight soak in solution.
3. Dry in vacuum oven at 25°C, 720mmHg vacuum, for 8 hours.

These unreacted stakes were only treated to one retention level for each modifying chemical, as opposed to four WPG's for the modified stakes. An unreacted retention close to the highest reacted WPG was achieved by adjusting the concentration of each modifying chemical in acetone. Retentions were calculated from the difference in weight of oven dry stakes and of treated stakes prior to vacuum drying. At the temperatures used during impregnation (<20°C) the solubility of phthalic and succinic anhydrides in acetone proved too low for high retentions to be achieved. In these cases a second impregnation was undertaken after the first drying step. The overnight soak was not repeated in order to avoid leaching of chemical already in the stakes.

Butyl isocyanate was not included in this part of the test due to the undesirability of handling and testing stakes impregnated with unreacted isocyanate.

8.2.2 Test conditions - fungal cellar

The fungal cellar was set up using five plastic bins (Nomad Box Company Ltd) measuring 100cm x 60cm x 55cm (depth). Drainage holes were drilled in the bottom of these and they were filled to a depth of 5cm with 10mm gravel. Top soil was mixed with sharp sand to achieve a water holding capacity of 30%. Lowering the WHC in this manner increased the drainage of the soil to give lower soil moisture contents favourable to basidiomycete decay. The soil was added to the bins to a depth of 30cm.

Throughout the test the bins were monitored and watered at regular intervals and kept to a soil moisture content at the top of the bin of approximately 15-20% (though the soil tended to get wetter deeper in each bin).

Stakes of each modification level and type were distributed amongst the bins and planted to a depth of 150mm. Pine needles from forest floor litter were scattered on top of the soil in each bin to provide an extra inoculum and carbon source for the system.

The fungal cellar was kept at a temperature and relative humidity of 27°C and 70-80%. The longest time for which stakes were exposed was 27 months.

8.2.3 Test conditions - field trial

Stakes for the field trial were exposed in the Hickson Corporation field test site in Conley, Georgia. This site is an aggressive decay site with lower termite activity than similar alternative sites. Field test stakes were exposed for 22 months.

The field trial work was much less extensive than the fungal cellar work. Due to space limitations only the first two levels of each modification were exposed. This work therefore serves only as a comparison to the fungal cellar test.

8.2.4 Assessment methods

Fungal cellar stakes were assessed in three ways:

1. Appearance grading. A non-destructive test in which the extent of decay was graded as 10 (no decay), 9, 7, 4, or 0 (failure to decay fungi). Stakes were removed at various time intervals, graded, and replaced in the bins. Results are presented on a scale of decay from 0 to 10 (as graded or an average of grades), though when considering results presented in this manner the subjective nature of this assessment needs to be taken into consideration.
2. Deflection testing. Another non-destructive test in which stakes were placed in a three point bending rig. Deflection was measured by a micrometer when a fixed load was applied. By measuring the same stake at various time intervals (including an early measurement when decay would have been minimal, but equilibrium moisture content would have been reached) a measure of strength loss was obtained. This method of assessment is a slightly cruder version of that described by Gray (1986).
3. Weight loss. This is a destructive method of measuring decay as the oven drying of stakes completely halts the decay process. Although re-exposure would be possible, the extra time required for decay fungi to become re-established would invalidate any comparison of the extent of decay over a particular time period. Several sets of stakes of each modification / weight gain were exposed to give the opportunity of removing sets for weight loss determination at various time intervals. Once a set of stakes had been oven dried they were not returned to the fungal cellar.

Field trial stakes were not monitored so extensively. These were removed in two sets (one after 12 months, one after 22 months) at which times they were visually graded and weight loss was determined.

8.3 Results and discussion - fungal cellar

8.3.1 Test conditions

Unmodified stakes were inserted into each bin at various time intervals as moisture content checks. These were removed after approximately two weeks. This was considered enough time to reach equilibrium moisture content but not for significant levels of decay to occur. Unmodified stake moisture content commonly varied between 30% and 50% though occasionally moisture contents up to 70% were recorded. These are measures of overall stake moisture content and do not take account of the variation in stake moisture content between the lower moisture content at the top of the stake (above ground) and the higher moisture contents at the base. In unmodified stakes it is likely that at some point along its length the moisture content of a stake would have been ideal for decay by the various types of wood decay fungi.

There was a wide variation in activity between different soil bins, and even between areas of the same bin. The main decay type in the fungal cellar was brown rot, commonly starting in the form of pocket rot. Soft rot was also evident towards the bottom of the stakes.

Where rotted, all stakes regardless of modification type were decayed by brown rot fungi, though there was no evidence of soft rot in acetic anhydride or butyl isocyanate modified stakes at any level of modification. Despite acetic anhydride and butyl isocyanate modified wood being susceptible to soft rot at lower WPG's (see chapter 7), it

seems that difficulties caused specifically to soft rot by modification allowed brown rot to predominate. The distribution of modification, in which the outside of the stakes were more heavily modified than the inside (see section 8.3.2), may have played some part in this.

Other than these cases there was no evidence of a particular preference of a type of decay for a particular modification type. Having said this, phthalic anhydride modified stakes were prone to a black staining fungus and succinic anhydride modified stakes were particularly susceptible to mould, in a very similar way to that described in chapter 7 for the unsterile soil soft rot test. As neither of these was seen to cause decay in wood they were disregarded when visually grading stakes.

Good rates of decay were observed in unmodified stakes. Weight losses of 10% of the weight of a stake (sometimes equating to high levels of localised decay in a stake) were recorded in under 3 months, and stake failures were common within 8 months.

8.3.2 Determination of performance

Varying degrees of success were achieved in assessing fungal cellar stakes using the three methods described above:

1. Appearance grading. This proved the most useful method of assessing decay in stakes. Examples of the five decay rates (10, 9, 7, 4 and 0) are shown in plates 8.1 to 8.5. Difficulties were encountered using this method due to patterns of distribution of decay similar to those described in previous chapters. Stakes reacted with acetic anhydride, butyl isocyanate and, to a lesser extent, alkenyl succinic anhydride tended to decay on the inside of the stake while leaving an outer skin of 1 - 2mm thick free from decay. Examples of this are shown in plate 8.6. When a stake was removed and oven dried, heavy decay became obvious due to the excessive shrinkage of the decayed

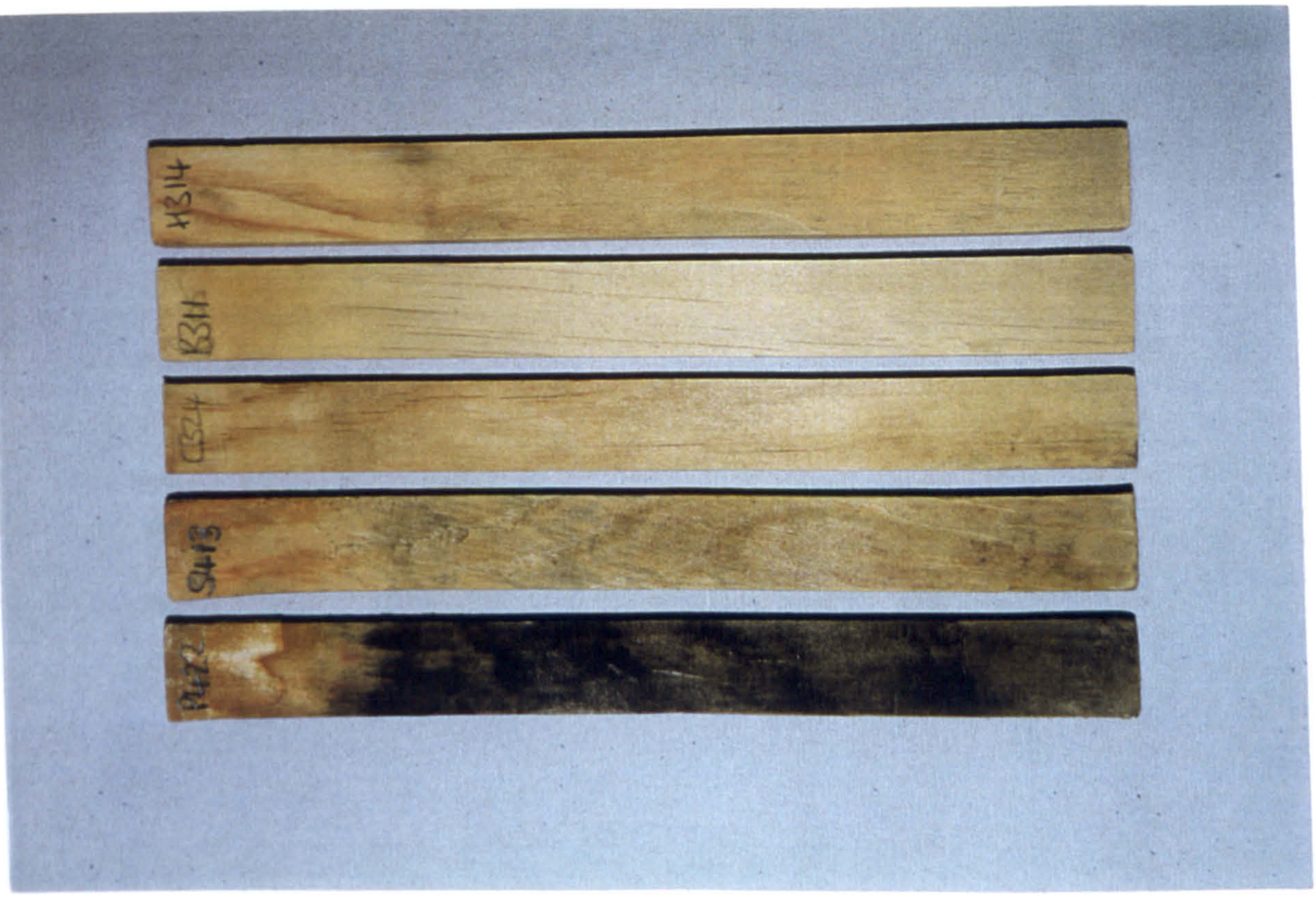


Plate 8.1 Example of stakes exposed in the fungal cellar test: Decay rating 10

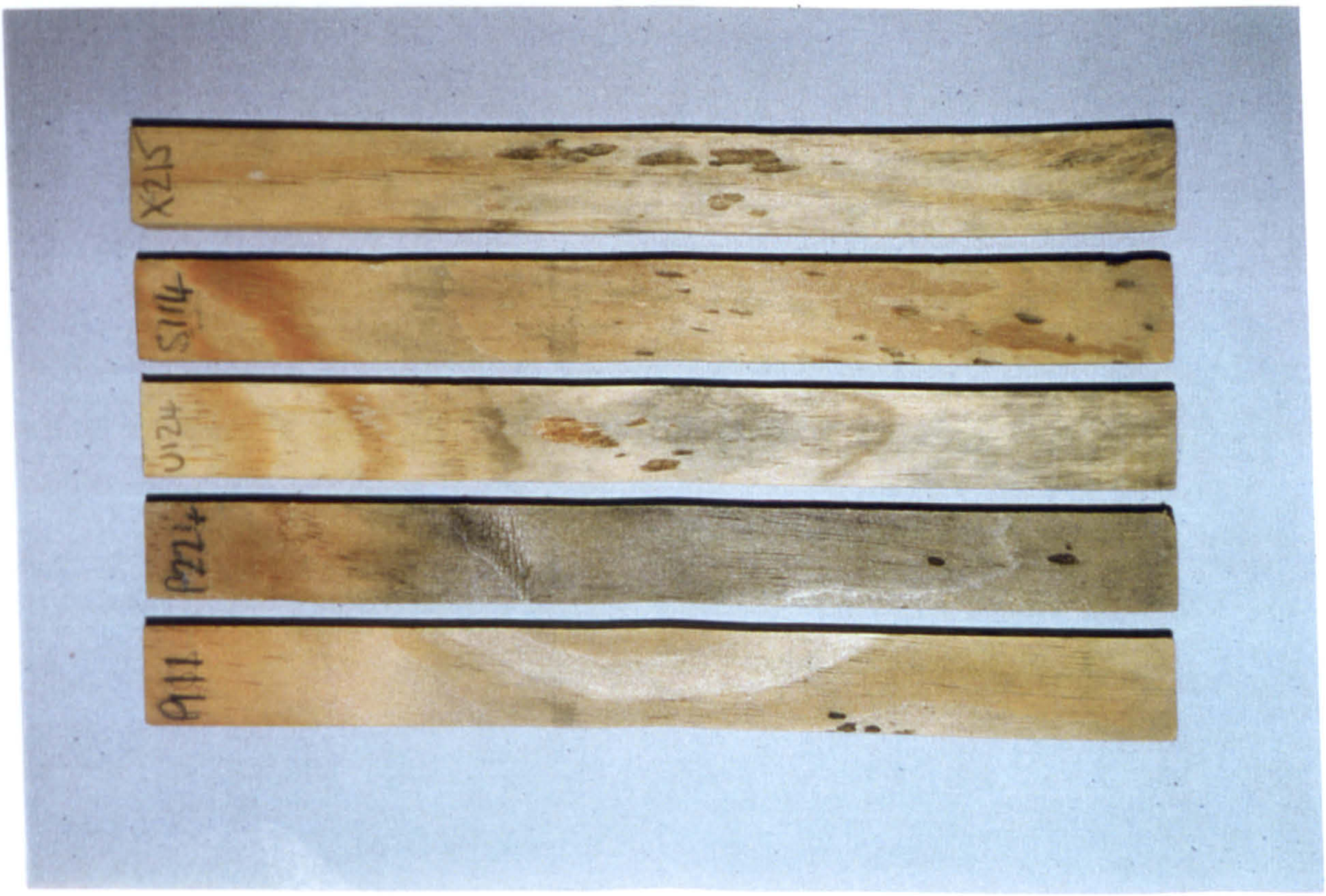


Plate 8.2 Example of stakes exposed in the fungal cellar test: Decay rating 9

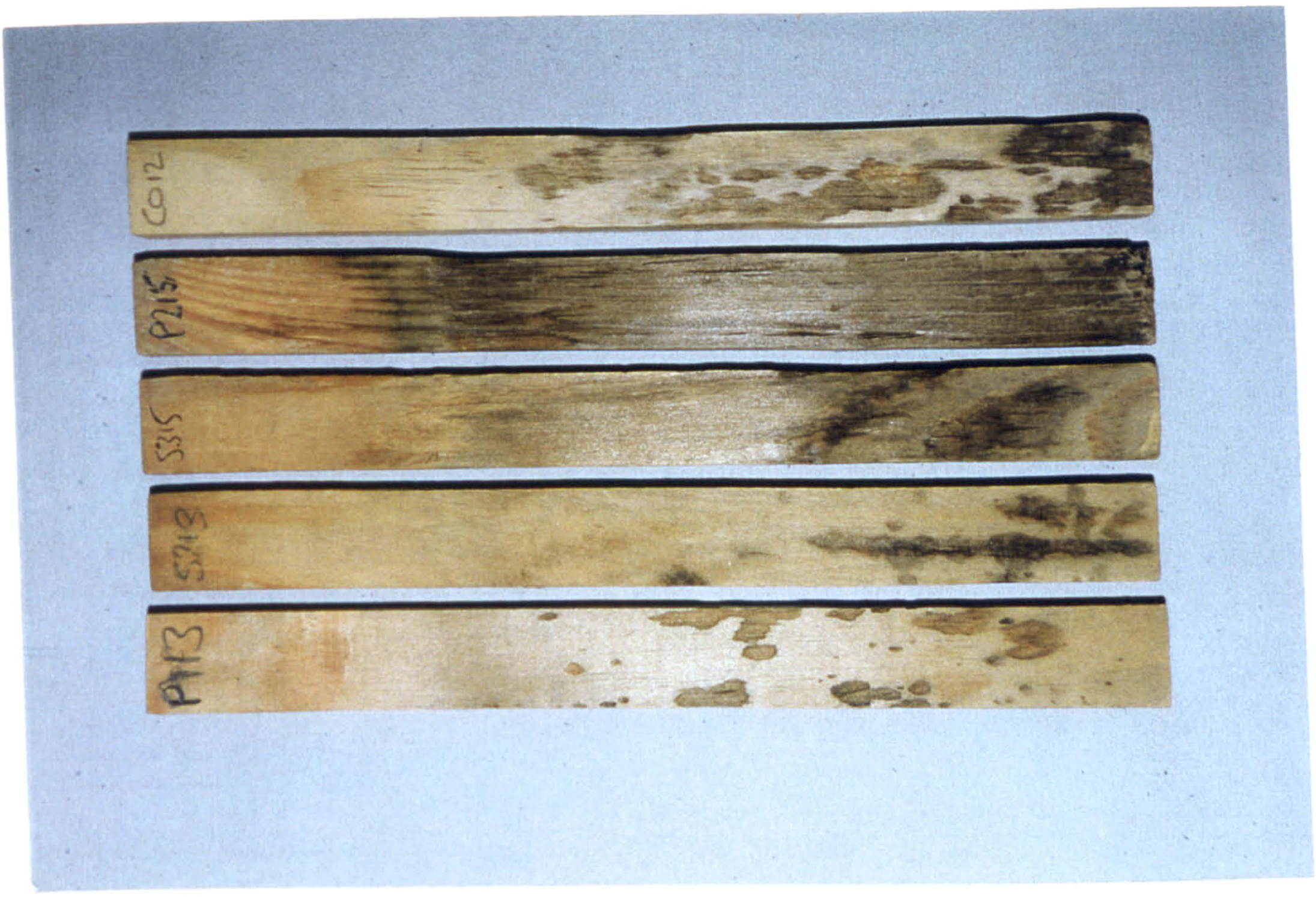


Plate 8.3 Example of stakes exposed in the fungal cellar test: Decay rating 7

Plate 8.4 Example of stakes exposed in the fungal cellar test: Decay rating 4

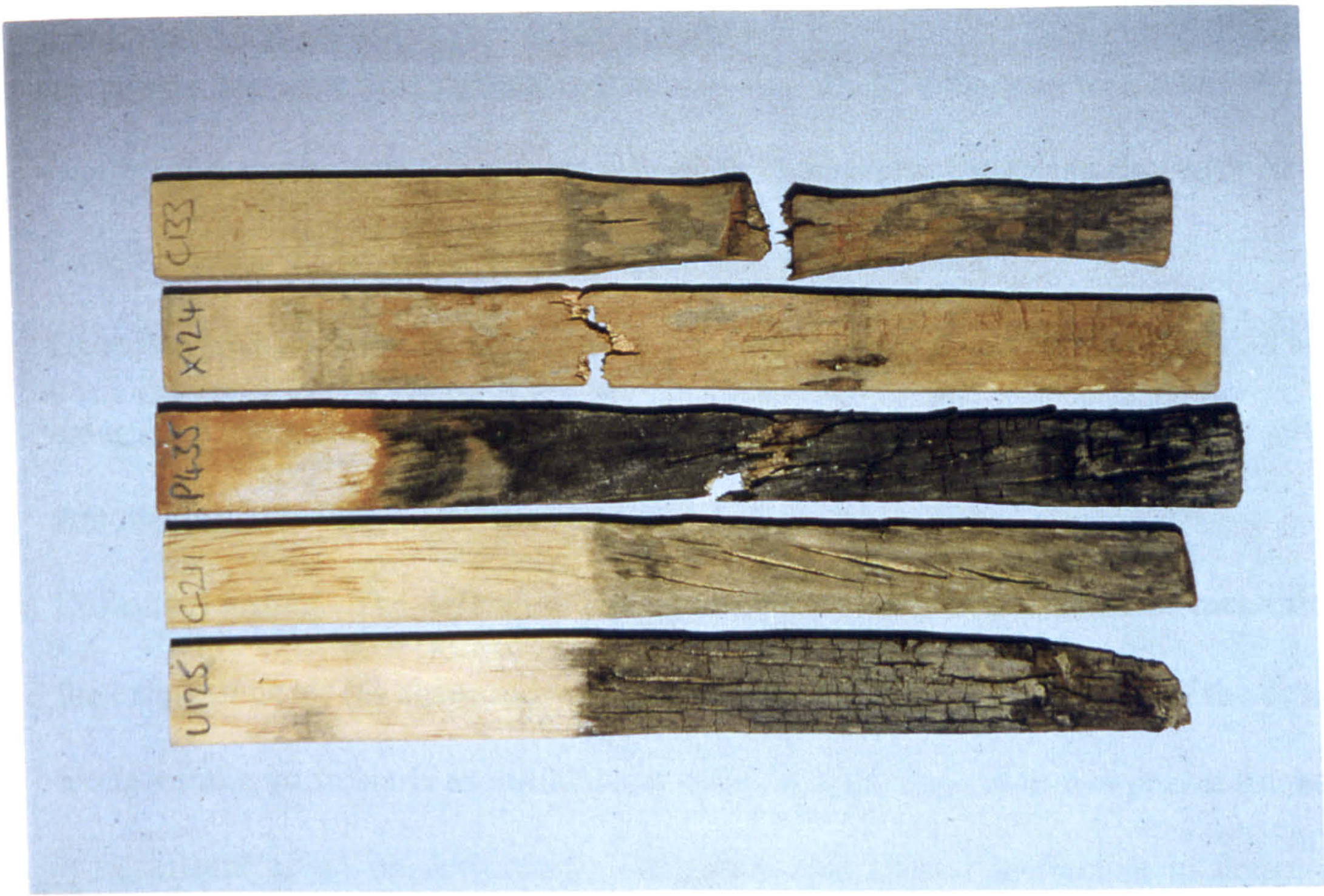


Plate 8.5 Example of stakes exposed in the fungal cellar test: Decay rating 0

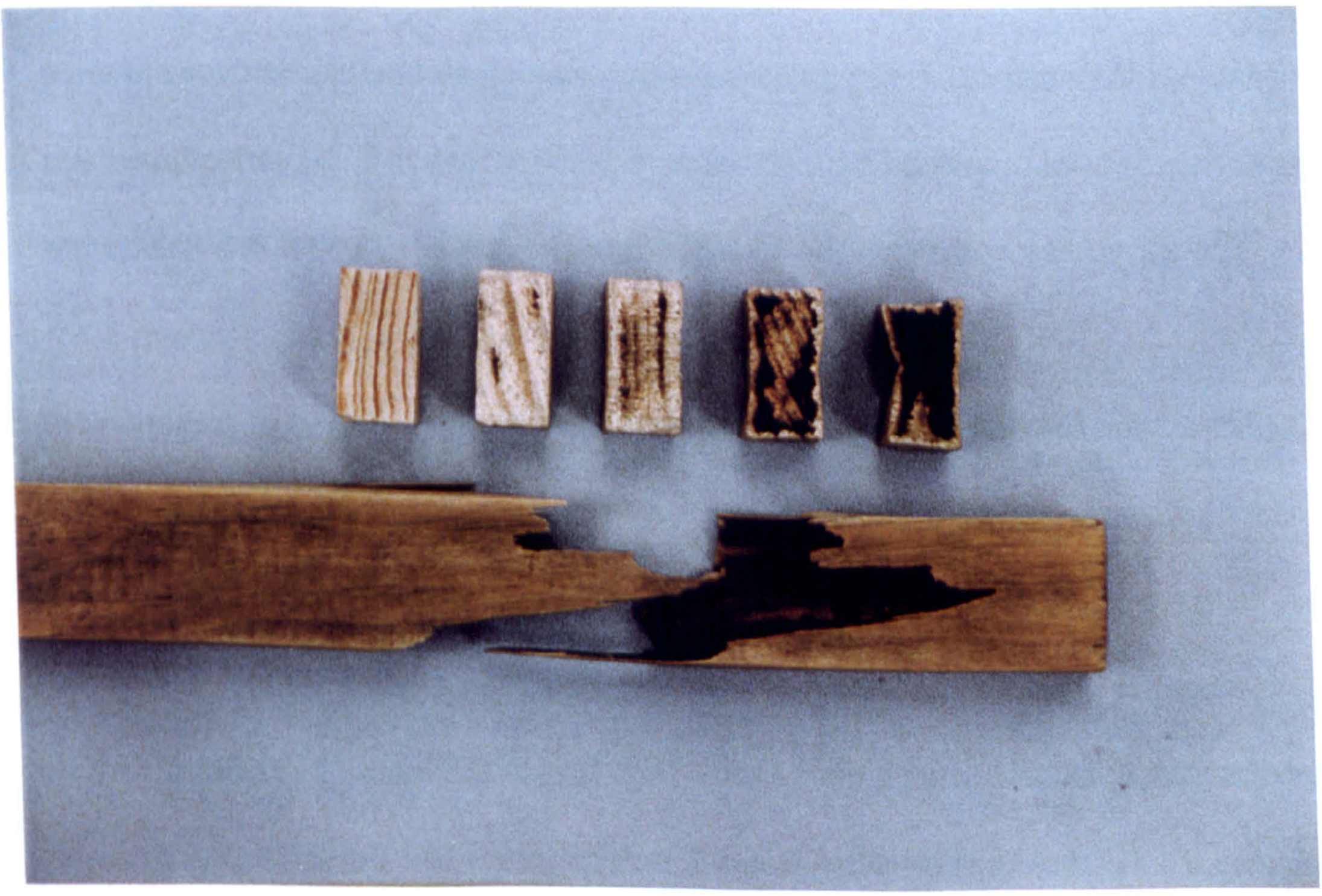


Plate 8.6 Interior brown rot decay of acetic anhydride modified stakes. The base of a broken test stake and five cross-sections (all approx. 8% WPG, varying weight loss). Left hand cross-section is from an unexposed acetylated stake.

interior, which caused the undecayed outer layer to crack. During continuous monitoring the stake was not allowed to dry, and decay only became evident if the interior of the stake became soft enough to be detected by squeezing the stake or to cause the stake to snap when it was being replanted in the soil bins.

Once removed and oven dried, all stakes were cut open to assess interior decay. This, along with weight loss figures, was taken into account when grading stakes after removal from the fungal cellar.

2. Deflection testing. The deflection testing proved an ineffective method of measuring the extent of decay (as measured visually or by weight loss). The position of the decay along a stake, particularly as initial decay often took the form of brown pocket rot, had a significant effect on deflection. Deflection also proved ineffective in detecting internal decay until late stages when decay became detectable by other means. When bending a stake, strength is tested to the greatest extent in the top surface (highest point of compression) and the bottom surface (highest point of tension) of the stake as it is being deflected. The fact that these remained sound seems to have restricted any increase in deflection to levels not measurable by the apparatus used in this test. For these reasons deflection data has not been presented in this work.
3. Weight loss. Due to the destructive nature of this test, assessment was only possible as sets of stakes were removed at various time periods for oven drying. However, when a stake not due for removal for oven drying failed when removed for visual and deflection testing, it could not be returned to the fungal cellar. Early removal of the failed and therefore more heavily decayed stakes from a particular set greatly affected average weight loss of that set at the time it was eventually removed. This meant that weight loss was not a valid method of assessment of decay over time in its own right, but proved a useful check of the validity of grading stakes (after removal from the

fungal cellar). As mentioned above, weight loss was taken into account in the assessment of the end of test grade, though this was by no means the only consideration. With the exception of occasional outlying results, the vast majority of end of test visual grades for acetic anhydride, butyl isocyanate and alkenyl succinic anhydride modified wood corresponded to weight losses as shown in table 8.1. Weight losses tended to be higher for a given grade in succinic and phthalic anhydride modified wood. This is probably due to loss of modifying chemical from the wood.

Table 8.1 Approximate relationship between visual grade and weight loss of unmodified pine sapwood stakes upon removal from the fungal cellar.

Visual grade	Weight loss (%)
10	<4%
9	<8%
7	4-15%
4	7-20%
0	15+%

8.3.3 Performance of chemically modified wood

In this section the results have been presented in two ways. In the first, decay rating is plotted against time for each weight gain of each modification. Secondly, end of test decay rating has been plotted against all sets of stakes still present in the fungal cellar when the test finished. Any stakes from these sets that failed prior to this stage are included as zero rating.

The graphs depicting average decay rating against time for each modification type and for CCA are given in figures 8.1 to 8.6.

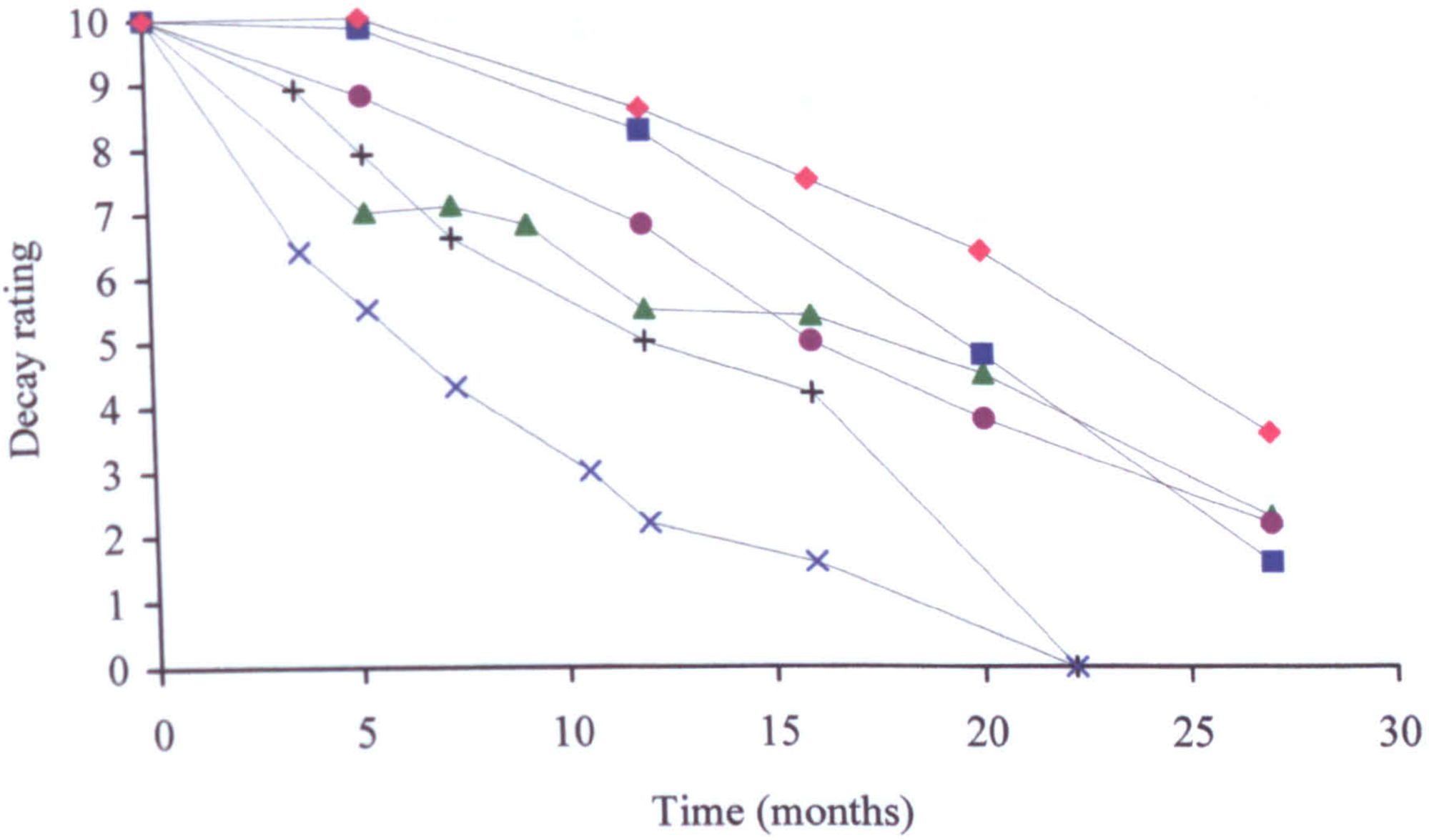


Figure 8.1 Decay rating of succinic anhydride modified stakes modified to various weight gains exposed in the fungal cellar

Untreated control (+); Pyridine control (×); 7.4% (▲); 11.8% (●); 23.9% (■); 31.2% (◆)

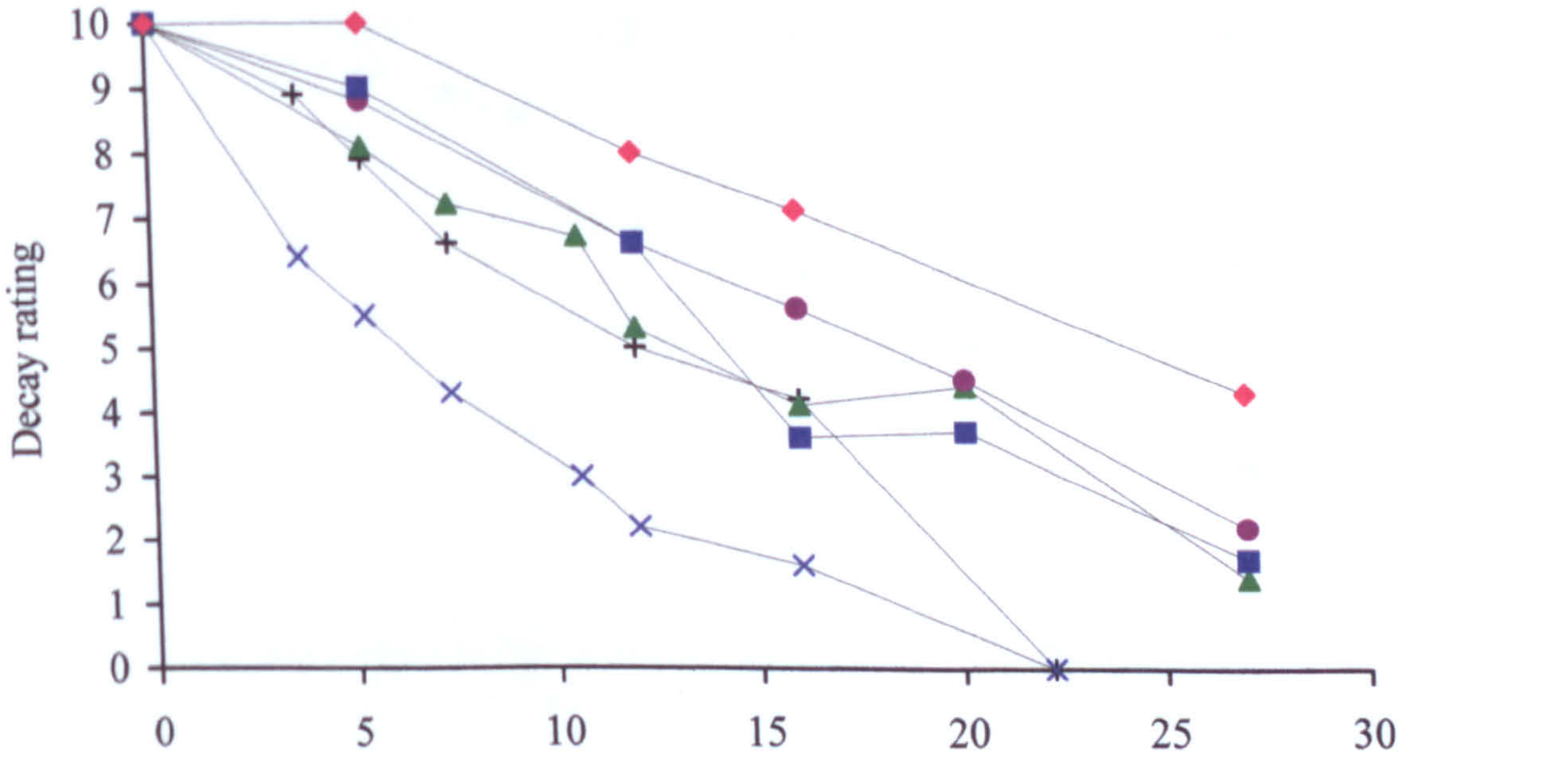


Figure 8.2 Decay rating of phthalic anhydride modified stakes modified to various weight gains exposed in the fungal cellar

Untreated control (+); Pyridine control (×); 6.8% (▲); 15.6% (●); 32.7% (■); 47.1% (◆)

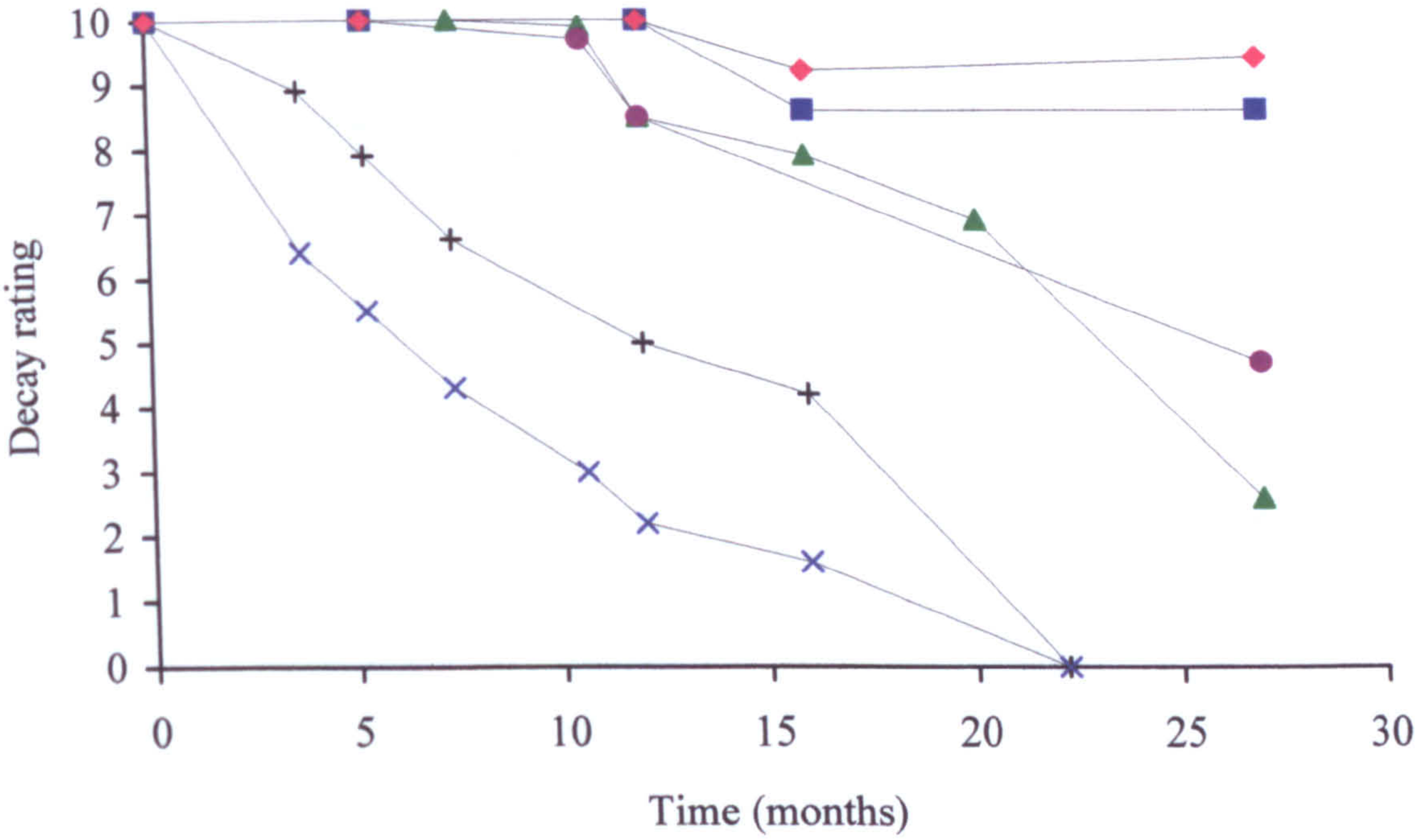


Figure 8.3 Decay rating of acetic anhydride modified stakes modified to various weight gains exposed in the fungal cellar

Untreated control (+); Pyridine control (×); 5.6% (▲); 7.9% (●); 14.4% (■); 17.8% (◆)

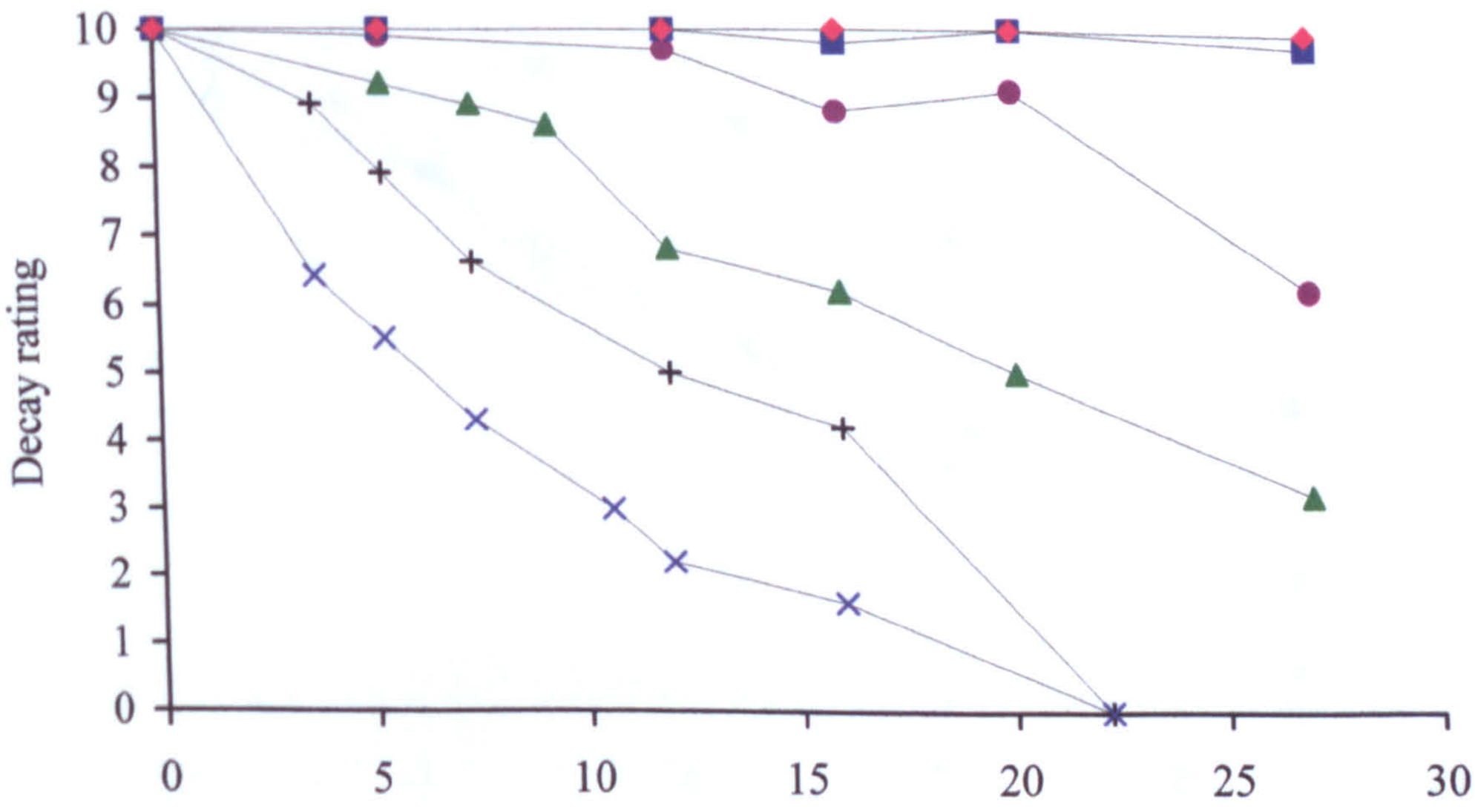


Figure 8.4 Decay rating of alkenyl succinic anhydride modified stakes modified to various weight gains exposed in the fungal cellar

Untreated control (+); Pyridine control (×); 5.8% (▲); 10.8% (●); 26.1% (■); 36.1% (◆)

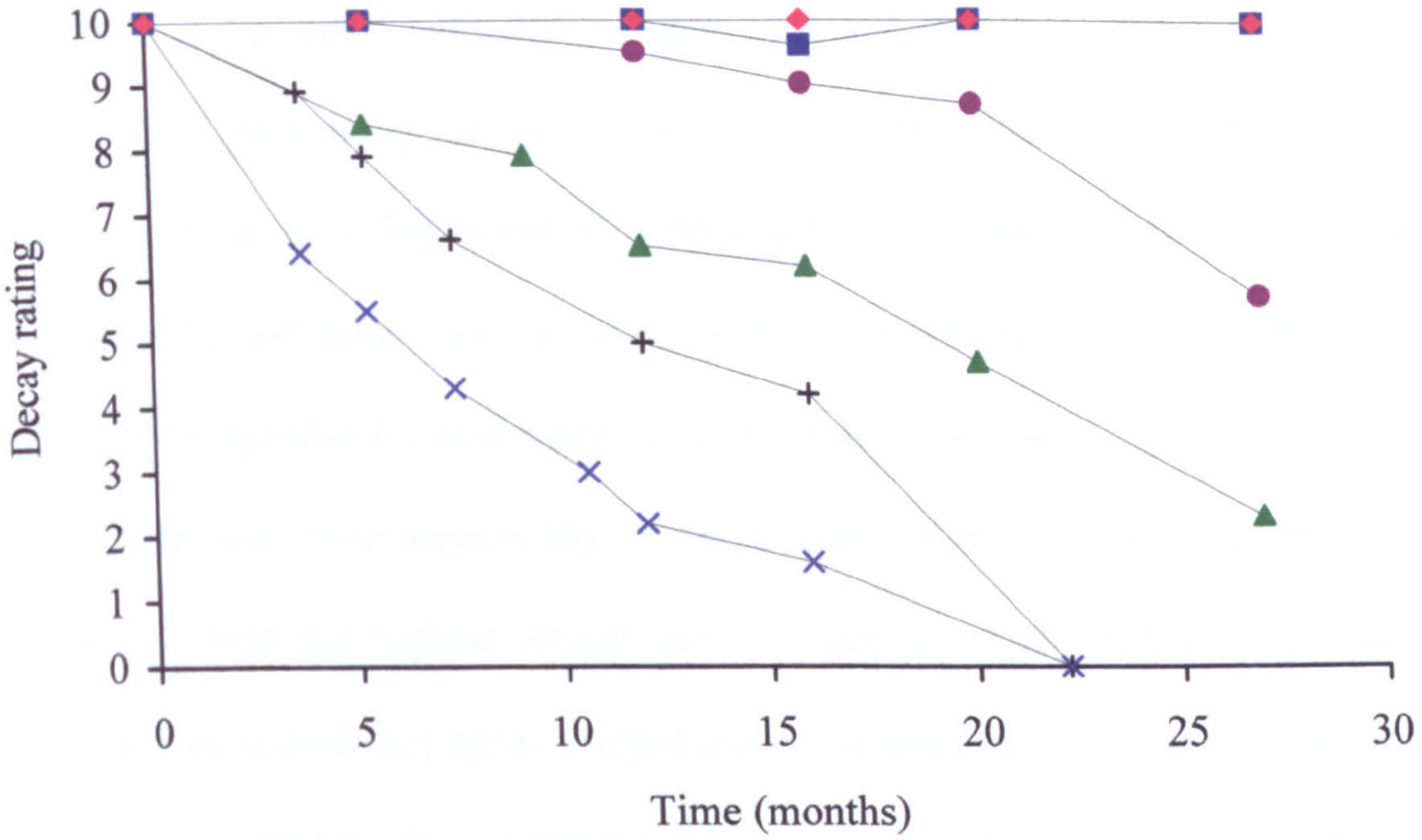


Figure 8.5 Decay rating of butyl isocyanate modified stakes modified to various weight gains exposed in the fungal cellar

Untreated control (+); Pyridine control (×); 2.1% (▲); 5.6% (●); 15.0% (■); 17.6% (◆)

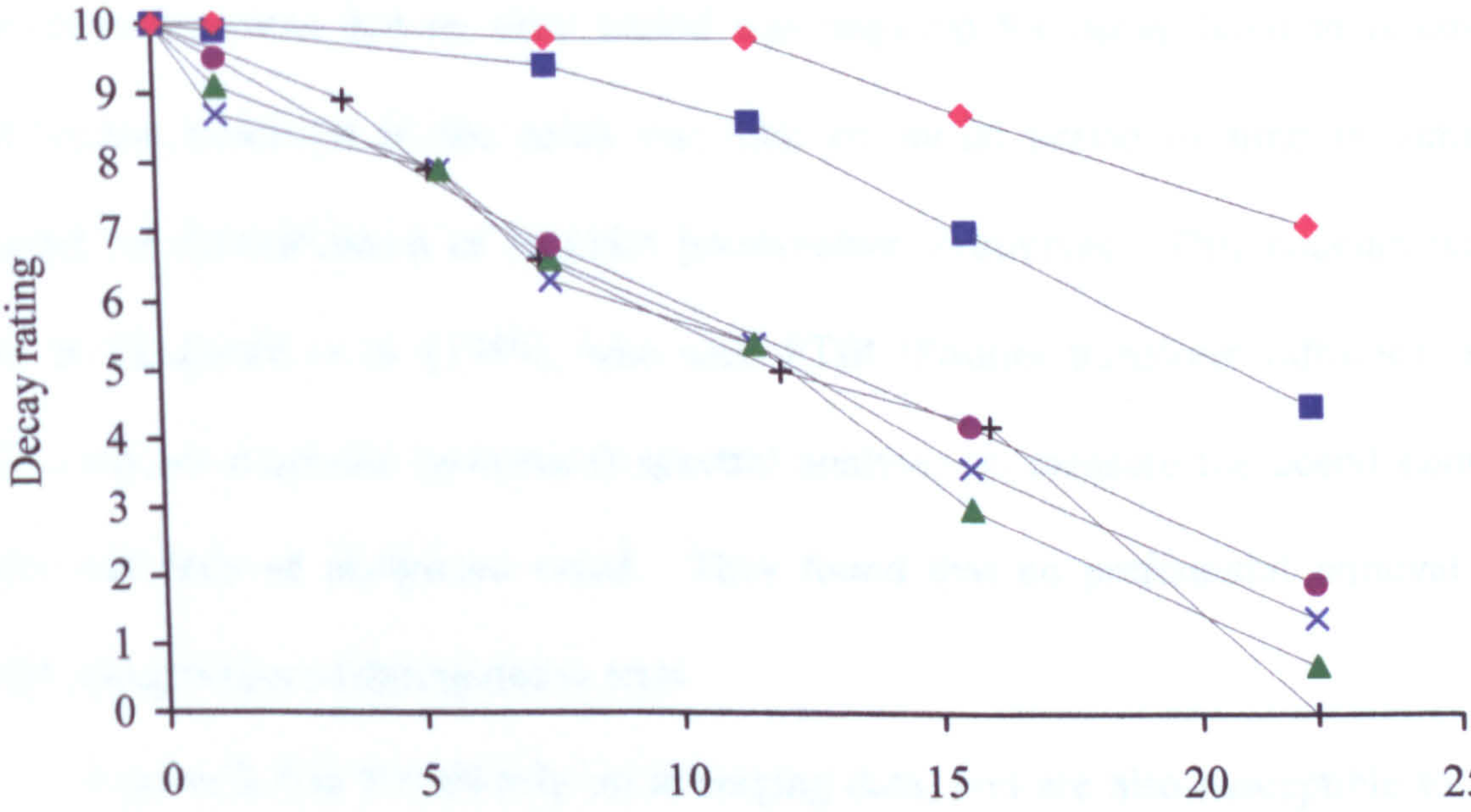


Figure 8.6 Decay rating of CCA treated stakes exposed in the fungal cellar

Untreated control (+); 0 kg/m^3 (water control) (×); 0.8 kg/m^3 (▲); 1.6 kg/m^3 (●); 3.2 kg/m^3 (■); 6.4 kg/m^3 (◆)

The first point that can be raised from these figures is that at the levels used in this test, succinic and phthalic anhydrides (figures 8.1 and 8.2) and CCA (figure 8.6) were unsuccessful at preventing decay. Acetic anhydride, butyl isocyanate and alkenyl succinic anhydride modified wood all performed well in the test, the most successful appearing to be the latter two, although WPG's of alkenyl succinic anhydride were significantly higher than for acetic anhydride and butyl isocyanate.

The second observation is that in cases where modified stakes were decayed (i.e. in all cases except the highest weight gains of acetic anhydride, butyl isocyanate and alkenyl succinic anhydride) decay started almost immediately, or after only a short lead time. The exception to this in the figures is that of acetic anhydride modification, in which internal decay was the most difficult to detect. Only after a year, when decay began to reveal itself, did the decay rating begin to drop. It is likely that in these stakes the internal decay also began immediately, but was initially undetected. Such an observation indicates that no time period was required for decay fungi to remove the modification treatment in the same way that an initial period of time is sometimes required for detoxification of biocidal preservative treatments. This concurs with the work of Takahashi *et al.* (1989), who used FTIR (Fourier transform infrared) and ^{13}C NMR (nuclear magnetic resonance) spectral analyses to measure the acetyl content of sound and decayed acetylated wood. They found that no preferential removal of the acetyl group occurred during decay tests.

Figures 8.1 to 8.6 all rely on averaging data, and are also susceptible to a more severe end of test assessment, at which point stakes were cut open to assess interior decay. The effect of the latter can be seen as sudden declines in decay rating at the final reading for some sets of stakes modified with acetic anhydride, alkenyl succinic anhydride and butyl isocyanate (figures 8.3, 8.4 and 8.5). These difficulties are overcome

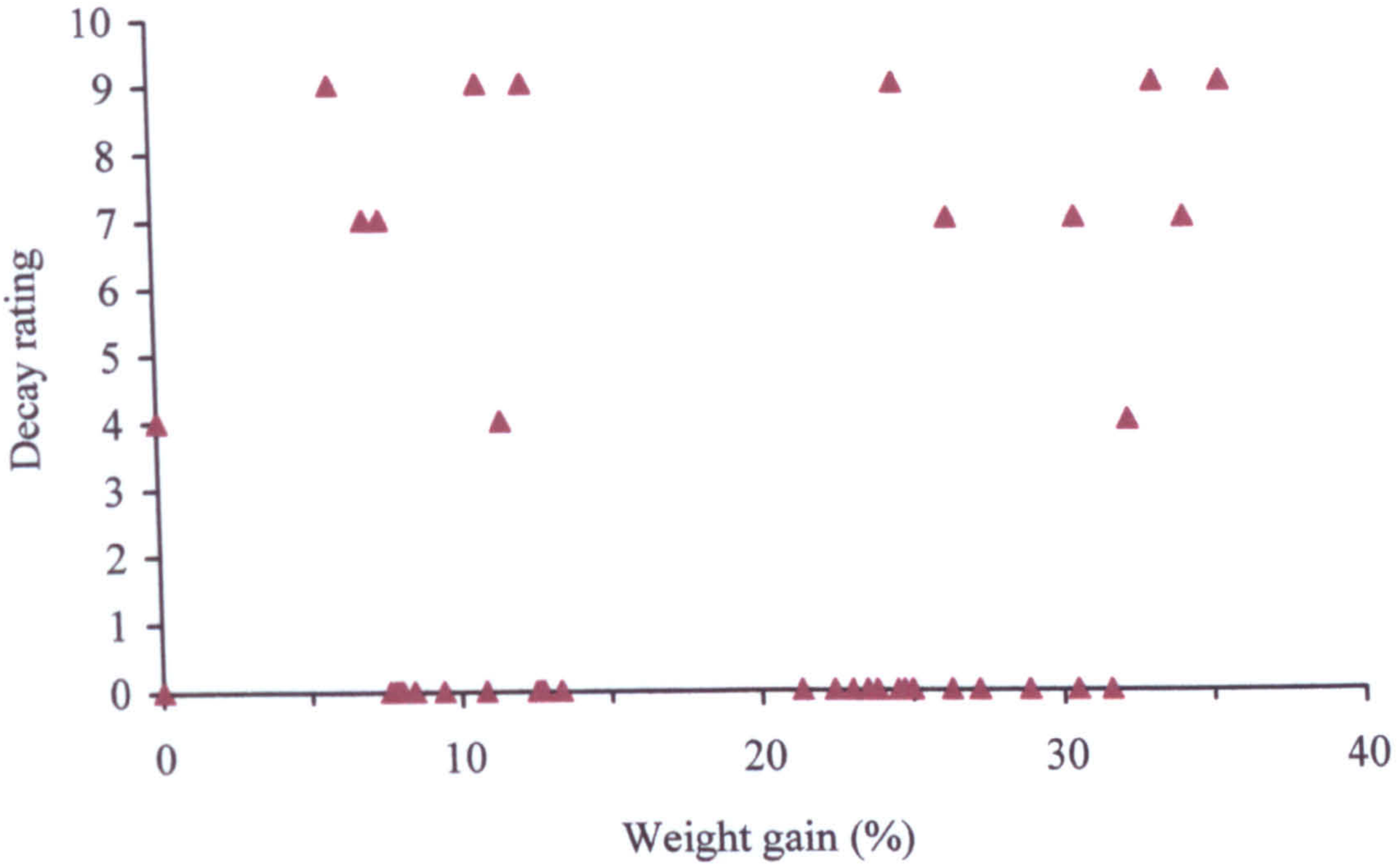


Figure 8.7 Decay rating of succinic anhydride modified stakes after 27 months exposure in the fungal cellar

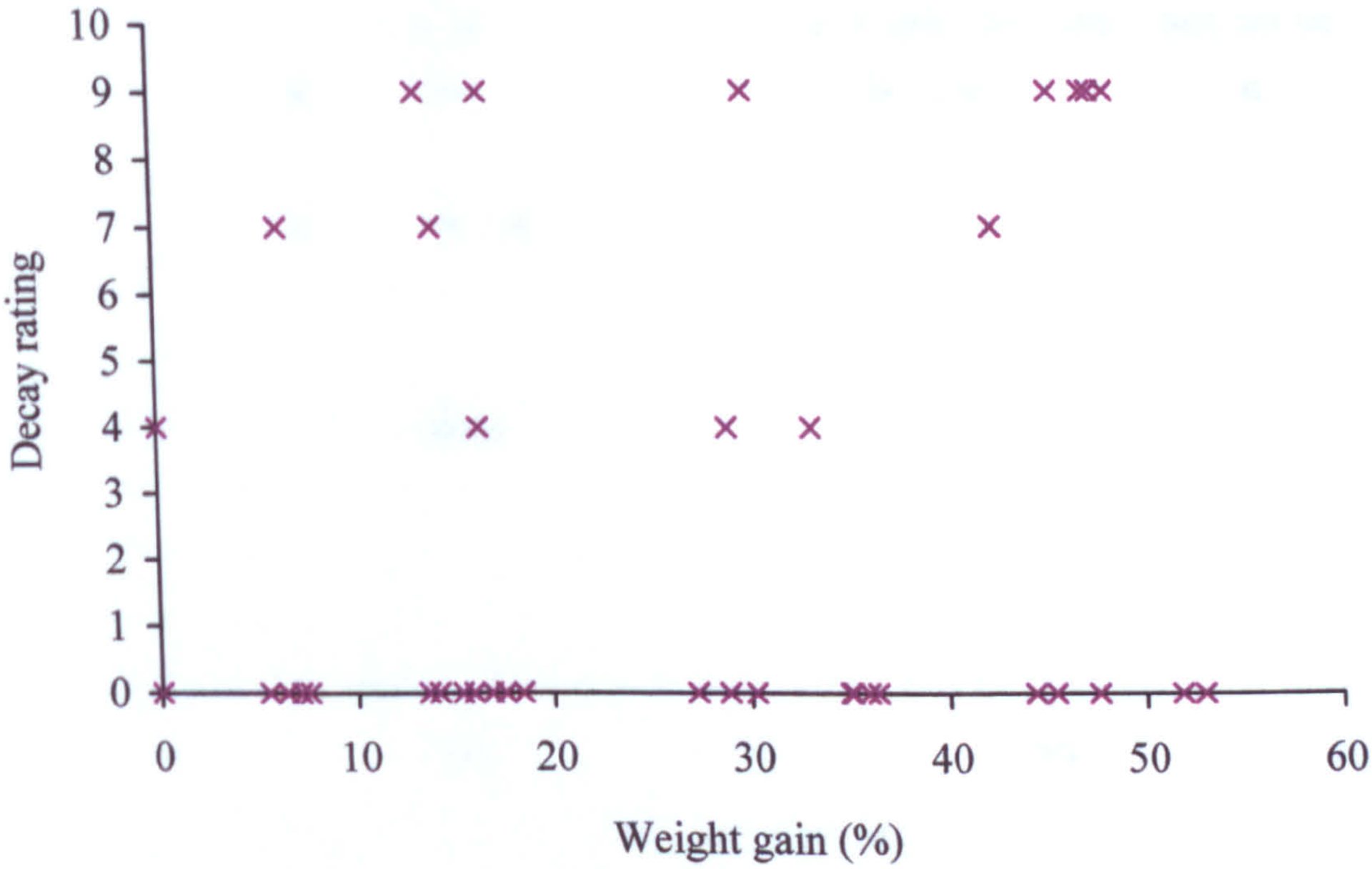


Figure 8.8 Decay rating of phthalic anhydride modified stakes after 27 months exposure in the fungal cellar

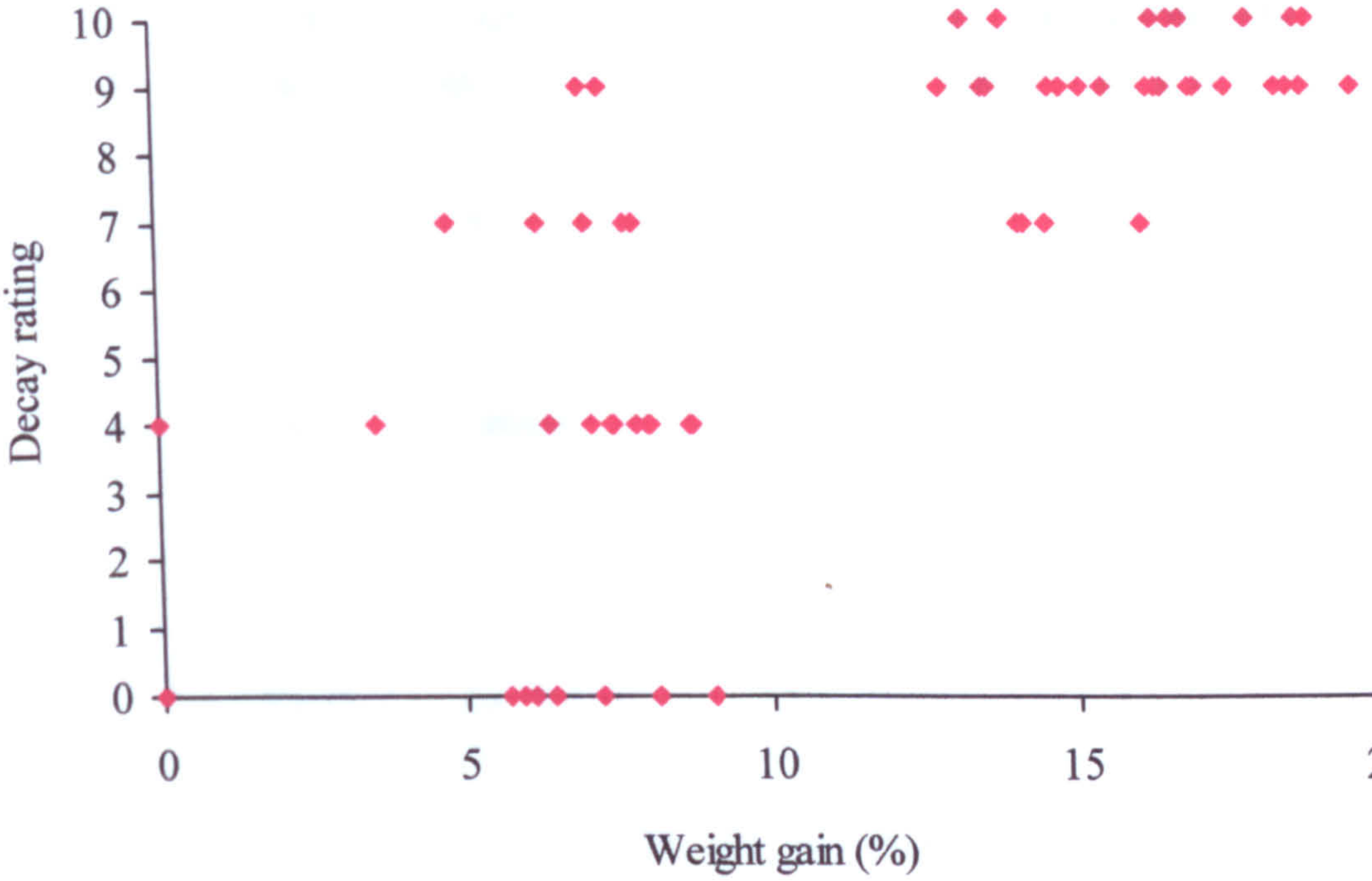


Figure 8.9 Decay rating of acetic anhydride modified stakes after 27 months exposure in the fungal cellar

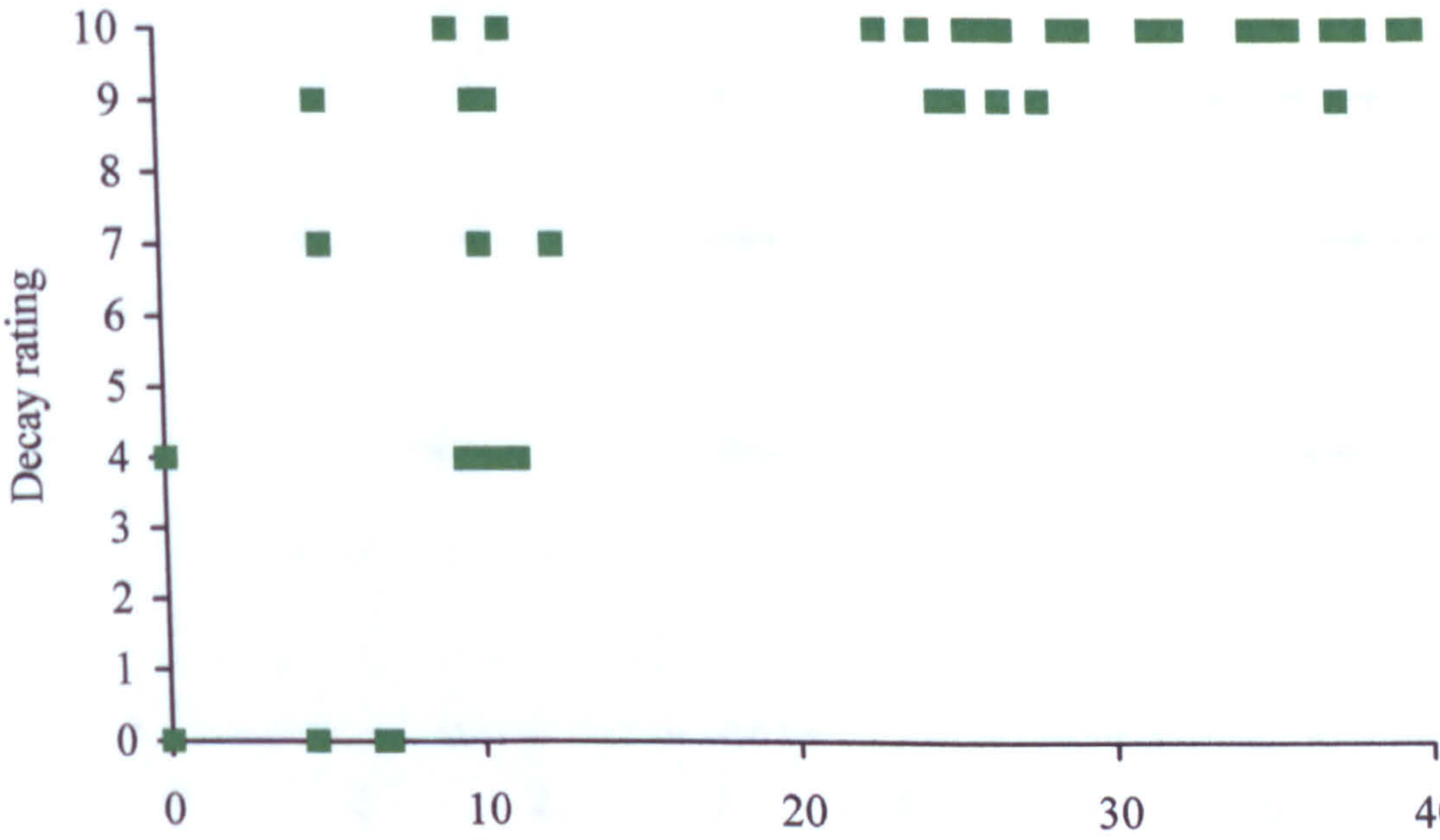


Figure 8.10 Decay rating of alkenyl succinic anhydride modified stakes after 27 months exposure in the fungal cellar

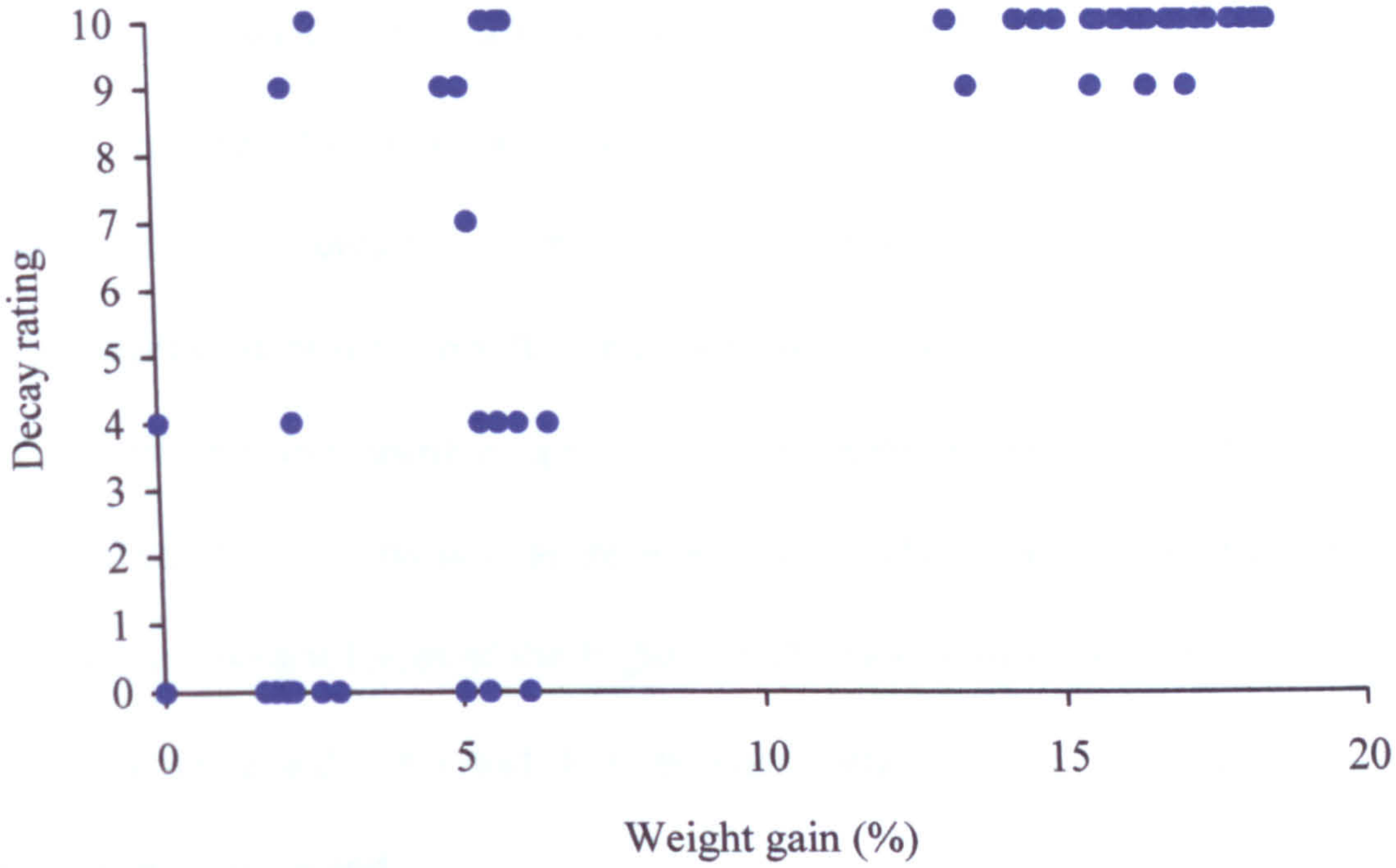


Figure 8.11 Decay rating of butyl isocyanate modified stakes after 27 months exposure in the fungal cellar

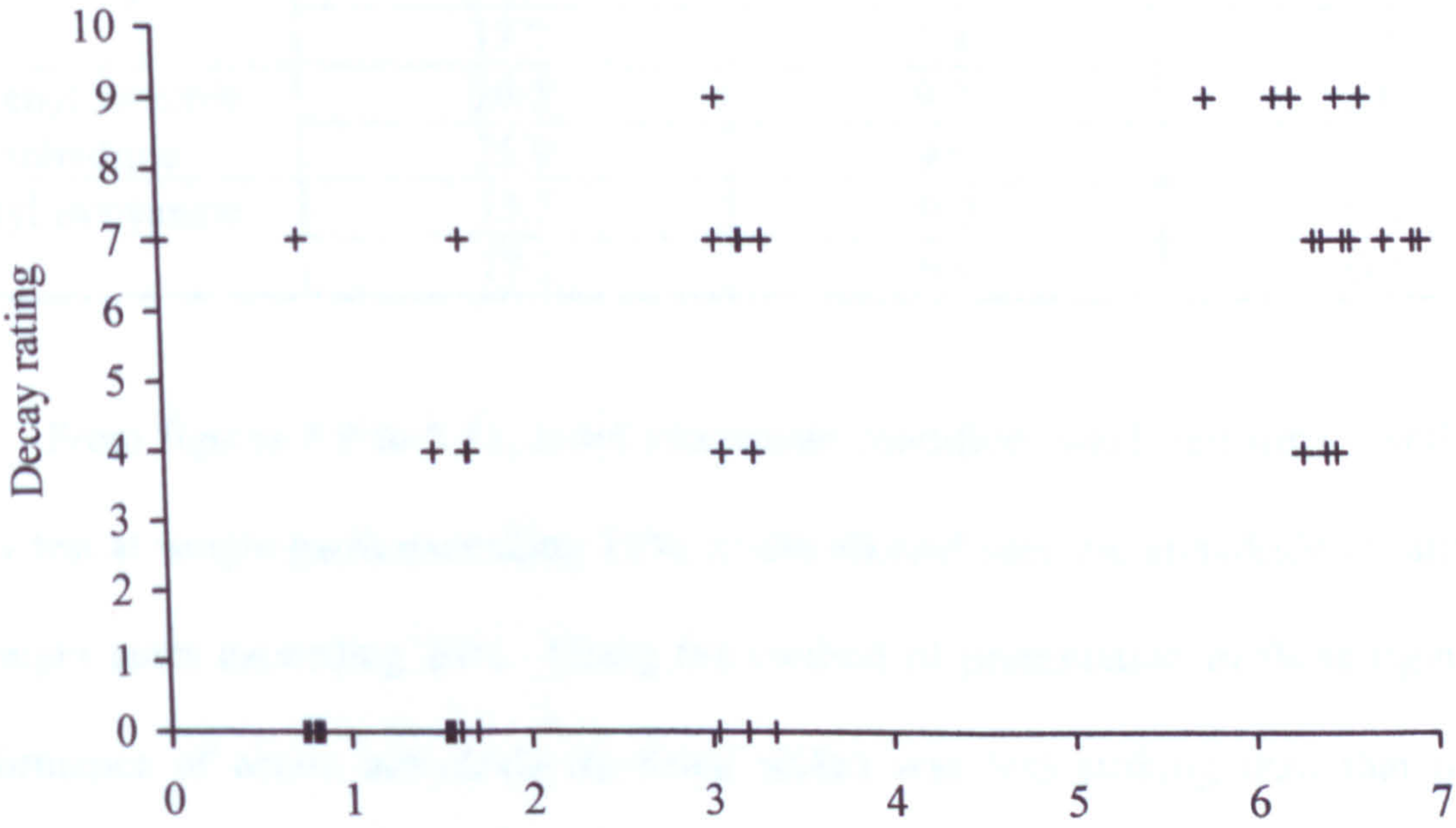


Figure 8.12 Decay rating of CCA treated stakes after 22 months exposure in the fungal cellar

by scatter plotting results for individual stakes at the end of the test, giving a weight gain - decay rating relationship. These are shown in figures 8.7 to 8.12.

The inability of succinic and phthalic anhydride modification to protect wood in this test is shown in figures 8.7 and 8.8. Any slight dose response seen in figures 8.1 and 8.2 is no longer evident when results are presented using this method.

The effect of increasing weight gain is more apparent for acetic anhydride, alkenyl succinic anhydride and butyl isocyanate modified wood (figures 8.9, 8.10 and 8.11). For comparison, the weight losses of the highest modification levels of these three chemicals are given in table 8.2. All had low average weight losses, most particularly butyl isocyanate modified wood.

Table 8.2 Comparison of weight losses and decay rating for stakes exposed in the fungal cellar for 27 months

Modification type	Average WPG	Average decay rating	Average weight loss (%)
Acetic anhydride	14.8	8.6	3.6
	17.7	9.4	2.3
Alkenyl succinic anhydride	26.5	9.7	2.6
	35.9	9.9	3.7
Butyl isocyanate	15.3	9.9	0.4
	17.2	9.9	0.8

From figures 8.9 to 8.11, butyl isocyanate modified wood performed well in the decay test at weight gains exceeding 12%, as did alkenyl succinic anhydride modification at weight gains exceeding 20%. Using the method of presentation in these figures, the performance of acetic anhydride modified stakes was less striking than that of butyl isocyanate and alkenyl succinic anhydride modifications at the weight gains achieved, though good levels of protection were still recorded.

Table 8.2 shows that alkenyl succinic anhydride modified wood lost an average of 3.7% of its weight even at high decay ratings. It is possible that internal soft rot occurred at the base of the stakes, and that visual grading failed to detect this (though no attempt was made to confirm this by microscopy). The susceptibility of alkenyl succinic anhydride modified wood to soft rot was reported in chapter 7.

No obvious pattern of variation of end of test moisture content with the extent of decay or with modification could be found. End of test moisture content tended to vary from bin to bin and also depended on when the stake was removed (a constant soil moisture content in the bins could not be strictly maintained, variation over time was inevitable). Excess moisture in heavily decayed stakes seems to have been lost as stake moisture content came into equilibrium with that of the soil during exposure. Even in the most heavily decayed stakes, the moisture content at the end of the test only occasionally exceeded 100% and was sometimes found to be as low as 35% (20% weight loss).

The moisture content of acetic anhydride, butyl isocyanate and alkenyl succinic anhydride modified stakes was occasionally below 20%, though this is a measure of the moisture content of the whole stake and it is likely that portions below ground were at a moisture content somewhat above this.

Because of these factors the end of test moisture content was not a useful measure in this test.

8.3.4 Performance of stakes treated with unreacted modifying chemicals

Stakes treated with unreacted modifying chemicals were exposed for only 7 months. Average weight loss and decay rating are given in table 8.3.

Table 8.3 Mean decay rating and weight loss of stakes treated with unreacted modifying chemicals after 7 months exposure in the fungal cellar.

Modification	WPG	Decay rating	Weight loss (%)
Untreated control	0	3.2	14
Acetone control	0	5.6	8
Acetic anhydride	17	4.3	24
Succinic anhydride	32	7	20
Phthalic anhydride	35	6.4	20
Alkenyl succinic anhydride	36	9	4

Stakes impregnated with acetic anhydride recorded similar decay ratings to control stakes, yet at a significantly higher weight loss (24% and 14% respectively). The higher weight loss was as expected due to the loss of unbound chemical.

Stakes impregnated with succinic and phthalic anhydride were again quickly colonised by mould and black staining fungi respectively. Weight losses were high due to the loss of these chemicals through leaching or through their use as a food source by mould or stain fungi. The decay ratings were higher than for acetic anhydride, particularly in the case of succinic anhydride. It is possible that in these cases the initial colonisation by mould and stain fungi retarded the subsequent invasion by more active decay fungi.

Unreacted alkenyl succinic anhydride was the best of the chemicals at protecting wood in this test. After 7 months a decay rating of 9 was recorded for all stakes. This was due to soft rotting of the stakes. Weight losses were lower than in other samples indicating that only a small amount of chemical, if any, was being lost from each stake. On cutting these stakes open, a slight discoloration of the exposed stakes to a depth of approximately 3mm was evident. Microscopy revealed that fungal hyphae had colonised this region, though decay was only superficial.

On comparison with estimated 7 month decay ratings from figures 8.1 to 8.5, decay rating was lower in stakes containing unreacted modifying chemical than it was for the equivalent weight gain of reacted chemical.

The distribution of chemical is likely to have been very different in this test than in modified wood. Pyridine is a better wood sweller than acetone, and modifying chemical would have had greater access to the cell wall in the pyridine system. Also, the cleanup stage in the modification procedure (chapter 3) would have removed excess chemical from the cell lumina of reacted stakes. Where modified chemical was impregnated and unreacted, acetone was merely dried off, leaving the modifying chemical were it in the cell walls or in the lumina. In the case of alkenyl succinic anhydride, in which no significant loss of chemical due to leaching was recorded, the difference in distribution of chemical within reacted and unreacted samples may have had an important bearing on the increased susceptibility of the unreacted wood. If unreacted alkenyl succinic anhydride had been distributed in an identical manner to that in the reacted wood, and had proved non-leachable, then decay resistance of these two sets of stakes may have been very similar. This is because only a relatively small number of substitutions take place for a given weight of chemical even when reacted, so the bulking effect of the chemical, rather than the reaction itself, may be the reason for decay resistance of alkenyl succinic anhydride modified wood. This is further discussed in chapter 9.

In the cases of smaller, less hydrophobic, leachable modifying chemicals, it appears that no long term protection was obtained through treatment with unreacted modifying chemical. This is particularly the case in this test for acetic anhydride, the action of which depends on its reaction with wood polymers, if only to secure it within the wood cell wall structure.

8.4 Results and discussion - field trial

Average decay ratings for the field trial stakes after 22 months exposure are given in table 8.4.

Termite attack proved a problem in this test, with several stakes being attacked, some heavily. Acetic anhydride, alkenyl succinic anhydride and butyl isocyanate modified stakes were less affected by termite attack, though were not completely resistant at the modification levels used in this test. In one case an acetylated stake had been completely hollowed out, leaving a 2mm thick skin unattacked with the exception of a single entrance point. This effect was recorded by Kumar and Agarwal (1983) in mango (*Magnifera indica* Linn.) blocks acetylated using thioacetic acid, and was similar to the pattern of fungal decay found in the fungal cellar. In tests on acetylated southern yellow pine blocks, Goldstein *et al.* (1961) found an apparent threshold level of protection against termites of 18% weight gain, though they reported that slight attack was in evidence in blocks acetylated to weight gains as high as 28%.

Other stakes (phthalic and succinic anhydride modified, CCA and controls) were commonly attacked and some completely destroyed by termites. This always took the form of attack from the surface of the stake towards the interior. Failures to termite attack included CCA treated stakes, though the maximum retention of preservative in the stakes exposed in the field trial was low, at only 1.7 kg/m^3 (hydrated salt retention).

Brown and white rot decay were both found in stakes exposed in the field trial. As with the fungal cellar stakes, no pattern of preference was found for different treatments and decay types. Acetic anhydride and butyl isocyanate modified stakes were once again decayed on the interior while leaving the very outside of the stakes largely intact. This was the case whether stakes had been decayed by brown or white rot fungi.

Table 8.4 Comparison of mean decay ratings for chemically modified wood exposed for 22 months in the field and the fungal cellar

Modification	Weight gain / retention	Field trial decay rating	Fungal cellar decay rating
Untreated control	0%	1.8	0.2
Pyridine control	-0.5%	1.4	0.2
Acetic anhydride	5.5%	8.4	5.8
	7.8%	8.3	6.4
Succinic anhydride	7.1%	4.5	4
	11.5%	2.3	3.4
Phthalic anhydride	7.0%	8	3.4
	15.6%	8.4	4
Alkenyl succinic anhydride	5.5%	7.6	4.6
	10.8%	9.8	8.2
Butyl isocyanate	2.2%	6	4.1
	5.6%	4.6	7.8
CCA	0.8 kg/m^3	8	0.8
	1.6 kg/m^3	4.6	2

Phthalic anhydride modified field stakes were subject to staining in a similar manner to the corresponding fungal cellar test stakes.

End of test moisture contents were noticeably higher than in the fungal cellar test, almost always in excess of 100%, irrespective of weight loss. The exceptions tended to be among those stakes modified with acetic anhydride (7.8% weight gain) or alkenyl succinic anhydride (10.8% weight gain). This may in part have been due to the test set-up. The above ground portion of the stake was completely covered in a wrap-around identification label, which also trapped moisture in the stake above the ground line. This caused higher overall moisture contents and several stakes to decay in the above ground portion, in a way not seen in the fungal cellar. It is unlikely that this could have been totally responsible for the higher moisture contents in the field trial; it seems that the field trial provided a significantly wetter environment for the stakes than in the fungal cellar.

The results in table 8.4 only include the decay rating of stakes; termite damage was not taken into account. Stakes attacked by termites to an extent where no decay

rating could be made were not included in the calculation of the average decay rating. Figures in the table are therefore an average of between three and five stakes.

Weight loss calculations have not been presented as the relatively widespread termite grazing meant that only in relatively few stakes was weight loss solely due to fungal degradation.

Appreciable improvements in decay resistance over the controls are evident for acetic and alkenyl succinic anhydride modified stakes even at the low modification levels being tested. It is probable that higher modifications would have performed better still. In a field trials of acetylated stakes in Sweden and Finland, Larsson Brelid, Simonson and Bergman (1997) found that acetylated stakes (*P. sylvestris*) performed well after 5 years exposure, after which all untreated stakes had failed. After this period slight decay was found even in some stakes reacted to achieve acetyl contents above 20% by weight.

Also included in table 8.4 is the estimated decay rating for the corresponding fungal cellar stakes exposed for 22 months (estimated from figures 8.1 to 8.6). In almost all cases the fungal cellar proved more aggressive than the field trial. This is only to be expected due to the accelerated nature of the fungal cellar (ideal conditions for decay 24 hours a day, with no seasonal variations). The most notable exception is the set of butyl isocyanate stakes modified to 5.6% weight gain, which performed worse in the field than in the fungal cellar. This was mainly due to two failures of stakes in this set. This is not a particularly significant result, however, as figure 8.10 shows that after 27 months some fungal cellar stakes also failed at this level of butyl isocyanate modification. It cannot be concluded from this restricted test that butyl isocyanate modification to higher weight gains would have been less effective in the field than it proved in the fungal cellar.

8.5 Summary of points raised in chapter 8

- Good rates of decay were recorded in the fungal cellar, where decay by brown and soft rot fungi was evident. The field trial also provided a white rot challenge, though only low WPG test stakes were exposed in this part of the study. The fungal cellar provided a more aggressive test than the field trial.

- The primary method of assessment was visual grading, though weight loss was taken into account once a stake had been removed from the test. Visual grading was difficult in cases where interior decay occurred, leaving a sound outer portion of the wood. The latter effect occurred in acetic anhydride, butyl isocyanate and alkenyl succinic anhydride modified wood, and was attributed to an uneven distribution of modifying chemical in the wood. Deflection testing did not prove a successful method of assessing decay in this test.

- Succinic and phthalic anhydride proved ineffective at protecting wood from decay. They were susceptible to the mould (succinic) and black stain (phthalic), as was reported in the soft rot test (chapter 7).

- Acetic anhydride, butyl isocyanate and alkenyl succinic anhydride all performed well at the higher modifications tested. The most successful appeared to be the latter two, although WPG's for alkenyl succinic anhydride were significantly higher than for acetic anhydride and butyl isocyanate.

- Where decay occurred, it seems to have started soon after exposure in the fungal cellar. A delay was recorded for acetic anhydride modified wood, but this was thought to be due to the problems in detecting decay in these stakes. No initial time period

was required for decay fungi to remove modifying chemical prior to decaying the wood.

- Stakes treated with unreacted alkenyl succinic anhydride were reasonably resistant to decay over the relatively short period of the test. Soft rot decay was evident, but no brown rot. It was thought that the difference between the efficacy of these stakes and those reacted with alkenyl succinic anhydride may only have been due to the distribution of the chemical within the wood cells, and not necessarily due to reaction itself.

- Stakes treated with unreacted acetic anhydride were extensively decayed. The action of this chemical depends on its reaction with wood polymers, even if only to secure it within the cell wall structure.

- Stakes treated with unreacted succinic and phthalic anhydride were also decayed, though not as extensively as in the case of those treated with unreacted acetic anhydride. Once again, these two chemicals were susceptible to mould (succinic) and stain (phthalic) fungi. It is possible that the initial colonisation by these fungi retarded the subsequent invasion by more active decay fungi, accounting for the better performance of these stakes over their acetic anhydride equivalents.

Chapter 9. General discussion

As described in chapter 1, the purpose of this study was to assess the decay resistance wood modified with a variety of chemicals, and to attempt to further understand the mechanism by which chemical modification protects wood from decay.

9.1 Biological performance of various modifying chemicals

Wood samples modified with five chemicals of varying structure were tested against decay fungi in pure culture and in unsterile conditions. In general, succinic and phthalic anhydrides were the least effective of the five, while acetic anhydride, alkenyl succinic anhydride and butyl isocyanate all showed the potential to protect wood from decay in both pure culture and unsterile environments.

Phthalic and succinic anhydrides were not effective as wood protection chemicals. It appears that this was because of the instability of the bond that these chemicals form with the wood polymers. This instability was detected in all experiments where wood samples modified with these chemicals were held in prolonged contact with water. Leaching results showed weight losses from wood modified with these two chemicals to be significantly higher than in the other cases (table 3.12). Despite the loss of chemical from the wood, phthalic anhydride performed well in the pure culture basidiomycete tests. The apparent lack of hyphal growth over or through blocks led to the conclusion that phthalic anhydride (or more likely its diacid, hydrolysed from the wood) has some biocidal activity (section 6.3.3). It was ventured that no long term protection would be achieved using this chemical as loss through leaching was so high. This was confirmed in the unsterile tests.

A further observation in the unsterile tests for wood modified with succinic and phthalic anhydride was the growth of mould and stain fungi. In the case of succinic anhydride it seems likely that mould fungi are able to utilise the succinate as an energy and carbon source. Similarly, the growth of a black stain fungus in phthalic anhydride modified wood could be due to the ability of this fungus to break down and utilise phthalate, but may also be due to the inhibition of competing fungi. If the former is the case, then this could contribute to the eventual failure of phthalic anhydride modified wood to basidiomycete decay fungi, as any biocidal protection afforded by phthalic anhydride is removed.

The other three modifying chemicals; acetic anhydride, butyl isocyanate and alkenyl succinic anhydride, were more successful in protecting wood from decay. In all tests, butyl isocyanate proved the most effective modifying chemical at a given WPG, though threshold protection levels in pure culture were close to those of acetic anhydride (table 6.2). Alkenyl succinic anhydride conferred good decay resistance to wood, though a significantly higher WPG was required for protection when compared with acetic anhydride and butyl isocyanate. At low weight gains, alkenyl succinic anhydride often appeared better at protecting wood in the pure culture tests than equivalent WPG's of acetic anhydride, though at WPG's in excess of 10%, acetic anhydride was the more effective chemical (Figures 6.5 to 6.15). This is further discussed in section 9.2.

In the soft rot test, alkenyl succinic anhydride was unable to completely protect wood from decay. It was suggested that this could be due to the inability of the large alkenyl succinic anhydride molecule to effectively penetrate the cell wall, and that this chemical could therefore not protect wood from fungal hyphae growing and forming cavities in the S_2 layer. This is an example of how the distribution of chemical within wood can affect decay resistance, even before the mechanism of that decay resistance is

considered. The effects of distribution of chemical in this study are discussed in the following section.

9.2 Distribution of modifying chemical within wood

Decay resistance could be affected by the distribution of chemical in two ways; uneven distribution in the gross wood structure and uneven distribution across an individual cell wall (as mentioned above). These are discussed in turn.

Problems were encountered regarding the distribution of modifying chemical through the gross wood structure. Decay patterns were observed in modified wood in which the interior of a sample was decayed leaving a sound outer layer, and more specifically in which the latewood was more susceptible to decay while earlywood was protected. The latter observation was made previously by Nilsson and Rowell (1982) and Cardias and Hale (1990) and was attributed to the low ratio of modifying chemical to cell wall material in the latewood in the central portion of the block during reaction. Evidence of these distribution effects were seen in wood modified with acetic anhydride, butyl isocyanate and alkenyl succinic anhydride. Wood modified with acetic anhydride appeared to be the most susceptible, with obvious interior failure of fungal cellar stakes at low WPG's and with interior latewood failure in pure culture, soft rot and fungal cellar tests (see sections 6.3.1, 7.3.3 and 8.3.2).

From the graph of hydroxyl substitution against molar concentration of the reaction solution (figure 3.4) it can be seen that acetic anhydride was significantly more reactive than butyl isocyanate and alkenyl succinic anhydride. Comparing acetic anhydride and butyl isocyanate modifications, approximately double the number of hydroxyls were substituted at each molar concentration of acetic anhydride than they were of butyl isocyanate. If butyl isocyanate in the reaction solution was being depleted

at a much slower rate than acetic anhydride, as seems to be the case, then the depletion of modifying chemical may not be so important a limiting factor for the extent of butyl isocyanate reaction in these conditions, and reaction time may be more important. If depletion of modifying chemical has a lesser effect on butyl isocyanate reaction, it would be expected that the difference in modification level between latewood and earlywood would be lower, and the distribution of modifying chemical more even. The greater molecular size of the butyl isocyanate adduct (Mw = approx. 99) in comparison to that for acetic anhydride (Mw = approx. 42) meant that in general, even at lower levels of substitution, higher WPG's were achieved at each molar concentration (tables 3.4 to 3.6).

The possibility of differences in the distribution pattern of each chemical makes comparison of WPG - weight loss relationships among different modifying chemicals difficult. It is quite possible that if acetic anhydride modification was more evenly distributed within wood then a 20% or lower weight gain would have completely protected wood as reported by other workers (see section 2.4.2). It may be that the reaction procedure used here was the cause of these areas within the wood (interior latewood) in which WPG was depressed sufficiently below this level for decay to occur. The fact that butyl isocyanate was more effective in protecting wood in this study could purely be due to better distribution of chemical.

To overcome distribution problems in acetic anhydride modified wood, higher concentrations of acetic anhydride in the reaction solution would be required, possibly undiluted acetic anhydride, so solution concentration would no longer be the limiting factor in reaction. Undiluted acetic anhydride has been used previously in modification reactions by workers such as Takahashi *et al.* (1989) and Beckers *et al.* (1994).

The distribution of chemical across an individual cell wall may also have varied between different modifying chemicals. Mentioned above is the example of the

susceptibility of alkenyl succinic anhydride to cavity forming soft rot fungi. This was attributed to the inability of the relatively large alkenyl succinic anhydride molecule to penetrate the cell wall effectively. However, size may not be the only governing factor with regard to distribution across the wood cell wall. The apparent lower reactivity of butyl isocyanate when compared to acetic anhydride may result in modifying chemical being able to diffuse further into the cell wall before reaction. This would become important as the modifying chemical within the wall is used up and diffusion from the lumen takes place. The more reactive acetic anhydride may react more extensively than butyl isocyanate at the cell wall surface, and less so within the cell wall. A difference in distribution across the cell wall could conceivably affect the decay resistance of the wood, though any effect of this is not readily seen as in the case of the earlywood - latewood pattern discussed above.

Distribution across the wall will also vary with WPG. Low WPG's may involve higher reaction at the lumen surface (due to the excess modifying chemical in the lumen) than within the cell wall, despite the pre-reaction impregnation procedure to make sure modifying chemical was within the cell wall. This could be the cause of the observed resistance to white rot fungi at lower WPG's. Although it has been reported that penetration of the cell wall is necessary for the initiation of simultaneous white rot decay, this is thought only to occur slightly ahead of the erosion trough, and not to diffuse deep into the cell wall as in the case of brown rot (Eriksson *et al.*, 1990; Evans *et al.*, 1991). Reaction at or near the lumen surface may therefore have a greater effect on white rot fungi when compared with brown rot fungi.

The distribution across the cell wall is also likely to be affected by the solvent /catalyst system. For example, uncatalysed acetic anhydride may provide a better distribution across the wall as reactivity is reduced and the modifying chemical is able to

diffuse further. The swelling solvent used in this study (pyridine) may have caused reaction to occur at sites in the cell wall otherwise unexposed during water soak. This could have caused threshold levels of protection to be higher in this study as modifying chemical is needlessly used up on otherwise inaccessible sites. The reaction of water inaccessible sites could have caused the increase in aggregate swell above that of the water soak at low WPG's (table 4.3).

9.3 Mechanisms of protection

The main purpose of the study was to try to further understand the mechanism (or mechanisms) by which chemical modification protects wood from decay. This is not a straightforward task, due in part to the fact that the actual mechanism by which fungi decay wood is itself not fully understood. Recent literature suggests that both brown and white rot decay is initiated at a distance from the hyphae, within the wood cell wall, where enzymes produced by these fungi are too big to penetrate. A variety of reactive low molecular weight compounds have been proposed as being responsible for decay (section 2.3).

The main protection mechanisms for chemical modification proposed by previous workers are discussed in section 2.4.4. These are generally separated into blocking mechanisms and the lowering of moisture content.

Blocking of wood polymers can be considered either as blocking of specific decay susceptible chemical groups on the wood polymers, or pore blocking by the bulk of the modifying chemical, restricting the access of degradative agents. Both of these were considered by Stamm and Baechler (1960). The former of these mechanisms concerns the removal of hydroxyl sites through reaction and the shielding of adjacent sites. This is

discussed in section 9.3.1. The latter is non-specific, and does not rely on actual reaction with hydroxyls. This is discussed in section 9.3.2.

The lowering of the moisture content of wood (or the wood cell wall) by chemical modification to levels below those required by decay fungi was again proposed by Stamm and Baechler (1960) and is a commonly quoted hypothesis for the mechanism of decay resistance. This is discussed in section 9.3.3.

9.3.1 Removal or blocking of decay susceptible chemical groups

Decay resistance through the blocking of sites has been thought to come about because the large site specific enzymes of decay fungi are unable to attach themselves to substituted wood polymers (Rowell, 1992). However, with the recognition of the involvement of small non-enzyme degradative agents, the blocking of specific enzyme binding sites is unlikely to affect depolymerisation reactions unless all potential reaction sites are shielded from these reactive non-specific species.

In figure 4.18 the available monomolecular adsorption sites (at 100% RH) are plotted against WPG for each modification. From these results it was found that for wood modified with butyl isocyanate to 22.9% WPG, 68% of the hydrophilic sites in unmodified wood are still available to adsorption. If a degradative agent such as the hydroxyl radical is responsible for depolymerisation reactions during decay, then these sites that are accessible to water will be accessible to the smaller hydroxyl radical. However, wood modified with butyl isocyanate to this level was found to be resistant to decay (see table 6.2).

The results from the sorption study seem to indicate that site blocking is not the mechanism of decay resistance, though things are not necessarily as straightforward as this. Takahashi *et al.* (1989) reported that acetylated wood showed increased resistance

to Fenton's reagent at WPG's in excess of 15%. At 22% WPG, which was found to protect wood against brown rot fungi, weight loss due to Fenton's reagent was at approximately 25% of its level in unmodified wood. Results in figure 4.18 indicate that over 50% of sorption sites would still be available at this level of acetylation. The drop in weight loss on exposure to Fenton's reagent to as low as 25% of that in unmodified wood (Takahashi *et al.*, 1989) could be due to a reduction in preferential reaction sites or to the distribution of modification. It might be expected that a greater proportion of the hydroxyl groups at the lumen surface (adjacent to the reservoir of modifying chemical within the lumen) would be reacted than those further into the cell wall. The fact that the remaining sorption sites are more remote may account for the results found by Takahashi *et al.* (1989). Whatever the reason, results here and those found by Takahashi *et al.* lead to two conclusions:

1. Modification with acetic anhydride caused a reduction in weight loss on exposure to Fenton's reagent, indicating that site substitution can protect wood from degradation. In this case, pore blocking would not stop the hydroxyl radical reaching sites accessible to water, neither could it be claimed that lack of water could be responsible for the observed protection.
2. The results in figure 4.18, in which a large proportion of hydroxyl sites remain available for sorption (and presumably are open to attack by the small hydroxyl radical), and the fact that threshold levels of acetylation (against brown rot fungi) did not completely protect wood from Fenton's reagent (Takahashi *et al.*, 1989), indicate that other mechanisms are involved in wood protection.

Wood polymers could be protected from small reactive degradative agents if substitution of hydroxyl groups by modifying chemicals alters the reactivity of the polymers. Protection could then occur even if all hydroxyls are not reacted. Rowell

(1980) suggested that a minimum of 22% of accessible holocellulose hydroxyls had to be reacted to protect wood from decay by *G. trabeum* (assuming 100% of hemicellulose hydroxyls and 35% of cellulose hydroxyls are accessible). However, results presented in figure 6.18 show that protection on the basis of substitution varies greatly between chemicals, with alkenyl succinic anhydride much more effective at protecting wood per hydroxyl reacted than other modifying chemicals. This demonstrates the importance of the size of the modifying chemical rather than just the number of hydroxyls reacted. Takahashi *et al.* (1989) proposed that preferential substitution of lignin in the reaction procedure (see section 2.4.4) could account for the lower WPG's required for the protection of wood from decay by white rot fungi. When the action of ligninolytic enzymes is considered this is quite plausible. An example of this would be the case of phenoloxidases or their mediators and their reported dependence on a phenolic substrate. If phenolic hydroxyls in the lignin are substituted through chemical modification then these enzymes or mediators might not be able to operate. Further, if the reaction mechanisms proposed by Pizzi *et al.* (1994) for acetylation are correct (section 3.2.1) then the substitution of a ring carbon in the lignin with a deactivating group could cause the aromatic nucleus to become less susceptible to oxidation even by lignin peroxidase or its mediators.

9.3.2 Restricting access of degradative agents through cell wall pore blocking

As mentioned above, current theory suggests that initial brown and white rot decay occurs within the cell wall at a distance from fungal hyphae. Modification might prevent decay if modifying chemical within the cell wall reduced pore size to an extent where degradative agents were excluded. For example, a popular theory of brown rot decay mechanism involves the movement of Fenton's reagent (H_2O_2 and Fe^{2+}) into the

wall (see section 2.3.2). Hydrogen peroxide is only slightly larger than water and would presumably not be excluded from the wall. The theory of Hyde and Wood (1997) involves the chelation of Fe^{2+} by oxalate, which then moves into the cell wall to a point where the pH is high enough for the reaction with H_2O_2 and the formation of the hydroxyl radical. However, if the chelation of Fe^{2+} caused it to become too large for penetration, then the Fe^{2+} would never reach the portions of the cell wall at which the pH is high enough to allow Fenton's reagent to form the hydroxyl radical, so the degradative chemical species thought to be responsible for depolymerisation in this case would never be produced. Access of iron chelating agents proposed by Goodell and Jellison (1997) and Enoki *et al.* (1992), and veratryl alcohol (proposed as a mediator for lignin peroxidase, see section 2.3.1) may also be restricted. This would also apply for Mn^{2+}, proposed as a mediator for manganese dependent peroxidase, if Mn^{2+} was chelated. In each of these cases, the sensitivity of a decay fungus to chemical modification would depend on the size of the degradative agent used to impregnate and depolymerise the cell wall. Differing sizes of degradative agents between different decay fungi would cause different threshold levels of protection of wood modified with a given chemical.

An attempt to measure pore size was made by using the pore size exclusion technique. A general reduction in space within the cell wall was measured, as would be expected due to the bulking effect of the modifying chemical. The change in size distribution of cell wall pores at low WPG's of acetic anhydride and butyl isocyanate modification could signal the narrowing of wider pores (section 5.3.2). This could lead to restricted access for degradative agents released by decay fungi. However, due to problems encountered during the test (described in chapter 5) no confident conclusion could be drawn from this work.

9.3.3 Suppression of moisture content

It is well documented that chemical modification can cause wood to become more hydrophobic and also cause it to become more resistant to decay (section 2.4). It has often been stated that the reduction in moisture content is the likely cause of increased decay resistance, though only circumstantial evidence appears to have been presented. The fact that wood becomes both more hydrophobic and more decay resistant does not necessarily mean that one is the cause of the other.

In this study an attempt was made to verify any link between the two by comparing the water relationships and decay resistances of wood modified with a variety of chemicals. Swelling and sorption characteristics of modified wood were measured, and moisture content was increased in part of the pure culture decay test to challenge modified wood in a high moisture content environment.

In the pure culture test (chapter 6) it was demonstrated that the end of test moisture content in the sterile jars was restricted by modification but was theoretically high enough to support decay, particularly in the higher moisture content part of the test. In the decay tests (high m.c. pure culture, soft rot and fungal cellar tests), the end of test moisture contents even in samples that had been protected from decay by modification were also theoretically high enough, and in many cases at a level regarded as being ideal for decay.

This overall moisture content gives no indication of the position of moisture within the wood. It could be that wood decay fungi require a certain amount of water in the cell wall itself and that overall moisture content is of lesser importance, a possibility suggested by Stamm and Baechler (1960). The maximum cell wall moisture content is the fibre saturation point. Two of the tests in this study provided measures of FSP; the adsorption test (chapter 4) and the pore size exclusion analysis (chapter 5). For reasons

described previously, adsorption gave relatively low measures of FSP and pore size exclusion gave relatively high measures of FSP. In the latter of these, several problems were encountered during measurement, and time limits restricted measurement to just two WPG's per modification type (see chapter 5). For these reasons FSP measures from pore size exclusion are not further considered here.

Figures 9.1 to 9.6 show weight losses in the pure culture tests (chapter 6) plotted against FSP of unexposed modified wood calculated from adsorption (chapter 4). It was expected that if the suppression of cell wall moisture content was the mechanism of increased decay resistance, then the relationships for the different modifications would be identical (i.e. the same weight loss at the same FSP, regardless of WPG and modifying chemical). Succinic and phthalic anhydride modifications have not been included in these figures. The lack of permanence of both chemicals and the suspected biocidal effect of phthalic anhydride modification make their inclusion meaningless.

When viewing the figures it should be taken into account that results from two different sets of blocks have been plotted against one another. Although the adsorption test blocks and the pure culture test blocks were both selected from the same reaction sets (see chapter 3), the variation in WPG within these sets caused the mean WPG's of the selected test sample sets to differ very slightly. This difference was found to be very small in comparison with the difference in WPG between reaction sets, so the plots in figures 9.1 to 9.6 are still considered valid.

In each case in figures 9.1 to 9.6, there was relatively little agreement between the curves for different modifications. From these results it appears that cell wall moisture content (FSP) is unable to explain resistance to decay in modified wood, and that other factors are at least partly responsible.

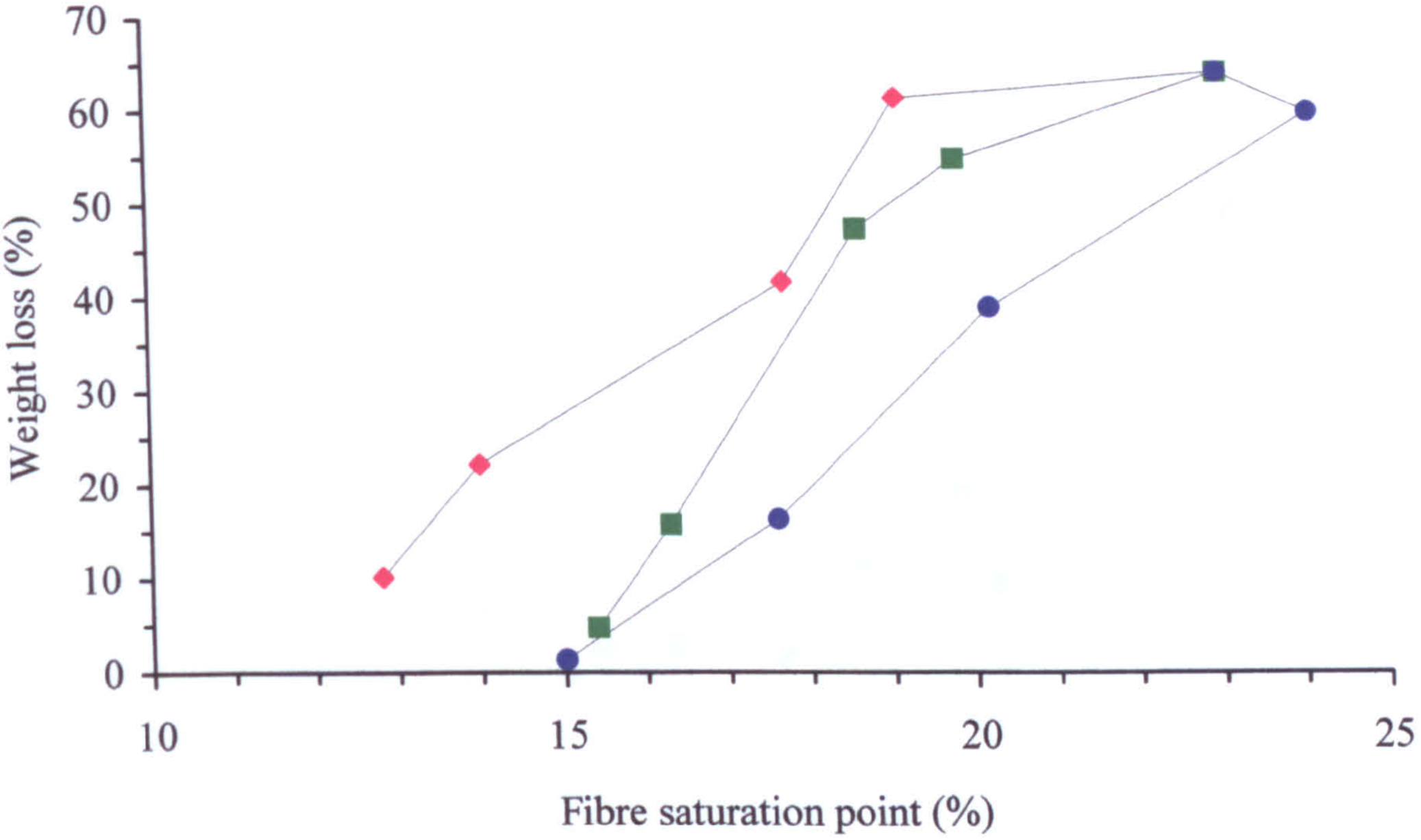


Figure 9.1 Weight loss in the *Coniophora puteana* (low moisture content) test plotted against FSP (from adsorption)

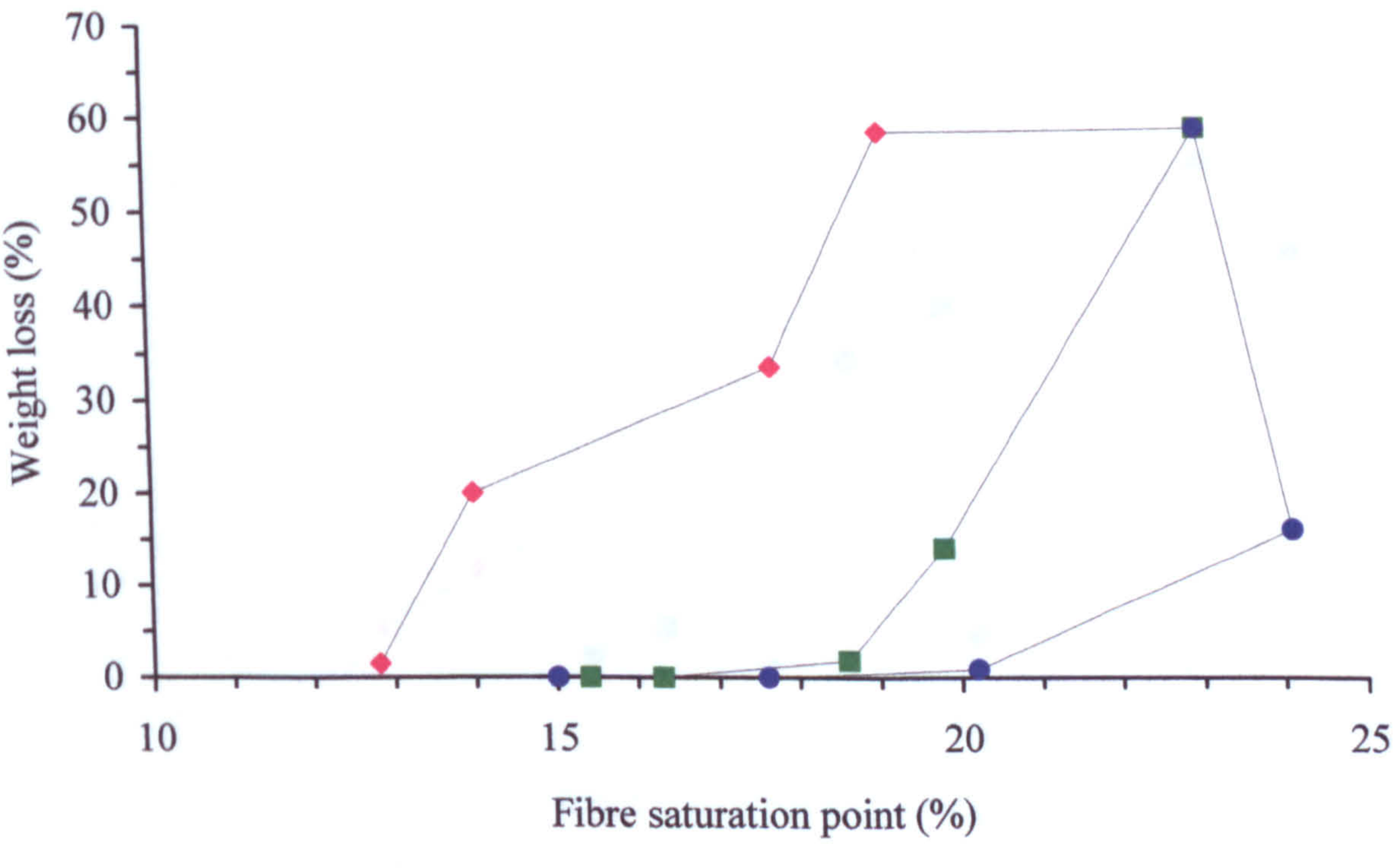


Figure 9.2 Weight loss in the *Coniophora puteana* (high moisture content) test plotted against FSP (from adsorption)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Butyl isocyanate (●)

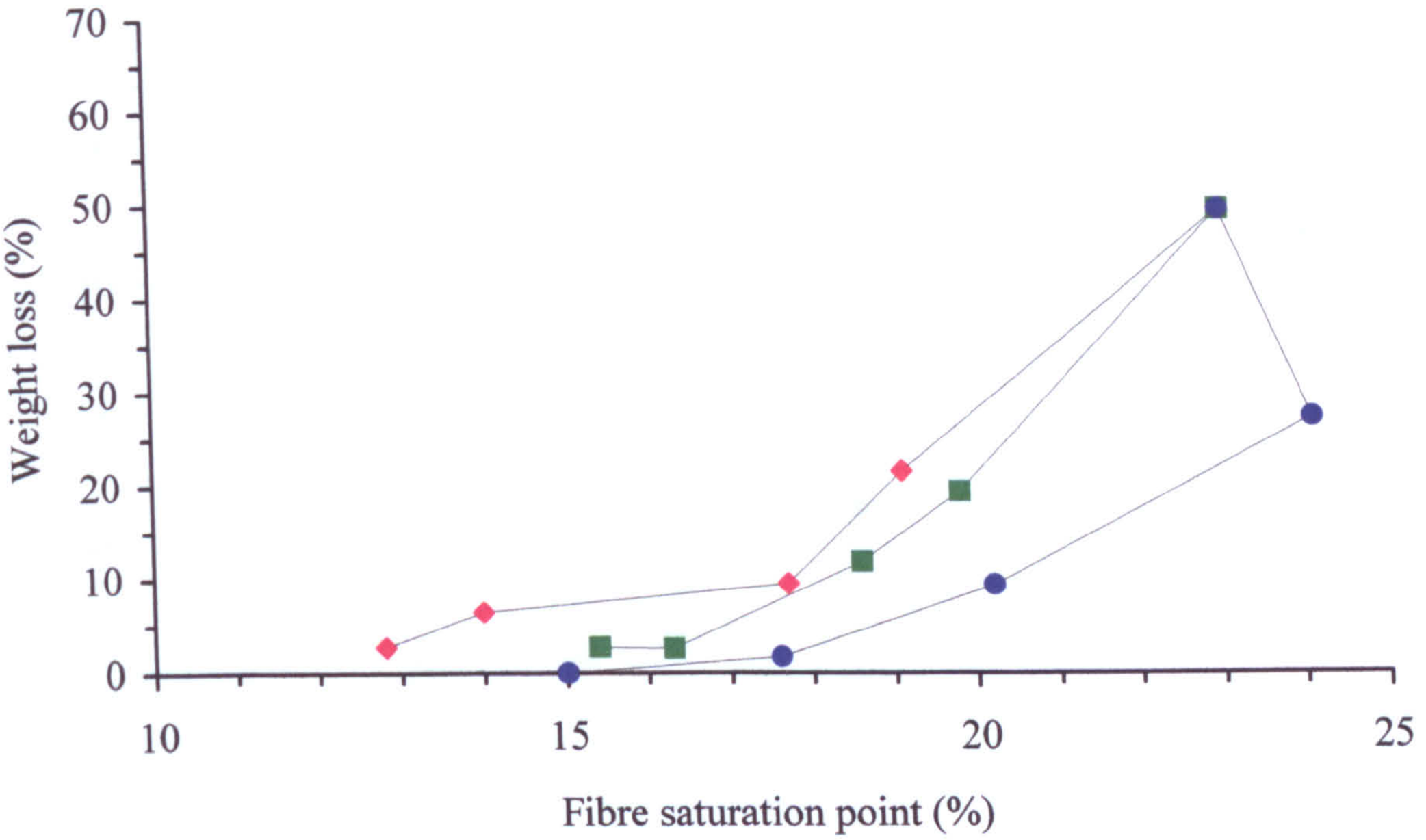


Figure 9.3 Weight loss in the *Gloeophyllum trabeum* (low moisture content) test plotted against FSP (from adsorption)

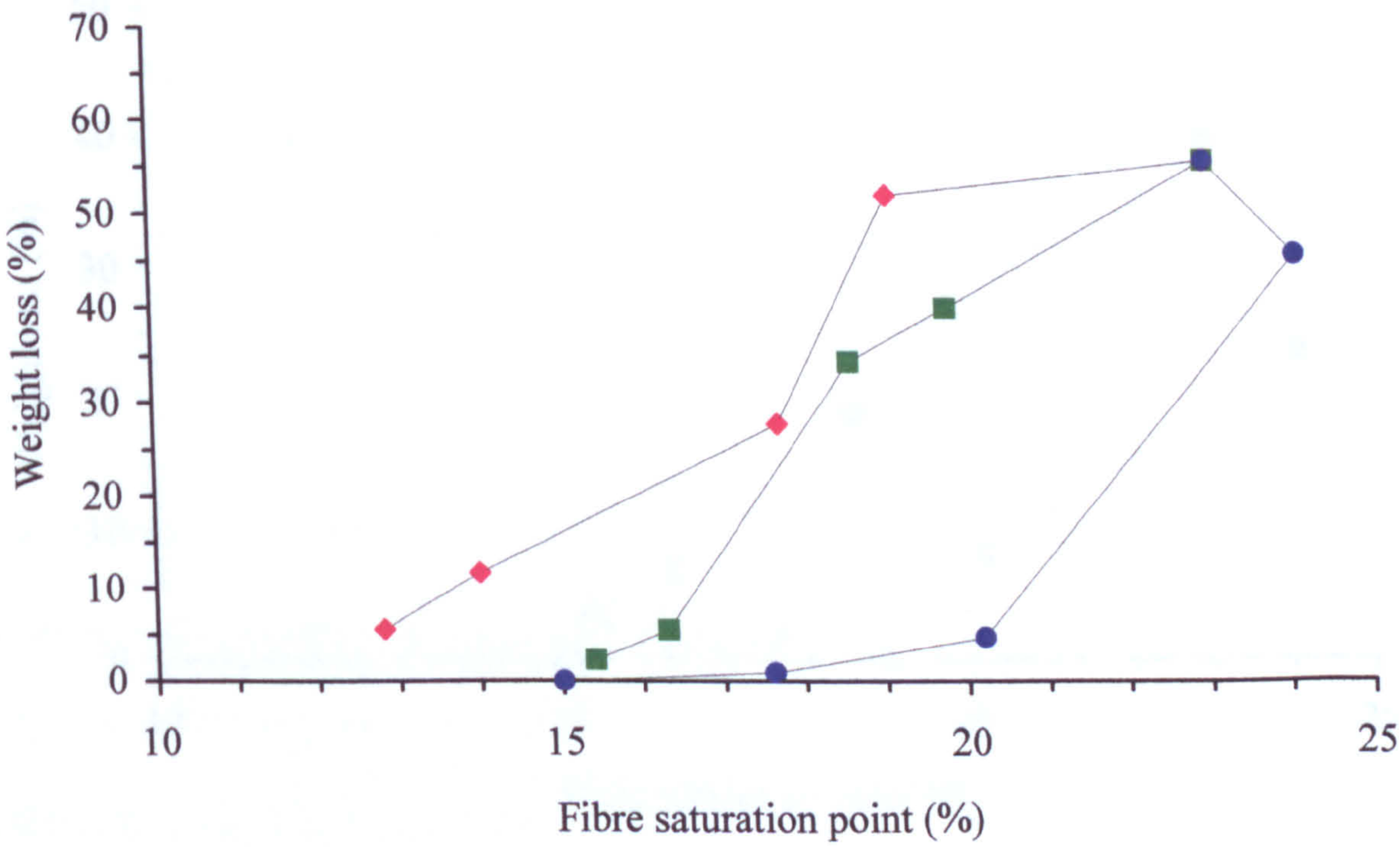


Figure 9.4 Weight loss in the *Gloeophyllum trabeum* (high moisture content) test plotted against FSP (from adsorption)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Butyl isocyanate (●)

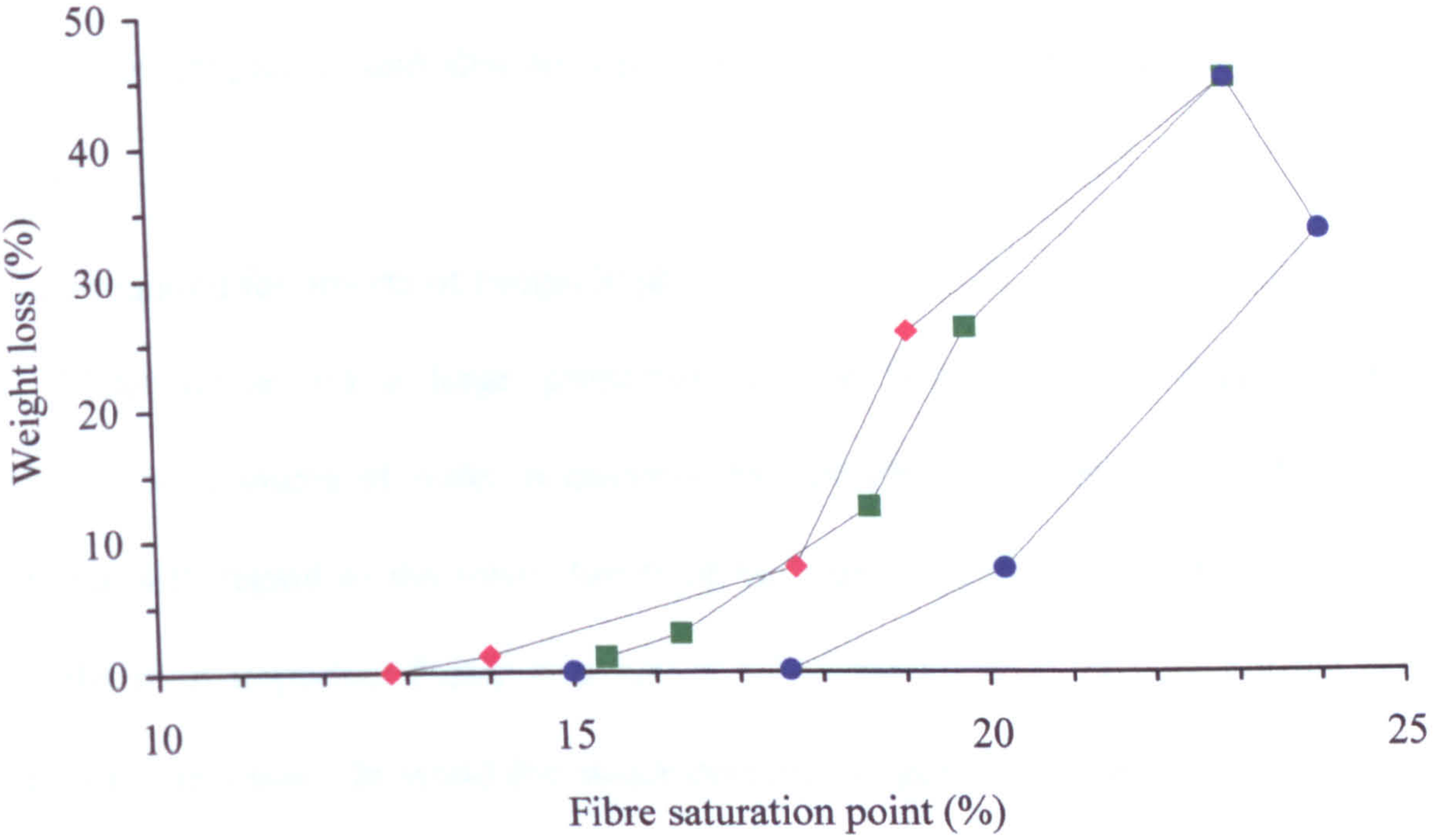


Figure 9.5 Weight loss in the *Trametes versicolor* (high moisture content) test plotted against FSP (from adsorption)

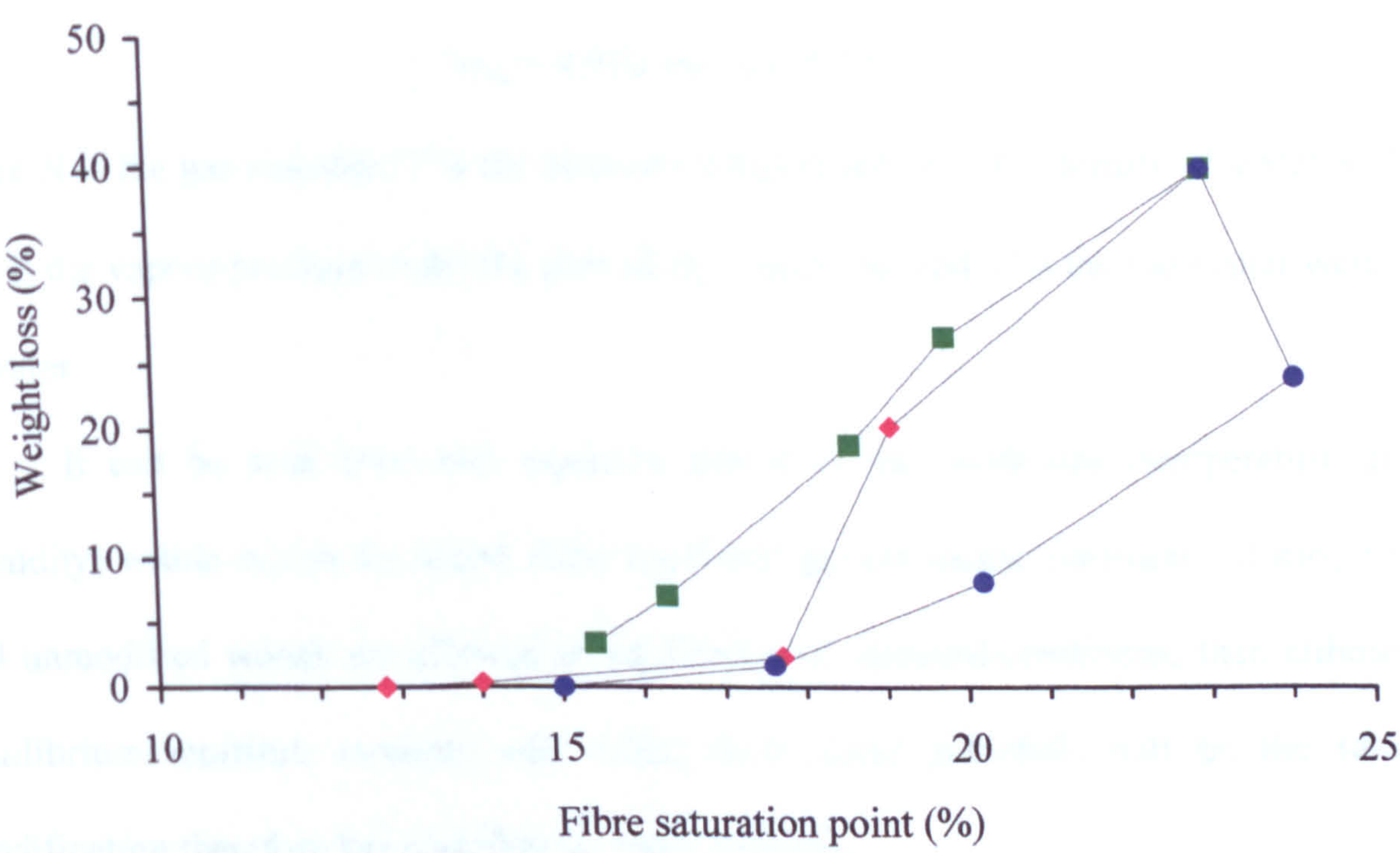


Figure 9.6 Weight loss in the *Pycnoporus sanguineus* (high moisture content) test plotted against FSP (from adsorption)

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Butyl isocyanate (●)

It is here useful to recall the four reasons why water is required by decay fungi, as introduced in chapter 1, and discuss each in the context of modification and decay resistance.

1. Water required for growth of fungal hyphae.

Water makes up a large proportion of the contents of the fungal hyphae themselves, so a source of water is essential for growth of the fungus. Griffin (1977) stated that with regard to the lower limits of moisture content required for growth and decay, the most important factor was not so much the moisture content but the water potential in the wood. In wood the water potential is generally governed by the matric potential, except in situations where large amounts of solutes occur, as in marine locations, where osmotic potential becomes important (Rayner and Boddy, 1988). Matric potential (ψ_m, expressed in MPa) is given by the following equation:

$$\psi_m = -(RT\rho \ln p/p_o)/10^7 M$$

where R is the gas constant, T is the absolute temperature, ρ is the density of water at T, p/p_o is the vapour pressure under the prevailing conditions, and M is the molecular weight of water.

It can be seen from this equation that it is the conditions (temperature and humidity) within which the wood finds itself that govern matric potential. If modified and unmodified woods are allowed to equilibrate in identical conditions, then although equilibrium moisture contents will differ, their water potentials will be the same. Modification therefore has no effect on water potential.

At higher moisture contents it was shown in decay tests that higher reaction levels of wood modified with certain chemicals were resistant to decay. In these blocks it was evident that there was plenty of free water in the cell lumina available for growth of

fungal hyphae. Modification does not protect wood by reducing the amount of water available for growth.

2. Water required for hydrolysis reactions.

The main carbohydrate degradation enzymes released by fungi have been found to result in the hydrolysis of linkages in wood oligomers or polymers, and without water would be unable to operate. If an extracellular hydrolytic enzyme or other degradative agent reaches a site of attack, it will have diffused through water to get there, and so water will presumably already be at any site of attack. The availability of water to a reaction would depend on the water potential as measured above, which is unaffected by modification. Finally it is generally recognised, certainly in the case of brown rot decay, that it is an oxidative system that is involved in initial depolymerisation reactions and hydrolytic enzymes cannot penetrate the wood cell wall even late in decay (Srebotnik and Messner, 1991). If these enzymes operate only at the lumen / cell wall boundary or in the hyphal sheath to break down depolymerisation fragments then this would be unaffected by the water content within the wood cell wall.

Again it appears that the reduction of cell wall moisture content does not affect this requirement for water.

3. Water required as a solvent for diffusion of degradative agents (or depolymerisation products).

If the moisture content of wood is above fibre saturation point, as was the case in the decay tests undertaken, then water is present adsorbed to hydrophilic sites within the wall (which are theoretically susceptible to decay), as polymolecular water within the cell wall, and as free water in the cell lumen. There is therefore water in all areas required to carry degradative agents to susceptible decay sites. If as discussed above, pores are too

small for the access of degradative agents, then this is due to the blocking effect of the modifying chemical and not to any change in the ability of the water to act as a solvent.

4. Water required to swell the wood cell wall.

In order for degradative agents to reach the site of attack and for depolymerisation to occur, water is required to swell the wood cell wall and open the transient capillary structure through which these degradative agents can diffuse. In the absence of sufficient water these pores would be too small to allow diffusion to occur.

Figures 9.7 to 9.12 show weight losses in the pure culture decay tests plotted against the volumetric swell after water soak (from figure 4.20). These figures have been constructed in the same way as those for the FSP plots in figures 9.1 to 9.6. On comparison with the FSP plots (figures 9.1 to 9.6), some very good agreements between modifications are seen in figures 9.7 to 9.12. These seem to indicate that volumetric swell has a significant effect on the decay process, and its reduction by chemical modification could (at least in part) be responsible for the resistance of modified wood to decay.

The point at which decay ceased in these figures is very close to 5% swell. This is equivalent in this study to an ASE of just over 70%. This is in good agreement with a figure presented by Stamm and Baechler (1960) in which ASE at threshold level of protection of acetylated wood against decay by *Lenzites trabea* (= *G. trabeum*) was approximately 70%.

The similarity between curves in figures 9.7 to 9.12 was not good in all cases, and was particularly poor in the high m.c. brown rot tests. As described in section 6.3.4, it is thought that the different performances of modifying chemicals in these tests could be due to the distribution of free water, and the ability of each fungus to cope with a high

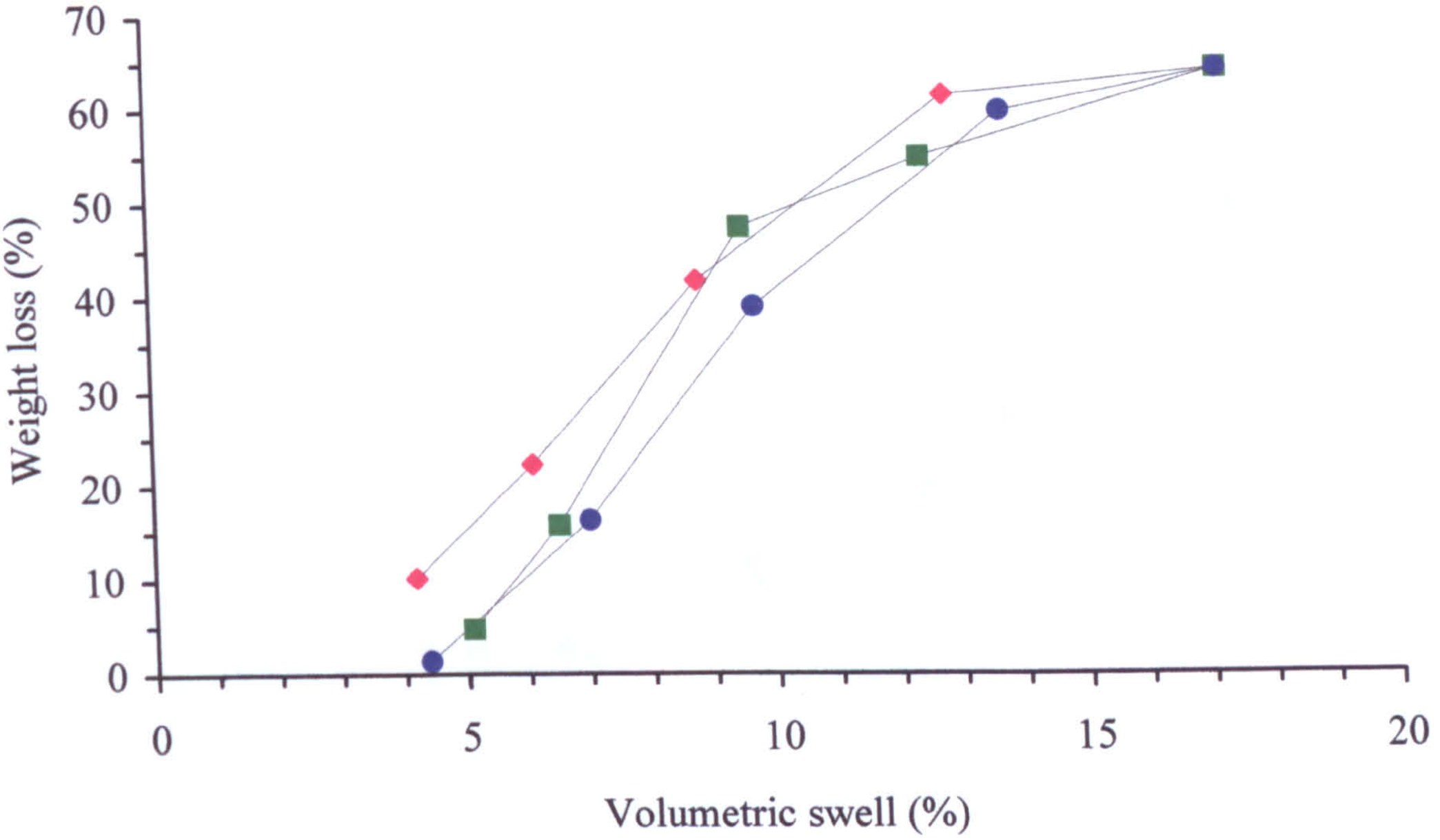


Figure 9.7 Weight loss in the *Coniophora puteana* (low moisture content) test plotted against maximum volumetric swell

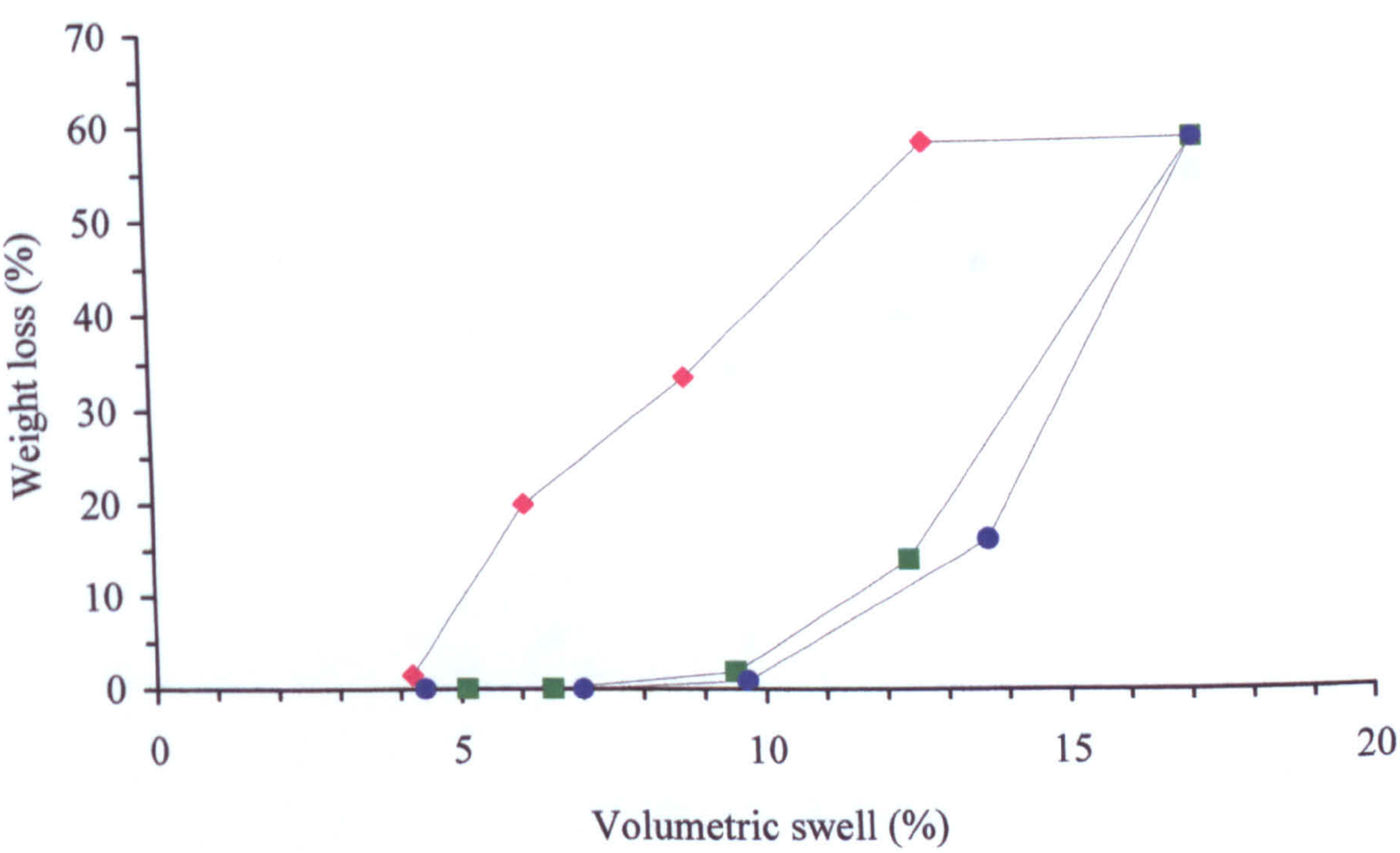


Figure 9.8 Weight loss in the *Coniophora puteana* (high moisture content) test plotted against maximum volumetric swell

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Butyl isocyanate (●)

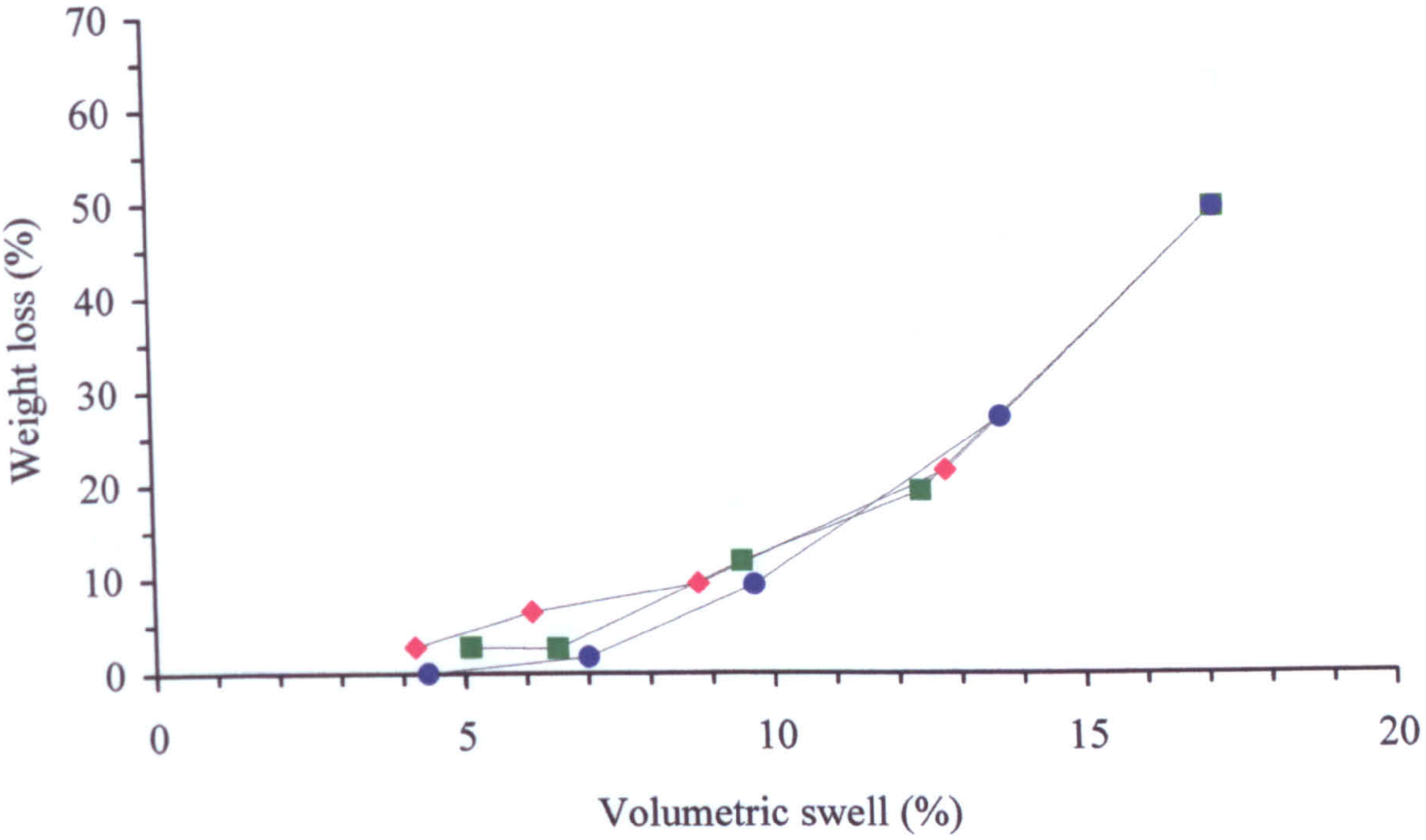


Figure 9.9 Weight loss in the *Gloeophyllum trabeum* (low moisture content) test plotted against maximum volumetric swell

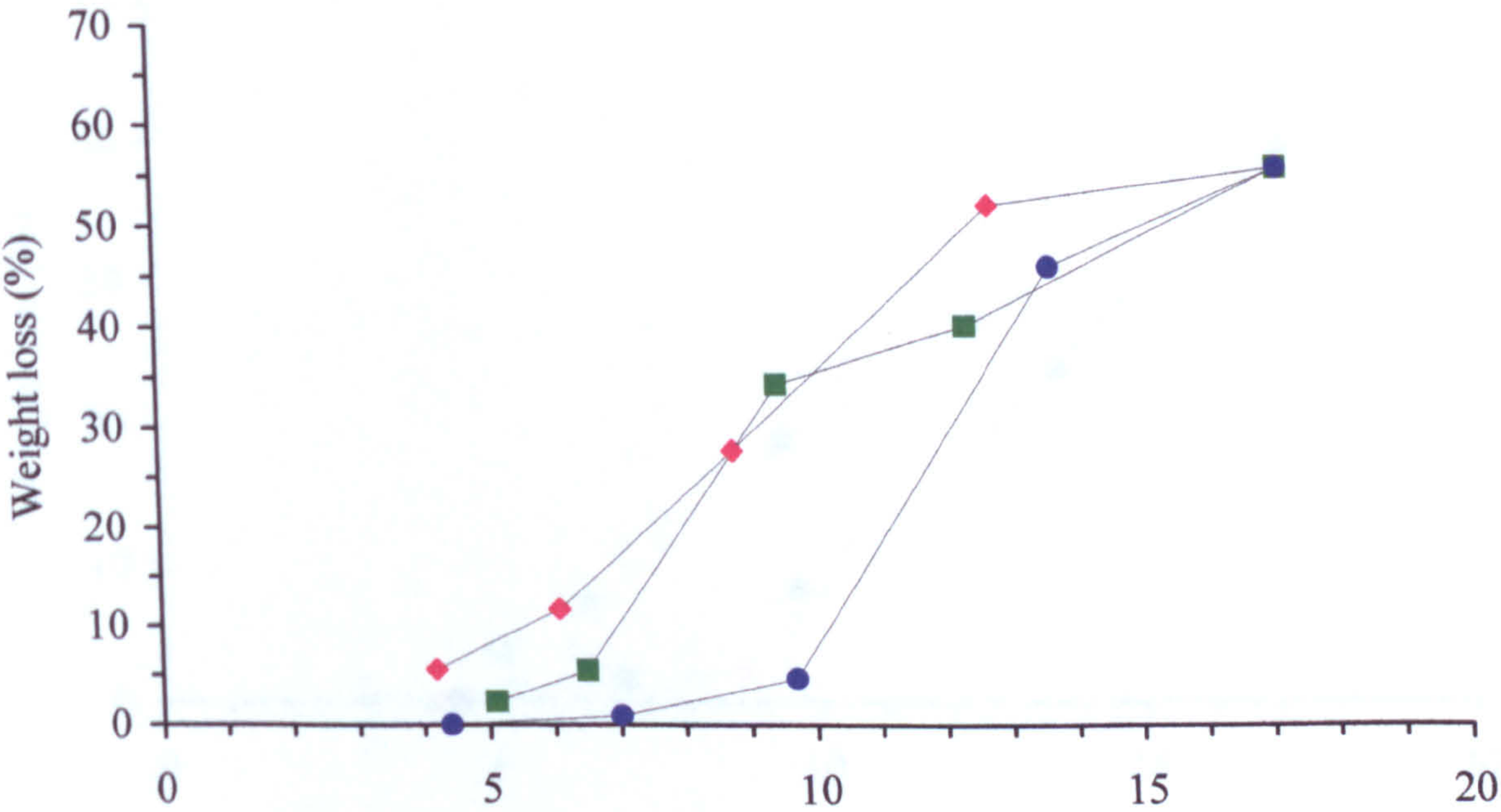


Figure 9.10 Weight loss in the *Gloeophyllum trabeum* (high moisture content) test plotted against maximum volumetric swell

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Butyl isocyanate (●)

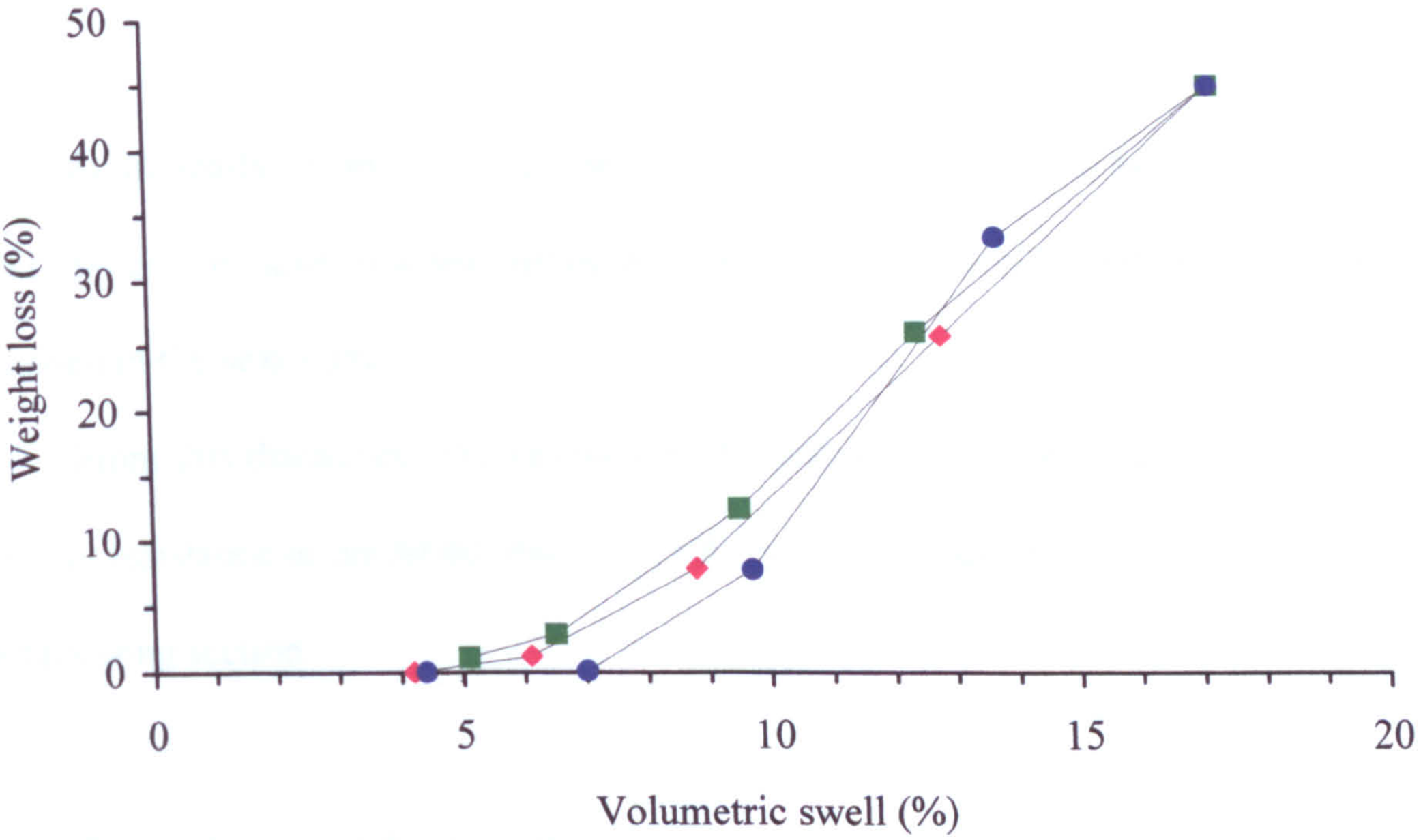


Figure 9.11 Weight loss in the *Trametes versicolor* (high moisture content) test plotted against maximum volumetric swell

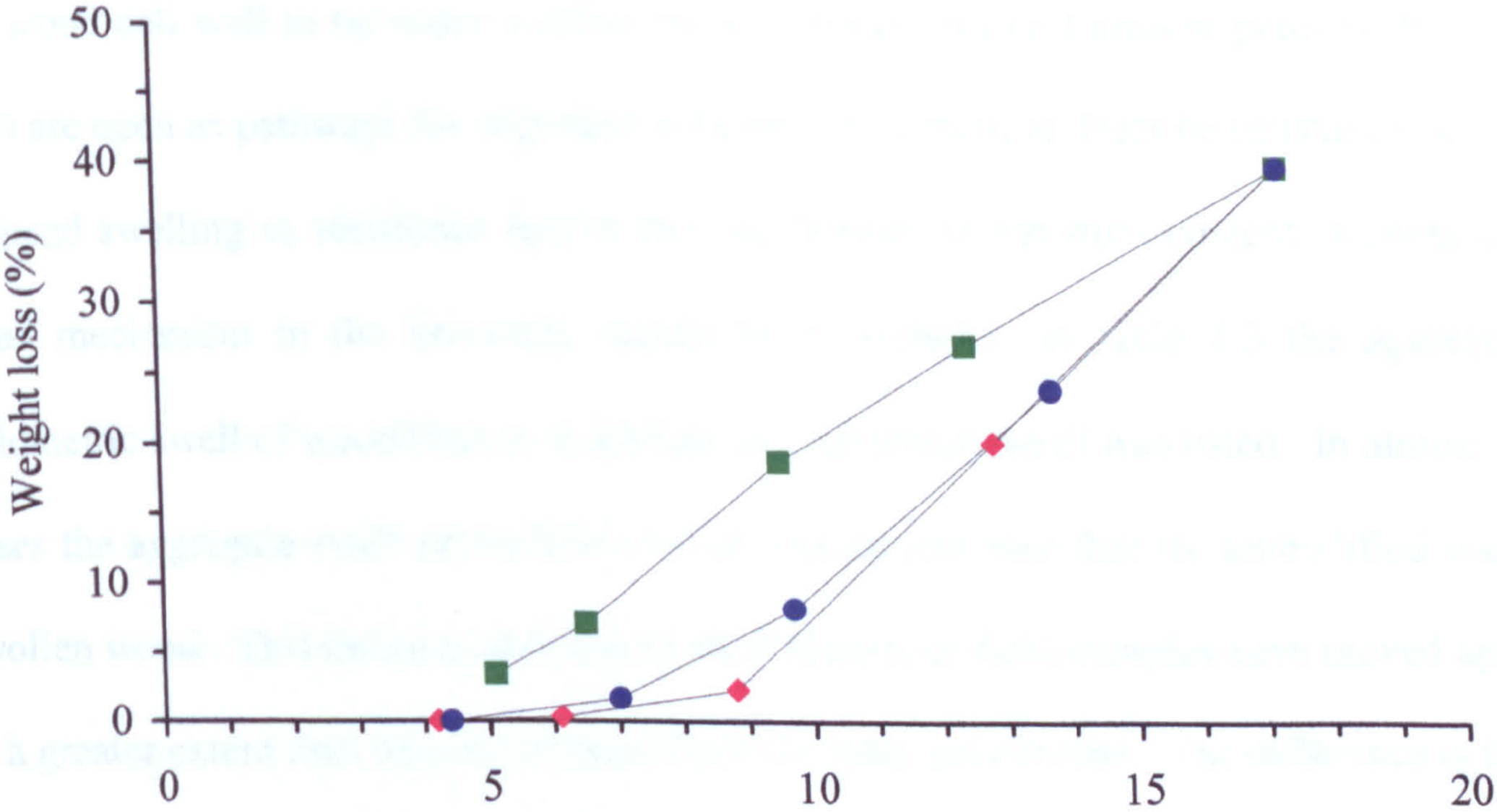


Figure 9.12 Weight loss in the *Pycnoporus sanguineus* (high moisture content) test plotted against maximum volumetric swell

Acetic anhydride (◆); Alkenyl succinic anhydride (■); Butyl isocyanate (●)

block moisture content. This could account for the differences observed in figures 9.8 and 9.10.

Additionally, in the *P. sanguineus* test the alkenyl succinic anhydride modified wood appears to have behaved differently from the other modified woods. This is discussed in the next section.

From this discussion, the restriction of swelling has emerged as a possible cause of decay resistance in modified wood. A full theory of decay resistance is discussed in the following section.

9.3.4 The mechanism of decay resistance to brown and white rot fungi

Figures 9.7 to 9.12 suggest (in certain cases) that reduction in swelling has a significant role to play in decay resistance of chemically modified wood. Fungi require the wood cell wall to be water swollen prior to decay so that transient pores within the wall are open as pathways for degradative agents. However, to describe resistance due to reduced swelling as resistance due to the suppression of moisture content, a popularly cited mechanism in the literature, would be a mistake. In table 4.3 the aggregate volumetric swell of wood (due to modification plus water swell) was listed. In almost all cases the aggregate swell of modified wood was greater than that for unmodified water swollen wood. This indicates that the wood polymers in these samples have moved apart to a greater extent than they would have done by water swell alone. The difference is that in the case of modified wood, volume in the pores that would have been solely occupied by water is now occupied by modifying chemical. The modifying chemical has displaced the water, and is causing a blockage in the transient pores. It is this blockage that appears to be increasing decay resistance. If this is the case, then it is not surprising that good correlations were found between weight loss and water swell in some of the cases in

figures 9.7 to 9.12. This is because the amount by which modified wood swells in water will govern the amount by which pores can widen, allowing degradative agents to bypass any blockage modification causes in the cell wall pores. Only in the case of high WPG's of acetic anhydride was there any evidence that reduced water swelling was partly caused by the removal of hydrophilic groups in wood (see section 4.5.2). However, even in this case by far the most important cause of reduction in water swell was through bulking of the cell wall with modifying chemical.

The above theory is supported by the fact that cell wall moisture content (FSP) does not provide a good explanation of decay resistance (figures 9.1 to 9.6). The difference between cell wall moisture content and the ability of the modified wood to swell was discussed at length in section 4.5. In this section the concept of free volume associated with modification (Vf) was introduced. The lack of correlation of decay loss with FSP, and the correlation of weight loss with volumetric swell, suggests that Vf is unimportant with regard to pathways within the wood cell wall and the access of degradative agents. If Vf is directly associated with the modifying group attached to wood, as appears to have been the original suggestion (Nakano, 1988; cited by Hill and Jones, 1998), then it is quite conceivable that this would be of little consequence to the opening up of transient cell wall pores.

The above theory also accounts for the much greater quantity of alkenyl succinic anhydride required for decay resistance. It was discussed in section 4.5.2 that the free carboxyl group could cause excess swelling of wood modified with cyclic anhydrides. If such excess swell occurs in alkenyl succinic anhydride modified wood, then transient pores may be opened wide enough to allow the ingress of fungal degradative agents despite the increased bulk within the wood cell wall.

As mentioned in section 9.3.3, correlation in figures 9.7 to 9.12 is not ideal in all cases. The effect of free water on decay in figures 9.8 and 9.10 was discussed in section 9.3.3. In the case of the *P. sanguineus* test, alkenyl succinic anhydride appears to have been less successful at protecting wood at a given level of volumetric swell. This may be because white rot is more sensitive to the substitution of hydroxyl sites than brown rot (see section 9.3.1), the substitution level in alkenyl succinic anhydride modified wood being significantly lower than with other chemicals. However, no similar effect was observed for *T. versicolor*.

In figure 9.7, the weight loss of acetic anhydride modified wood to *C. puteana* is still at 10% when the volumetric swell is reduced below 5%. The most likely explanation is that this is due to the distribution effects and is discussed in section 9.2. Alternatively the effect in figure 9.7 could be due to chain length of the modifying chemical. The acetyl group introduced onto wood polymers during acetylation is a small chemical group fixed closely to the polymer. The only movement possible is rotation about the ester linkage (see figure 3.2a). When acetylated wood swells and wood polymers move apart, the acetyl groups also move apart. Butyl isocyanate is a significantly larger chemical with a 4 carbon length chain. As wood swells, the flexibility of this chain may allow it to move into the newly opened transient pores and continue to hinder any the movement of degradative agents. In this case, despite equivalent swelling, butyl isocyanate modification still has the greater ability to block transient pores than acetic anhydride modification. This does not appear to hold for the much larger alkenyl succinic anhydride, which requires significantly higher weight gains to protect wood. This could be due to the increased swell caused by the free carboxyl groups, though may also be affected by the low substitution level (i.e. fewer introduced chemical groups within the

wall). If this is the case there may be an optimum chain length for a modifying chemical (a possibility originally discussed in section 6.3.3).

The shortcomings in the pore size exclusion analysis (chapter 5) mean that the above theory cannot be fully confirmed.

9.3.5 The mechanism of decay resistance to soft rot fungi

With no pure culture test, and the control of conditions involved with it, the analysis of the mechanism of protection of chemically modified wood against soft rot fungi is not easy. Added to this is the fact that even less is understood about the decay mechanisms of soft rot fungi than for brown and white rot fungi, though it is thought that degradation through the action of enzymes at the cavity surface is largely responsible for decay (see section 2.3.3). If this is the case, and the penetration of degradative agents into the wood cell wall away from the hyphae is not required, then it is unlikely that the same mechanism of protection proposed for decay by basidiomycete fungi is relevant to decay by soft rot fungi. Neither does it seem logical that the lowering of the moisture content is responsible for protection, as much of the discussion in section 9.3.2 (with the exception of swelling) also holds for soft rot fungi. This and the proposed involvement of enzymes in depolymerisation reactions in the cell wall lead to the conclusion that the substitution of wood polymers in the cell wall is most likely be the major factor in protecting wood from decay by soft rot fungi. However, experimental data do not confirm this. Butyl isocyanate proved the most effective chemical at protecting wood against soft rot (section 7.3.1), though was less effective than acetic anhydride at reducing the number of monomolecular sorption sites (figure 4.18) and had a lower level of substitution at any given WPG. It would be expected that both of these latter measures would give an indication of the susceptibility of modified wood to decay. The

explanation of this anomaly could lie in the distribution of modifying chemical across the wood cell wall. In the case of alkenyl succinic anhydride, the lack of any further reduction in the level of decay once the WPG exceeded approximately 8% was attributed to the poor penetration of this chemical into the cell wall. Discussed in section 9.2 was the possibility that the high reactivity of acetic anhydride could have caused lower reaction deeper within the wall, which may in turn account for the lower resistance of wood modified with this chemical to decay by soft rot fungi.

The above discussion is largely based on the assumption that depolymerisation of wood polymers by soft rot fungi is due to the action of enzymes. It is probably fair to say that the mechanism of the decay resistance afforded to wood by chemical modification will not be fully understood until more is known of the decay mechanism itself.

Chapter 10. Conclusions and further work

10.1 Conclusions

- Butyl isocyanate was the best of the modifying chemicals at controlling decay in wood. Its superiority over acetic anhydride may in part have been an effect of poor distribution of the latter chemical in wood. The depletion of acetic anhydride in the reaction solution, particularly in the latewood within the sample, caused pockets of decay susceptible modified wood. To overcome this, the concentration of solutions would have to be increased to a level where reaction is no longer limited by chemical concentration.
- In comparison with butyl isocyanate and acetic anhydride, higher WPG's of alkenyl succinic anhydride were required to protect wood. This is thought to be because of excess volumetric swell (due to water soak) caused by the free carboxyl group, creating wider pathways in the wood cell wall through which degradative agents could pass. It was suggested that the low substitution level of wood modified with this chemical may also slightly reduce its decay resistance (at a given WPG) in comparison with butyl isocyanate and acetic anhydride modified wood, specifically to white rot decay.
- Even at WPG's in excess of 40%, alkenyl succinic anhydride was unable to completely protect wood against decay by soft rot fungi. This is thought to have been due to the size of the alkenyl succinic anhydride molecule preventing it from penetrating the cell wall sufficiently to protect it from soft rot cavity attack.
- Phthalic anhydride was unable to fully protect wood from decay. This chemical proved highly labile, and relatively high weight losses were recorded in leaching and

in sterile soil jar tests. Observations (and good results) in the pure culture test suggested that this chemical had some biocidal activity, though results were poor in unsterile environments. The poor performance in unsterile tests was probably due to leaching of chemical from the wood and/or by the action of a black stain fungus, which could have been acting to metabolise phthalate.

- Succinic anhydride was also unable to protect wood from decay, and also proved labile. Very heavy mould growth was observed on samples in unsterile environments, leading to the conclusion that this chemical could provide an energy and/or carbon source for certain fungi. The participation of succinate in the tricarboxylic acid (Krebs) cycle indicates that this is a distinct possibility.
- Upon analysis of the mechanism of decay resistance, neither the overall wood moisture content nor the cell wall moisture content (measured from the adsorption trial) appeared to provide a good explanation of the extent of decay. However, the relationships between volumetric swell (after water soak) and weight loss (in pure culture decay tests) in some cases showed very good agreement between wood modified with acetic anhydride, butyl isocyanate and alkenyl succinic anhydride; three very different modifying chemicals. It was concluded from this that swelling has an important part to play in the protection of wood from decay.
- A mechanism involving pore blocking was proposed for the resistance of modified wood to decay by basidiomycete fungi. Chemical modification bulks the wood cell wall and takes up space within the wall that would otherwise be filled with water. This restricts the access of degradative agents released by decay fungi, and could even account for the resistance to the oxidative system thought to be employed by brown rot fungi. If chelated iron is too large to move through the occluded cell wall pores in modified wood, then it may never reach areas within the wall where Fenton's reagent

formation of the hydroxyl radical can take place, then the degradative chemical species thought to be responsible for depolymerisation will not be produced. The amount by which wood swells is important in this theory since this will determine by how much transient pores in modified wood can open, and whether enough space is created to bypass this blocking effect.

- The possibility of the role of site substitution in decay resistance was not discounted. It was thought that this might contribute to the decay resistance of modified wood, particularly against white rot fungi.
- The mechanism of resistance to decay by soft rot fungi is thought not to be the same as that for basidiomycete fungi. In this case it was expected that site substitution and shielding will be more important, though test results did not confirm this. It was suggested that decay by soft rot fungi is sensitive to the distribution of modifying chemical across the wood cell wall, and that any differences in this distribution between different modified woods could account for variation in the results.

10.2 Further work

- In this study a largely unsuccessful attempt was made to measure the cell wall pore size in modified wood. It would be interesting to repeat this with a wider range of WPG's and probes. By modifying wood with a series of linear anhydrides or isocyanates, any effect of chain length could be assessed. It may be possible to use HPLC (high pressure liquid chromatography) to give a precise measure of the probe concentration, and so overcome the problem of extractives affecting measurement.
- A similar pore size study to the above could be undertaken using chelated iron species similar or identical to those thought to be involved in the decay process (see section 2.3). If modified wood was soaked in a solution of chelated iron (a pH close to that

expected during decay may be required), then any impregnation of iron into the wood cell wall could be assessed. For example, it may be possible to prepare these samples for EDXA (energy dispersive x-ray analysis) or autoradiography.

- Further analysis of the FSP of wood modified to different WPG's of different modifying chemicals could be undertaken. Methods such as the heat of wetting or moisture content associated with swelling could be used (Stamm, 1964; Skaar, 1988).

- A similar study to that reported by Takahashi *et al.* (1989) could be undertaken, in which modified wood is exposed to Fenton's reagent. It would again be interesting to use different WPG's of modifying chemicals with different chain lengths. This should provide information on the ability of different substitution levels of different size chemicals to protect wood from oxidation.

- Codd (1997) found that a combination of low WPG's of alkenyl succinic anhydride and low retentions of copper protected wood from decay by brown rot fungi. In view of the inability of alkenyl succinic anhydride to protect wood against soft rot in the present study, the alkenyl succinic anhydride – copper combination would need to be exposed in a soft rot test.

- In section 6.3.4, the possible effect on decay of a variation in water repellency between the different modifications was discussed. It may be possible to measure such an effect if wood modified with different chemicals was subsequently treated with a water repellent (a non-modifying chemical) that would stay at the lumen surface. The additional effect of this water repellent could be assessed by testing these samples against decay fungi in differing moisture content regimes, as described in chapter 6.

Appendix 1. Isotherm fitting

Table A1.1 Constants calculated for the Hailwood - Horrobin adsorption isotherms

Modifying chemical	WPG	Kh	Kd	Mo
Untreated control	0	5.792	0.750	5.994
Pyridine control	-2.6	5.397	0.759	5.809
Acetic anhydride	5.4	4.984	0.744	5.150
	11.7	4.748	0.763	4.411
	16.2	4.772	0.765	3.451
	20.4	3.768	0.754	3.355
Succinic anhydride	6.5	4.389	0.733	5.986
	16.8	4.205	0.748	5.609
	29.4	4.415	0.755	5.751
	38.6	3.789	0.744	6.085
Phthalic anhydride	6.5	4.257	0.758	5.643
	17.5	3.985	0.785	4.948
	32.9	4.424	0.792	4.839
	45.1	4.395	0.821	4.696
Alkenyl succinic anhydride	4.5	3.917	0.740	5.514
	12.0	3.789	0.754	4.878
	22.4	3.612	0.742	4.506
	31.8	3.668	0.732	4.456
Butyl isocyanate	2.1	4.592	0.769	5.881
	8.2	3.951	0.762	5.090
	15.6	3.807	0.751	4.679
	22.9	3.158	0.721	4.637

Appendix 2. Pure culture decay tables

Table A2.1 Mean weight losses (%) of chemically modified wood exposed to the brown rot fungi *Coniophora puteana* and *Gloeophyllum trabeum* in pure culture decay tests (standard deviation in brackets).

Chemical	Weight percent gain	Low moisture test			High moisture test		
		Sterile control	*C. puteana*	*G. trabeum*	Sterile control	*C. puteana*	*G. trabeum*
Untreated wood	0	1.1 (.5)	59.3 (4.8)	40.2 (10.6)	1.8 (.2)	49.1 (12.7)	58.7 (9.9)
Pyridine control	-2.2	1.0 (.1)	64.9 (3.0)	50.3 (13.5)	1.9 (.2)	61.2 (8.0)	57.6 (6.3)
Acetic anhydride	5.9	0.7 (.1)	62.0 (4.3)	22.2 (8.4)	1.2 (.1)	59.7 (7.3)	53.0 (3.9)
	12.2	0.6 (.1)	42.3 (4.8)	10.1 (7.4)	1.1 (.2)	34.7 (7.0)	28.7 (10.0)
	17.0	0.5 (.1)	22.7 (2.8)	7.1 (5.0)	0.4 (.1)	20.4 (6.3)	12.1 (6.3)
	20.3	0.3 (.3)	10.4 (2.2)	3.0 (2.2)	0.3 (.1)	1.8 (2.4)	5.9 (5.5)
Succinic anhydride	5.3	1.5 (.2)	60.2 (4.5)	28.3 (10.0)	3.7 (.3)	59.4 (10.7)	58.7 (7.3)
	16.2	2.4 (.4)	59.4 (2.0)	32.0 (10.4)	5.3 (.2)	59.7 (3.9)	59.7 (7.3)
	30.2	3.6 (.5)	36.7 (9.2)	11.8 (6.3)	6.7 (.3)	14.0 (3.3)	55.0 (7.4)
	38.2	5.5 (.4)	31.4 (11.5)	7.9 (1.6)	7.9 (.2)	16.3 (2.9)	39.8 (4.6)
Phthalic anhydride	7.4	1.6 (.1)	34.9 (15.4)	4.5 (3.0)	5.4 (.7)	30.0 (11.3)	33.0 (6.1)
	16.5	2.9 (.3)	5.9 (0.5)	4.1 (.5)	8.3 (.8)	27.7 (10.6)	17.9 (6.1)
	31.5	3.1 (.3)	7.3 (1.2)	4.7 (.9)	12.0 (.8)	14.9 (1.9)	16.2 (1.5)
	39.1	3.2 (.3)	6.4 (0.9)	4.0 (1.2)	13.1 (1.0)	18.2 (1.3)	17.4 (1.7)
Alkenyl succinic anhydride	4.4	1.2 (.2)	56.0 (2.0)	20.5 (5.5)	1.2 (.2)	15.2 (11.4)	41.1 (7.6)
	10.2	0.6 (.1)	48.0 (4.8)	12.4 (5.2)	0.5 (.2)	2.3 (2.1)	34.7 (7.9)
	23.4	0.5 (.1)	16.2 (4.5)	3.1 (2.8)	0.6 (.1)	-0.2 (.2)	6.1 (5.5)
	31.8	0.6 (.1)	5.3 (3.3)	3.3 (4.3)	0.7 (.1)	-0.1 (.2)	3.0 (2.0)
Butyl isocyanate	2.8	1.5 (.3)	61.1 (3.3)	28.7 (4.4)	1.8 (0.3)	18.0 (9.8)	47.6 (15.5)
	9.0	0.7 (.1)	39.7 (6.3)	10.0 (4.2)	1.0 (.3)	1.9 (1.1)	5.7 (4.0)
	16.3	0.4 (.1)	16.7 (7.9)	2.1 (.9)	0.5 (.1)	0.3 (.1)	1.4 (1.3)
	21.8	0.3 (.0)	1.6 (1.2)	0.2 (.2)	0.6 (.1)	-0.1 (.2)	0.2 (0.3)

Table A2.2 Mean weight losses (%) of chemically modified wood exposed to the white rot fungi *Trametes versicolor* and *Pycnoporus sanguineus* in pure culture decay tests (standard deviation in brackets).

Chemical	Weight percent gain	Sterile control	*T. versicolor*	*P. sanguineus*
Untreated wood	0	1.8 (.2)	28.4 (5.8)	32.5 (3.3)
Pyridine control	-2.2	1.9 (.2)	46.7 (5.4)	41.6 (3.5)
Acetic anhydride	5.9	1.2 (.1)	27.0 (4.4)	21.3 (2.8)
	12.2	1.1 (.2)	7.3 (3.8)	3.4 (.9)
	17.0	0.4 (.1)	1.6 (.7)	0.7 (1.0)
	20.3	0.3 (.1)	0.0 (.3)	-0.1 (.4)
Succinic anhydride	5.3	3.7 (.3)	36.1 (10.3)	18.3 (4.5)
	16.2	5.3 (.2)	29.8 (5.2)	18.4 (6.1)
	30.2	6.7 (.3)	13.7 (2.9)	12.0 (2.4)
	38.2	7.9 (.2)	14.0 (3.6)	11.2 (1.4)
Phthalic anhydride	7.4	5.4 (.7)	5.4 (.3)	5.7 (.6)
	16.5	8.3 (.8)	8.4 (.6)	9.3 (.5)
	31.5	12.0 (.8)	13.5 (1.1)	14.6 (1.0)
	39.1	13.1 (1.0)	15.4 (1.3)	16.9 (.9)
Alkenyl succinic anhydride	4.4	1.2 (.2)	27.3 (6.0)	28.1 (2.4)
	10.2	0.5 (.2)	13.0 (1.8)	19.2 (1.7)
	23.4	0.6 (.1)	3.5 (1.9)	7.7 (1.7)
	31.8	0.7 (.1)	15.4 (1.3)	4.1 (1.5)
Butyl isocyanate	2.8	1.8 (.3)	35.1 (3.8)	25.6 (2.0)
	9.0	1.0 (.3)	8.8 (3.6)	9.2 (1.2)
	16.3	0.5 (.1)	0.6 (.3)	2.2 (.8)
	21.8	0.6 (.1)	0.3 (.1)	0.2 (.3)

Table A2.3 Mean end of test moisture content (%) of chemically modified wood exposed to the brown rot fungi *Coniophora puteana* and *Gloeophyllum trabeum* in pure culture decay tests (standard deviation in brackets).

Chemical	Weight percent gain	Low moisture test		High moisture test	
		C. puteana	*G. trabeum*	*C. puteana*	*G. trabeum*
Untreated wood	0	152.2 (30.1)	69.5 (17.1)	203.4 (36.2)	342.6 (142.0)
Pyridine control	-2.2	169.7 (55.2)	89.2 (28.0)	267.4 (54.6)	283.8 (57.6)
Acetic anhydride	5.9	71.4 (7.1)	44.2 (8.5)	181.1 (28.6)	242.7 (20.4)
	12.2	53.3 (17.7)	25.1 (7.4)	147.8 (21.1)	136.0 (17.1)
	17.0	28.8 (3.2)	20.9 (5.7)	126.7 (24.1)	103.6 (9.8)
	20.3	24.7 (4.3)	15.9 (2.3)	82.8 (6.1)	101.4 (8.8)
Succinic anhydride	5.3	104.0 (31.0)	61.4 (14.7)	241.8 (40.9)	267.7 (48.7)
	16.2	83.8 (13.1)	57.4 (10.4)	179.4 (26.8)	257.3 (39.6)
	30.2	62.7 (7.3)	39.1 (9.2)	126.9 (7.1)	238.7 (72.5)
	38.2	60.2 (10.8)	37.7 (6.5)	126.5 (10.8)	169.5 (9.7)
Phthalic anhydride	7.4	57.3 (13.9)	33.9 (6.5)	204.9 (36.1)	210.1 (23.5)
	16.5	36.0 (2.7)	29.5 (2.0)	159.6 (40.5)	145.5 (26.1)
	31.5	32.8 (3.4)	30.6 (4.0)	109.0 (15.0)	121.8 (10.7)
	39.1	32.1 (4.4)	25.8 (3.4)	128.5 (7.6)	119.2 (19.4)
Alkenyl succinic anhydride	4.4	106.4 (25.5)	43.0 (6.6)	134.8 (28.9)	195.7 (30.0)
	10.2	55.6 (6.7)	28.9 (1.8)	82.1 (17.8)	138.2 (14.5)
	23.4	38.7 (4.5)	17.9 (2.0)	71.9 (9.2)	86.1 (9.3)
	31.8	26.9 (4.5)	16.7 (3.4)	65.3 (13.0)	57.4 (12.9)
Butyl isocyanate	2.8	96.0 (34.1)	40.4 (2.1)	170.4 (25.1)	246.1 (43.5)
	9.0	53.3 (11.8)	24.7 (1.8)	125.0 (17.4)	114.9 (16.4)
	16.3	33.1 (6.5)	21.1 (2.3)	95.3 (8.6)	78.1 (17.9)
	21.8	19.7 (2.4)	15.2 (1.0)	47.7 (2.3)	62.8 (14.4)

Table A2.4 Mean end of test moisture content (%) of chemically modified wood exposed in sterile control test conditions, calculated on a modified wood and original unmodified wood basis (standard deviation in brackets).

Chemical	WPG	Low moisture test	High moisture test
Untreated wood	0	29.8 (0.7)	95.3 (10.3)
Pyridine control	-2.2	31.6 (1.2)	77.0 (7.2)
Acetic anhydride	5.9	25.8 (1.3)	46.2 (4.0)
	12.2	20.8 (0.7)	54.0 (6.3)
	17.0	17.0 (1.6)	48.2 (10.9)
	20.3	14.3 (1.3)	42.7 (1.6)
Succinic anhydride	5.3	34.8 (5.7)	63.2 (3.7)
	16.2	41.2 (7.8)	64.2 (4.0)
	30.2	48.4 (9.2)	63.7 (5.1)
	38.2	60.0 (19.9)	66.1 (4.3)
Phthalic anhydride	7.4	26.6 (1.0)	71.7 (4.6)
	16.5	25.8 (1.7)	65.6 (7.7)
	31.5	27.2 (3.5)	59.8 (6.8)
	39.1	27.3 (3.2)	70.5 (10.9)
Alkenyl succinic anhydride	4.4	25.3 (0.9)	49.9 (6.1)
	10.2	21.3 (1.2)	35.0 (1.3)
	23.4	16.4 (0.5)	33.8 (3.4)
	31.8	14.4 (1.1)	29.3 (3.2)
Butyl isocyanate	2.8	27.3 (0.9)	67.0 (3.5)
	9.0	21.4 (1.0)	53.7 (5.2)
	16.3	16.9 (1.0)	43.8 (5.6)
	21.8	14.5 (1.1)	45.5 (7.7)

Table A2.5 Mean end of test moisture content (%) of chemically modified wood exposed to the white rot fungi *Trametes versicolor* and *Pycnoporus sanguineus* in pure culture decay tests (standard deviation in brackets).

Chemical	**Weight percent gain**	***T. versicolor***	***P. sanguineus***
Untreated wood	0	187.8 (33.6)	216.7 (42.0)
Pyridine control	-2.2	233.7 (37.0)	260.7 (44.4)
Acetic anhydride	5.9	182.4 (22.7)	165.6 (9.1)
	12.2	122.7 (15.3)	127.2 (9.9)
	17.0	110.9 (8.0)	114.5 (11.5)
	20.3	107.4 (10.8)	107.5 (10.0)
Succinic anhydride	5.3	241.0 (45.0)	179.1 (11.2)
	16.2	192.9 (22.9)	160.1 (15.9)
	30.2	127.4 (8.6)	128.6 (8.8)
	38.2	116.0 (16.7)	127.2 (7.0)
Phthalic anhydride	7.4	109.9 (32.1)	139.0 (17.4)
	16.5	98.8 (20.8)	115.1 (16.9)
	31.5	112.9 (11.3)	105.6 (9.1)
	39.1	117.0 (9.4)	125.4 (7.3)
Alkenyl succinic anhydride	4.4	132.1 (22.2)	171.2 (14.0)
	10.2	82.8 (10.8)	119.8 (9.7)
	23.4	41.9 (7.9)	76.3 (9.6)
	31.8	37.5 (7.3)	65.3 (12.7)
Butyl isocyanate	2.8	208.0 (26.3)	198.8 (14.9)
	9.0	113.9 (16.8)	112.9 (13.2)
	16.3	79.0 (13.2)	84.7 (11.2)
	21.8	67.7 (7.9)	84.8 (15.9)

Table A2.6 Mean weight losses (%) of CCA treated wood exposed to the brown rot fungus *Coniophora puteana* in pure culture decay tests (standard deviation in brackets).

CCA retention (kg/m^3)	Low moisture test			High moisture test		
	Sterile control	*C. puteana*	*G. trabeum*	Sterile control	*C. puteana*	*G. trabeum*
0.0	0.7 (.2)	63.4 (2.5)	37.6 (7.5)	1.6 (.2)	55.7 (4.2)	60.7 (8.7)
0.5	0.9 (.3)	40.5 (14.4)	45.1 (17.9)	1.9 (.2)	22.5 (9.3)	64.6 (6.0)
1.0	0.9 (.4)	12.6 (12.4)	43.3 (18.4)	2.0 (.2)	8.5 (5.7)	72.2 (3.2)
2.0	0.6 (.1)	1.0 (.3)	5.9 (5.4)	1.7 (.3)	1.6 (.3)	59.4 (8.9)
4.0	0.1 (.1)	0.3 (.3)	2.0 (3.0)	1.2 (.2)	1.3 (.6)	30.8 (10.9)

Table A2.7 Mean weight losses (%) of CCA treated wood exposed to the white rot fungi *Trametes versicolor* and *Pycnoporus sanguineus* in pure culture decay tests (standard deviation in brackets).

CCA retention (kg/m^3)	Sterile control	*Trametes versicolor*	*Pycnoporus sanguineus*
0.0	1.6 (.2)	34.7 (5.1)	33.5 (1.8)
0.5	1.9 (.2)	8.6 (5.2)	2.2 (.6)
1.0	2.0 (.2)	1.1 (.3)	2.1 (.4)
2.0	1.7 (.3)	1.1 (.5)	1.6 (.7)
4.0	1.2 (.2)	1.1 (.4)	1.4 (.4)

Table A2.8 Mean end of test moisture content (%) of CCA treated wood exposed to the brown rot fungi *Coniophora puteana* and *Gloeophyllum trabeum* in pure culture decay test (standard deviation in brackets).

CCA retention (kg/m^3)	Low moisture test		High moisture test	
	C. puteana	*G. trabeum*	*C. puteana*	*G. trabeum*
0.0	168.9 (21.7)	59.7 (9.2)	259.2 (65.6)	268.2 (65.2)
0.5	81.0 (30.7)	56.2 (12.3)	182.4 (24.6)	180.8 (28.3)
1.0	53.2 (20.0)	67.5 (33.8)	165.6 (16.1)	331.3 (34.3)
2.0	37.7 (1.1)	36.1 (3.1)	119.1 (17.9)	274.3 (103.7)
4.0	38.5 (4.0)	34.4 (3.0)	96.6 (26.6)	193.3 (22.3)

Table A2.9 Mean end of test moisture content (%) of CCA treated wood exposed in sterile control test conditions, calculated on a modified wood and original unmodified wood basis (standard deviation in brackets).

CCA retention (kg/m^3)	Low moisture test	High moisture test
0.0	29.6 (.5)	63.9 (7.8)
0.5	30.3 (1.3)	74.3 (5.4)
1.0	30.4 (1.4)	75.6 (8.3)
2.0	29.9 (0.8)	74.1 (9.5)
4.0	29.8 (1.2)	76.6 (5.4)

Table A2.10 Mean end of test moisture content (%) of CCA treated wood exposed to the white rot fungi *Trametes versicolor* and *Pycnoporus sanguineus* in pure culture decay tests (standard deviation in brackets).

CCA retention (kg/m^3)	***T. versicolor***	***P. sanguineus***
0.0	209.1 (22.6)	218.9 (19.9)
0.5	138.0 (14.4)	142.8 (18.7)
1.0	92.4 (13.6)	113.1 (21.1)
2.0	97.9 (10.4)	115.9 (26.0)
4.0	83.0 (11.3)	100.3 (24.1)

Appendix 3. Metabolism of cellulose

If wood breakdown results in the complete metabolism of cellulose to carbon dioxide and water, then this can be represented as:

$$C_6H_{10}O_5 + 6O_2 \rightarrow 6CO_2 + 5H_2O$$

where the anhydroglucose monomer ($C_6H_{10}O_5$) is used as a model for the relative quantities of carbon, hydrogen and oxygen in cellulose.

Taking molecular weights of carbon, hydrogen and oxygen to be 12, 1 and 16 respectively, then the quantity of water formed as a proportion of the weight of the original cellulose is:

$$\frac{5 \times 18}{342} = 0.56\,(2dp) = 56\%$$

Appendix 4. Soft rot decay tables

Table A4.1 Mean weight loss (%) and end of test moisture content (%) of chemically modified wood exposed in unsterile soil (standard deviation in brackets).

Modifying Chemical	Weight percent gain	13 months exposure		20 months exposure	
		Weight loss	Moisture content	Weight loss	Moisture content
Unreacted wood	0	24.4 (6.3)	187.2 (35.5)	32.8 (6.1)	254.9 (33.2)
Pyridine control	-1.9	27.6 (3.9)	173.9 (41.3)	33.6 (4.3)	247.7 (62.2)
Acetic	5.2	16.6 (2.5)	156.7 (27.5)	21.3 (3.2)	190.1 (42.0)
anhydride	8.6	12.0 (3.0)	124.6 (17.7)	16.2 (2.7)	172.3 (17.4)
	12.1	6.8 (1.9)	98.5 (15.5)	8.9 (2.4)	130.5 (22.4)
	14.0	6.9 (1.3)	90.1 (11.3)	8.4 (1.8)	131.4 (15.0)
Succinic	6.7	24.7 (4.8)	165.9 (46.1)	32.7 (4.0)	249.6 (40.4)
anhydride	12.3	17.2 (3.6)	174.7 (22.3)	24.0 (5.8)	212.0 (35.2)
	22.4	13.2 (1.0)	151.0 (13.9)	20.4 (2.6)	172.8 (23.6)
	29.6	13.1 (1.6)	134.5 (20.3)	24.3 (6.5)	188.7 (42.5)
Phthalic	9.8	25.5 (4.9)	169.6 (37.2)	33.7 (5.1)	251.0 (31.2)
anhydride	15.9	21.8 (3.6)	167.8 (32.4)	29.1 (3.5)	212.7 (30.0)
	28.0	22.0 (2.3)	156.2 (15.7)	27.4 (3.8)	176.9 (16.7)
	35.3	22.5 (1.4)	152.5 (7.0)	30.2 (2.3)	185.5 (18.9)
Alkenyl	2.8	16.9 (2.8)	116.3 (26.1)	25.4 (3.8)	187.9 (35.7)
succinic	7.7	11.4 (3.2)	81.9 (22.9)	12.4 (4.1)	130.0 (24.5)
anhydride	12.1	10.3 (3.6)	81.6 (19.5)	13.1 (3.0)	134.2 (19.5)
	22.5	12.0 (3.5)	83.7 (21.7)	15.7 (3.3)	134.3 (10.9)
Butyl	0.4	25.1 (2.5)	160.2 (33.1)	34.4 (2.5)	269.0 (35.2)
isocyanate	2.5	18.2 (3.6)	112.6 (24.1)	19.9 (3.7)	183.7 (29.6)
	7.3	8.0 (3.0)	78.9 (25.7)	10.7 (3.4)	149.2 (25.0)
	12.4	1.6 (0.5)	42.4 (6.2)	1.5 (0.7)	72.2 (12.6)

Table A4.2 Mean weight loss (%) and end of test moisture content (%) of CCA treated wood exposed in unsterile soil (standard deviation in brackets).

CCA	13 months exposure		20 months exposure	
retention (kg/m^3)	Weight loss	Moisture content	Weight loss	Moisture content
0	26.4 (4.6)	189.5 (30.0)	30.6 (6.5)	230.4 (34.8)
0.9	25.5 (5.4)	176.2 (36.5)	28.6 (6.8)	222.2 (46.0)
1.8	20.1 (5.6)	144.7 (25.8)	28.1 (5.2)	195.6 (32.0)
3.5	10.0 (5.1)	69.2 (20.1)	19.6 (5.1)	132.4 (31.7)
7.0	3.9 (1.3)	47.2 (2.5)	7.6 (3.0)	63.8 (12.1)

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