Studies on soluble nutrient components in wood and their influence on decay susceptibility and preservative efficacy.

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ABSTRACT

The work described in this thesis was undertaken to determine the nature and identity of soluble carbohydrate and nitrogenous components which migrate and accumulate at evaporative surfaces of dried wood and the influence these nutrients have on wood decay and preservative performance.

Specific soluble carbohydrates and amino acids were shown to redistribute and accumulate at surface regions of wood during drying. Analysis of dried wood showed that soluble carbohydrates constituted 2-5% of the dry mass of wood at surface regions, and that soluble nitrogenous components constituted < 0.5% in the same areas. The soluble sugars which redistributed and accummulated at surface regions during drying were mainly reducing in nature in the softwoods. Glucose and fructose were the predominant sugars in these woods. In lime, sucrose was the predominant sugar.

Soluble amino acids contributed to a significant proportion of the nitrogen content at surface regions of softwoods. In pine and spruce soluble amino acids constituted 30% and 40% of the total nitrogen content, but in lime, concentrations of soluble amino acids constituted only 6% of the total nitrogen content. The major amino acids observed in pine, spruce and lime were aspartic acid, glutamine and arginine.

Soil burial studies undertaken highlighted the problems encountered when trying to mimic natural wood of high nutrient status. Test blocks impregnated with soluble sugars and amino acids displayed loss of these added nutrients on emplacement in soil, and the effect of added substrates could not be evaluated individually.

The results of soil burial studies using CCA treated wood which was also impregnated with amino acids, showed that the latter influenced wood decay and preservative stability in lime. Weight losses in preserved lime were shown to correlate with increasing arginine and glutamine concentrations. A substantial copper loss was recorded in hardwoods and softwoods treated at sub-toxic levels with CCA and also treated with glutamine. Soluble sugars incorporated into preserved wood did not influence wood decay or preservative efficacy.

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CONTENTS

ABBREVIATIONS

asp	aspartic acid
asn	asparagine
thr	threonine
ser	serine
glu	glutamic acid
gln	glutamine
pro	proline
gly	glycine
ala	alanine
val	valine
ile	isoleucine
leu	leucine
tyr	tyrosine
phe	phenylalanine
lys	lysine
his	histidine
arg	arginine
ATP	adenosine triphosphate
ADP	adenosine diphosphate
NADP	nicotinamide adenine dinucleotide phosphate

CHAPTER 1

INTRODUCTION

1 Introduction

The interaction of wood and the soil environment is an area of considerable interest due to the failure of wood as a consequence of fungal degradation. One of the most serious drawbacks to the use of wood as a construction material, is that under favourable conditions it easily decays and thus loses its strength and elasticity. This decay destroys annually, wood worth large sums of money and wood decay thus represents a very serious economic problem. Estimates for import of wood and wood products in the United Kingdom alone, are more than £2,800 million pounds annually (Levy and Dickinson, 1981) and today it is approaching £5,000 million pounds.

Timber is an attractive and extensively used building material. It has good strength characteristics, weight for weight being stronger than steel, and in contrast to the finite reserves of steel, wood stocks with careful management are renewable (King, 1981). In modern buildings, apart from strength and aesthetic factors, there is a need to consider economy in the use of materials, the suitability of newly developed products and the demands of occupants for thermal comfort and convenience. In these circumstances timber products may be more highly stressed and used more closely to their design performance limits. Besides stress factors, other factors such as temperature, water, weathering and interaction with associated materials e.g. fire retardants, metal plate fasteners, also affect the life expectancy of a timber product. Most of these factors are influenced to some extent by the moisture content of the timber. In practice, control of moisture content is probably the single most important factor in obtaining a long and satisfactory service life (Morgan, 1986).

Wood is made up mainly of three polymeric materials: cellulose, the hemicelluloses and lignin (Kirk, 1973). Other substances such as nitrogenous materials, pectin, starch, low molecular weight sugars, and minerals are also present. In addition, extraneous materials such as lignans, terpenes etc., are found in varying amounts. Pectin, starch and low molecular weight carbohydrates may be especially important as initial carbon sources for the establishment of micro-organisms in wood. It has been suggested that removal of these carbohydrates may be a way to prevent decay under some circumstances.

Nitrogenous materials, present usually in minimal amounts in wood, are essential to the growth and activities of wood destroying organisms and therefore exert considerable influence on the rate of decay (Cowling, 1970).

Decomposition of wood is practically exclusively a biological process caused by organisms that digest wood and use it as a nutrient. The principal agents of decay in wood are fungi and insects (Findlay, 1985). Two main groups of insects are destroyers of wood, namely, termites and wood-boring beetles. In temperate countries, wood-boring insects can cause serious damage to certain timbers in buildings. Attack by wood-boring insects arise mainly through the introduction of infested material into buildings rather than through time related exposure (Morgan, 1986). In the tropics and sub-tropics, termites are often the major cause of timber destruction (Findlay, 1985 op.cit). They can be divided into two main groups, namely, dry wood termites and subterranean termites. Dry wood termites live in dry seasoned wood and do not require any contact with soil, and subterranean termites live either wholly or partly in the ground, and always maintain a connection with the soil. Both groups of insects i.e. termites and wood-boring beetles, exhibit a form of symbiosis with bacteria or fungi, either as gut flora, food nurturing young larvae or nymphs, or partially to decay wood before infestation by the insects.

The micro-organisms that decay wood can be divided into four major groupings; bacteria, mould and stain fungi, soft rot fungi and basidiomycetes, (Levy, 1969; 1971; Liese, 1970). These micro-organisms occur in different ecological situations and can produce greater or lesser degrees of decomposition dependant on the suitability of the surrounding environment. Bacteria normally require wood to be water-laden before extensive bacterial proliferation can take place. The staining and mould fungi usually colonise only freshly felled wood and do not recolonise it after it has dried, even if rewetted (Findlay, 1966; King and Eggins, 1973). Soft rot fungi require high moisture levels, and considerably more protein than is found in wood to produce significant amounts of decay. The basidiomycetes may totally decay wood under relatively dry conditions, and require less protein than soft rot fungi to decay wood.

Bacteria have been shown to be the initial colonisers of both unpreserved and preserved wood in soil (Banerjee and Levy, 1971; Clubbe and Levy, 1982). Bacteria from several genera are known to attack wood as a nutrient source (Levy, 1967; Greaves, 1971), with Bacillus and Pseudomonas being especially important in these events. Greaves (1971) grouped bacteria which are isolated from wood into four categories determined by their functions once invasion of the wood had occurred. Bacteria of Group 1 were those which affected the permeability of the wood to liquid penetration but had no significant effect on strength properties; bacteria of Group 2 had the capability to attack the wood structure, thereby reducing the strength of the wood; bacteria of Group 3 were those which in association with other wood decay micro-organisms could cause total breakdown, and bacteria of Group 4 were the "passive" colonisers which had no effect upon the wood, but which had a marked influence on the other wood decay organisms by their antagonistic activities. Greaves (1971, op.cit) also noted that bacteria and actinomycetes were probably the micro-organisms most abundant in wood during decay, and Baecker and King (1981) showed that actinomycetes can occur in large numbers in decaying lime and pine.

A group of bacteria known as tunnelling bacteria have been shown to be able to degrade a wide variety of decay resistant timbers (Nilsson and Daniel, 1983). This group of single-cell bacteria are characterised by their ability to tunnel within the secondary cell walls of wood fibres. Attack is initiated by a single bacterium which attaches itself to the S_3 layer of a wood fibre. The bacterium sinks into the cell wall though lytic action and produces a minute cavity with pointed ends. Division of the bacterium into two bacteria enlarges the cavity and further division of these bacteria results in the formation of small cavities or tunnels which radiate out from the original cavity. Tunnelling bacteria have been reported to attack CCA-treated vineyard poles in New Zealand but not CCA-treated poles in Sweden (Nilsson and Daniel, 1983, op.cit). The inability of these bacteria to attack transmission poles led the authors to postulate that tunnelling bacteria require higher moisture levels than normally found in transmission poles, and that these bacteria are strongly stimulated when wood is exposed in fertilised soils. Significant decay by cavitation bacteria have also been observed in a large number of CCA-treated pine posts in horticultural soils in New Zealand (Nilsson and Singh, 1984).

This group of bacteria have been defined to be able to degrade wood through formation of discrete cavities within the wood cell walls. Unlike tunnelling bacteria where the enzymatic activity is confined to the immediate vicinity of bacterial cells, cavitation bacteria are able to produce diffusable wood-degrading enzymes. These bacteria like soft rot fungi and tunnelling bacteria confine their attack to the S_2 layer of wood cell walls.

Until the work of Savory (1954), cellulolytic and lignocellulolytic basidiomycete fungi were considered to be the sole causative agents of large scale fungal degradation of timber. Savory however demonstrated that Ascomycetes and Fungi Imperfecti were responsible for the degradation of timber in water cooling towers. Decay by these fungi was shown to be restricted to S_2 layer of the secondary cell walls of tracheids and fibres and regulated by cellulose activity. This form of decay is characteristically recognised by the surface softening of timber and Savory (1954, op.cit) proposed the term "soft-rot" to describe such decay. It has been shown (Krapivina, 1960; Merrill, 1966) that a number of staining and mould fungi can act as soft rot fungi. Work on the successions of fungi which colonise dried wood (Merrill and French, 1966; Butcher, 1968; Levy, 1969; Banerjee and Levy, 1971) has shown that many mould-staining and soft rot fungi are associated with the decay process. The work of Banerjee and Levy (1971) has shown that soft rot is largely confined to the peripheral regions of fence posts and that basidiomycetes are capable of developing beyond the 5mm depth and towards the centre of the post where competition is low. These authors (Banerjee and Levy, 1971 op. cit.) noted that colonisation by maximal numbers of fungal species occurred in the outer 5mm of wood, an area in wood in which migration of soluble nitrogenous materials was shown by King (1975).

Wood-destroying fungi obtain the carbon needed for their metabolic activities from the carbon-rich polymers, which make up the cell walls. It has been shown in pure culture studies (Henningsson, 1967), that fungi that decay wood develop more slowly on cellulose than on simple sugars. As long as soluble carbohydrates remain, there may be no stimulus for cellulase production (Bravery, 1968a). Generally, enzymes which hydrolyse cellulose are produced when growth is restricted (Hulme and Stranks, 1970). Enzymatic attack on hemicellulose proceeds in a manner similar to that of cellulose; resulting in the formation of dimers and monomers which are then able to enter fungal cells.

Working with isolated polysaccharides, Takahashi and Nishimoto (1973) found that the soft rot fungi, Chaetomium globosum was capable of degrading xylans and mannans, the former being degraded at a slightly greater rate than the latter. A similar observation was recorded with basidiomycetes, in which the fungi tested exhibited growth on the xylans, (Henningsson, 1967 op. cit.). This led the authors, Takahashi and Nishimoto, to postulate that the higher xylan content in hardwoods may contribute to its decay susceptibility.

Wood-destroying fungi satisfy their nitrogen requirements primarily from the wood itself. Nitrogen is an essential constituent for growth and development of wood-inhabiting fungi. In fungal hyphae and spores, nitrogen is a major constituent of the amino acids, peptides and proteins and the nucleotide bases. Enzymes are protein molecules, and extracellular enzymes regulate all wood polymer breakdown. Thus, for active decay of wood to take place, micro-organisms must acquire nitrogen for enzyme synthesis.

The nitrogen content of wood occurs in many forms. The cambium and rays, contain proteins, peptides, amino acids, nucleic acids, lipoprotein membranes and other nitrogenous constituents. Wood fibres and dead parenchyma cells also contain protoplasmic residues. Young cell walls contain proteins; and a protein rich in the amino acid hydroxyproline has been found in the primary walls of sycamore (Lamport and Northcote, 1960) and also in Scots pine protein (Laidlaw and Smith, 1965). The bulk of nitrogen in wood is in the form of cell wall protein and is thus not immediately available to colonising organisms. Because of the low levels of nitrogen in wood, its availability to wood decay fungi may become a major limiting factor to rates at which decay can proceed.

Wood has nitrogen contents of between 0.03% - 0.10% (w/w) with C:N ratios varying between (350-500):1, (Cowling and Merrill, 1966). Soluble nitrogen is considered only as a fraction of a minor wood component. Cowling and Merrill (1966, op. cit.), had shown that some of the nitrogen in wood was in a soluble form. The work of Cowling et. al. was confirmed by Baker, Laidlaw and Smith (1970), who showed that slight amounts of soluble amino acids were present in the sapwood of Scots pine. King (1975) showed that greatest amounts of soluble nitrogen were in the outer sapwood regions with lesser amounts in the heartwood regions.

King, Oxley and Long (1974) demonstrated that these soluble nitrogenous materials migrated and accumulated at evaporative faces of wood during drying.

The composition of soluble nitrogenous components in wood has been investigated in several studies. Free amino acids were shown to be present in sapwood in abundance and in heartwood in trace amounts (Fukuda, 1963; Merrill and Cowling, 1966). Most of the amino acids detected by Fukuda and Merrill and Cowling were common protein amino acids but included none of the aromatic acids. Investigations have also shown that most of the nitrogen in the xylem sap of many tree species occur as organic compounds, rather than as nitrate or ammonia (Bollard, 1957a, b, c; Barnes, 1963). The main organic nitrogen compounds in tree xylem saps are amino acids and ureides. Glutamine and arginine were demonstrated to be the most abundant amino acids in the investigations of Bollard (1957c) and Barnes (1963). These amino acids were also the predominant amino acids in the buds, shoots, apices and leaves of white spruce saplings (Durzan, 1968a). Merrill and Cowling (1966) demonstrated that various extracts from aspen wood (Populus grandidentata) contained aspartic acid, glutamic acid, serine, glycine, alanine, valine, leucine, isoleucine and threonine. These amino acids found by Merrill and Cowling were utilised by all of the birch and aspen - attacking fungi tested by Henningsson (1967). In his investigation, Henningsson showed that the monoamino monocarboxy acids (glycine , alanine, leucine and valine), the acidic amino acids (aspartic and glutamic acids) and the hydroxyamino acids (serine, threonine) were easily assimilated by the basidiomycete fungi tested. Nitrate proved to be a poor source of nitrogen for most of the fungi attacking birch and aspen; but amino and amide nitrogen were good nitrogen sources for all the fungi tested.

Findlay (1934) demonstrated that increasing wood nitrogen content by additions of inorganic salts increased decay rates of Sitka spruce by Trametes serialis. Lundstrom (1972) recorded maximum decay by soft rot fungi in hardwoods when test veneers were incubated in vermiculite moistened with nutrient solution containing ammonium nitrate. Butcher and Drysdale (1974) noted that nitrogen source as well as concentration were important factors influencing the decay of wood by soft rot fungi. These authors demonstrated that decay rates were accelerated in pine sapwood veneers which had been impregnated with lowest nitrogen concentrations.

The C:N of the veneers used by Butcher and Drysdale were approximately 120:1 and these authors considered such ratios to be of more importance in determining decay than absolute amounts of nitrogen. Later, Butcher (1976) confirmed that the higher values of nitrogen, impregnated as soluble salts, caused decrease in the decay process, and estimated a C:N ratio of 250:1 to be optimal for soft rot decay. Soil studies undertaken by King et al (1981a) also indicated that nitrogen contents were important as determinants of decay. These authors showed that weight loss in blocks became significant only when nitrogen contents had increased to an estimated C:N ratio of 200:1 i.e. approximately 0.2% w/w of the wood.

In spite of the low nitrogen contents and high C:N ratios in wood, basidiomycetes are able to decay wood. It is postulated that such fungi utilise their own autolytic products to conserve nitrogen (Levi et al, 1968) and can adapt their physiologies by preferential allocation of limited amounts of nitrogen to production of enzymes for substrate utilisation (Levi and Cowling, 1969). In some conditions of exposure, the nitrogen requirements of wood-destroying fungi can be satisfied by sources outside the wood itself. Under conditions of soil contact, for example, the fungi could assimilate nitrogen directly from the soil. Levy (1968) considered that nutrient salts moved with water into wooden fence posts in ground contact and presumably there, act as nitrogen sources. Three mechanisms have been described which facilitate nitrogen increases in wood in soil. These are wick movement, (Baines and Levy, 1979; Uju et al ,1981); nitrogen fixation, (Sharp and Millbank, 1973; Levy et al, 1974; Baines and Millbank, 1976); and microbial transfer (King et al , 1981a).

Baines and Levy (1979) demonstrated the wick effect of water by immersing one end of a small wooden stake in water and leaving the other end above the surface of the water. Water movement through the wood to the exposed end of the stake would take place provided a difference in water availability existed between the two ends. This 'wick action' depended on the evaporation of water from the wood above the water surface, and these authors considered that for wood in soil, materials soluble in soil solution e.g. minerals, salts, soluble nutrients etc., would be deposited in the wood at or above the groundline. Uju et al, (1981), using sterile Scots pine stakes half inserted into sterile soil; the soil previously having been augmented with inorganic nitrogen salts, showed that

nitrogen accumulation occurred not only in that portion of the stake below the groundline but also in wood above the soil surface. Major deposition of soluble salts took place at the groundline portion of the stake, the region at which most extensive decay is usually observed. Levy and Dickinson (1981) suggested that at the groundline, conditions of moisture and oxygen availability favour fungal activity, and it is here that organisms received a continuous supply of nutrients by wick action.

A second process which can contribute to nitrogen increases in wood is bacterial nitrogen fixation. Sharp and Millbank (1973) and Levy et al, (1974) have demonstrated that nitrogen fixation can occur in wood. Baines and Millbank (1976) demonstrated that these nitrogen fixing bacteria penetrate Scots pine sapwood along the rays. The presence of these nitrogen fixing bacteria may therefore augment the low nitrogen content of wood thus making it more susceptible to decay.

King, Oxley and Long (1976) postulated that the nitrogen movement to unpreserved wood observed by them was primarily in a biological form. These authors found that nitrogen increases had not taken place in those parts of wood blocks buried in soil, which had been wetted by soil moisture but were as yet uncolonised, though colonised parts of wood had increased nitrogen values. Waite and King (1979) showed that nitrate analysis of buried blocks and also blocks exposed to pure culture, showed no significant movement or accumulation of nitrogen in wood in this form. In pure culture studies undertaken with both fungi and bacteria (King and Waite, 1979; King, Henderson and Murphy, 1980), nitrogen transfer to wood was demonstrated to be part of a biological invasion process. The importance of nitrogen therefore, to decay of wood in soil and pure culture systems, suggests that it may be of exceptional importance to decomposition processes.

Soft rot fungi are the major agents for the failure of copper chrome arsenic (CCA) treated wood in soil. Henningsson and Nilsson (1976) reported enhanced nitrogen contents of preserved transmission poles in Sweden, and Friis-Hansen (1976) showed increased decay in CCA treated transmission poles situated in cultivated fertilised fields. Friis-Hansen (1976, op. cit.) also noted that where transmission poles were not in contact with soil, because of the presence of a rock backfill, decay did not occur.

Soft rot fungi are able to tolerate higher amounts of toxic compounds if they grow on a substrate rich in nutrients. It has been shown in laboratory experiments that an increase in nitrogen levels also increases the tolerance of soft rot fungi to toxicants (Henningsson, 1976; Hulme and Butcher, 1977c). The importance of soluble nutrients on the effectiveness of CCA preservative was studied by King et al, (1981b) using wood of high and low nutrient status. It was postulated that the soluble nutrients accelerate decay of preserved wood. The rate of increase of nitrogen content, during decay was shown to be stimulated by the presence of soluble nutrients concentrated at wood surfaces. Nitrogen accumulation in these superficial zones of wood either by redistribution of soluble nutrients or by autolysis of 'sacrificial colonists', have been shown to be associated with the reduction of toxic limits of preservatives (King, Smith and Bruce, 1980). It is known that microbial metabolites solubilise preservatives in wood (Levi, 1969; Bravery, 1976). Stimulation of decay by soluble nutrients may cause a further increase in solubilisation, as a result of the greater fungal activity in the wood.

The transfer of microbial biomass initiated by the chemostimulatory nature of wood, was demonstrated in pure culture studies by Mowe, King and Senn, (1983). In these experiments, it was observed that some wood decomposing fungi orientate their growth towards wood blocks used as bait; apparently attracted by wood volatiles. This chemotropic response of fungi to wood was not affected by the presence of CCA (Mowe, 1983). Bacteria have also been shown to respond chemotactically to aqueous extracts of wood and to 'swim' in the direction of concentration gradients of these materials (Mowe, 1983 op. cit.). The presence of high concentrations of soluble nutrients in wood has been shown to influence nitrogen transfer and preservative losses in wood (Briscoe, 1987). It may therefore be hypothesised that increases in microbial biomass at soil regions adjacent to wood surfaces indirectly increases leaching of preservatives through the action of fungal metabolites, and autolytic products of bacteria e.g. amino acids may chelate heavy metal ions thus rendering them inactive as toxic materials, and leaving the wood unprotected.

Mowe and King (1981) demonstrated that the response of soft rot fungi to the presence of preservative treated wood, differed between fungal species, wood preservatives, and wood species. Carey, Bravery and Savory (1981) considered that early organisms in the ecological sequence may affect the susceptibility of timber to decay and also preservative toxicity. Detailed analysis of microbial succession in both an untreated and CCA treated hardwood (birch), and a softwood (Scots pine), have been undertaken by Clubbe and Levy (1982). The successional events for untreated hardwood and softwood were essentially the same; however, the time course for the decay of the softwood was longer. Basidiomycetes were the climax micro-flora in both species of untreated wood. Initial colonisers were bacteria, and these were followed sequentially by primary moulds, 'soft rots' and basidiomycetes, with secondary moulds increasing in importance with the establishment of gross decay. The effect of treating sapwood of both species with CCA was to eliminate the establishment of basidiomycetes, the climax micro-flora, and the substitution of soft-rot fungi in this role and to confer greater protection on the softwood to soft rot decay.

The microflora of soil surrounding wood stakes treated with CCA was investigated by Murphy (1982). These investigations (both soil bed and field trials) concentrated on the occurrence of copper tolerant fungi in soil adjacent to untreated and preservative (CCA) treated birch stakes. An increase in the inoculum of copper tolerant fungi was observed in soil surrounding preservative treated stakes. However, in soil adjacent to untreated stakes, the occurrence of copper tolerant fungi was less. Copper tolerant fungi such as the soft rot Phialophora spp. and the sap-stain fungi Cladosporium spp., predominated at the soil region adjacent to preservative treated wood. Murphy considered that the changes in soil microflora in response to untreated and CCA treated wood, would subsequently lead to the development of a localised soil microflora, adapted towards a wood inhabiting species, and a preservative tolerant species. Fungi isolated from soil were also isolated from wood, confirming the observations of King et al (1980), of the biotic connections maintained between wood and adjacent soil.

Murphy (1982, op. cit.) also found that after insertion of the stakes in soil, there was a period of adaptation in which increased inoculum of fungi capable of attacking wood, and fungi tolerant of preservatives, was found in soil adjacent to untreated and treated stakes respectively. Murphy considered that, after this period, the decay hazard to which wood was exposed was increased in comparison with ordinary soil. This period of adaption proposed by Murphy is similar to the juvenile and induction phases described for preserved wood by Bravery (1968b) and Smith (1980). Smith (1980, op. cit.) considered the induction phase to be the period of time which elapsed before measurable decay of wood took place, and which increased with preservative concentration. The initiation of this induction phase occurred outside the wood and was determined by the unfixed portion of the preservative, which diffused into the soil.

CCA is very effective against soft rot when used as a preservative in softwoods. However, despite the use of higher loadings of preservative than used in softwoods, many hardwoods treated with CCA fail prematurely in soil contact. From the many studies conducted, three main schools of thought have developed in order to explain the variable performance of hardwood timbers when in ground contact.

The hypothesis of substrate susceptibility was developed by Hulme and Butcher (1977 a, b, c,) when investigating the relationship between timber substrates and CCA preservative. In a series of laboratory experiments they found than an increased preservative retention could compensate for the increased susceptibility of a particular species towards soft rotting fungi. Butcher (1979) proposed after a detailed anatomical study of selected hardwoods and data relating to their toxic thresholds, that decay potential and preservative requirement could be related to the proportion of fibres within the wood tissue of a particular species.

The second hypothesis interprets the cause of premature decay as being due to the poor microdistribution of the copper, chromium and arsenic between cell types as well as between differing layers of the fibre cell wall (Dickinson, 1974; Greaves, 1974a; Dickinson, Sorkhoh and Levy, 1976; Greaves and Levy, 1978; Levy and Greaves, 1978). In summary it is suggested that an intrinsic fault of CCA preservatives, with certain hardwood species, is that pockets of fibres may be left inadequately treated and that the penetration

of preservative into the cell wall is not sufficient to give protection against soft rotting fungi. This is in contrast to the treatment of softwoods in which an even distribution of preservative is found combined with good penetration of the tracheid walls (Dickinson, Sorkhoh and Levy, 1976). Drysdale, Dickinson and Levy (1980) noted that disproportionation of the copper, chromium and arsenic within the cell wall may be of significance in preservative performance.

Recent studies on the mechanisms of fixation of CCA preservatives have focused attention on a third hypothesis in which lignin and hemicellulose, rather than cellulose, provide the main fixation sites for inorganic multisalt preservatives (Pizzi, 1982a,b,c; Pizzi and Conradie, 1986; Plackett, 1983). If CCA is bound to lignin it is not likely to be available in a soluble form in the cellulose layers in which soft rot occurs. The hypothesis of Butcher and Nilsson (1982) and Nilsson (1982) on the role of lignin in offering fixation sites for copper, might provide an explanation for the susceptibility of a timber to decay by soft rot fungi.

These authors suggested that if CCA complexes with lignin, high lignin content wood should be able to complex sufficient CCA to mask all T branch initiation sites, whereas a low lignin content wood could never complex enough CCA to permanently mask such sites. Thus high lignin content woods like the softwoods, should be protected from soft rot decay at sub-toxic levels, whereas low lignin content woods like the hardwoods, cannot. If the hypothesis of Butcher and Nilsson (1982, op. cit.) is correct, high susceptibility wood species such as lime can only be protected from decay if the concentration of CCA within the cellulose fraction, especially the S_2 layer is high enough to be directly toxic to micro-organisms. This may be achieved in practice by treating wood to very high retentions of CCA. Butcher and Drysdale (1978) found that the effective CCA retention is species related and grouped hardwoods into broad categories dependant upon the amount of copper required to prevent attack. Drysdale, Dickinson and Levy (1980) and Lewis and Brooks (1983) confirmed the basis of this type of classification. Purslow and Williams (1979) reported that at least four times more CCA preservative is required in hardwoods than softwoods in order to obtain equivalent protection. In conclusion, these authors point out that the susceptibility of a timber is important with a small increase in the natural resistance of the substrate giving a considerable increase in the protection afforded by a CCA preservative.

Purpose of Study

It has been established, from the literature review, that nitrogen sources are major limiting factors to wood decomposition by both fungi and insects and that soluble nitrogenous compounds, redistributed during drying of wood, enhance the nutrient status at evaporative surfaces of wood, which are those first exposed to micro-organisms. Soluble carbohydrates are also suggested to migrate and accumulate and such high nutrient surfaces have been shown to enhance wood decay in both hardwoods and softwoods; reduce toxic limits of CCA preservatives; stimulate sacrificial colonisation by micro-organisms and decrease preservative stability in preserved forms of hardwoods and softwoods. Combinations of a diverse range of nitrogenous and carbohydrate components are implicated in this phenomena. In view of the importance of soluble nitrogenous components and carbohydrate nutrients, especially those in simple forms on microbial activity, this thesis was initiated to provide basic information on the role that individual nutrients might play in the early colonisation and decay of preserved and unpreserved wood. The particular objectives were:

1. to determine the soluble amino acid and soluble carbohydrate composition of redistributed soluble nutrients in hardwoods and softwoods.

2. to evaluate the distribution of the soluble carbohydrate and nitrogenous components, on an annual ring basis in undried (green) and dried wood.

3. to determine the individual roles of soluble carbohydrates and amino acids on wood decay and nitrogen transfer from soil to wood.

4. to determine the influence of these materials on preservative stability of preserved forms of hardwoods and softwoods.

CHAPTER 2

MATERIALS AND METHODS

2. Materials and Methods

The experiments undertaken as part of this thesis are described in this chapter in two sections. The first section includes all experiments, undertaken to determine the soluble carbohydrate and nitrogenous nutrient components, present in two softwoods and two hardwoods. The second section includes all soil burial experiments, undertaken to evaluate the biological influence of selected nutrients on decay of both unpreserved wood and wood treated with a sub-toxic level of copper chrome arsenic (CCA) preservative.

2.1. Studies to determine the soluble components in wood.

Introduction

Four wood types were used in the qualitative and quantitative determinations of the soluble carbohydrate and nitrogenous components in wood. These were spruce (Picea sitchensis, Carr), pine (Pinus sylvestris, L), lime (Tilia vulgaris, Hayne) and kempas (Koompasia malacensis, Maing). Spruce, pine and lime were obtained from the local forests at Tentsmuir and Dunkeld, and kempas was supplied to this laboratory by Hicksons Timber Products Ltd, at Castleford, Yorks. The softwoods were chosen as they are of considerable economic importance to the United Kingdom. Spruce constitutes nearly 40% of the forest stocks in Britain and is extremely difficult to preserve. Pine and lime were selected as a considerable amount of work has been undertaken at this laboratory on these species, which had shown that redistributed soluble nutrients had considerable effects on wood decay and preservative performances (Briscoe, 1987). Furthermore, all three woods have been shown to have surface accumulation of nitrogen (King, Oxley and Long 1974; King, Smith and Bruce, 1980). Kempas was selected as it is a tropical hardwood of significant economic importance in Malaysia, and has been shown to contain high concentrations of soluble nitrogen (King et al,1980 op.cit.).

Four major experiments were undertaken to determine the soluble nutrients present in wood. These were:

1) a preliminary experiment to determine the distribution of soluble carbohydrate and nitrogenous components in the outer sapwood, inner sapwood and heartwood regions of green spruce.

This experiment was also used to determine if redistribution of soluble nutrients occurred during drying,

2) a detailed study of the distribution of soluble nutrients in green spruce and pine,

3) a detailed study of the distribution and redistribution of soluble nutrients in dried spruce and pine,

and

4) a study to determine the soluble nutrients present in the outer sapwood region of surface and sub-surface samples of dried spruce, pine, lime and kempas.

2.1.1. Preparation of wood.

Bolts, approximately a metre long were removed from breast height regions of mature standing trees of spruce, pine and lime. These bolts were then immediately converted by quarter sawing, after which, the resultant quarter sawn planks of dimensions 75cm in length, 36cm in width and 5cm thick, were stored in a 'green' condition in a deep freeze at -18°C (Figure 2.1). Planks were removed from the deep freeze and allowed to thaw at room temperature for twenty-four hours, prior to conversion to smaller planks of dimensions 40cm in length, 36cm in width and 5cm thick. This conversion was to facilitate drying the planks in a fan oven, in the later experiments. A total of three spruce planks, two pine planks, and one of lime, were used in the experiments described in this section.

2.1.1.1. Preparation of green and dried wood samples (Experiment 1).

A plank of spruce of dimension 40cm x 36cm x 5cm, was cut longitudinally into two opposing halves through the centre of the pith (Figure 2.2). One half was kept in an undried 'green' form and the other half was dried in a fan oven at 40°C for two weeks. This was to allow for any redistribution of soluble nutrients to the surface of planks, which might take place during drying (King, Oxley and Long 1974). The undried spruce plank was cut radially to provide two smaller sections, each with a similar distribution of ring groups (Figure 2.2). Each section was further divided into three major sub-sections, approximating to outer sapwood, inner sapwood and heartwood.

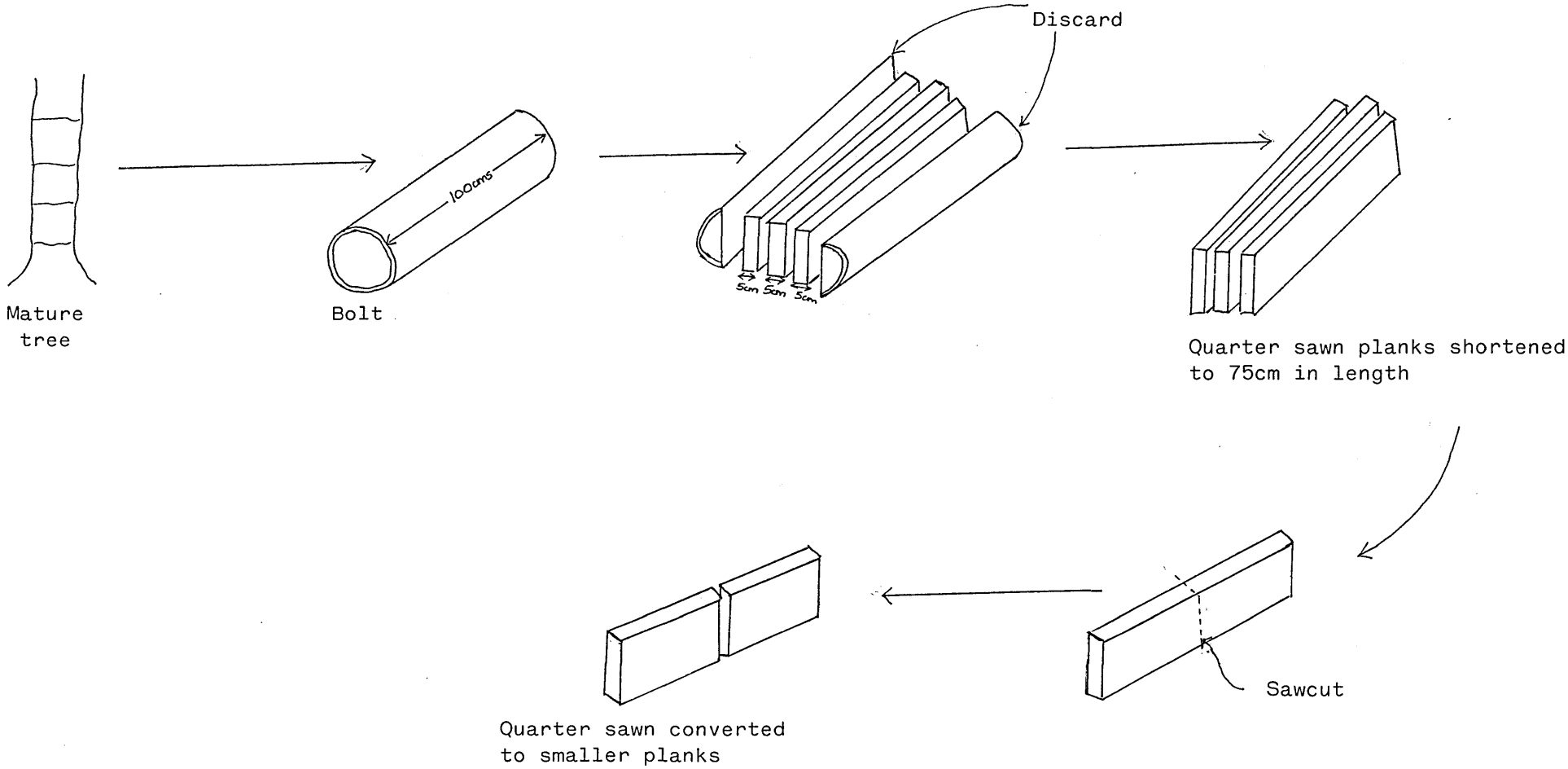
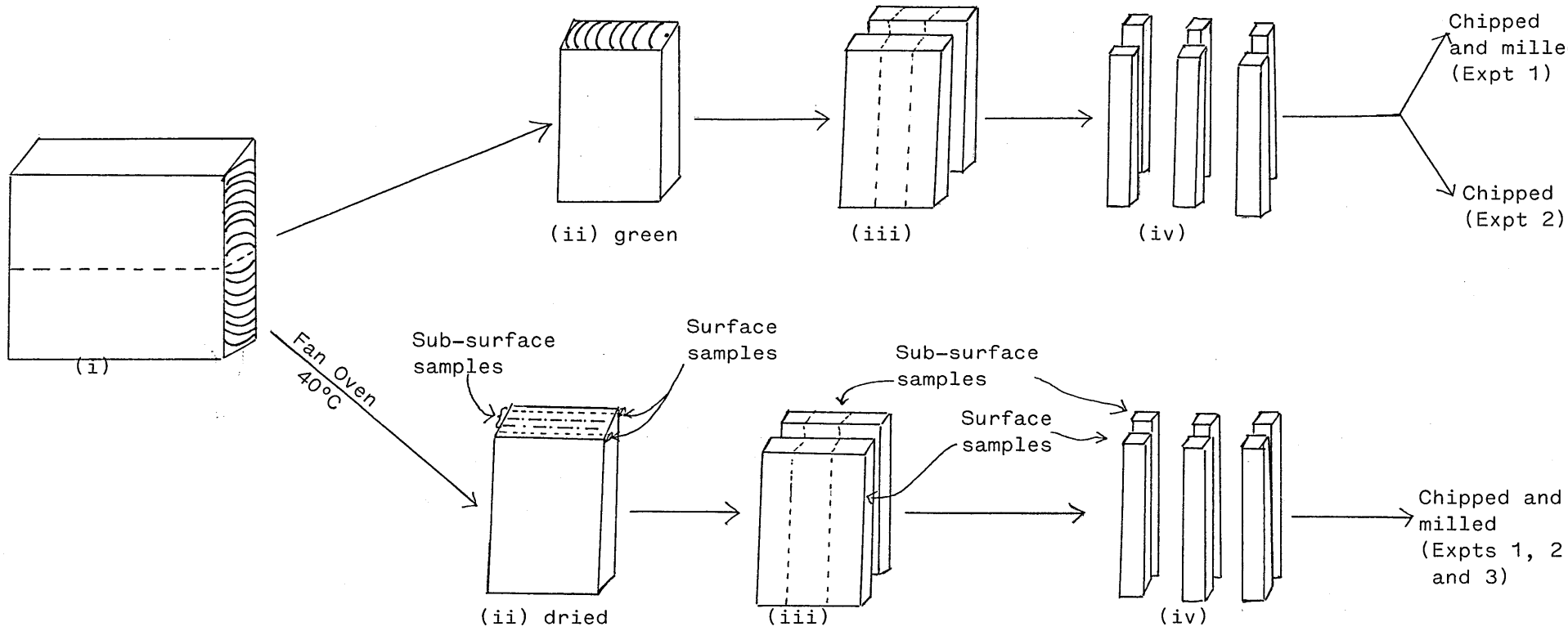


Fig 2.1 Conversion of bolt to planks



(i) Debarked quarter sawn plank
(ii) Opposing halves of quarter sawn plank
(iii) Sections divided into ring groupings
(iv) Strips containing ring groupings

Fig 2.2 Conversion of plank to samples containing ring groups

The first sub-section contained ring groupings 8 - 20 measured from the cambium (outer sapwood) and the second and third sub-sections, contained ring groupings 21 - 35 and 36 - 45, which approximated to inner sapwood and heartwood respectively. Samples from matched ring groupings of each sub-section were converted to small chips and milled in an undried form in a Wiley micro-hammermill through a 0.5mm sieve. All millings including residues in the mill were collected.

The remaining half of the green spruce plank was removed from the oven after drying at 40°C for two weeks. Entire radial surfaces of the plank were removed to a depth of 3mm from the evaporative face. Radial sub-surface samples, removed at a depth of 10mm from the evaporative faces, were also prepared. These were converted to similar ring groupings, corresponding to outer sapwood, inner sapwood and heartwood, as described for green spruce. Samples from each ring grouping were converted to small chips, and milled through a Wiley micro-hammermill, to pass through a 0.5mm sieve. Milled wood from each ring grouping, and from both surface and sub-surface regions, were stored in stoppered jars until required for analysis.

2.1.1.2. Preparation of green and dried wood samples (Experiments 2 and 3).

A plank of spruce and a plank of pine, each of dimensions 40cm by 36cm by 5cm (Length x width x thickness), were prepared in a manner similar to that described for spruce in Experiment 1 (Figure 2.2). One half of each plank was retained in an undried form and the remaining half was dried in a fan oven at 40°C for two weeks. Surface sections approximately 5mm thick, containing the entire sapwood and heartwood, were removed from the radial faces of green spruce and pine. Sections containing 10 ring groupings for spruce, and 5 ring groupings for pine (both measured from the cambium), were prepared from these radial faces. In spruce, ring groupings 1 - 10, 11 - 20 approximated to the outer sapwood region, ring groupings 21 - 30 the inner sapwood region, and ring groupings 31 - 40 and 41 - 50 the heartwood region. In pine, ring groupings 1 - 5, 6 - 10, 11 - 15, 16 - 20 approximated to the outer sapwood region, ring groupings 21 - 25, 26 - 30 the inner sapwood region, and ring groupings 31 - 35, 36 - 40, 41 - 45 and 46 - 50 the heartwood region. Each ring grouping was converted to narrow strips which were chipped to provide small specimens.

Half of the quantity of "chipped" green wood from each ring grouping was dried in an oven at 50°C for 48 hours. This was to determine if any qualitative difference existed between green wood and green wood dried in chipped form, when wood was subjected to drying procedures. The dried chipped wood sections were milled in a Wiley micro-hammermill to pass through a 36 mesh test sieve (British Standard Sieve Series BS410:1986), of approximately 0.5mm aperture size.

Thin radial sections of approximately 3mm thick, were removed from the evaporative surfaces of the section of the spruce and pine planks which had been dried at 40°C in the fan oven. Further sections of wood were also removed from the sub-surface region, 10mm below the surface of the plank. These surface and sub-surface samples were converted into 10 ring groupings for spruce, and 5 ring groupings for pine, as described earlier for the green spruce and pine sections. Samples from each ring grouping were converted to small chips, and milled to pass through a 36 mesh test sieve. Milled wood from each ring grouping, and from both surface and sub-surface samples, were stored in air-tight storage jars until required for analysis.

2.1.1.3. Preparation of dried wood samples (Experiment 4).

In addition to the distribution studies (Experiments 1, 2 and 3), another experiment (Experiment 4), using dried spruce, pine, lime and kempas was undertaken. Quarter sawn planks of spruce measuring 40cm by 36cm by 5cm, pine measuring 40cm by 36cm by 5cm, and lime measuring 40cm by 35cm by 5cm, were dried in unconverted forms in a fan driven oven at 40°C for two weeks. Surface samples containing redistributed soluble nutrients were removed to a depth of 3mm from the outer sapwood regions, (rings 0-25 as measured from the cambium), of the dried planks. Sub-surface samples removed at a depth of 10mm below the surfaces of matched planks, were also prepared. Kempas was supplied to this laboratory in the form of dried sticks of dimensions 6.0 cm x 2.5 cm x 2.5 cm. Surface and sub-surface regions were not differentiated in this wood due to the method of preparation of this test material. All wood samples were converted into small chips to facilitate milling through the Wiley micro-hammermill. The milled wood fractions were collected and sieved to pass through a 72 mesh sieve (aperture size 212 μm). Larger fractions were collected and milled again until all fractions passed through the 72 mesh sieve.

The milled wood fractions from each wood type, and from each region, were stored in separate air-tight containers until required for analysis.

2.1.2. Determination of moisture contents.

The moisture contents of dried milled wood were determined as described in Tappi Standards T264-om-82.

2 grams of milled wood was weighed to an accuracy of $\pm$0.001g in a tared weighing bottle. The sample was dried in an oven at 103°C $\pm$2°C for three hours and cooledin a dessicator. Prior to weighing, the stopper was opened momentarily to equalise the air pressure and the bottle weighed. The weighing bottle was returned to the oven for a further hour; and the cooling and weighing process was repeated for successive hourly periods, until constant weight was achieved. This was when successive weighing did not change by more than 0.002g.

$$\text{Moisture Content (\%)} = \frac{\text{Initial dry weight of wood} - \text{Final dry weight of wood}}{\text{Final dry weight of wood}}$$

Duplicate determinations of moisture contents were undertaken for each wood type. Moisture contents of green wood samples in Experiments 1 and 2 were not undertaken as the interest in the work was then qualitative.

2.1.3. Extraction procedures

A number of extraction procedures were employed in the determination of soluble carbohydrate and nitrogenous components in wood. In the experiments investigating the distribution of soluble nutrients in green and dried spruce and pine, samples were extracted in cold water. In the later experiments (Experiment 4), extractions with alcohol, hot water and cold water were employed to determine the soluble nutrient components in dried surface and sub-surface samples. Extractions performed in the later studies conformed to the standard procedures described in Tappi standards.

2.1.3.1. Aqueous extraction

All wood samples, both green and dried from the distribution studies (Experiments 1, 2 and 3), were subjected to an aqueous extraction in deionised water.

An aqueous extraction was chosen as this would indicate the nutrients that were readily available to primary colonising organisms. Preliminary experiments had been undertaken to determine the soluble carbohydrate content in wood, extracted in cold water for different time periods. Extracts were removed at five minute intervals up to thirty minutes, and then at hourly intervals up to 6 hours. Extracts were assayed for the total carbohydrate content. It was found that a large proportion of the soluble carbohydrates was extracted within thirty minutes. An increase in extraction time did not yield significantly higher quantities of soluble carbohydrates (McFarlane, pers.comm). Accordingly, all wood samples both chipped and milled, 'green' and dried, were extracted in cold water in wood to water ratios of 1:15.

Wood samples were extracted in Erlenmeyer flasks (500ml), placed in a water bath set at 30°C. Usually 6g of wood was extracted in 100mls of deionised water. The flask and contents were allowed to equilibrate for five minutes and the contents were stirred by an overhead stirrer. After thirty minutes, the contents were filtered through filter paper (Whatman's No 1), and then refiltered twice through a membrane filter (pore size, 0.45μm) to remove any residual wood debris. The volume of the filtrate was measured and the filtrate was freeze dried. The freeze dried contents were later redissolved in deionised water, to give a 10-fold concentration of the original extract. The concentrated samples were stored frozen until required for analysis.

2.1.3.2. Alcohol extraction (Experiment 4).

The method used for the extraction of milled wood in alcohol is similar to that used by Baker, Laidlaw and Smith (1970). The method of extraction is as that described in Tappi T264-om-82.

Milled wood ($2g \pm 0.5g$) was weighed and extracted in an extraction crucible (pore size 100 - 120 μm). A small cone of fine mesh screen wire was placed in the top of the crucible to prevent the loss of wood during extraction. The crucible and its contents were weighed and extracted in a soxhlet apparatus with 70% aqueous ethanol (200ml) for six hours. After this period, the contents of the crucible was rinsed with small amounts of 70% aqueous ethanol. The rinsings were collected and added to the extract in the flask.

The combined extract and rinsings were filtered through a membrane filter (0.45μm) to remove any residual sawdust. The filtrate was then transferred to a pre-weighed round bottom flask and rotary evaporated to dryness under reduced pressure at 40°C (Long, 1978). The flask was dried in a dessicator for 18 hours and weighed again prior to the residue being taken up in deionised water (20ml). The extracts were kept frozen until required for analysis. Duplicate extractions were carried out for each wood species. The milled wood, retained in the crucible was dried in an oven at 103°C$\pm$2°C until constant weight. From the weights obtained, the weight loss of the sample, the weight of alcohol solubles, and the weight of cold water solubles recovered from the dried alcohol extracts could be determined.

2.1.3.3. Hot water extraction (Experiment 4).

Milled wood samples which had been previously extracted in 70% aqueous ethanol, were subjected to a further extraction in hot water. The extraction procedure employed was as described in Tappi T207-om-81 for the water solubility of wood and pulp.

Samples were transferred from the crucibles to pre-weighed flasks. The flasks and contents were weighed. The contents were then extracted in hot water (100ml) under reflux for 3 hours. After this period, the insoluble material was recovered and rinsed with hot water (200ml). The rinsings and extract were combined and reduced in volume to 100ml, by evaporation over a hot plate. The reduced filtrate was cooled prior to freeze drying in a pre-weighed flask. The flask and dried contents were later placed in a dessicator to dry before being weighed again. The freeze dried material was redissolved in deionised water (10ml), filtered through a membrane filter (0.45μm), and stored frozen. The extracted wood samples was dried (103°C$\pm$2°C) until constant weight was achieved. From the weights obtained, the weight loss of the sample and the amount of material recovered after freeze drying could be determined. Duplicate extractions were undertaken for each wood type.

2.1.3.4. Cold water extraction (Experiment 4).

Milled wood samples were extracted in cold water using a modified method of Tappi T207-om-81 for the solubility of wood in water. Preliminary experiments undertaken following Tappi procedures highlighted a number of problems.

Extracts showed signs of microbial contamination towards the end of the extraction period (48 hours). This would have resulted in inaccuracies in the analysis of the soluble nutrient concentrations. Also, the time taken to concentrate samples by freeze drying was lengthy, as the freeze dryer could not operate at maximum capacity for continuous periods. The extraction procedure was therefore modified to avoid these problems. Samples were extracted in smaller volumes of water (50ml), but in the same wood to water ratio as that used in Tappi. The extraction time was reduced to half an hour, as earlier studies had shown that the bulk of the soluble carbohydrate had been extracted within this period. The procedure used for the extraction in cold water is outlined below.

Milled wood ($0.33g \pm 0.1g$) was placed in a beaker and extracted with deionised water (50ml) at ambient temperatures for half an hour. The contents in the beaker were stirred constantly by a magnetic stirrer. Extracts were filtered through a membrane filter (0.45 µm). The volume of the filtrate was measured before being freeze dried in a preweighed flask. After further drying in a vacuum dessicator (18 hours), the flask and freeze dried contents were reweighed. The freeze dried contents were redissolved in deionised water (5ml), to give a 10-fold concentration of the original extract. The concentrated extracts were stored frozen until required for analysis. The extracted wood sample was dried in an oven ($103°C \pm 2°C$) until constant weight was achieved. The weight loss of the sample, and the weight of cold water solubles recovered after freeze drying, were calculated from the weights obtained. Duplicate extractions were undertaken for each wood species.

2.1.4. Hydrolysis of extracts

Acid hydrolysis was undertaken to determine the soluble protein content of the outer sapwood region (rings 1 - 20) of green spruce and pine. The polypeptide chain is generally hydrolysed to the constituent amino acids by 6M HCl at 110°C for 16 - 72 hours. With this procedure, most of the amino acids are recovered quantitatively and can be detected using the amino acid auto-analyser.

The sample (200µl), in 6M HCl (AR grade), was placed in a hydrolysis tube and flushed with oxygen-free nitrogen for ten minutes. The sample was de-aerated using a water pump and then sealed. After incubating at 110°C for 18 hours, the hydrolysate was placed in a round bottomed flask and the hydrochloric acid removed by rotary evaporation.

The hydrolysate was dissolved in a small volume of deionised water and the resulting solution was evaporated to dryness. The solubilisation and drying was repeated until all traces of acid were removed. The residual extract was redissolved in deionised water (2ml) and stored frozen.

2.1.5. Analyses of extracts.

A number of assays were performed in the analysis of the soluble nutrient composition of the wood extracts. General assays such as the phenol-sulphuric acid assay, dinitro-salicylic acid assay, protein assay and the ninhydrin assay were employed in the quantification of total carbohydrate content, total reducing sugar content, protein content and soluble amino acid content respectively. Specific enzymatic assays were undertaken to determine the glucose and fructose contents of samples. Composition of amino acids in samples were determined using an amino acid auto-analyser, and in the later stages of the experimental analysis, wood sugars were determined by high performance liquid chromatography (HPLC).

2.1.5.1. Total carbohydrate content assay.

This assay is based on a colorimetric method for the determination of sugars (Dubois et. al., 1956). Simple sugars, oligosaccharides, polysaccharides and their derivatives including the methyl ethers with free or potentially free reducing groups, give an orange-yellow colour when treated with phenol and concentrated sulphuric acid. The reaction is sensitive, and the amount of colour produced at a constant phenol concentration, is proportional to the amount of sugar present.

Several dilutions of a glucose standard (0.2mM) containing from 18 - 72 µg/ml glucose were prepared. 2mls of the standards and appropriately diluted samples were placed in test-tubes. Aqueous phenol (80% w/v, 50µl) was added to each of the tubes. Analar concentrated sulphuric acid was dispensed into each tube using a calibrated automatic dispenser. The contents of the tube were mixed and left to stand for a period of 15 minutes, at ambient temperatures. The absorbance of the tubes were measured at 485nm against a reagent blank. A calibration graph was obtained by plotting the absorbances against the standard sugar concentrations. The concentration of carbohydrates in the sample was calculated (as glucose) from these graphs.

2.1.5.2. Reducing sugar assay

This is a colorimetric assay used for the qualitative and quantitative determination of reducing sugars (Sumner, 1925). The assay involves the reduction of 3, 5 - dinitrosalicylic acid by reducing sugars, to form a coloured amino compound. The intensity of the colour formed is proportional to the concentration of the reducing sugars present.

Solutions of a glucose standard containing between 0.1 mg/ml and 0.5 mg/ml were transferred to separate test-tubes. 3, 5-dinitrosalicylic acid (1ml) was added to each tube, and the contents mixed before being incubated in a water bath (100°C) for 5 minutes. Condensers were placed over the top of the tubes to prevent evaporation of the contents. After incubation, the contents were cooled and deionised water (4ml), was added to each tube. The contents were mixed again before the absorbances of the solutions were measured at 540nm against a reagent blank. Samples of wood extract were assayed for total reducing sugar content in a similar manner to that described for the glucose standard. A calibration graph of absorbances against glucose standard concentrations was plotted, and concentrations of reducing sugars in sample extracts were determined by interpolation.

2.1.5.3. Enzymatic determination of glucose and fructose.

This enzyme specific assay is based upon the conversion of glucose to glucose-6-phosphate by ATP in the presence of hexokinase (EC No. 2.7.1.1.) (Klotzsch and Bergmeyer, 1965). The product of this reaction becomes the substrate for the coupling reaction that is catalysed by glucose-6-phosphate dehydrogenase (EC No. 1.1.1.49).

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate} + \text{ADP} \qquad (1)$$

$$\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow[\text{dehydrogenase}]{\text{Glucose-6-phosphate}} \text{6-phosphogluconic acid} + \text{NADPH} + \text{H}^+ \qquad (2)$$

The glucose-6-phosphate is oxidised with glucose-6-phosphate dehydrogenase and $NADP^+$, to give 6-phosphogluconic acid, NADPH and H^+ (equation 2). As NADPH has a high extinction coefficient (6.22×10^3 l mol^{-1} cm^{-1}) at 340nm, and $NADP^+$ has no absorbance at this wavelength, the progress of the coupled reaction is followed by measuring the increase in absorbance at 340nm. The glucose concentration is determined directly from absorbance readings without the need of standards or calibration curves.

The increase in absorbance (at 340nm) due to the formation of NADPH is directly proportional to the concentration of glucose. The equilibrium constant for the reaction in equation (2) is very high and lies to the right of equation. When equilibrium is reached in this reaction, the concentration of fructose in the sample can be determined. The fructose-6-phosphate originally in the sample, and also the frustose-6-phosphate formed according to equation (1) from fructose, is converted on addition of phosphoglucose isomerase (EC No 5.3.1.9.) to glucose-6-phosphate.

$$\text{Fructose-6-phosphate} \xrightleftharpoons[\text{isomerase}]{\text{phosphoglucose}} \text{glucose-6-phosphate} \qquad 3$$

The glucose-6-phosphate is estimated according to equation (2). The equilibria of reactions (1) and (2) lie far to the right. The equilibria of reaction (3) is not important since the glucose-6-phosphate formed reacts immediately according to equation (2). As a result, all three reactions proceed stoichiometrically.

A Sigma Glucose 10 Assay vial was used in the determination of glucose and fructose present in the extracts. The vial was reconstituted with deionised water (31ml) to obtain the following reagents:

ATP	1mmol/l
β - $NADP^+$	0.5mmol/l
Hexokinase (yeast)	800U/l
Glucose-6-phosphate dehydrogenase (yeast)	500U/l
Mg^{2+}	2mmol/l
Buffer Salts	pH7.5

(U = a unit which is the amount of enzyme which converts 1 μmole of subtrate in 1 minute at 25°C).

A reagent blank (1ml) was pipetted into a quartz cuvette. In a second matched cuvette, the reagent (0.9ml) and the sample to be analysed (100μl) were added. The contents in the cuvette were mixed and the absorbance of the sample was monitored until no further increase occurred, and the resulting value was noted. The cuvette was removed from the spectrophotometer (Perkin Elmer, (UV-vis) and phosphoglucose isomerase (0.5μl) was added to the reaction mixture. The cuvette was returned to the spectrophotometer and the reaction was monitored until equilibrium was reached again. The difference in the two absorbance readings allows the concentration of fructose to be determined. The amount of sugar present in the cuvette was calculated from the following equation:

$A = Ecl$ where A = absorbance

E = extinction coefficient (6.22×10^3 $lmol^{-1}$ cm^{-1})

c = concentration

l = light path (1cm)

All assays were carried out in triplicate.

2.1.5.4. Protein assay

The BioRad protein microassay is used in instances where concentration of the protein is < 25 µg/ml. The dye binding microassay is based on the differential colour change of a dye in response to various concentrations of proteins (Bradford, 1976). The dye, Coomasie Brilliant Blue G250, exists in two different forms, red and blue. The red form is converted to the blue form upon binding with protein. The binding of the dye to the protein causes a shift in the absorption maximum of the dye from 465nm to 595nm. The absorption at 595nm is monitored.

Several dilutions of a protein standard bovine serium albumin (25 µg/ml) containing from 1 to 25 µg/ml were prepared. The standards and appropriate dilution of the samples were placed in test tubes. Deionised water (0.8ml) was used as a reagent blank. The BioRad Dye Reagent Concentrate (0.2ml) was added to each test tube. The contents in the tubes were mixed and left for a period of 5 minutes, after which absorbances at 595nm were measured against the reagent blank. Standard calibration graphs were plotted from the absorbance and concentration of protein, in the assay of fixed volume. Concentrations of protein in the samples were determined from the graph.

2.1.5.5. Amino Acid Assay.

This colorimetric assay is based on a modified procedure for the analysis of compounds with an amino group of the α-carbon (Rosen, 1957). These compounds include amino acids, imino acids, amino alcohols and primary amides. In the reaction of amino acids with ninhydrin, ammonia and hydrindantin (a reduced form of the ninhydrin) are produced. The ammonia reacts with an additional molecule of ninhydrin and the hydrindantin, to yield a purple substance (Ruheman's purple) which absorbs maximally at 570nm. This absorption is a linear function of the concentration of α-amino groups present in a sample.

Several dilutions of a threonine standard containing 20 - 80 μmoles of the amino acid were prepared in test-tubes. Ninhydrin solution (1ml) prepared using ethanediol as a solvent (Moore, 1968) was added to each tube. The contents were mixed before being incubated in a water bath (100°C) for 15 minutes. After the incubation period, the tubes were cooled before aqueous ethanol (50% v/v, 2ml) was added to the contents in the tubes. The solutions were mixed and their absorbanc es read at 570 nm against a reagent blank. A calibration graph of absorbances against amino acid concentration was plotted. Samples of wood extracts were assayed in a similar manner to that described for the amino acid standard. Concentrations of amino acids in the wood extracts were evaluated from the calibration graphs.

2.1.5.6. Determination of amino acids using an amino acid auto-analyser.

In addition to the ninhydrin assay used in the quantification of amino acids (2.1.5.5), the amino acids in the extracts were also determined using an amino acid auto-analyser. In this method, a mixture of amino acids is loaded onto a column of an analytical ion-exchange resin. Buffers of increasing pH and varying ionic strength are pumped sequentially through the column to effect the separation of the amino acids.

The amino acid analyses were performed on an LKB 4101 amino acid analyser. An automatic sampler injector (LKB 4104) was connected to the main instrument. A block diagram showing the components of the amino acid auto-analyser is presented in Figure 2.3. The amino acids were separated on an Ultropac 10 resin, employing a sodium three buffer system:

(i) 0.2M sodium citrate buffer, pH 3.25 (containing 2% v/v isopropanol)
(ii) 0.2M sodium citrate buffer, pH 4.25
(iii) 1.2M sodium citrate buffer, pH 6.45.

2% v/v isopropanol was added to the first buffer to improve the separation of threonine and serine. Thiodiglycol was added to buffers (i) and (ii) to prevent the oxidation of methionine, and phenol (0.1% w/v), was added as a preservative to all three buffers to inhibit the growth of micro organisms.

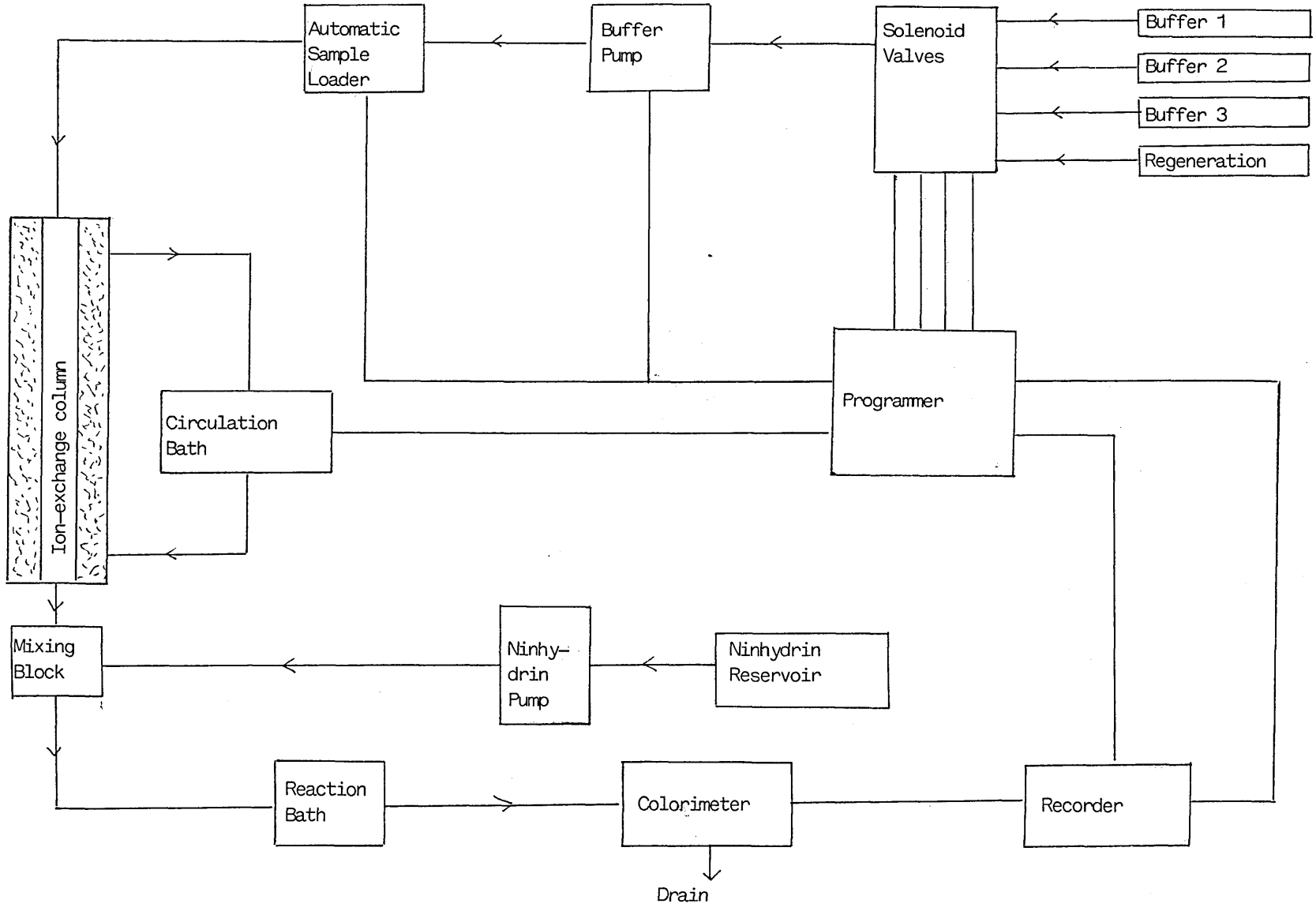


Fig 2.3 Block diagram of the amino acid auto-analyser

During the analysis of amino acids, the column eluant is mixed with ninhydrin solution and the mixture is pumped through a coil of PTFE tubing immersed in a bath of boiling water. The ninhydrin reacts with the amino acids present in the eluant, forming coloured compounds. On leaving the reaction bath, the mixture is passed through a two channel colorimeter where the absorption of the coloured compound is measured at two wavelengths, 570nm and 440nm. The changes in light absorption are registered on a two channel continuous writing recorder where the amino acids are represented by peaks in a chromatogram. The position of the peaks on the chart represent the column retention times for the individual amino acids. Measurement of the area under each peak gives quantitative information on the amount of each amino acid present in the sample. At the end of each analysis, the ion-exchange resin is regenerated by pumping a strong base through the column and then equilibrated by pumping the first elution buffer for a time after which the analyser is ready for another run.

A typical automated procedure for the amino acid analysis is outlined in Table 2.1.

Table 2.1 Run conditions used in the amino acid auto analyser.

Resin:	Ultropac 10	
Analysis:	0.2M sodium citrate buffer, pH3.25	4 mins
	0.2M sodium citrate buffer, pH4.25	38 mins
	1.2M sodium citrate buffer, pH6.45	78 mins
Regeneration:	0.4M sodium hydroxide	5 mins
Equilibration:	0.2M sodium citrate buffer, pH3.25	66 mins
Column temperature:	50°C for 40 minutes, then at 70°C for remainder of the run	
Flow Rates:	buffer system 50mls/hr	
	ninhydrin system 25mls/hr	

The quantitation of amino acids in a sample was determined against a standard containing known amounts of given amino acids. The standard protein hydrolysate mixture (LKB) contained 12.5nmol/ml in 0.2M sodium citrate buffer (pH2.2) of the following amino acids: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, ammonia and arginine. (Cystine was present at a concentration of 6.25nmol/ml). Because of their instability, both glutamine and asparagine are not added to any commercially available standard.

Standards of aspartic acid and asparagine, glutamic acid and glutamine, and aspartic acid, glutamic acid and glutamine (each at a concentration of 12.5nmol/ml) were run separately to provide standard calibration chromatograms of these amino acids.

For the manual evaluation of the results, the following procedure was followed. The baseline and total height value of each amino acid peak was determined. Subtracting the baseline value from the peak height, gave the net height. If the baseline was not parallel to the chart grid, the peak area could still be calculated as described above except that the height should be drawn perpendicular to the sloping basline as shown schematically in Figure 2.4.

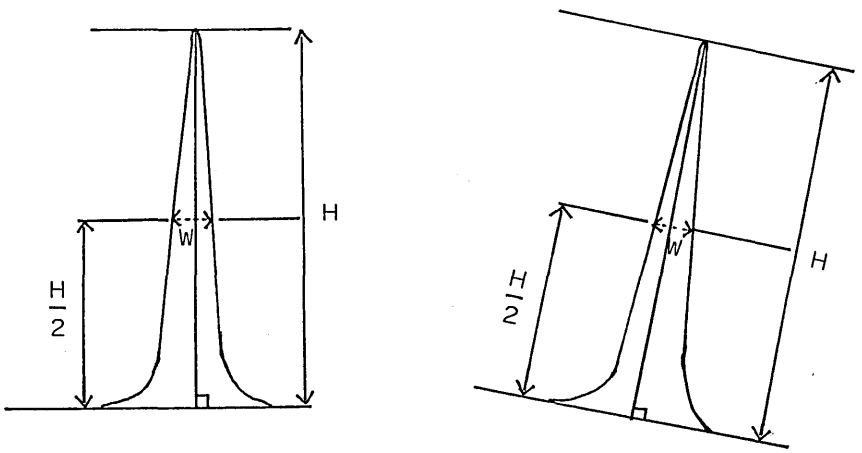


Fig 2.4 Procedure for manual evaluation of amino acid concentration

The peak area is calculated by multiplying the peak height (H), with the peak width (W), at half peak height (H/2). By calculating the area (A) for a known concentration (C) of an amino acid from a standard run, a constant (Caa) corresponding to the amino acid can be determined

$$Caa = \frac{A}{C} \text{ nmoles}^{-1}\text{ml}$$

Chromatograms from samples containing unknown quantities of amino acid are evaluated by comparing retention times. The areas under these peaks are calculated and the concentration of the amino acid is determined by dividing the peak area with the constant obtained for the amino acid. The standard protein hydrolysate was analysed every six sample runs.

2.1.5.7. Separation of wood sugars using high performance liquid chromatography (HPLC)

The separation of wood sugars in the extracts of dried spruce, pine, lime and kempas (Experiment 4) were undertaken using HPLC. The equipment was available in the Department during this stage of the experimental analysis. This method of analysis was selected in preference to others (assays and chromatographic procedures), as it had the advantage of high resolution, speed, sensitivity and automatic operation.

Components of a HPLC system and its mode of operation

The separation of wood sugars was performed in a BioRad HPLC system. The system comprises of five separate components, viz, a pump (mobile phase delivery system), an injector, a column, a detector and a recording and data handling system. A schematic diagram of the system in shown in Figure 2.5.

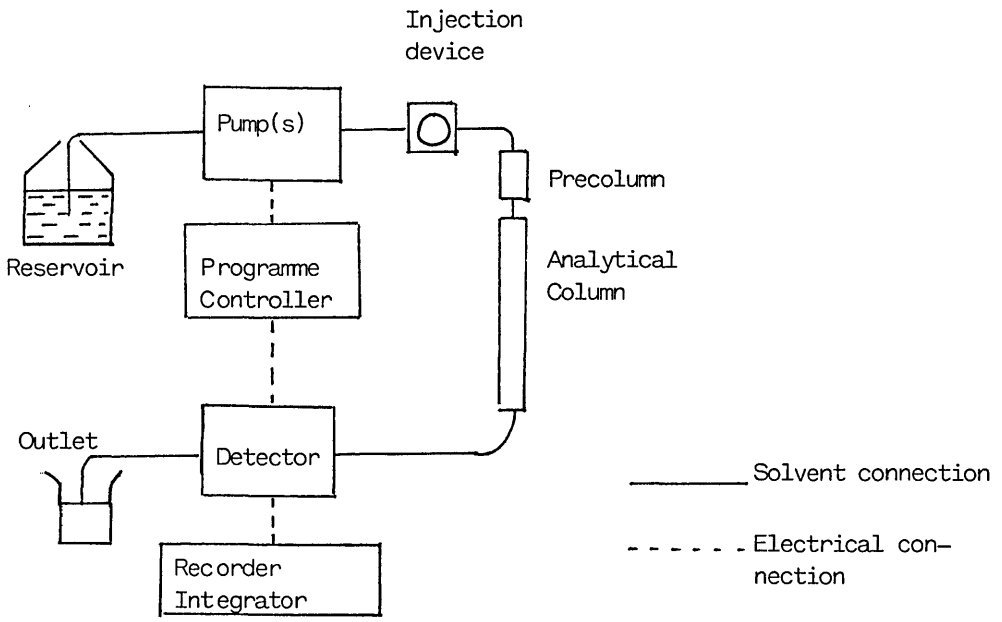


Figure 2.5. Schematic diagram of a typical HPLC system.

In the system used, the pump produces a reproducible flow of solvent through the column at high pressure. The injection utilises a valve which contains a loop of fixed volume into which the sample is injected. After the sample is loaded, the valve is mechanically actuated to bring the loop (and sample) in line with the HPLC system. All of the sample is then discharged onto the top of the column.

The column used is usually constructed of a good grade stainless steel because of the relatively high pressures employed in HPLC analysis. A small pre-column is introduced between the injector and the analytical column. The precolumn is packed with microparticulate materials similar to those in the analytical column. It protects the analytical column from degradation due to particulate matter and aggressive reagants in the sample or solvent.

The column used in the separation of wood sugars is an Aminex HPX-87P column packed with 8% cross linked cation exchange resin in the lead form. This column was selected as it provides good resolution when disportionate carbohydrate quantities are present. The column is an Ion Moderated Partition (IMP) column and allows the use of an isocratic HPLC system with no derivatization of the compounds. IMP techniques use mechanisms of ion exclusion, ion exchange, ligand exchange, size exclusion, reverse phase and normal partitioning. The major mode of separation achieved on the Aminex HPX-87P column is by the mechanism of ligand exchange. The column is heated by a column heater insulated by a column jacket. For carbohydrate analysis, the resin columns are heated to 85°C to optimise operating conditions.

The detector used is a refractometer which monitors the difference in refractive index between the pure mobile phase and the mobile phase plus sample as it elutes from the column. The difference in refractive index between the sample and the mobile phase produces a voltage which is collected and processed using a Chromatochart interface (P.K. Warme, Interactive Microwave, Inc.) with an Apple II microcomputer. The voltage produced is continuous and the chromatochart is programmed to collect these signals at regular time intervals. These electrical signals are connected to an analogue-digital (A/D) converter value to a number on a pre-set scale and the data is then stored in the memory of the computer. Table 2.2 outlines the conditions used in the HPLC analysis.

An Overview of the Chromatochart

The chromatochart program is divided into eight functional modules listed in the Main Menu options. These eight modules intercommunicate via data arrays and disk files. The module used most frequently in the analysis of wood carbohydrates was the method file. The method file contains instructions that control all aspects of the chromatochart's analysis. Through this file the conditions of the run, along with choices of whether or not to save the raw data, baseline and peak files on disk can be controlled.

Integration parameters such as percentage change in width allowed from one peak to the next, and the minimum area of a valid peak can be defined. Data collection with external events such as signals to and from autosamplers pumps etc. can also be synchronized via the options available in the method file.

The chromatochart does not integrate peaks during the data collection phase but defers the baseline calculation until the complete data are available. This also makes it possible to reintegrate the peaks after changing certain options. The chromatochart program recognises a baseline when a valley is wider than the specified minimum baseline width and when no changes in slope is larger than the slope threshold within that section of the chromatogram. Before each peak is integrated, a straight line is drawn between adjacent baseline points and the area below the baseline is subtracted from the peak area. The height of the peak is determined by fitting a least squares quadratic curve to five points surrounding the peak. The exact retention time of the peak is computed as the position of maximum along this fitted curve and its height is computed as the height at the maximum. This approach is more accurate than using the highest data point value to determine the height and peak position. The chromatochart is also programmed to correct for background noise. At the end of the integration phase, after all the peaks have been integrated, the retention time, height, width and area are displayed on the screen and related to a printer.

Sample preparation and evaluation

Sugar standards (1mg/ml) were prepared from the following: stachyose, sucrose, raffinose, glucose, xylose, galactose, rhamnose, mannose, arabinose and fructose. Standards containing mixtures of sucrose, glucose, xylose, galactose and mannose (1mg/ml) and raffinose, rhamnose, arabinose and fructose (1mg/ml) were also prepared. Four replicate runs of each sugar standard were undertaken. Their mean retention times and peak areas were used to identify wood sugars in the sample analysis. A diagram showing the retention times of the sugar standards is presented in the results section (Figure 3.31) of this thesis.

Four replicate runs were also undertaken on the sample extracts. The concentration of the sugar in the sample is evaluated from the response factor determined for each sugar in the standard solution. The response factor is a constant obtained by dividing the peak area of the sugar standard with the concentration of the sugar.

The concentration of sugar in the sample can be determined by dividing the peak area of the sample sugar with the response factor of the corresponding sugar in the standard mixture. The total concentration of the sugar in the wood extract is expressed as a percentage of the initial dry weight of wood.

Table 2.2 Run conditions used in HPLC analysis.

Column: Aminex HPX-87P, 300mm x 7.8mm (9μm mean particle size)
Eluant: Degassed deionised water
Flow Rate: 0.6ml min^{-1}
Temperature: 85°C
Pressure: 1000 p.s.i.
Sample size: 20 μl
Detector: Refractometer 2X

The Bio-Rad HPLC system was used under isocratic conditions. Data was collected and processed using a Chromatochart interface with an Apple II microcomputer.

2.2. Soil Burial Studies

Introduction

The biological influence of soluble carbohydrates and soluble amino acids on wood decay and preservative toxicity and stability are described in this section. Soil burial tests were chosen as a method of investigation. Softwoods are attacked more rapidly in soil burial tests than in pure culture methods and, as test specimens are usually buried in small containers, environmental conditions such as temperature and moisture content of the soil can be controlled. Test specimens are also exposed to a wide spectrum of indignous soil microflora present in the non-sterile soil used (Savory and Bravery, 1971). Test specimens used in this laboratory are wood blocks of dimensions 10mm x 10mm x 5mm, with the 10mm x 10mm face in radial section, and the 10mm x 5mm faces in transverse and tangential sections. The chosen size of the wood blocks is to facilitate decay to be measured more rapidly than would be otherwise possible in larger specimens.

In the experiments undertaken in this section, wood specimens of low nutrient status were impregnated with soluble sugars, amino acids and a mixture of amino acids and sugars to replicate wood of high nutrient status i.e. surface regions of dried wood. In studies using preserved wood, test specimens were impregnated with 0.5% w/v copper chrome arsenic preservative prior to a second impregnation with soluble sugars or amino acids. Four wood species were used in the soil burial tests. These were spruce (Picea sitchensis, Carr), pine (Pinus sylvestris, L), lime (Tilia vulgaris, Hayne), and beech (Fagus sylvatica, L). Spruce, pine and lime were chosen, as analysis on the composition of soluble nutrient components of these woods had been undertaken in Experiment 4 (2.1.3). These woods have also been used in soil burial studies undertaken at this laboratory. Beech was selected as it is a wood of low nutrient status and is easily decayed. Kempas was omitted from the soil burial studies as experiments undertaken on this wood showed low concentrations of soluble nutrients.

The soil burial experiments undertaken were divided into two sections, i.e. those using unpreserved wood and those using preserved wood. In the soil burial studies using unpreserved wood, the following experiments were included:

1) a soil burial study with soluble sugars incorporated into softwoods and hardwoods as the sole additional source of soluble carbohydrate,

2) a soil burial study with soluble amino acids incorporated into softwoods and hardwoods as the sole additional source of nitrogen,

3) a soil burial study with an amino acid incorporated into a hardwood species and buried at 100% moisture content,

4) a soil burial study with a mixture of soluble sugar and amino acid incorporated into a hardwood species,

and

5) a short term soil burial experiment to investigate the leaching of added amino acids from buried wood blocks.

In studies using preserved wood, the following experiments were undertaken:

6) a soil burial study using preserved hardwood blocks impregnated with arginine,

7) a soil burial study using preserved softwoods and hardwoods impregnated with glutamine,

and

8) a soil burial using a preserved softwood and hardwood species impregnated with sugars.

All these studies (with the exception of experiments 4 and 5) were conducted over a soil burial period of twelve weeks. The parameters monitored during the soil burial tests were moisture content of test specimens, as an indication of moisture conditions in the soil, weight loss as a measurement of decay in test specimens and nitrogen content as an indication of microbial presence in the wood (King et al ,1981a).

2.2.1. Preparation of wood.

Quarter sawn planks of spruce, pine and lime of dimensions similar to those described in 2.1.1.3. were dried in a fan oven (40°C) for two weeks. Sapwood sections containing rings 3-25 measured from the cambium were removed from the dried planks (Figure 2.6).

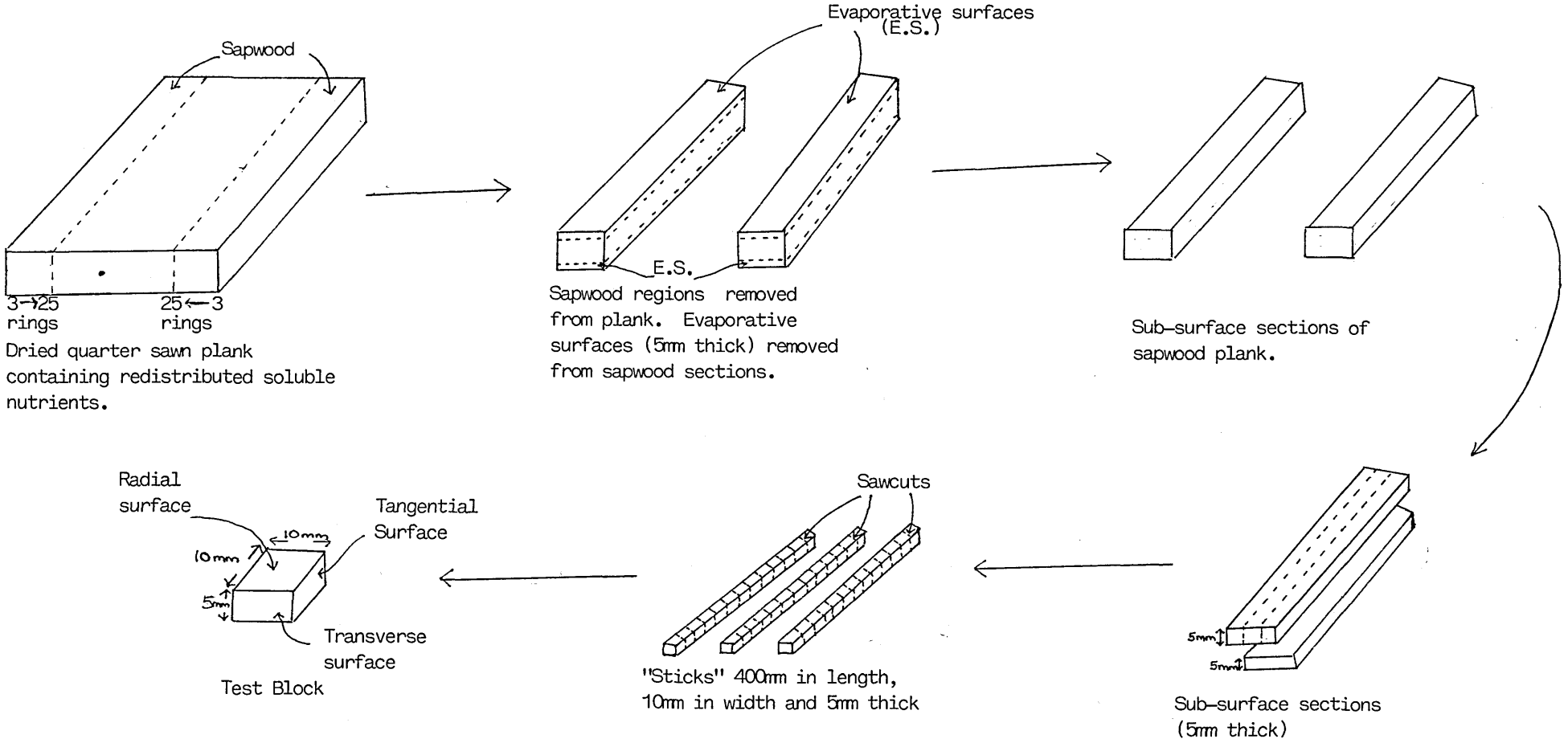


Fig 2.6 Conversion of dried plank to test blocks

The radial evaporative faces containing redistributed soluble nutrients were removed to a depth of 5mm from the sapwood sections. Sub-surface samples removed 10mm beneath the evaporative faces were also prepared. Wood blocks of dimensions 10mm x 10mm x 5mm, with the 10mm x 10mm face in radial section and the 10mm x 5mm faces in transverse and tangential sections, were prepared from these sub-surface samples. Beech blocks prepared to similar dimensions as those of spruce, pine and lime were supplied in a dried form by the Building Research Establishment in Princes Risborough and by Penarth Research Station in Winchester, Hampshire. All test blocks were numbered, oven dried (103°C) to constant weight and weighed. In Experiment 4 of the soil burial studies using unpreserved wood, test blocks prepared from the radial surface regions of sapwood sections were also included in the experiment. A total of 2540 blocks were analysed in the soil burial studies undertaken as part of the experimental analysis for this thesis.

2.2.2. Impregnation of wood blocks

2.2.2.1. Impregnation with soluble sugar and amino acid solutions

In the soil burial experiments using unpreserved wood, impregnation of wood blocks were undertaken in a beaker (1l), in which wood blocks were retained on the base by covering with weighted glass slides. The beaker was placed in a vacuum dessicator and a vacuum was drawn for 15 minutes, after which the appropriate treating solution was introduced. The vacuum was released and the wood blocks were left submerged for 30 minutes. After this period, the blocks were removed from the solution, blotted dry to remove excess solution and weighed. All blocks were impregnated with the appropriate treating solution in ascending order of concentration. Softwood blocks were impregnated separately to hardwood blocks. Control blocks consisted of wood blocks not treated with the soluble sugar or amino acid solutions.

2.2.2.2. Impregnation with 0.5% w/v CCA solution

In the preservative studies, wood blocks were impregnated with 0.5% w/v CCA solution (BS4072:1974). Wood blocks were placed in litre beakers and weighted down with glass slides. Each beaker was placed in turn, in a vacuum dessicator and a vacuum was pulled for 15 minutes.

After this period, the CCA solution was introduced and the residual vacuum was released (BS6009:1982). The wood blocks were submerged for 30 minutes in the treating solution before being removed and blotted dry. The wet weights of the blocks were recorded. Softwood test blocks were impregnated separately to hardwood blocks.

2.2.2.3 Curing and fixation of preserved wood blocks

The preserved wood blocks were cured and fixed in large glass dessicators in which the dessicant had been removed.

Wood blocks were placed with their 10mm x 10mm faces in horizontal plane in glass petrie dishes. These dishes were then placed on top of the wire mesh in the dessicator. Deionised water (10ml) and a small amount of xylene in a glass vessel were placed at the base of the dessicator. The dessicators were kept sealed for two weeks, partially open for a third week and fully opened for the final week. Wood blocks were turned onto alternate 10mm x 10mm faces twice weekly. After the fourth week, petrie dishes were removed from the dessicator and wood blocks were allowed to dry at laboratory temperatures for a further week.

2.2.2.3. Impregnation of preserved wood blocks with soluble amino acid and sugar solutions

Preserved wood blocks were subjected to a second impregnation with either amino acid or sugar solutions. The impregnation procedure employed was similar to that described in 2.2.2.1. However, in this instance, wood blocks were submerged in the appropriate treating solution for 5 minutes, to prevent loss of preservative to the solution. After this period, blocks were removed from the solution, blotted dry and weighed. In the preservative studies, control blocks consisted of unpreserved wood blocks not treated with CCA and, preserved wood blocks which were not subjected to impregnations with either amino acid or sugar solutions.

2.2.3. Modifications to soil burial experiments

Soil burial experiments were modified in each instance in order to investigate the role of individual soluble nutrient components (carbohydrates and amino acids) on decay of wood and preservative efficacy. The modifications made to each experiment are described in the next section.

2.2.3.1. Soil Burial experiments using unpreserved wood

a) Experiment 1

In this study the role of simple sugars on the decay susceptibility of hardwoods and softwoods was investigated. Wood blocks were impregnated with sugar solutions containing glucose and fructose in ratios of 1:1. Glucose and fructose were chosen as these sugars were shown to be the predominant sugars in surface regions of dried wood. Test specimens were impregnated with appropriate concentrations of sugars to attain in the blocks, approximate weight increments of 1%w/w, 3%w/w, 5%w/w and 7%w/w in spruce, pine and lime blocks, and 1%w/w, 3%w/w 4%w/w and 6%w/w in beech blocks. These retention values obtained are probable values determined by the liquid uptake of sugar solutions. The treating concentration of the sugar solutions and the mean percentage weight increment in each group of blocks are presented in Appendix 2. After impregnation with sugar solutions, blocks were dried at laboratory temperatures, for 48 hours prior to soil burial. Five replicate blocks of each treatment (including controls) were analysed at sampling periods of 0, 3, 6, 9 and 12 weeks. A total of 500 blocks were used in this study.

b) Experiment 2

The purpose of this study was to determine if amino acids incorporated into wood blocks as sources of nitrogen influenced decay in hardwoods and softwoods. Solutions containing equimolar quantities of aspartic acid, glutamine and arginine were used to impregnate the wood blocks. These amino acids were selected as they were the major amino acids present in the woods examined (2.1) and also represent an acidic, neutral and basic amino acid respectively. A sample calculation of the procedure used to obtain the amino acid concentrations and a table showing the treating concentrations of amino acids are presented in Appendices 3 and 4 respectively.

In this study, blocks were impregnated with varying concentrations of the amino acid solution to provide 'amino acid blocks' of the following added nitrogen contents, 0.2% 0.3%. 0.5% and 0.9%. Nitrogen content of test blocks were raised to 0.2% and above as soil burial studies at this laboratory showed that significant mass losses (>3%) were observed in test specimens when nitrogen contents were at 0.2% (Mowe, 1983).

High nitrogen contents of 0.5% and 0.9% were included to determine if these concentrations would stimulate more rapid colonisation of wood by micro-organisms, since nitrogen is no longer a limiting factor. After impregnation, wood blocks were blotted dry, weighed and then dried in a fan over at 40°C for 48 hours. Six replicate blocks of each treatment were analysed at the following sampling periods, 0, 3, 6, 9 and 12 weeks. A total of 600 blocks were analysed in this study.

c) Experiment 3

In the previous study with amino acids blocks after treatment were allowed to dry at 40°C prior to soil burial. Amino acids are known to react with sugars at high temperatures to form dark coloured products (Ellis, 1959). This may render the amino nitrogen unavailable as a source of nitrogen. Though no dark colouration was observed in the dried wood blocks in the first amino acid study, a second study using blocks buried at 100% moisture content was undertaken to investigate the effect drying may have on the amino acids in the block. Lime was chosen as a test species for this study as it is highly decay susceptible. Wood blocks were impregnated with a solution of amino acids (3.60%w/v) containing equimolar concentrations of aspartic acid, glutamine and arginine. At this amino acid concentration, nitrogen contents of the blocks were increased to 0.9%. Blocks were treated with the highest concentration of amino acid to allow decay to occur more rapidly in the blocks, since nitrogen is no longer a limiting factor. Control blocks in this experiment were impregnated with deionised water. After impregnation, all blocks were blotted dry, weighed and their wet weights recorded. Blocks were weighed again periodically until block weight showed 100% moisture content when evaluated on the initial dry weights of the block. Six replicates of each treatment were sampled at each sampling period over the 12 week soil burial period. A total of 60 blocks were used in this study.

d) Experiment 4

As studies undertaken to this point had only investigated the effect of either sugars or amino acids on wood decomposition, this study examined the effect of a mixture of these two soluble components on the decay of wood.

Lime was chosen as a test species as decay is seen rapidly in this wood species. Wood blocks were impregnated with a solution containing a mixture of sucrose (6%w/v) and arginine (2%w/v), both these components being major soluble nutrient components in lime. The treating concentration used resulted in wood blocks attaining an 8% weight increment due to sugar, and a nitrogen content of 0.8%, both these retention values being probable values determined by liquid uptake. This treating concentration was chosen so that the soluble nutrients were not limiting factors to decay of wood by micro-organisms. Control blocks were blocks impregnated with deionised water. All blocks after impregnation were buried at 100% moisture content. Five replicates of each treatment were analysed at sampling periods, 0, 1, 3 and 6 weeks. The sampling periods were reduced as previous studies showed the loss of the added nutrients to the soil within three weeks of emplacement of wood blocks in the soil. A total of 60 blocks were used in this study.

e) Experiment 5

The results of the soil burial experiments undertaken showed that soluble nutrients artificially incorporated into wood blocks were lost to the soil system during soil burial. This was apparent in studies in which blocks were impregnated with amino acid solutions. As the nitrogen contents of these blocks decreased significantly during the first three weeks of soil burial, the purpose of this experiment therefore, was to investigate the time taken for the amino acids to leach from wood blocks after emplacement in the soil. Pine was chosen as a test species as this wood species has a high permeability and high durability status. Thus the loss of amino acids measured from the nitrogen contents can be monitored as the results obtained would be real and not artefacts of weight loss. Pine blocks containing redistributed soluble nutrients were also included in this study. This provided comparisons between blocks with high nutrient status and those with added nutrients to achieve a similar status.

Wood blocks were impregnated with a solution of arginine (1.9%w/v) to raise the nitrogen contents of the wood blocks to 0.8%. Arginine was chosen as this amino acid is a major amino acid component in pine and has a high nitrogen content.

Blocks containing redistributed soluble nutrients were impregnated with deionised water for 5 minutes to prevent loss of nutrients. Half the quantity of these blocks and those impregnated with arginine were air dried at ambient temperature to 100% moisture content. The remaining half were air dried at ambient temperature to approximately 10% moisture content. Control blocks i.e. those without redistributed soluble nutrients or arginine, were buried at 100% moisture content. A total of 150 wood blocks were used in this experiment, with six replicate blocks of each treatment analysed at sampling periods of 0, 4, 8 and 12 days.

2.2.3.2. Soil burial experiments using preserved wood

a) Experiment 6

This preliminary study was undertaken to investigate the influence of amino acids on the decay susceptibility of preserved wood blocks. Lime was selected as a test species as it is more decay susceptible than either beech, spruce or pine, and arginine was selected as the amino acid to be tested as it has a high nitrogen content and is also a major soluble amino acid component in lime. Preserved wood blocks were impregnated with varying concentrations of arginine to achieve, in the wood blocks, nitrogen contents of 0.3%, 0.5% and 0.7%. A tabular summary of the amino acid concentrations used is presented in Appendix 5. Blocks after impregnation were buried at 100% moisture content. The sampling periods for this experiment were 0, 3, 6 and 12 weeks. Five replicates of each treatment were analysed at these sampling periods. Control blocks in this study consisted of unpreserved wood blocks (i.e. blocks not treated with CCA), and preserved blocks which were not impregnated with arginine.

b) Experiment 7

The results of the preliminary study prompted a detailed investigation into the effect of amino acids on preserved wood. In this study, the influence of glutamine on preserved wood blocks of lime, beech, pine and spruce were investigated. Glutamine was selected to allow comparisons to be made with arginine, the other major soluble amino acid component in wood extracts. Preserved wood blocks were impregnated with concentrations of glutamine to raise nitrogen contents in blocks to 0.2%, 0.3%, 0.5% and 0.7%.

The concentration of glutamine used to achieve these levels of nitrogen are presented in Appendix 5. Control wood blocks in this study were unpreserved wood blocks and preserved wood blocks without glutamine impregnations. A total of 720 wood blocks were used in this experiment, with six replicates of each treatment analysed at sampling periods of 0, 3, 6, 9 and 12 weeks. Analysis of copper and chromium contents of unburied and buried preserved wood blocks were also undertaken. Wood blocks in this study were buried in an air-dried condition to allow comparisons to be made with other CCA soil burial studies undertaken at this laboratory.

c) Experiment 8

Concurrent with the glutamine study (Experiment 7), a soil burial study to investigate the influence of soluble sugars on preserved blocks of lime and pine was also undertaken. Lime and pine were chosen as a representative hardwood and softwood species respectively. Lime test blocks were impregnated with sucrose (a major soluble carbohydrate component in the extracts of lime) and pine with glucose (a major soluble carbohydrate component in the extracts of pine) to obtain in both wood types, sugar concentration of 0.5%w/w, 1%w/w, 2%w/w and 4%w/w. The treating concentration of sugars used is presented in Appendix 5. Wood blocks after impregnation were buried in an air dried condition. A total of 320 blocks were analysed at sampling periods of 0, 3, 6, 9 and 12 weeks.

2.2.4. Soil burial

2.2.4.1. Preparation of soil

The soil used was obtained from an unfertilised site at the Scottish Crop Research Institute in Invergowrie. The soil was taken back to the laboratory in large containers and sieved through a 2mm mesh sieve to remove stones and litter. The water holding capacity of the sieved soil was then determined (Savory and Carey, 1973) on four samples of approximately 200 grams each, from each container of soil.

2.2.4.2. Burial of test blocks

Preweighed plastic food boxes measuring 28cm in length, by 20cm in width and 10cm deep were used as soil containers.

Each container was filled to a depth of 3.5cm with sieved soil and taken up to the appropriate water holding capacity. Soil in containers designated for hardwoods were taken up to 80% water holding capacity, while soil in containers designated for burial of softwood blocks were taken to 100% water holding capacity. Paper templates were prepared and these covered the surface of the containers and were used to record the position of the buried blocks, so that they could be recovered at the end of the burial period. Each box contained blocks of different treatments placed randomly within the box. Hardwood and softwood blocks were buried in separate containers. The blocks were pressed firmly into the soil with the 10mm x 10mm radial face of the blocks in horizontal plane. The position of each block in its container was recorded and all containers were then filled with another 3.5cm of sieved soil. The soil in each container was levelled and the containers were reweighed and wetted evenly over the surface with deionised water, to bring the soil to 80% of its water holding capacity for boxes of lime and beech, and to 100% water holding capacity for boxes of spruce and pine. Lids were placed loosely over each container to reduce the rate of moisture evaporation and also to permit gaseous exchange. All containers were incubated in the dark at 25°C and weighed twice weekly and where necessary, deionised water was added evenly over the surface to maintain the soils at the required water holding capacity.

2.2.4.3. Sampling

Test blocks were removed from the soil containers at sampling periods of 3, 6, 9 and 12 weeks. Unburied blocks of each wood species containing 5 - 6 replicates of each treatment were designated as unburied controls. At each sampling period, templates were used to relocate the buried blocks. Adhering soil was removed from the blocks and the blocks were weighed prior to drying. The blocks were dried to constant weight in an oven at 103°C, cooled in a dessicator and weighed again.

2.2.5. Analyses of test blocks

2.2.5.1. Moisture contents

Moisture contents of buried blocks were determined on the post-burial dry weight of the blocks and calculated by the formula:

$$\text{Moisture Content (\%)} = \frac{\text{Wet weight of block} - \text{Final dry weight of block}}{\text{Initial dry weight of block}} \times 100$$

2.2.5.2. Weight loss

Weight losses of blocks in Experiment 1 of the soil burial studies were determined on the preburial weight of the block and also the preburial weight of the block and the weight of added sugar. Weight losses of test blocks in subsequent studies (Experiments 2 - 8) were determined on the preburial weight of the blocks alone. The formulae used in the calculation of these weight losses are:

a. $$\text{Weight loss (\%)} = \frac{\text{Initial weight of block} - \text{Final weight of block}}{\text{Initial weight of block}} \times 100$$

b. $$\text{Weight loss (\%)} = \frac{\text{Weight of block and sugar} - \text{Final dry weight of block}}{\text{Weight of block and sugar}} \times 100$$

2.2.5.3. Nitrogen content analysis

The nitrogen content of the blocks were determined using the micro-kjeldahl technique (Humphries, 1956). A modification of this technique has been developed at this laboratory in which a single wood digest can be used to determine both the nitogen content and also the copper, chrome and arsenic contents in preservative treated blocks (King et al, 1981b). This method involves the use of hydrogen peroxide (100 volumes) as an oxidant in the concentrated sulphuric acid digest. The procedure used in the nitrogen analysis is described below.

a) Acid digestion of test blocks

Wood blocks to be analysed were chipped using a Stanley Knife and the wood chips were placed in micro-kjeldahl flasks (30ml). Analar nitrogen free concentrated sulphuric acid (2ml, 18.4M) was added to each flask, followed by twelve drops (from a pasteur pipette) of hydrogen peroxide. The flasks were heated gently over gas in a fume cupboard. Digestion was stopped when fumes of sulphuric acid were observed. Flasks were cooled and a further twelve drops of hydrogen peroxide was added and the heating resumed. This was repeated until the digest in the flask was clear. The time for complete digestion varied from 60 - 90 minutes. A digest blank containing concentrated sulphuric acid (2ml) was included in the analysis.

b) Distillation

After the completion of the acid digestion of wood blocks, the flasks were allowed to cool and the contents were transferred to the distillation chamber of a Markham apparatus. The digest blank was first steam distilled through the apparatus. The micro-kjeldahl flasks were rinsed three times with deionised water (3ml) and each time the rinsings were transferred to the chamber. Ammonia was steam distilled from the chamber after addition of 40%w/v NaOH. The distillate from this chamber was collected in Erlenmeyer flasks (100ml capacity) containing 2%w/v boric acid (5ml) and five drops of the kjeldahl indicator (methylred/ethanolic bromocresol green indicator). The distillation was continued in all cases until the volume in the Erlenmeyer flasks reached the 25ml mark. Ammonia - Nitrogen in the distillate was determined by titration with standard 0.01MHCL (1ml of 0.01MHCL=0.14mgN). The final titres of the samples were corrected by subtracting the titre from the digest blank. This corrects for any nitrogen that might be present in minimal quantities in the reagents used.

2.2.5.4. Analysis of the copper and chromium contents in wood digests

Analysis of copper and chromium contents were undertaken on wood blocks from Experiment 7 of the soil burial studies. This experiment was a detailed study into the influence of amino acids on decay and preservative toxicity and stability in hardwoods and softwoods. Analysis of arsenic contents were not undertaken due to technical problems. Results of arsenic analysis performed by co-workers at this laboratory showed low sensitivity and large variability. As the time remaining for the experimental analysis was insufficient to allow for these problems to be solved, it was decided to omit arsenic analysis from this study.

In each instance, the wood digest after nitrogen determinations was collected via an outlet tube into a 100ml - Erlenmeyer flask, containing 12 drops of the kjeldahl indicator. The inner chamber of the Markham apparatus was rinsed with small amounts of deionised water. All rinsings were added to the Erlenmeyer flask. Approximately 75mls of the solution was collected in each flask. The solution when cooled, was acidified with sulphuric acid (2.5M, 10 - 15mls) until a colour change from yellow to pink was observed.

The acidified digests were then filtered (Whatman's No. 1) into 100ml volumetric flasks. The solution in each flask was taken up to the mark with deionised water. All glassware used in the analysis were of standard 'A' grade glassware.

The copper and chromium contents were determined by atomic absorption spectrometry using the method of standard additions. The digest (10ml) from the 100ml volumetric flask was pipetted into each of three 25ml volumetric flasks labelled A, B and C. In flasks B and C of the 25ml volumetric flasks, a standard containing 25ppm copper and chromium and 250ppm arsenic was added. 1ml and 2mls of this standard was added to flasks B and C respectively. The standard solution was not added to the contents in flask A. After addition of the standard solution, all flasks were taken up to the 25ml mark.

The detection of the metal elements in the solution was undertaken using an atomic absorption spectrophotometer (Perkin Elmer). Copper was determined using a hollow copper cathode lamp (λ = 324.8nm) and chromium was detected using a hollow chromium cathode lamp (λ = 357.9nm). For the detection of each element, the machine was maximised prior to analysis with a machine standard (BS 5666:Part 3:1979). For copper, the standard was at a concentration of 1ppm and for chromium the concentration was at 2ppm. During the analysis, the spectrophotometer was aspirated with deionised water between each set of three 25ml volumetric flasks. A mean of six readings for each flask was taken. The sensitivity of the spectrophotometer was checked at regular intervals and maximised using the appropriate machine standard to regain sensitivity. The concentration of the elements in the solution can be evaluated using the formula below

$$Cu = \frac{S}{\frac{(S_1-S) + (S_2-S)}{3}} = X\ \mu g/ml$$

where S = absorbance reading from flask A

S_1 = absorbance reading from flask B

and S_2 = absorbance reading from flask C

Total concentration of Cu in 100mls digest solution = $X \times \frac{25}{10} \times 100$

$$\% Cu\ (w/w) = \frac{X \times 250}{10000 \times \text{wt of block}}$$

The % Cr (w/w) was evaluated in a similar manner.

CHAPTER 3

RESULTS

3.0 Results

The results of all the experiments undertaken as part of this thesis are described in this chapter in two sections. The first section (3.1) details the results obtained from the experiments undertaken to determine the soluble carbohydrate and nitrogenous components in softwoods and hardwoods. The second section (3.2) describes the results obtained from the soil burial studies undertaken to evaluate the biological influence these materials have on wood decomposition.

3.1. Studies to determine the soluble nutrient components in wood

3.1.1. The distribution and redistribution of soluble nutrients in the sapwood and heartwood regions of spruce (Experiment 1)

This preliminary experiment was undertaken to determine the distribution of soluble carbohydrate and nitrogenous materials in the outer sapwood, inner sapwood and heartwood regions of green spruce. The experiment was also undertaken to determine if redistribution of these soluble nutrients occurred during drying.

Results of the analysis of soluble nutrients in green and dried spruce, soluble proteins and soluble amino acids are presented in Figures 3.1, 3.2 and 3.3. A tabular summary of these results is presented in Appendix 6. Results from the determinations of soluble carbohydrates and soluble proteins are expressed as a percentage of the initial dry weight of wood, and results from the determination of soluble amino acids are expressed as μmoles amino acid per gram of wood. Greenwood samples were corrected for moisture from the moisture content figure presented in Appendix 1.

In general, soluble carbohydrates constituted a greater proportion of the wood mass than either soluble protein or soluble amino acids in both green and dried wood. Concentrations of these soluble nutrients displayed a radial distribution pattern in which highest concentrations were observed in the outer sapwood regions and lowest in the heartwood regions. Dried wood displayed redistribution and accumulation of soluble nutrients at the surface regions, and concentrations of soluble materials at these regions were higher than those found at sub-surface regions.

In both green and dried wood, reducing sugars contributed to a significant proportion of the total carbohydrate content. Glucose and fructose were the predominant sugars in each instance.

Results of the analysis of green spruce for soluble carbohydrates, soluble proteins and soluble amino acids are presented in Figure 3.1. The total carbohydrate content in green spruce ranged from 0.60% in the outer sapwood to 0.20% in the heartwood region. Reducing sugars contributed 70% of the total carbohydrate content in the outer sapwood regions but to a lesser amount in the inner sapwood and heartwood regions. Concentrations of glucose and fructose were broadly similar to each other at each region of the wood tested. The combined concentrations of glucose and fructose ranged from 0.2% in the outer sapwood region to 0.02% in the heartwood region. Glucose and fructose concentrations contributed approximately 50% of the total reducing sugar content in green spruce. In contrast, soluble protein concentrations constituted 0.06% of the mass of wood in the outer sapwood regions. These materials displayed concentrations ranging from 0.06% in the outer sapwood to 0.01% in the heartwood region. Soluble amino acids displayed similar radial distribution patterns as observed for soluble carbohydrates. Concentrations of soluble amino acids ranged from 1.6 μmol/g in the outer sapwood region to 1.30 μmol/g in the inner sapwood region to 0.30 μmol /g in the heartwood region.

Results of the analysis undertaken on the outer sapwood, inner sapwood and heartwood regions of dried surface and sub-surface samples of spruce are presented in Figures 3.2 and 3.3 and in a tabular form in Appendices 6 and 7. The analyses undertaken were the same as those described for green spruce, viz., total carbohydrate content, reducing sugar content, glucose and fructose concentrations, soluble protein content and soluble amino acid content. Concentrations of these materials followed a similar pattern to those observed in the green material i.e. decreasing concentrations with increasing distance from the cambium. Total carbohydrate content displayed highest concentrations followed by reducing sugar, glucose and fructose, soluble protein and soluble amino acids.

The total soluble carbohydrate content in surface samples of dried spruce was twice the concentration found in sub-surface samples.

Differences in concentrations of soluble materials in the sapwood and heartwood regions were more marked in surface samples than in sub-surface samples. In surface samples, values for the total carbohydrate content ranged from 1% in the outer sapwood regions to 0.42% in the heartwood regions. In sub-surface samples, values for the total carbohydrate content ranged from 0.50% in the outer sapwood to 0.30% in the heartwood regions. Reducing sugars contributed 70% of the total carbohydrate content in surface samples but only 32% in sub-surface samples. As with greenwood, glucose and fructose were the predominant sugars.

The concentration of soluble protein in surface samples of spruce ranged from 0.17% in the outer sapwood to 0.03% in the heartwood region. Soluble protein concentrations at surface regions of dried spruce were three times those of greenwood and three times those of sub-surface regions. Soluble amino acids displayed concentrations of 3.0 μmol/g wood in the outer sapwood to 0.10 μmol/g wood in the heartwood. The overall concentration of soluble amino acids in the surface regions was three times the concentrations at sub-surface regions and twice the concentrations found in green spruce. Interestingly, concentrations of soluble nutrients in sub-surface samples of dried spruce were broadly similar to those seen in green spruce.

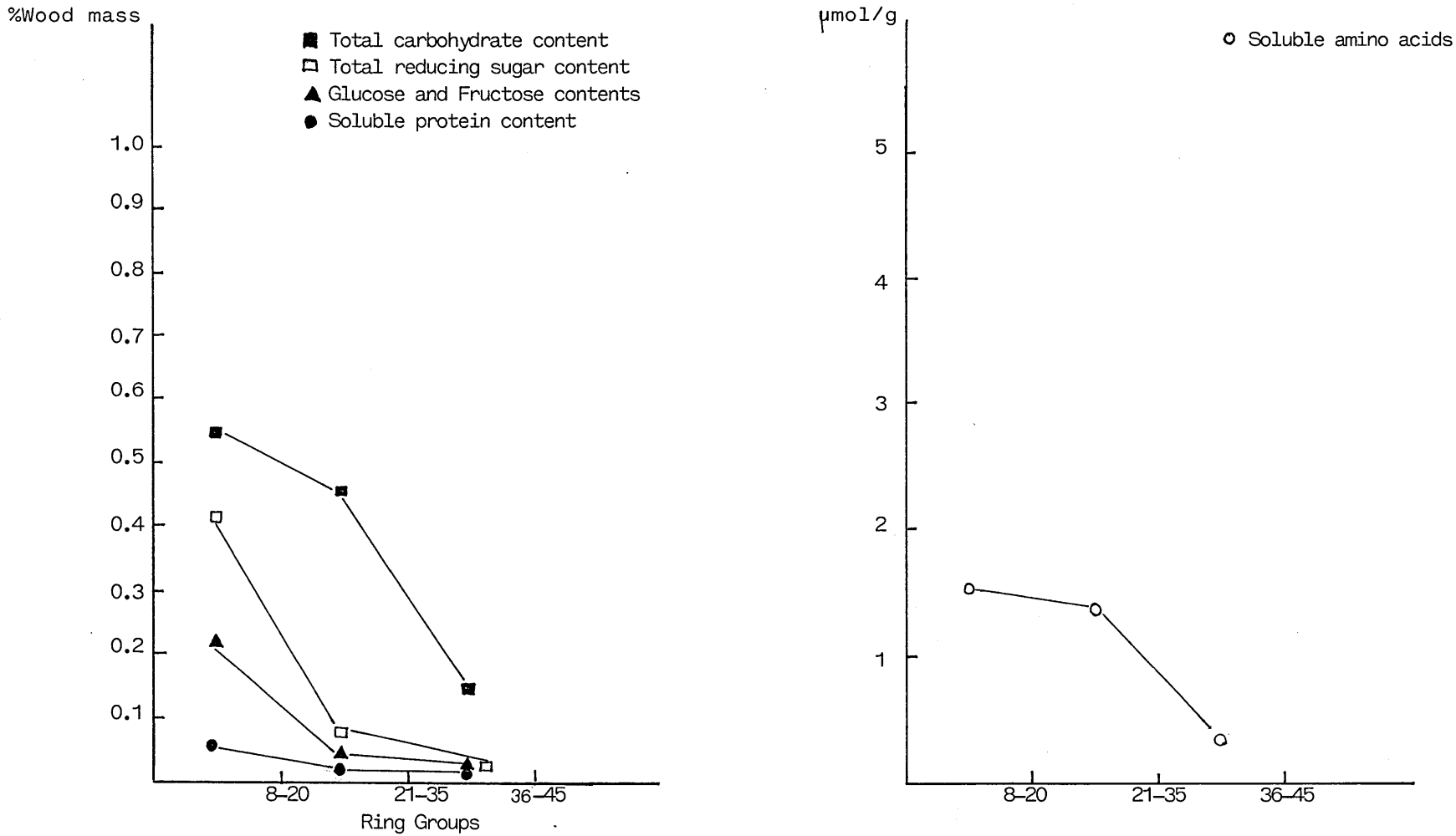


Fig 3.1 Soluble nutrient components in aqueous extracts of green spruce.
Results of soluble carbohydrate and soluble protein contents are expressed as a percentage of the initial weight of wood.
Results of the soluble amino acid contents are expressed as µmoles amino acid per gram of wood.

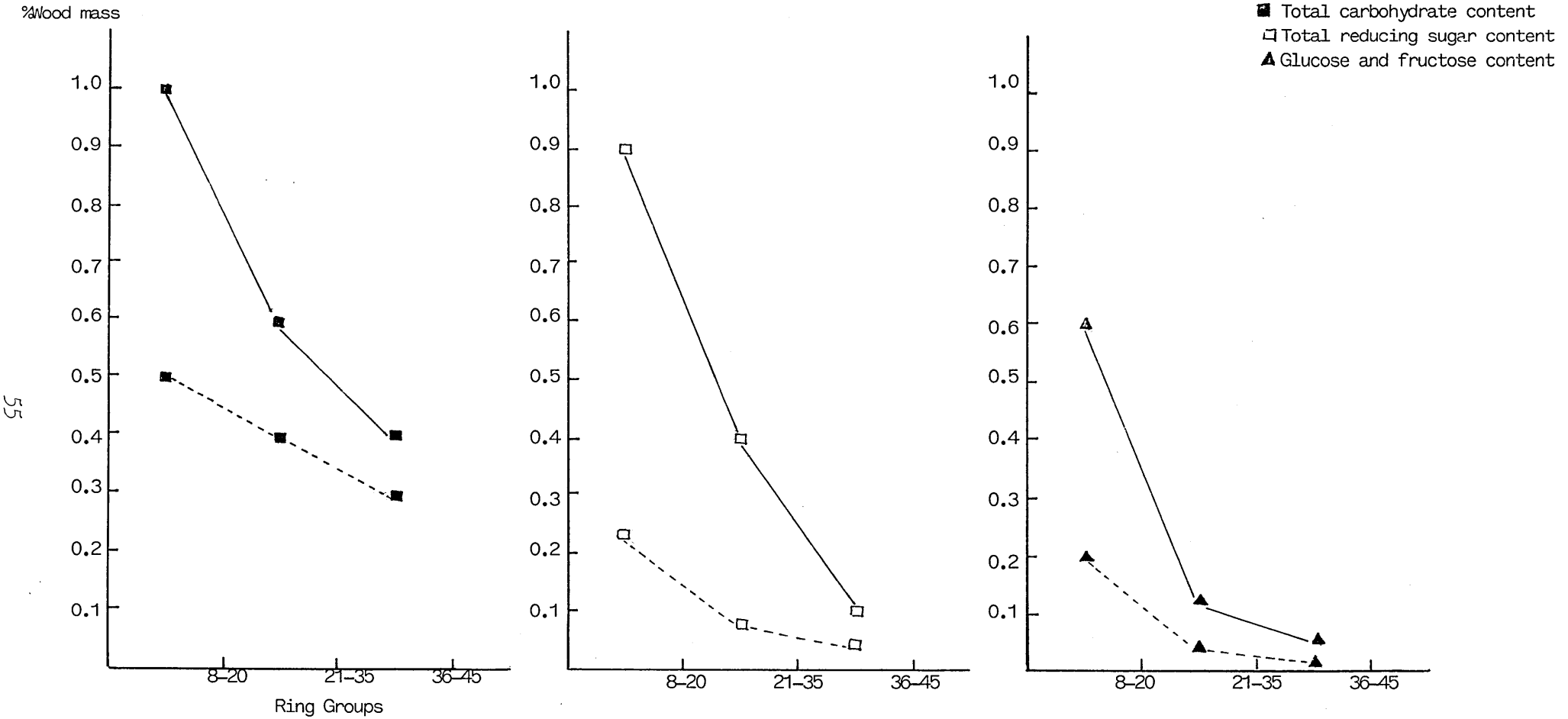


Fig 3.2 Soluble carbohydrates in surface (-) and sub-surface (---) samples of dried spruce. Results are expressed as a percentage of the initial dry weight of unextracted wood.

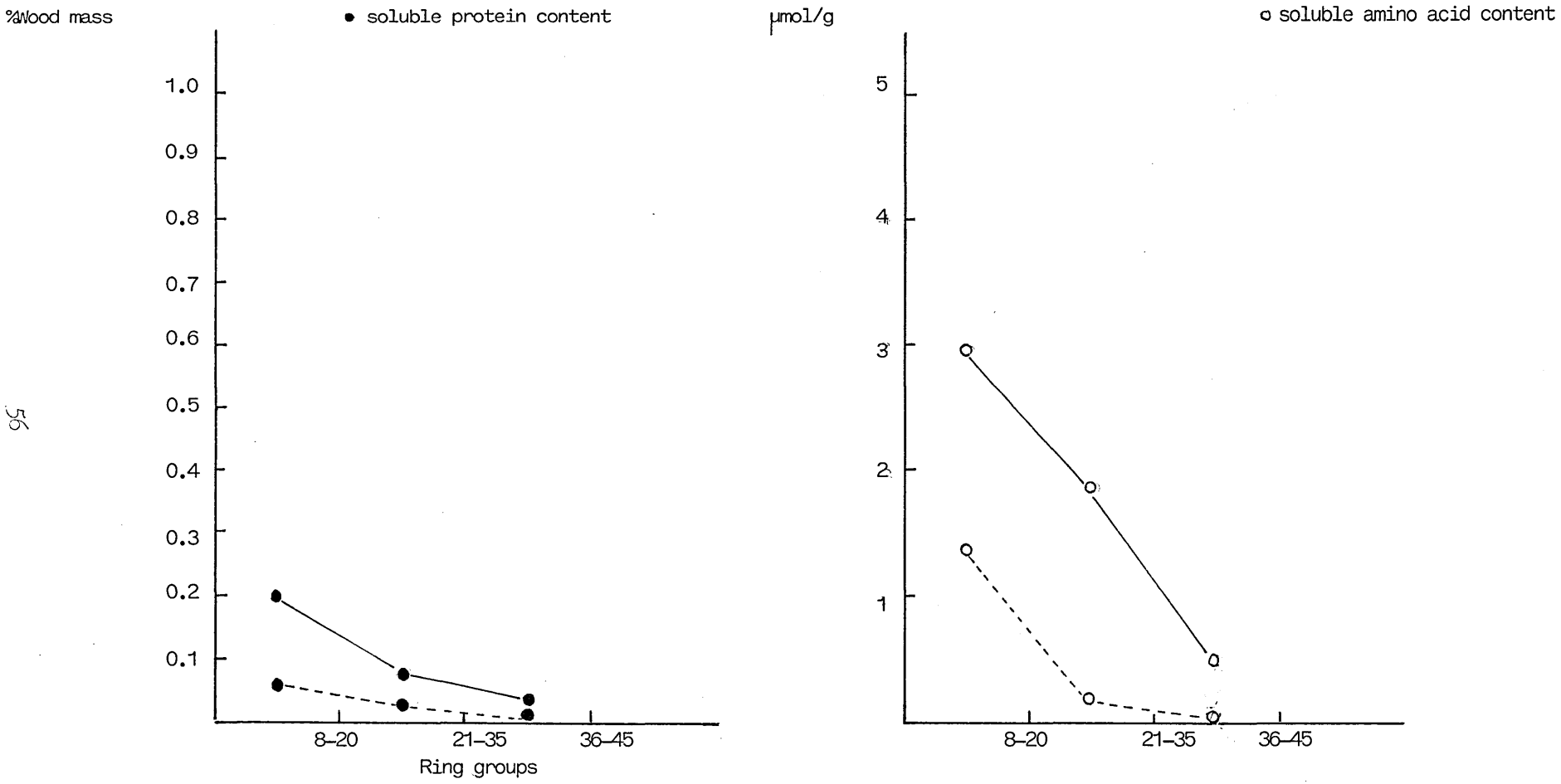


Fig 3.3 Soluble nitrogenous components in surface (-) and sub-surface (---) samples of dried spruce. Soluble protein contents are expressed as a percentage of the initial mass of wood. Soluble amino acid contents are expressed as µmole amino acid per gram wood.

3.1.2. Distribution of soluble carbohydrates and soluble amino acids in green spruce and pine (Experiment 2).

The preliminary experiment undertaken (3.1.1) showed that soluble nutrients migrate and accumulate at surface regions during drying. Nutrient gradients were also shown to occur across the sapwood and heartwood regions in greenwood. The interesting nature of the results prompted investigation of these aspects in greater detail in both spruce and pine. For this purpose, green spruce samples were divided into 10 ring groupings and green pine samples into 5 ring groupings. The greenwood samples were chipped for this experiment to determine comparability of results of analysis from milled and chipped materials. Pine was included in the experiment, to see if the redistribution effects observed with spruce, were reproducible with another softwood species. All samples were analysed for total carbohydrate content, total reducing sugar content and glucose and fructose contents. The concentrations of these soluble carbohydrate materials were expressed as a percentage of the initial dry weight of wood. Samples were also analysed for soluble amino acids by the ninhydrin assay method and by the amino acid auto-analyser. Results obtained by the former method were expressed as μmoles amino acid per gram wood and those by the latter method as μgram amino acid per gram wood. Individual amino acids were determined using the amino acid auto-analyser and concentrations of these materials were calculated using the molecular weights of the amino acids. In the ninhydrin assay, the composition of individual amino acids were not differentiated and therefore concentrations were expressed in μmol/g wood. Soluble proteins were not determined in this series of experiments. It was decided that emphasis should be given to the determinations of the major soluble free amino acids in wood, which could later be incorporated as sole sources of nitrogen in test blocks in the soil burial studies (3.2). All green and dried wood specimens were corrected for moisture from the moisture contents of wood tabled in Appendix 1.

3.1.2.1. Distribution of soluble carbohydrates and soluble amino acids in green spruce.

a) Soluble carbohydrates

The distribution of soluble carbohydrates in green spruce and in green spruce dried in chipped form are presented in Figures 3.4 and 3.5 respectively. These results are also presented in a tabular form in Appendix 8.

Graphical representation of the results were drawn to the same scale so that differences in concentrations of soluble materials between green and dried wood, and later between wood types are apparent.

The results of this study are in keeping with the trends observed in the preliminary study (3.1.1). As with the preliminary study, a steady decline in concentrations from the outer sapwood region to the heartwood region was observed for all the parameters measured. Division of the wood specimens into smaller ring groupings facilitated a clearer picture of the distribution of the soluble sugars in the outer sapwood, inner sapwood and heartwood regions. Marked differences between the concentrations of the total carbohydrate content and the total reducing sugar content were observed in the outer sapwood regions. However, in the inner sapwood and heartwood regions, the total carbohydrate content, the total reducing sugar content and the combined glucose and fructose contents were broadly similar to each other. Comparison of the concentrations of soluble carbohydrate in the preliminary study (3.1.1) to those obtained in this study, showed that the total soluble carbohydrate content in the first sugar study was three times that in the second. However, reducing sugar concentrations and the combined glucose and fructose concentrations were broadly similar in both studies.

The soluble carbohydrates present in green spruce dried in chipped form (Figure 3.5) displayed radial distribution patterns similar to green spruce. Radial distribution patterns of the total carbohydrate concentration displayed gentler gradients than those of the reducing sugar and glucose and fructose concentrations. The total carbohydrate concentration ranged from 0.6% in the outer sapwood region to 0.4% in the heartwood region. The results showed that concentrations of soluble carbohydrates were higher in the dried samples than in the green samples. Reducing sugars accounted for 90% of the total carbohydrate content in the outer sapwood regions of dried spruce chips and lesser amounts in the inner sapwood and heartwood regions. As with green spruce, the combined glucose and fructose concentrations paralleled closely to those of the total reducing sugars in both the sapwood and heartwood regions.

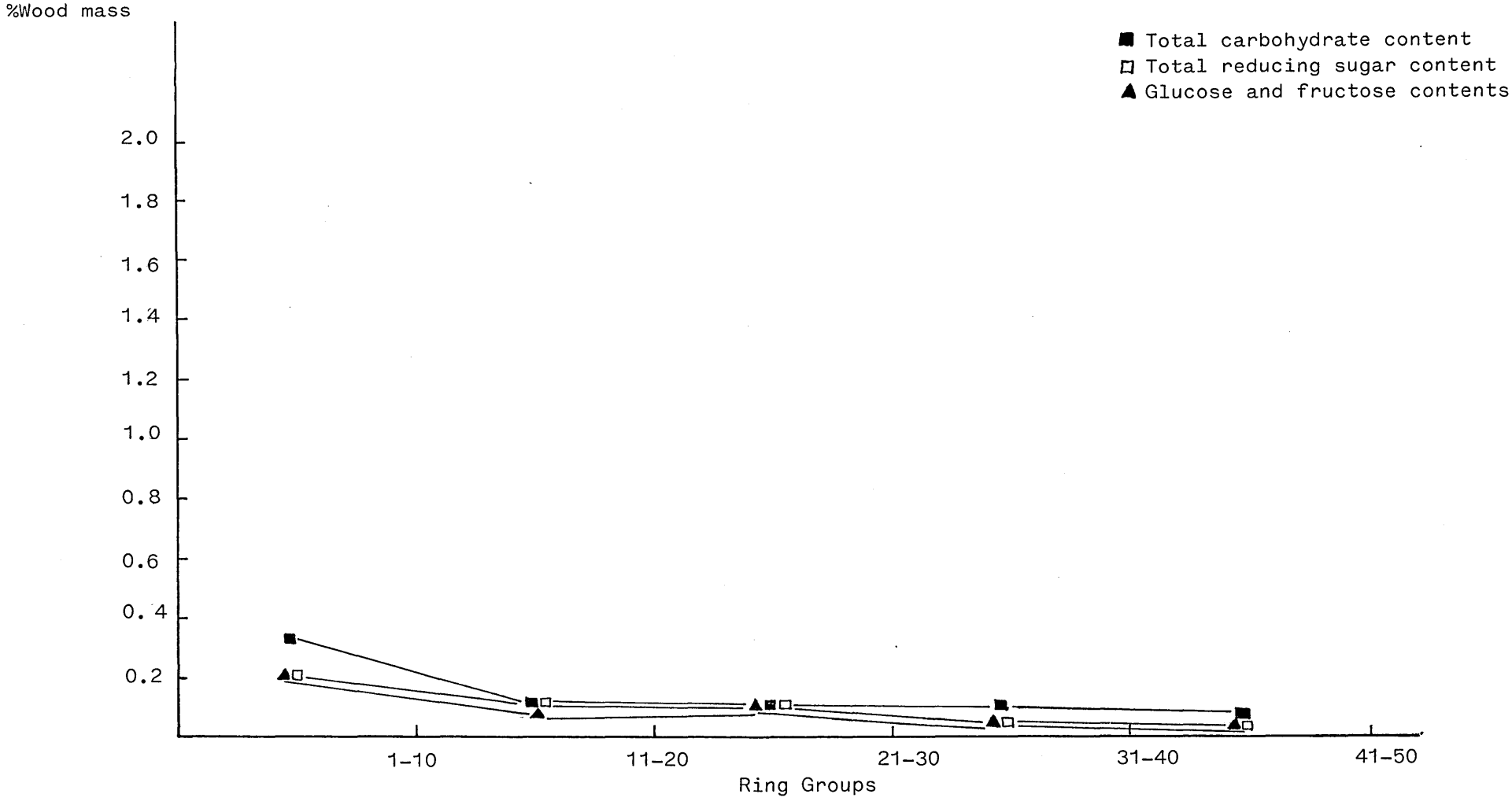


Fig 3.4 Distribution of soluble carbohydrates in green spruce.

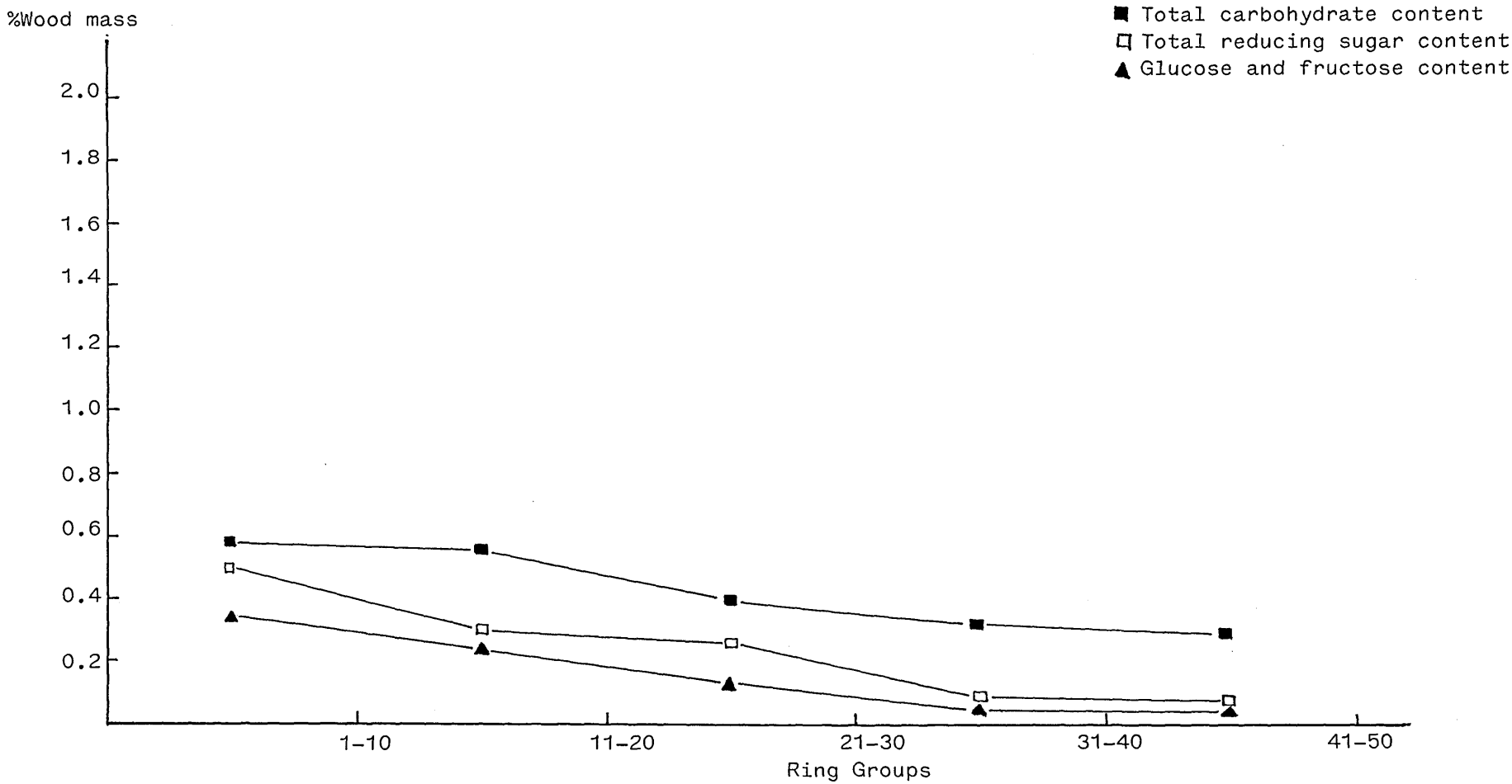


Fig 3.5 Distribution of soluble carbohydrates in green spruce dried in chipped form.

b) Soluble amino acids

The distribution of amino acids in green spruce and green spruce dried in chipped form is presented in Figure 3.6 and in a tabular form in Table 3.1. Radial distribution of amino acids were similar to those observed for soluble carbohydrates. Highest concentrations were recorded in the outer ring groups with lower concentrations in the heartwood. In green spruce, broadly similar concentrations of soluble amino acids were obtained by the two methods of amino acid analysis, the ninhydrin assay method and the amino acid auto-analyser, (Table 3.1). A comparison of the two methods of analysis could not be made for dried spruce samples, as technical difficulties with the amino acid auto-analyser prevented the completion of analysis of dried samples. Green spruce samples in this study displayed amino acid concentrations twice those of green spruce in the preliminary study. The total soluble amino acid concentration in dried spruce were one and half times that in green spruce. Amino acids in rings 41 - 50 were not detected as these were present in concentrations below the sensitivity of the assay.

The composition of amino acids as determined by the amino acid auto-analyser is presented in Table 3.2 for green spruce. Owing to technical difficulties with the auto-analyser, analysis of green spruce dried in chipped form of both spruce and pine could not be completed. Poor resolution and poor sensitivity of some of the chromatograms resulted in difficulty in the qualitative and quantitative evaluations of the amino acids. However, in spruce qualitative evaluations of ring group 1 - 10 and ring group 21 - 30 of the dried samples were made.

In green spruce, aspartic acid, glutamine, phenylalanine and arginine, were shown to be the major amino acids present in all ring groupings. Minor quantities of glycine, alanine, valine, isoleucine and leucine were also detected. These amino acids were present in trace quantities in the heartwood region. All amino acids showed a decrease in concentration from the sapwood to heartwood regions. Analysis of amino acids in ring grouping 11 - 20 was not undertaken as this sample was lost during the analysis.

The total amino acid concentrations in green spruce was 254µg/g wood. This value was calculated from the mean of the sum total of amino acid concentrations in each ring grouping. Soluble amino acids were shown to contribute to a very small percentage (∿0.03%) of the mass of wood. The calculated soluble amino nitrogen from the amino acid composition was 56µg/gwood or ∿0.006% nitrogen. Qualitative analysis of green spruce samples dried in chip form showed similar composition of amino acids to those in green spruce. Aspartic acid, glutamine and arginine were the major amino acids detected. Trace quantities of glycine, alanine, valine, isoleucine, leucine and phenylalanine were also detected.

Soluble protein concentrations as determined by the micro-assay method in 2.1.5.4. showed low concentrations of these materials in green spruce (3.1.1.). Acid hydrolysis of extracts from outer sapwood regions (rings 1-20) of green spruce in this study were undertaken to determine the soluble protein concentrations in these regions. The amino acid concentrations were determined by the amino acid auto-analyser.

The composition of amino acid present in unhydrolysed and hydrolysed samples of green spruce is presented in Table 3.3. The composition of amino acids in the hydrolysates of green spruce were similar to those in unhydrolysed spruce. The major amino acids in the hydrolysate of spruce were aspartic acid, glutamic acid, glycine and arginine. Glutamine, a major amino acid in unhydrolysed samples of green spruce was, on hydrolysis, deaminated to glutamic acid. The hydrolysates also yielded minor quantities of threonine, serine, proline and lysine which were not detected in the unhydrolysed samples. Concentrations of each amino acid detected were higher in the hydrolysates than in the unhydrolysed samples. The total amino acid concentrations in the hydrolysates were one and half times those of the unhydrolysed samples. As the total amino acid concentration indicates the soluble protein concentration in green spruce, it is clear that soluble protein is present in low concentrations.

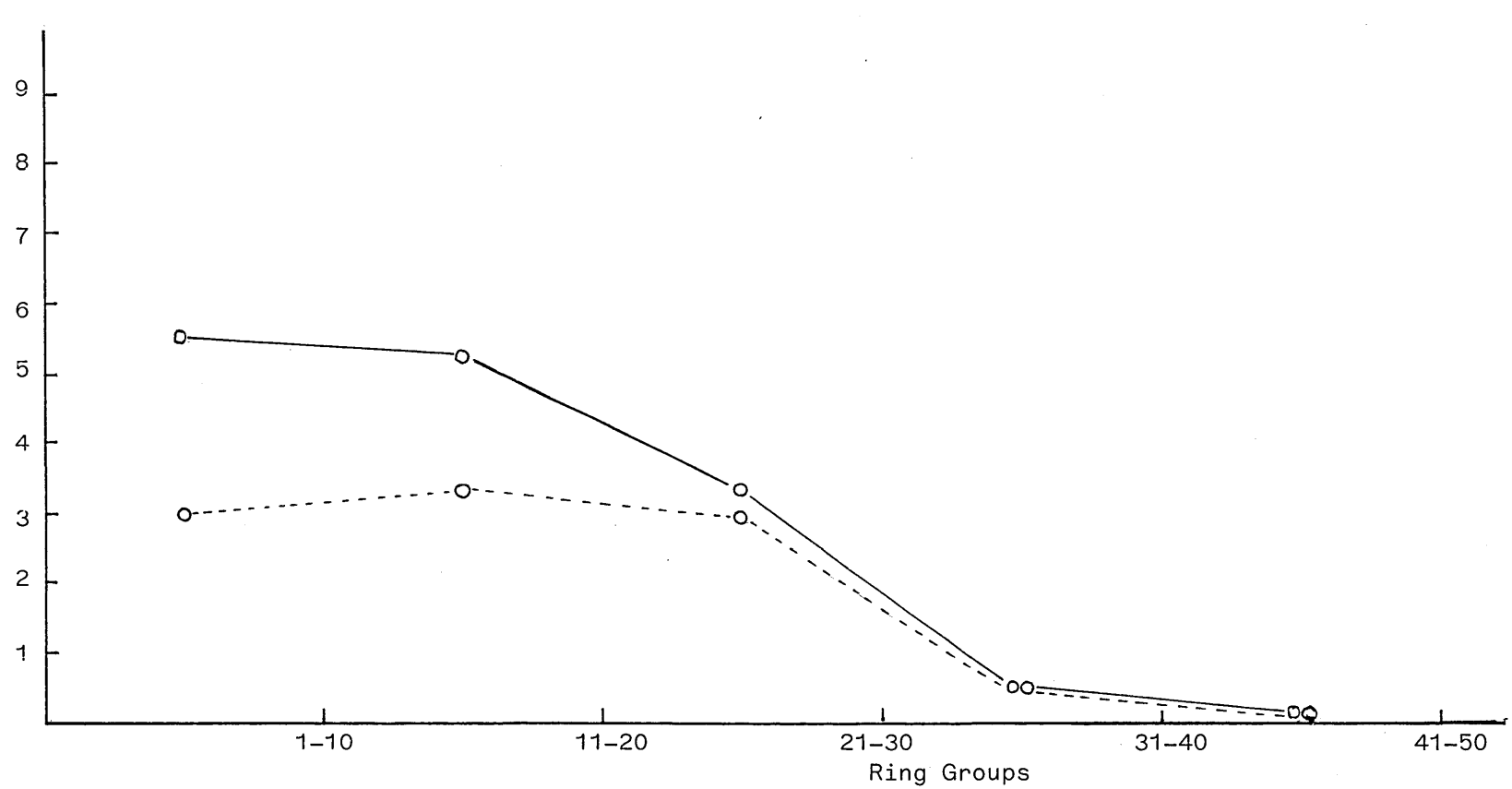


Fig 3.6 Distribution of soluble amino acids in green spruce (---) and green spruce dried in chipped form (—).

Table 3.1 Soluble amino acid concentrations in green spruce and in green spruce dried in chip form

Ring Groups	µmoles amino acid per gram wood		
	Green Spruce		Green spruce dried in chip form
	Ninhydrin assay	Amino acid autoanalyser	
1 - 10	3.00	4.35	5.56
11 - 20	3.35	NT	5.24
21 - 30	3.00	2.16	3.31
31 - 40	0.45	0.12	0.53
41 - 50	-	-	-
Average	2.45	2.21	3.66

NT no trial
\- not detected

Table 3.2. Soluble amino acid composition in green spruce

Ring Group / Amino Acid	µgram amino acid per gram wood				
	1-10	11-20	21-30	31-40	41-50
asp	98	94.43	NT	6.65	6.25
gln	129	85.26	"	5.84	5.84
gly	2.25	2.25	"	tr	tr
ala	15.93	6.23	"	tr	tr
val	7.02	4.68	"	tr	tr
ile	5.24	3.98	"	tr	tr
leu	11.79	6.55	"	tr	tr
tyr	-	-	"	-	-
phe	59.40	19.80	"	tr	tr
lys	-	-	"	-	-
his	-	-	"	-	-
arg	339	88.84	"	6.21	1.74
Total	669	312	"	18.70	13.83
Average µgaa/gwood	253.38				
Soluble amino nitrogen	56.44				

tr <1 µg/gwd
- not detected
NT No trial

Table 3.3 Soluble amino acid in unhydrolysed and hydrolysed outer sapwood samples of green spruce.

Amino Acid	µg amino acid/g wood	
	Unhydrolysed sample	Hydrolysed sample
asp	96.22	105.19
thr	-	11.18
ser	-	27.41
glu	-	145.23
pro	-	29.44
gly	2.25	115.27
ala	11.08	40.94
val	5.85	11.29
ile	4.86	8.58
leu	9.17	16.37
phe	39.60	49.70
lys	-	17.50
gln	129	-
arg	213.87	218.37
Total amino acid content	512	796.62
Total soluble amino nitrogen	109.81	218.39

- not detected

3.1.2.2. Distribution of soluble carbohydrates and soluble amino acids in green pine.

a) Soluble carbohydrates

Results of the soluble carbohydrate analysis undertaken for green pine and green pine dried in chipped form are presented in Figure 3.7 and Figure 3.8 respectively. A tabular summary of these results is presented in Appendix 9.

Soluble carbohydrates in green pine showed a radial distribution pattern similar to that observed in green spruce. Concentrations of these materials decreased with increasing distance from the cambium. In contrast to green spruce, a greater amount of extractable soluble carbohydrate was obtained from green pine. Concentrations of these materials were twice those found in green spruce. The total carbohydrate content in green pine ranged from 0.55% in the outer sapwood region to 0.36% in the heartwood region. Interestingly, the total carbohydrate content showed small increases in concentration in the heartwood regions. This feature was not observed in spruce.

The reducing sugar content constituted a significant proportion of the total carbohydrate content in the sapwood region. However in the heartwood region, reducing sugars contributed a smaller amount of the total carbohydrate content. Glucose and fructose were the predominant reducing sugars and concentrations of these sugars followed closely to those of the total reducing sugar content. As with spruce, glucose and fructose concentrations occurred in similar amounts. Concentrations of glucose and fructose in samples from ring groups 1 - 5 displayed higher values than either the total carbohydrate content or the total reducing sugar content. This discrepancy cannot be easily explained, as previous results showed that glucose and fructose concentrations were lower than those of the total carbohydrate content and the total reducing sugar content.

The radial distribution of soluble carbohydrates in green pine dried in chipped form is presented in Figure 3.8. As with spruce, dried samples displayed higher concentrations of soluble carbohydrates than green samples.

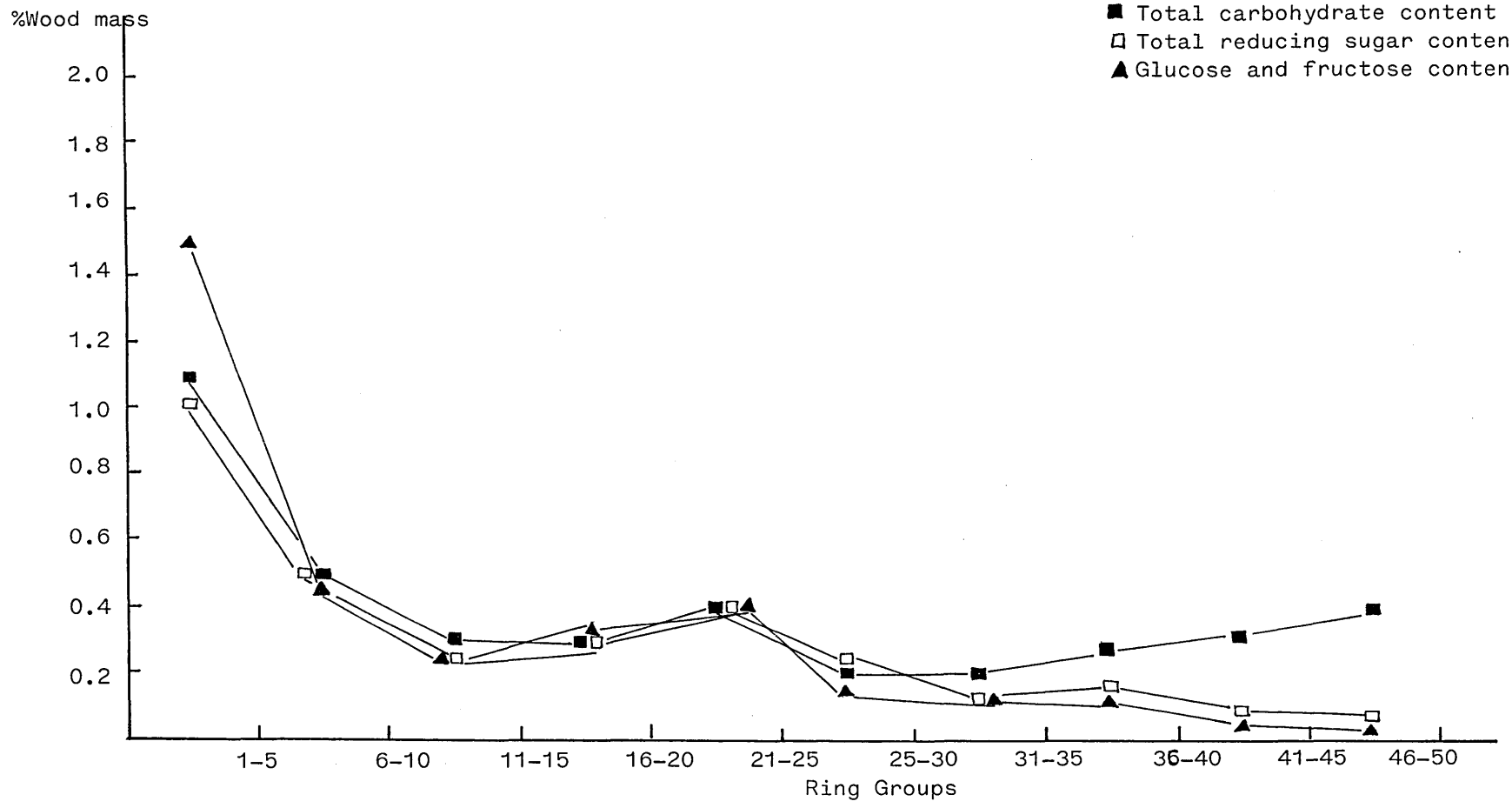


Fig 3.7 Distribution of soluble carbohydrates in green pine.

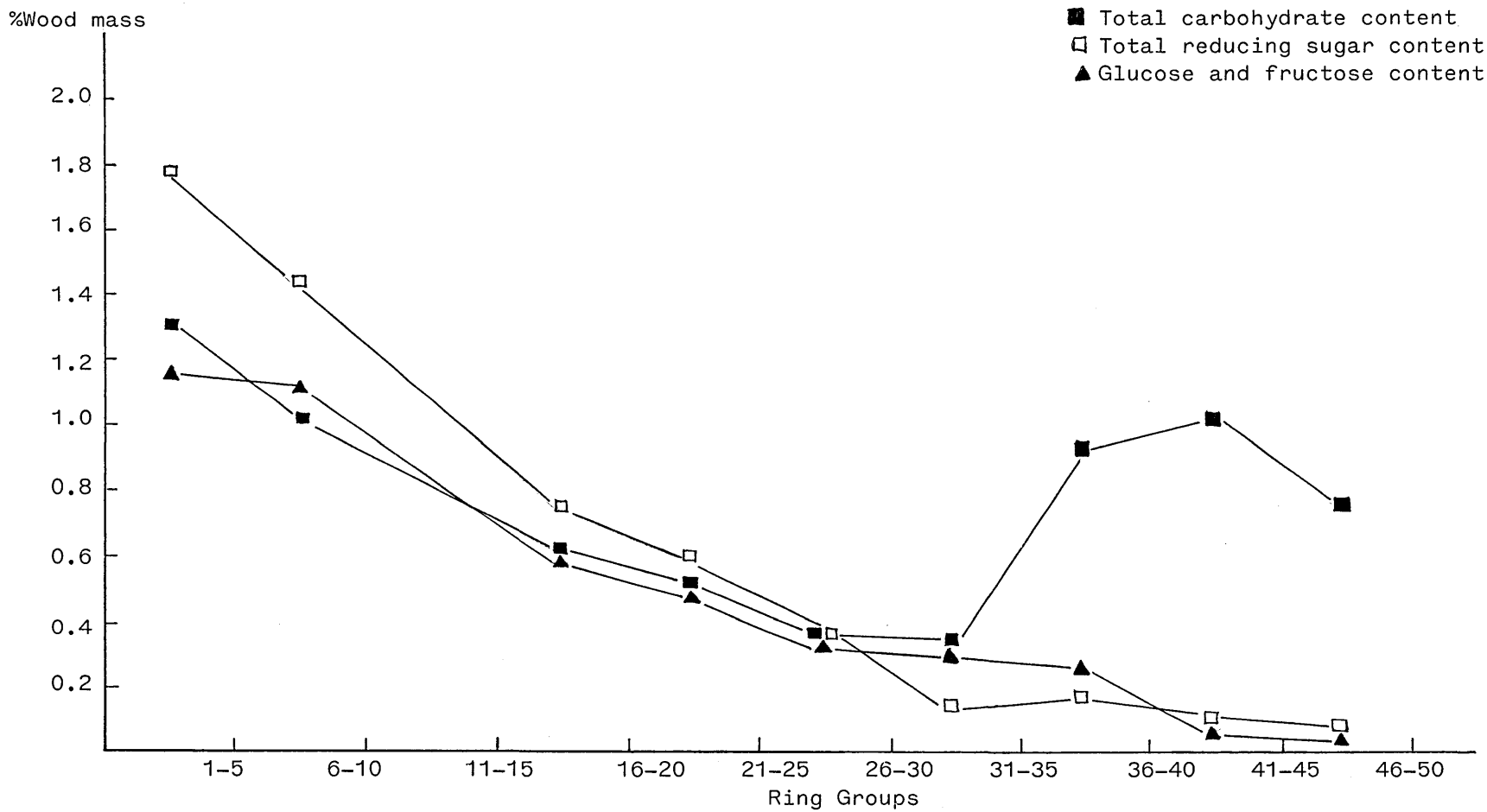


Fig 3.8 Distribution of soluble carbohydrates in green pine dried in chipped form

Increases in the total carbohydrate content in the heartwood regions were also observed in dried pine. The total reducing sugar content was seen to be higher than the total carbohydrate content in the outer and inner sapwood regions. However, glucose and fructose concentrations were similar to those of the total carbohydrate content. As glucose and fructose account for the majority of the soluble carbohydrates, it is likely that the high values observed for the reducing sugars are attributable to the presence of other substances that are capable of reducing the 3, 5 - dinitrosalicyclic reagent. Glucose and fructose concentrations ranged from 1.2% in the outer sapwood to 0.70% in the heartwood regions. These sugars contributed to 70% of the total soluble carbohydrate content.

b) Soluble amino acids

Concentrations of free amino acids in both green and dried samples of pine showed radial distribution patterns similar to those described in spruce (Figure 3.9). In green pine, concentrations of amino acids ranged from 4 μmol/g wood in the outer sapwood region to 1 μmol/g in the heartwood region. In dried pine, concentrations of amino acids ranged from 6 μmol/g wood to 2 μmol/g wood from the outer sapwood to heartwood region respectively. Differences in concentrations between green and dried pine were not as large as those observed in spruce. However, like spruce, larger differences in amino acid concentrations were observed in the outer sapwood regions of green and dried wood than in the heartwood regions. Comparison of amino acid concentrations by the ninydrin assay method and those calculated from analysis using the amino acid auto-analyser, showed concentrations in the latter method to be higher (Table 3.4). Analysis of amino acids by the auto-analyser were more specific and sensitive and showed higher concentration of amino acids in the inner sapwood and heartwood regions which were not observed in analysis using the ninhydrin assay method.

The composition of amino acids present in green pine is presented in Table 3.5.

The major amino acid present in all ring groupings are aspartic acid, glutamine, phenylalanine and arginine. Glycine, alanine, valine, isoleucine, leucine, tyrosine and lysine were present in minor quantities. Trace quantities (<1µg/gwood) of histidine were also detected. In general, concentrations of amino acids decreased with distance from the cambium. However, increased concentrations of amino acids were observed in the inner sapwood region i.e. ring groupings 21-25 and 26-30. Glutamine displayed large increases in this region.

Concentrations of soluble amino acids in green pine were higher than those in green spruce. The total soluble amino acid content in green pine (Table 3.5) contributed approximately 0.05% of the mass of wood. Soluble amino nitrogen calculated from the amino acid composition was 0.01%. The qualitative analysis undertaken on samples of green pine, dried in chipped form, showed that arginine and glutamine were the major amino acids. Minor quantities of aspartic acid, serine, glycine and alanine were detected and trace quantities of isoleucine, leucine, tyrosine and lysine were also present.

The amino acid content of unhydrolysed and hydrolysed samples of the outer sapwood regions (ring grouping 1-20) of green pine is presented in Table 3.6. The major amino acids present in the hydrolysates and unhydrolysed samples were aspartic acid, phenylalanine and arginine. Glutamine, a major amino acid in unhydrolysed pine, was, on hydrolysis, deaminated to glutamic acid. Other amino acids present in the hydrolysates were glycine, alanine, valine, isoleucine, leucine and lysine. Trace quantities of serine, threonine and proline were also present. These were not detected in the unhydrolysed samples. The overall concentration of amino acids in the hydrolysates was lower than the concentrations of amino acids in the unhydrolysed samples. This result is inconsistent with the expected increase in the total amino acid concentration in the hydrolysates. An explanation for this result is offered in the discussion chapter.

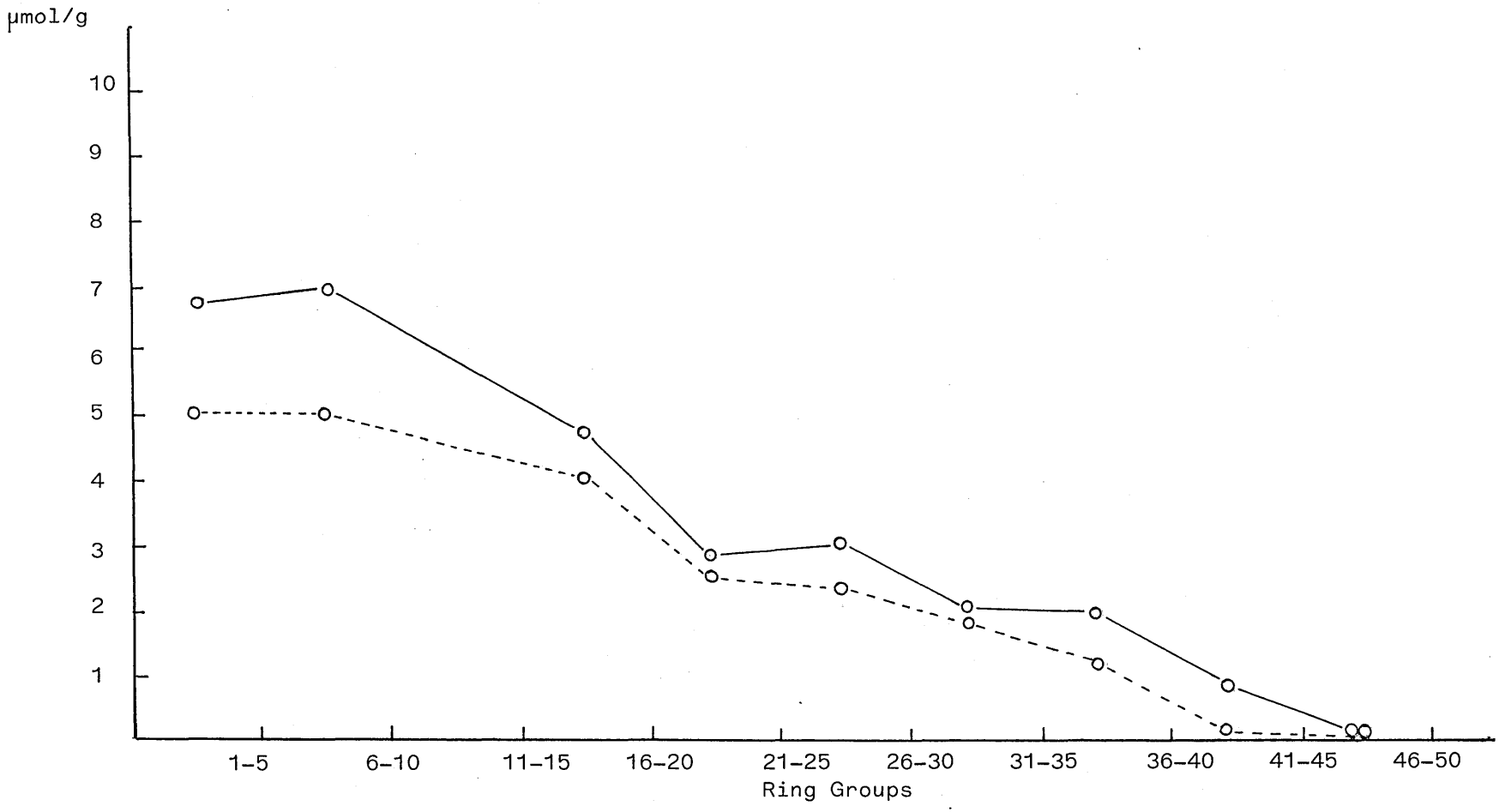


Fig 3.9 Distribution of soluble amino acids in green pine (---) and green pine dried in chipped form (—)

Table 3.4 Soluble amino acid concentrations in green pine and in green pine dried in chip form

Ring Groups	µmoles amino acid per gram wood		
	Green Pine		Green Pine dried in chipped form
	Ninhydrin Assay	Auto-analyser	
1 - 5	4.90	6.74	6.58
6 - 10	5.00	6.11	6.80
11 - 15	4.43	1.89	NT
16 - 20	2.57	2.31	4.65
21 - 25	2.90	3.76	2.81
26 - 30	1.80	5.82	3.20
31 - 35	1.05	2.14	2.05
36 - 40	0.84	1.64	2.00
41 - 45	-	0.24	-
46 - 50	-	-	-
Average	2.93	3.80	4.01

NT no trial
\- not detected

Table 3.5 Soluble amino acid composition in green pine

Ring Group / Amino Acid	μgram amino acid per gram wood									
	1-5	6-10	11-15	16-20	21-25	26-30	30-35	36-40	41-45	46-50
asp	46.50	41.22	15.70	15.96	25.27	33.25	10.24	11.97	1.33	-
gln	152	153	56.94	105	322.6	241	155.9	62.78	16.35	-
gly	15.75	17.25	6.75	8.25	10.50	79.50	9.37	8.25	1.87	-
ala	48.95	38.27	11.57	16.02	6.23	31.15	14.24	14.77	2.22	-
val	26.91	23.28	3.51	4.68	12.87	15.21	16.38	7.02	-	-
ile	17.03	14.90	2.62	5.24	7.86	7.86	5.63	tr	-	-
leu	31.44	26.06	5.24	9.17	13.10	17.03	11.26	tr	-	-
tyr	28.96	13.51	tr	-	-	-	-	-	-	-
phe	285.40	219.0	75.90	112	179.8	237.6	100.6	105.10	11.38	-
lys	37.96	30.36	10.22	10.20	11.68	8.76	-	-	-	-
his	tr	tr	-	-	-	-	-	-	-	-
arg	316.68	342	88.70	48.70	45.24	13.90	8.70	3.65	3.65	-
Total	1008	916	277	335	635	685	332	213	37	-
Average	493									
Soluble amino Nitrogen (μgN/gwood)	113									

tr trace quantity (< 1, μgg-1 wood)
- not detected

Table 3.6 Soluble amino acids in unhydrolysed and hydrolysed outer sapwood samples of green pine

Amino Acid	µg aminoacid g^{-1} wood	
	Unhydrolysed sample	Hydrolysed sample
asp	29.84	41.99
thr	-	10.92
ser	-	21.81
glu	-	80.62
pro	-	21.44
gly	11.25	17.50
ala	28.70	26.74
val	14.59	13.13
ile	9.94	10.73
leu	17.97	18.64
tyr	21.23	-
phe	173	89.31
lys	22.18	22.41
gln	116.7	-
arg	199	133
Total amino acid content	644.40	508.24
Total soluble amino nitrogen	118.50	78.51

3.1.3. Distribution of soluble carbohydrates and soluble amino acids in surface and sub-surface samples of dried spruce and pine (Experiment 3).

Greenwood samples showed radial distribution patterns of soluble carbohydrates and soluble amino acids from outer sapwood regions to heartwood regions. This experiment was undertaken to investigate distribution patterns of these soluble nutrients in surface and sub-surface samples of dried wood, and also to determine quantitative differences between these samples. Dried samples of spruce and pine were used. The surface and sub-surface samples were divided, in spruce, into 10 ring groupings, and in pine, into 5 ring groupings, as described in 2.1.1.2. All samples were analysed for total carbohydrate content, total reducing sugar content and glucose and fructose contents. The concentrations of soluble carbohydrates were expressed as a percentage of the dry wood mass. The samples were also analysed for amino acid content by the ninhydrin assay method and the composition of amino acids in the samples were determined using the amino acid auto-analyser. All dried wood specimens were corrected for moisture from the moisture contents of wood tabled in Appendix 1.

3.1.3.1. Distribution of soluble carbohydrates and soluble amino acids in surface and sub-surface samples of dried spruce.

a) Soluble carbohydrates

Results from the analysis of soluble carbohydrates in surface and sub-surface samples of spruce are presented in Figures 3.10 to 3.12. These results are also presented in a tabular form in Appendix 10.

A similar radial distribution pattern of total carbohydrate content, total reducing sugar content and glucose and fructose contents were seen in surface samples as for green samples in 3.1.2.1. Soluble carbohydrate concentrations in surface samples were five times those in sub-surface samples. Concentrations of these soluble nutrients were highest in the outer sapwood regions, and decreased with increasing distance from the cambium. Unlike green wood samples and surface samples, sub-surface samples of spruce did not display radial distribution of total carbohydrate content, total reducing sugar content and glucose and fructose contents.

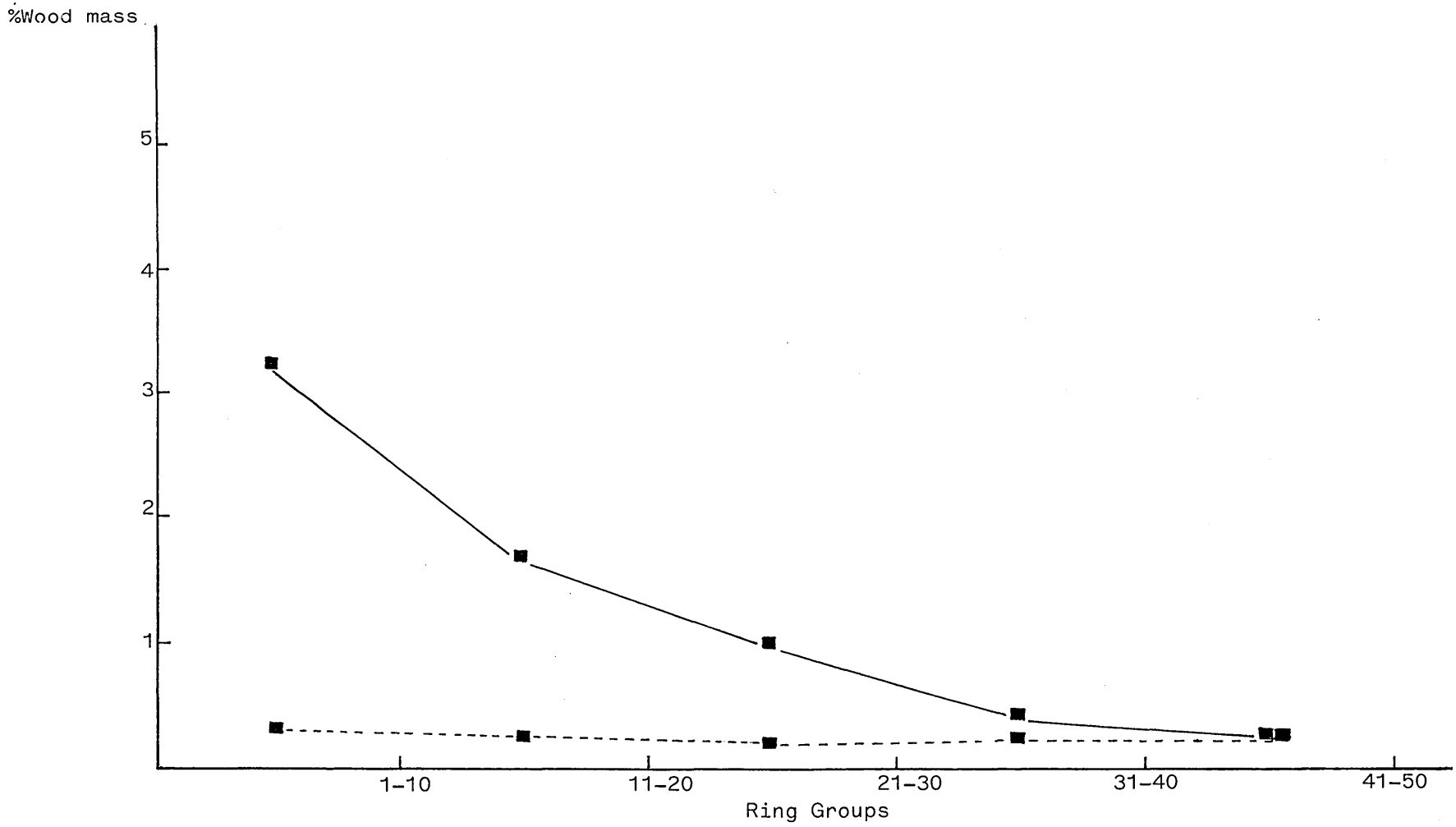


Fig 3.10 Distribution of the total carbohydrate contents in surface (—) and sub-surface (---) samples of dried spruce.

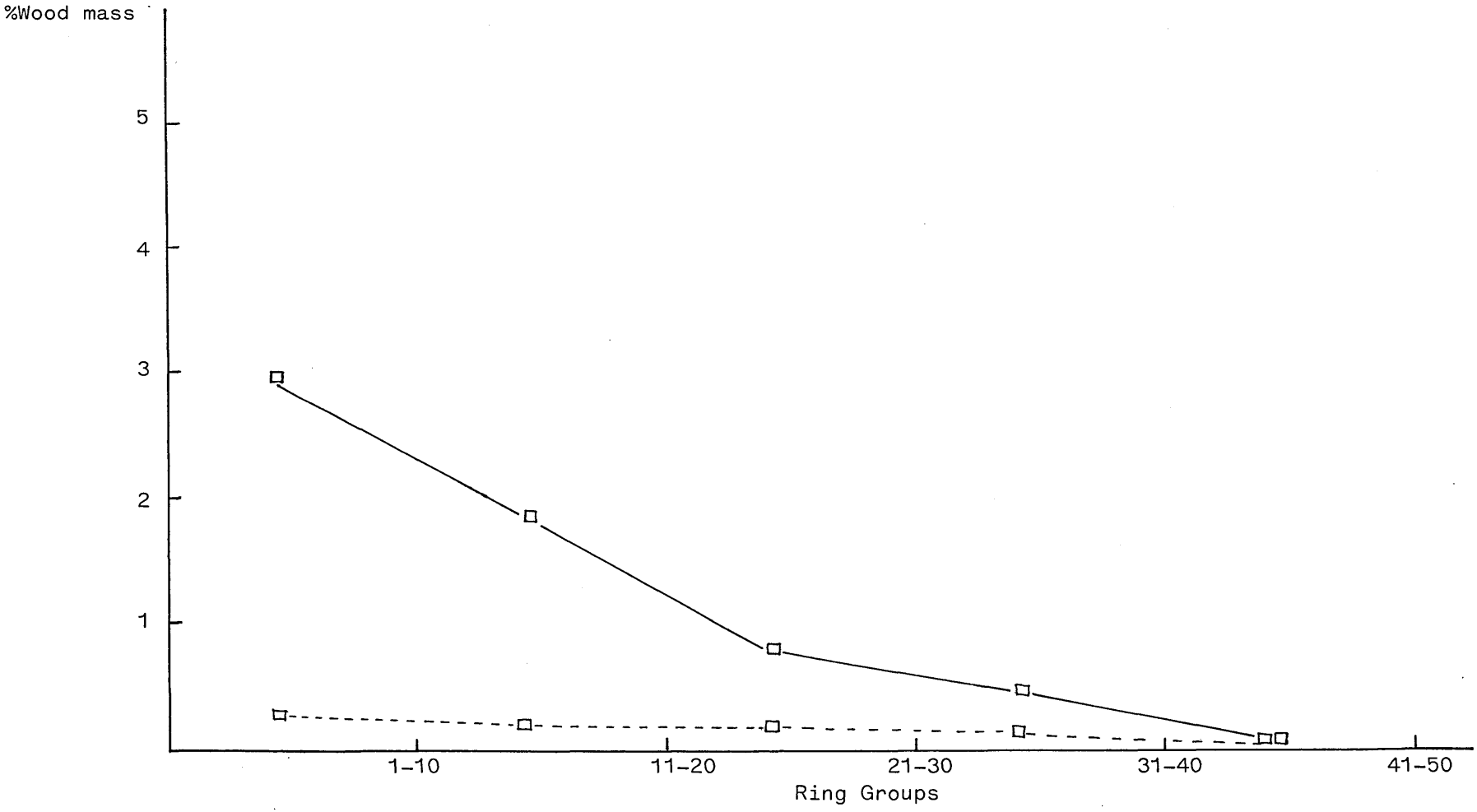


Fig 3.11 Distribution of total reducing sugar contents in surface (—) and sub-surface (---) samples of dried spruce.

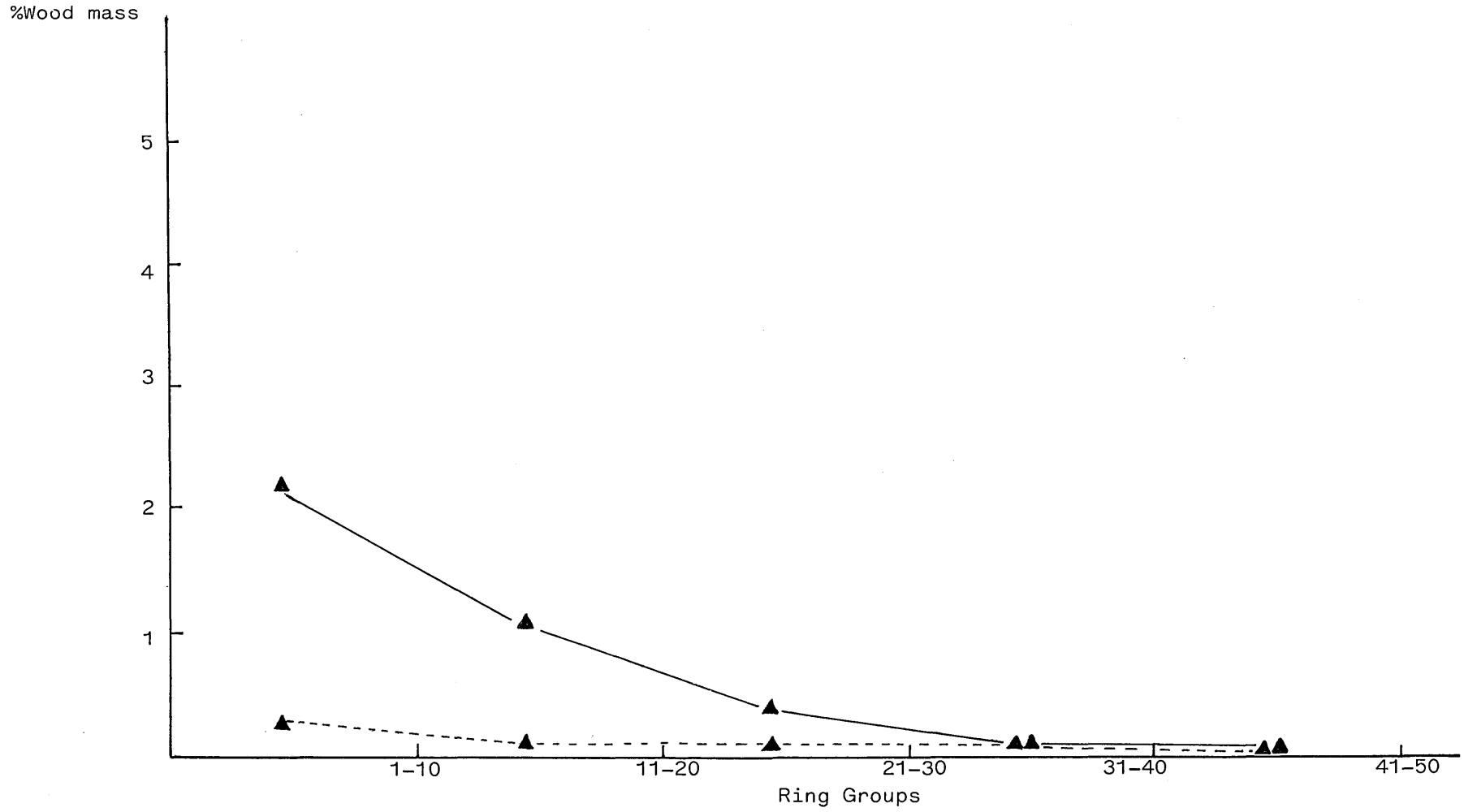


Fig 3.12 Distribution of glucose and fructose contents in surface (—) and sub-surface (---) samples of dried spruce.

Concentrations of these soluble carbohydrate materials were broadly similar in both the sapwood and heartwood regions of the sub-surface samples.

In surface samples, the total carbohydrate content constituted 1% of the mass of wood. Concentrations of these materials were significantly higher in the outer sapwood region than in the heartwood regions. In surface samples, the total reducing sugar content contributed 90% of the total carbohydrate content. Similarly, in sub-surface samples, the total reducing sugar content also constituted a large proportion (80%) of the total carbohydrate content. Glucose and fructose were the predominant reducing sugars in both these samples, constituting 75% of the total reducing sugar content. Concentrations of total carbohydrate content, total reducing sugar content and glucose and fructose contents were broadly similar in the heartwood regions of both surface and sub-surface samples.

A comparison of the soluble carbohydrate concentrations in green wood and dried wood is presented in Table 3.7. This table summarises results of the soluble carbohydrate analysis recorded in spruce from experiments 3.1.2.1. and 3.1.3.1. The results clearly show that drying increases the concentrations of soluble carbohydrates in the wood. Dried surface samples of spruce displayed concentrations ten times greater than those found in green spruce. The large difference in concentration between dried surface samples and green wood was apparent at each wood region i.e. in the outer sapwood, inner sapwood and heartwood regions. Sub-surface samples displayed soluble carbohydrate concentrations broadly similar to those in green spruce dried in chip form. Comparison of soluble carbohydrate concentrations in surface and sub-surface samples of this study with similar samples in the preliminary study (3.1.1) showed higher concentrations of soluble carbohydrates in the second study. The differences in concentrations may be explained by the greater detail employed in the analysis of samples of smaller ring groupings.

b) Soluble amino acids

The distribution of soluble amino acids in surface and sub-surface samples of spruce is presented in Figure 3.13 and in a tabular form in Table 3.8. The patterns of amino acid distribution in surface and sub-surface samples were similar to those described for green spruce (3.1.2.1).

Sample	Green Spruce			Green Spruce dried in chip form			Dried Spruce Surface samples			Dried Spruce Sub-Surface samples		
	Total Carbohydrate content	Total Reducing Sugar Content	Glucose and Fructose content	Total Carbohydrate content	Total Reducing Sugar content	Glucose and fructose content	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose and Fructose Content	Total Carbohydrate Content	Total Reducing Sugar content	Glucose and fructose content
Outer Sapwood (1-20)	0.21	0.17	0.15	0.57	0.39	0.27	2.48	2.46	1.88	0.30	0.27	0.22
Inner Sapwood (21-30)	0.10	0.12	0.10	0.39	0.26	0.11	0.97	0.83	0.19	0.21	0.20	0.07
Heartwood (31-50)	0.07	0.02	0.01	0.31	0.07	0.04	0.37	0.27	0.02	0.29	0.14	0.06
Average	0.13	0.10	0.09	0.42	0.24	0.14	1.27	1.19	0.69	0.26	0.20	0.12

Table 3.7 Soluble carbohydrate concentrations in outer sapwood, inner sapwood and heartwood regions of green spruce, green spruce dried in chip form and surface and sub-surface samples of dried spruce. Results from green wood samples were corrected for moisture. All results are expressed as a percentage of the initial dry weight of wood.

Concentrations of amino acids decreased with increasing distance from the cambium. Soluble amino acid concentrations in surface samples of spruce were five times those of sub-surface samples and also those of green wood samples. Large differences in concentration of soluble amino acids existed between the outer sapwood regions of surface and sub-surface samples, but soluble amino acid concentrations in the heartwood region of these samples were broadly similar. In surface samples, amino acid concentrations ranged from 30 μmol/g to 2 μmol/g in the outer sapwood to heartwood regions respectively. Concentrations of amino acid in sub-surface samples, ranged from 4 μmol/g in the outer sapwood region, to 1 μmol/g in the heartwood region.

The composition of soluble amino acids in surface samples of dried spruce is presented in Table 3.9. Analysis of samples in ring groups 21 - 30 and 41 - 50, as well as those of sub-surface samples were not undertaken due to technical problems with the amino acid auto-analyser. The results showed similar composition of soluble amino acids at surface regions of dried spruce as in green spruce. Aspartic acid, glutamine and arginine were the major amino acids detected in all ring groupings. Other amino acids detected in minor quantities were threonine, serine, glycine, alanine, valine, isoleucine, leucine and phenylalanine. Threonine and serine were not detected in green spruce samples. In spite of the incomplete analysis of surface samples, concentrations of soluble amino acids in these regions were approximately one and a half times the concentrations seen in green spruce (Table 3.2). Soluble amino nitrogen concentrations in surface samples were also higher than the soluble amino nitrogen concentration in green spruce.

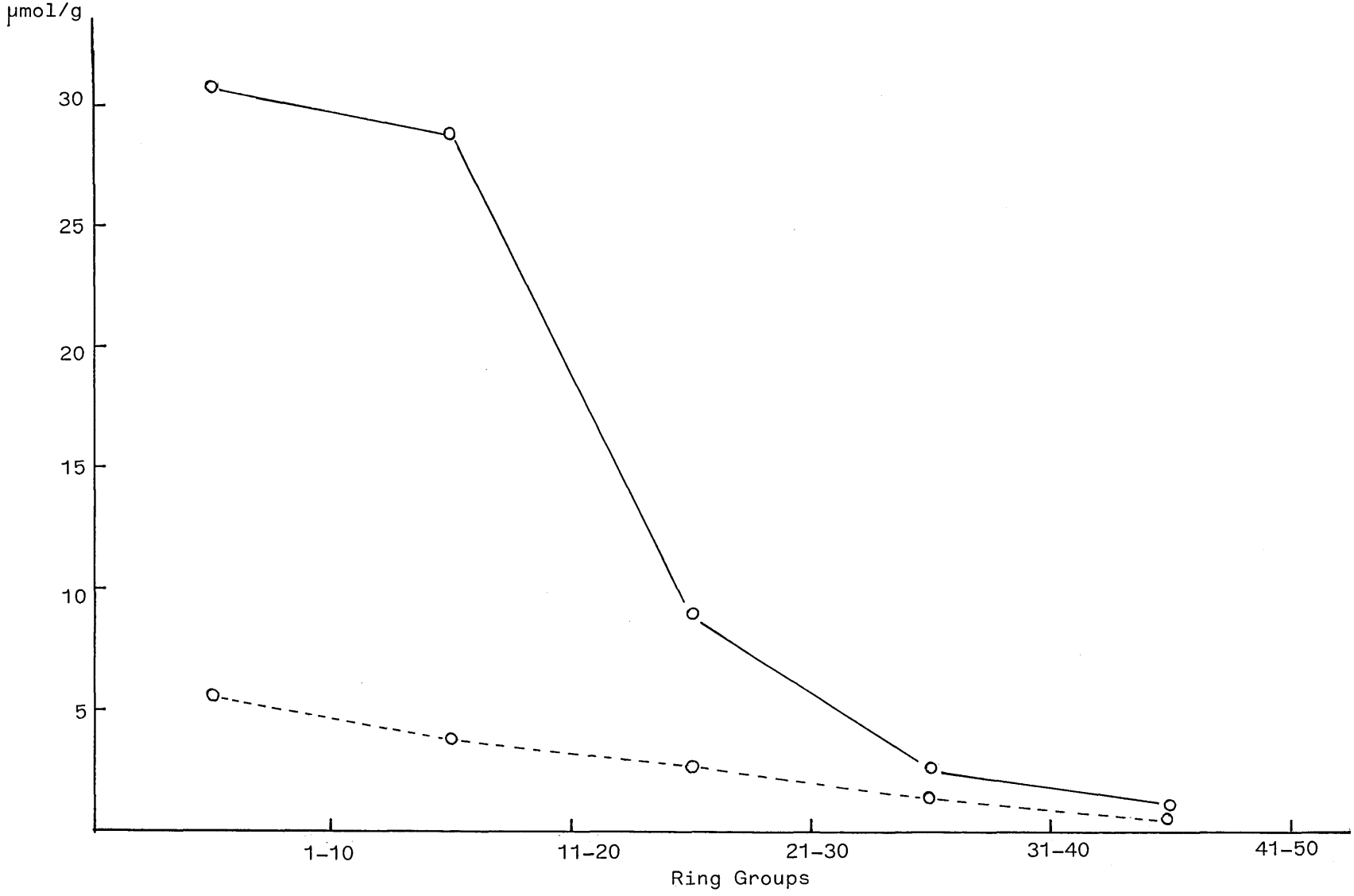


Fig 3.13 Distribution of soluble amino acids in surface(—) and sub-surface (---) samples of dried spruce.

Table 3.8 Soluble amino acid concentrations in surface and sub-surface samples of dried spruce.

Ring Groups	μmoles amino acid/g wood	
	Surface samples	Sub-Surface samples
1 - 10	30.88	5.21
11 - 20	28.85	3.72
21 - 30	8.96	2.65
31 - 40	2.48	1.32
41 - 50	0.91	0.72
Average	14.42	2.72

Table 3.9 Soluble amino acid composition in surface samples of dried spruce.

Ring Group / Amino Acid	µgram amino acid per gram wood				
	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50
asp	62.98	71.55	NT	24.87	NT
thr	8.19	8.56	NT	3.46	NT
ser	7.37	8.08	NT	3.04	NT
gln	82.06	52.41	NT	41.76	NT
gly	3.58	4.80	NT	8.70	NT
ala	6.38	5.87	NT	-	NT
val	11.01	16.03	NT	-	NT
ile	4.60	5.89	NT	-	NT
leu	5.71	7.21	NT	-	NT
phe	27.82	tr	NT	-	NT
lys	-	-	NT	-	NT
his	-	-	NT	-	NT
arg	256.70	267.9	NT	49.76	NT
Total	460.80	448.40	-	131.59	-
Average (µg aa/gwood)	346.93				
Soluble Amino Acid Nitrogen (µg N/g wood)	82.33				

tr trace quantities (<1 µgg^{-1} wood)
- not detected
NT no trial

3.1.3.2. Distribution of soluble carbohydrates and soluble amino acids in surface and sub-surface samples of dried pine.

a) Soluble Carbohydrates

Results of the analysis of soluble carbohydrates in surface and sub-surface samples of dried pine are presented in Figures 3.14 - 3.16. The radial distribution patterns of soluble carbohydrates in these samples were similar to those observed in green pine. Like green pine, the total soluble carbohydrate content decreased from the outer sapwood region to the inner sapwood region, but increased again in the heartwood region in both surface and sub-surface samples of dried pine. However, total reducing sugar content and glucose and fructose concentrations in surface samples, showed radial distribution patterns of decreasing concentration with increasing distance from the cambium. Radial distribution of total reducing sugar concentrations and glucose and fructose concentrations were not observed in sub-surface samples. Concentrations of these sugars were broadly similar in the sapwood and heartwood regions. Also, heartwood regions of surface and sub-surface samples showed similar concentrations of total soluble carbohydrate content, total reducing sugar content and glucose and fructose concentrations.

The concentrations of total soluble carbohydrates in surface samples of pine were four times those of sub-surface samples and constituted approximately 2% of the mass of wood. As with spruce, reducing sugars contributed to a significant proportion of the total soluble carbohydrate content (70%), in both surface and sub-surface samples. Glucose and fructose were detected as the major reducing sugars in all ring groupings.

Comparison of the results of the soluble carbohydrate analysis from experiments 3.1.2.2. and 3.1.3.2. are summarised in Table 3.10. Concentrations of soluble carbohydrate in surface samples of dried wood were five times those found in green wood. Concentrations of these materials in sub-surface samples of pine were broadly similar to those in green pine but lower than those of green pine dried in chipped form. Overall, concentrations of soluble carbohydrates in pine were higher than those in spruce of similar preparations.

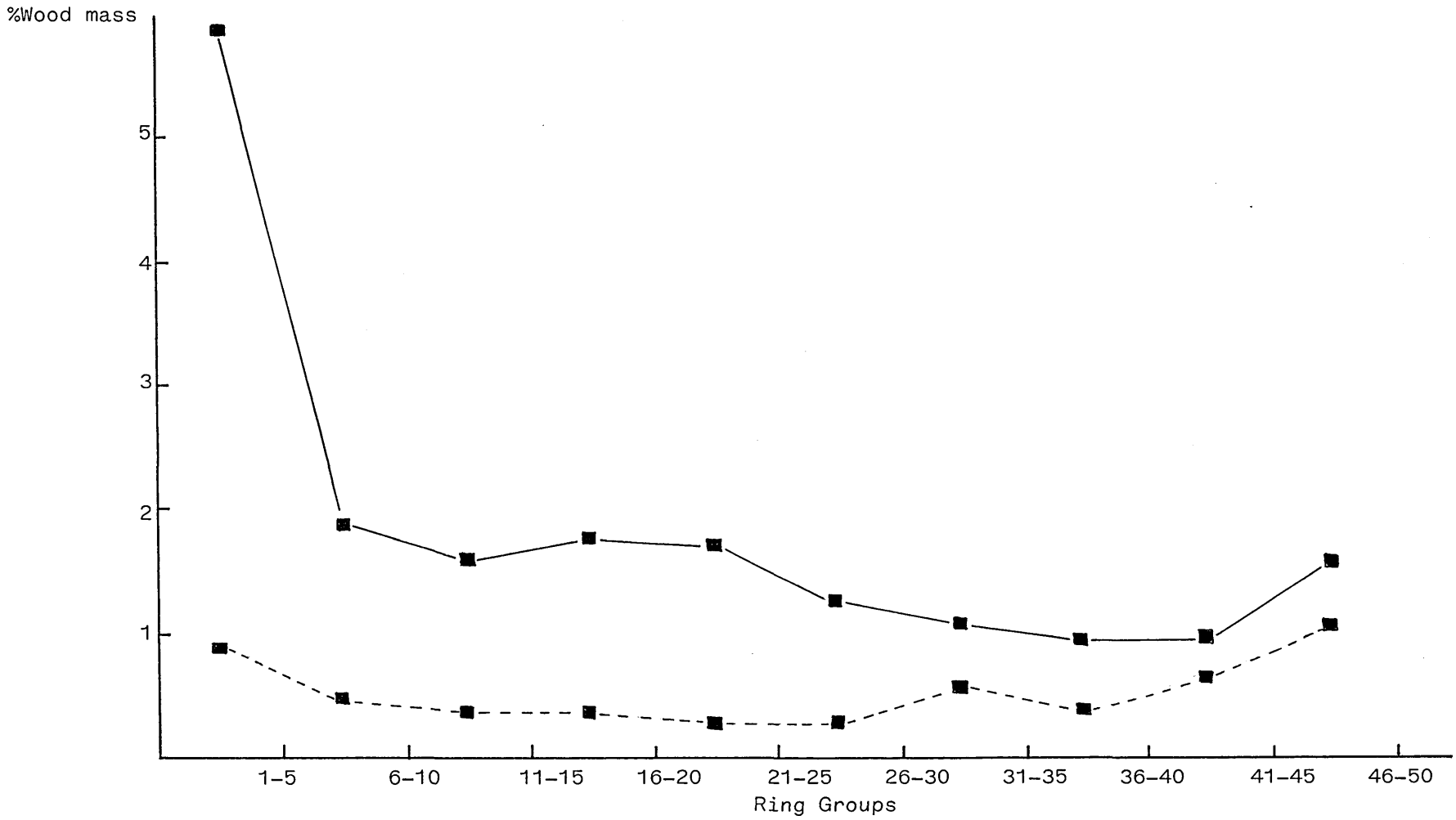


Fig 3.14 Distribution of total carbohydrate contents in surface (—) and sub-surface (---) samples of dried pine.

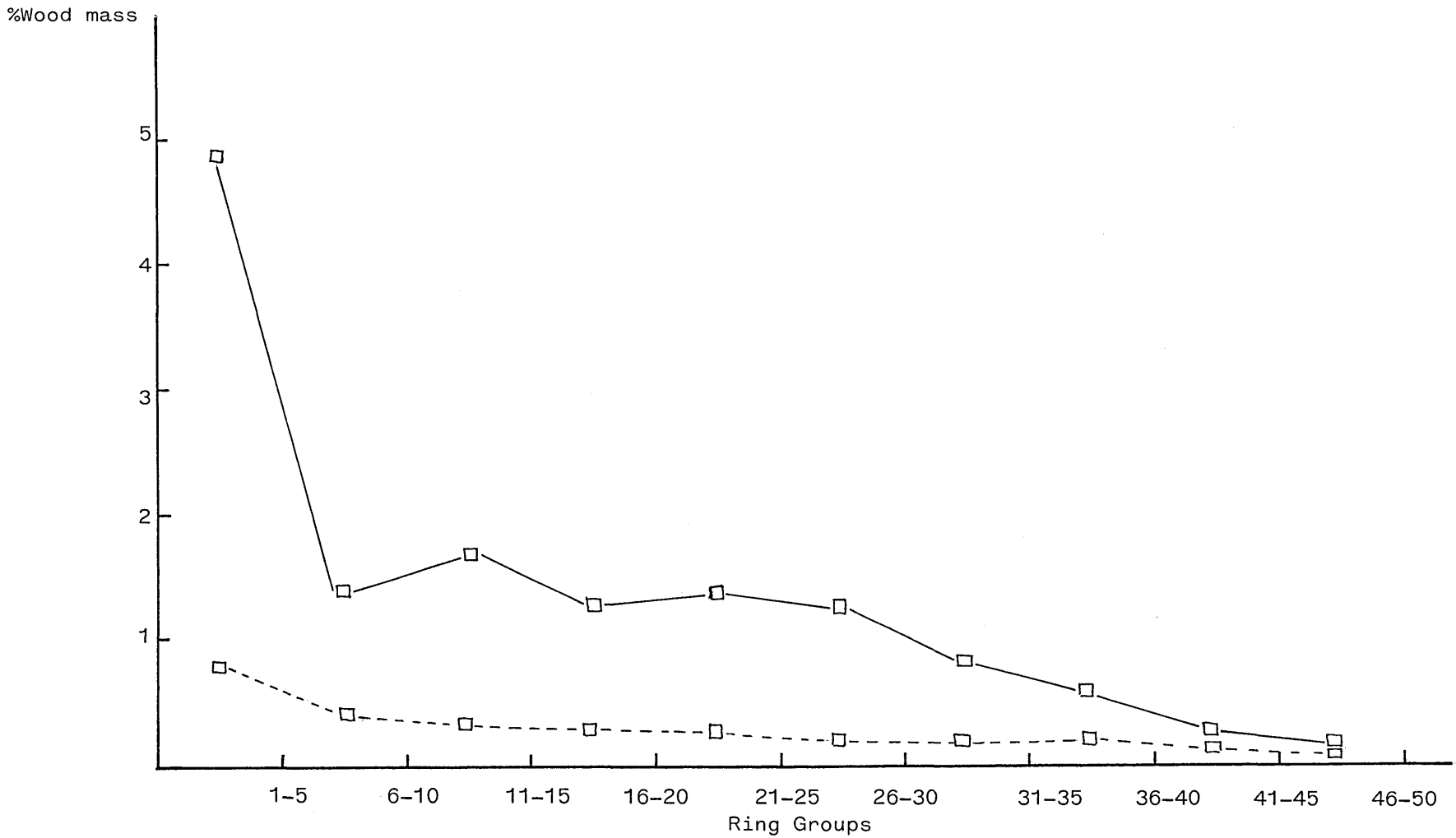


Fig 3.15 Distribution of total reducing sugar contents in surface (—) and sub-surface (---) samples of dried pine.

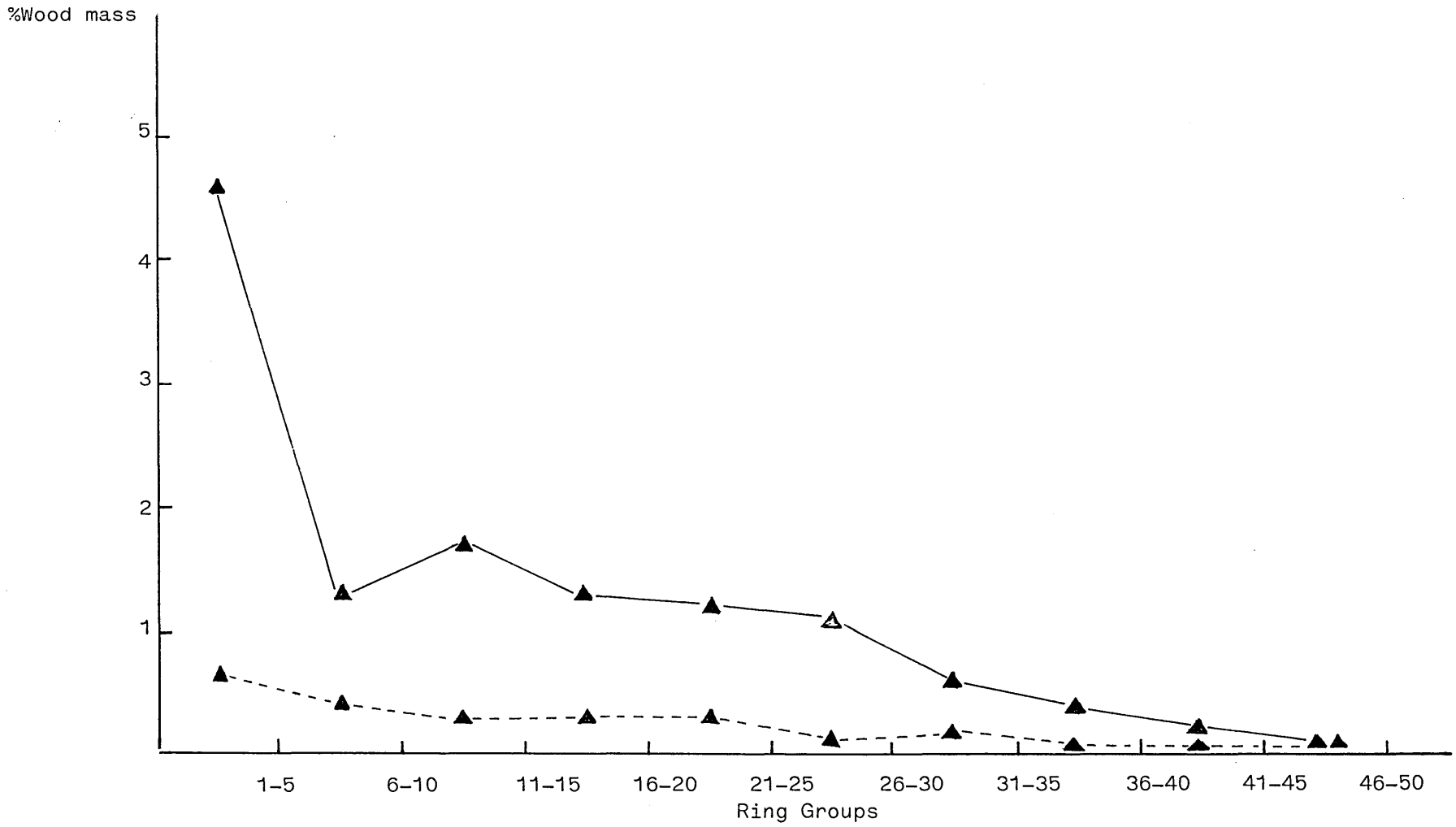


Fig 3.16 Distribution of glucose and fructose contents in surface (—) and sub-surface (---) samples of dried pine.

Sample	Green Pine			Green Pine dried in chip form			Dried Pine Surface Samples			Dried Pine Sub-surface samples		
	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose and fructose content	Total Carbohydrate content	Total Reducing sugar content	Glucose and fructose content	Total Carbohydrate content	Total Reducing sugar content	Glucose and fructose content	Total Carbohydrate content	Total Reducing sugar content	Glucose and fructose content
Outer Sapwood (1-20)	0.55	0.52	0.62	1.00	1.32	0.91	2.77	2.32	1.83	0.51	0.44	0.39
Inner Sapwood (21-30)	0.30	0.31	0.28	0.44	0.47	0.37	1.53	1.34	1.16	0.29	0.26	0.18
Heartwood (31-50)	0.28	0.11	0.07	0.80	0.11	0.17	1.19	0.46	0.30	0.72	0.19	0.09
Average	0.38	0.31	0.32	0.75	0.63	0.48	1.83	1.37	1.10	0.51	0.29	0.22

Table 3.10 Soluble carbohydrate concentrations in outer sapwood, inner sapwood and heartwood regions of green pine, green pine dried in chip form and surface and sub-surface samples of dried pine. All results are expressed as a percentage of the initial weight of drywood. Results from greenwood samples were corrected for moisture.

b) Soluble amino acids

The radial distribution patterns of soluble amino acids in surface and sub-surface samples of dried pine were similar to those in green pine. These radial distribution patterns are presented in Fig 3.17 and in a tabular form in Table 3.11. Concentrations of soluble amino acids in surface samples were four times those in sub-surface samples. Greater differences in concentrations between surface and sub-surface samples were observed in the outer sapwood regions than in the heartwood regions. Soluble amino acid concentrations were broadly similar in the sapwood and heartwood regions of sub-surface samples. Unlike the soluble carbohydrates, increases in concentrations of soluble amino acids were not observed in the heartwood regions of surface and sub-surface samples. Dried surface samples displayed concentrations of soluble amino acids twice those found in green wood samples. However, sub-surface samples of dried pine displayed lower concentrations of soluble amino acids to green pine.

Analysis of amino acid composition in surface samples of dried pine were undertaken on a limited number of ring groups (ring groups 11-15, 21-25, 26-30 and 31-35). Analysis of the remaining ring groups and those of sub-surface samples of dried pine were not undertaken because of technical problems with the auto-analyser. No reliable quantitative data could be obtained and the results of the analysis undertaken could only be used on a qualitative basis. The results showed that glutamine, phenylalanine and arginine were the major amino acids present in the samples analysed. Other amino acids present in minor quantities were aspartic acid, threonine, serine, glycine, alanine, isoleucine, leucine and lysine. The composition of amino acids in dried pine was broadly similar to that observed in green pine.

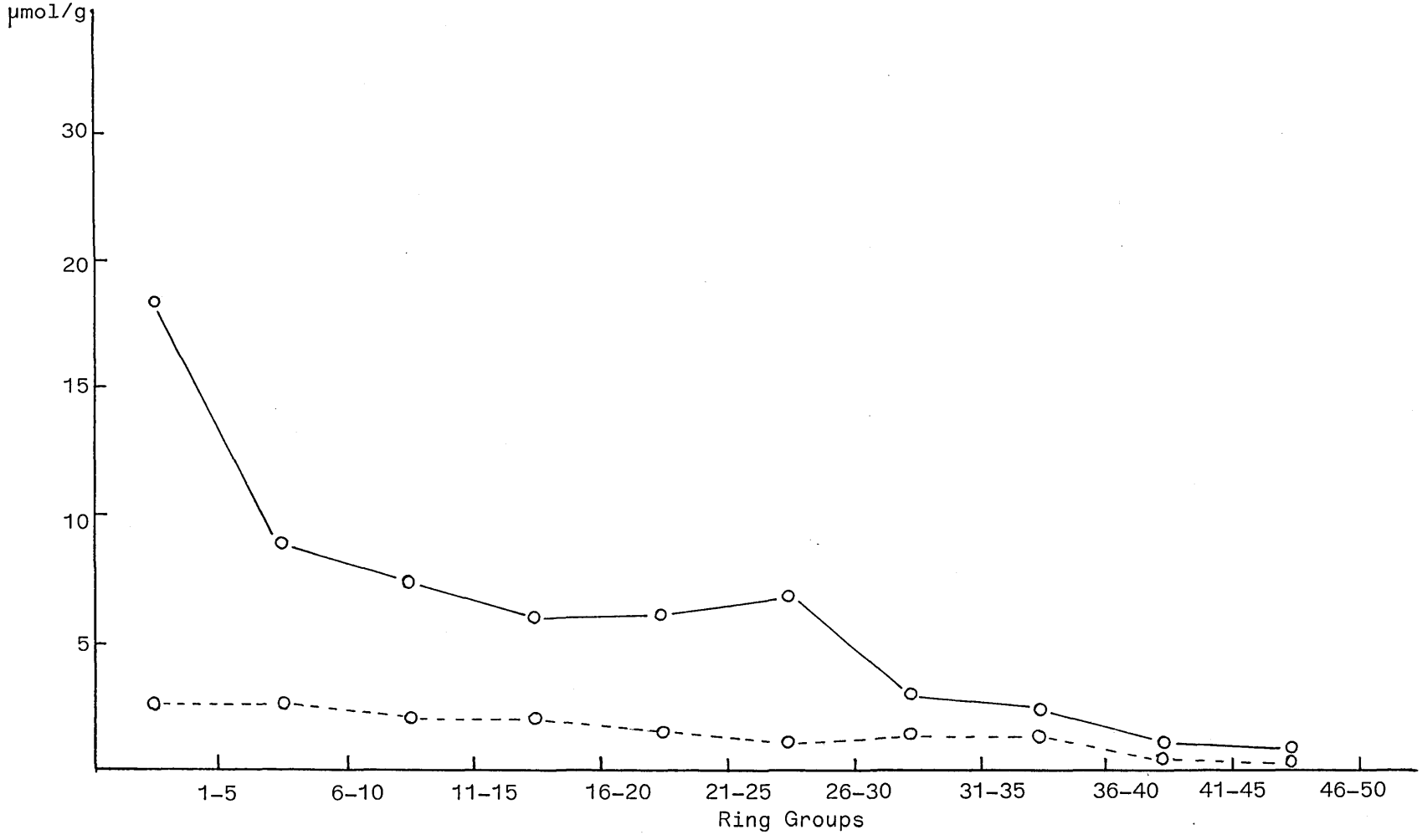


Fig 3.17 Distribution of soluble amino acids in surface (—) and sub-surface (---) samples of dried pine.

Table 3.11 Soluble amino acid concentrations in surface and sub-surface samples of dried pine.

Ring Groups	μmole amino acid/g wood	
	Surface samples	Sub-surface samples
1 - 5	18.61	2.65
6 - 10	9.10	2.66
11 - 15	7.65	1.89
16 - 20	5.98	1.93
21 - 25	5.99	1.42
26 - 30	6.73	1.12
31 - 35	2.90	1.22
36 - 40	0.91	0.94
41 - 45	0.61	0.42
46 - 50	-	-
Average	6.49	1.58

Conclusions

The conclusions drawn from this series of experiments were:

1) radial distribution patterns of soluble carbohydrates and soluble amino acids were observed in both green and dried samples of spruce and pine. Concentrations of these soluble nutrients decreased with increasing distance from the cambium;

2) migration and accumulation of soluble carbohydrates and soluble amino acids to surface regions occurred during drying of wood. Concentrations of these materials were higher in surface regions than in sub-surface regions. Concentrations of soluble nutrients were also higher in dried wood than in green wood;

3) reducing sugars accounted for the bulk of soluble carbohydrates in both green and dried wood. Glucose and fructose were the predominant reducing sugars;

4) soluble proteins constituted a small proportion of the soluble nutrients in wood;

5) green and dried wood showed similar compositions of amino acids. The major amino acids appeared to be aspartic acid, glutamine, phenylalanine and arginine.

3.1.4. Qualitative and quantitative determinations of soluble carbohydrate and soluble amino acids in surface and sub-surface samples of spruce, pine, lime and kempas (Experiment 4).

In these experiments, milled wood samples were subjected to extraction with different solvents and under different conditions of temperature and time. Samples from surface regions of dried spruce, pine and lime were subjected to two extraction procedures:

(i) an extraction in which samples were successively extracted with 70% aqueous ethanol and hot water,

and

(ii) an extraction with cold water alone.

Samples from sub-surface regions of spruce, pine and lime were extracted in cold water alone. Kempas was provided to this laboratory by Hickson's Timber Products. Surface and sub-surface regions were not differentiated in this wood due to the method of preparation of this test material. Whole samples of kempas were milled and extracted by the extraction procedures previously described. To concentrate samples, the extracts from each extraction procedure were dried either by rotary evaporation (alcohol extracts), or by freeze drying (aqueous extracts), and then made up in standard volumes of cold water for analysis.

The weight losses produced in milled, surface samples of spruce, pine, lime and kempas, after extraction, are presented in Figure 3.18. These weight losses are expressed as a percentage of the original dry weight of wood. Largest weight loss (12%) was observed in lime after extraction in alcohol. Kempas showed smallest weight losses at 4%, and the softwoods spruce and pine displayed broadly similar weight losses of 5% and 6% respectively. In the further extractions of the alcohol extracted samples with hot water, spruce, pine and kempas showed weight losses similar to those resulting from the extractions in alcohol. Lime however, displayed a smaller weight loss of approximately 3%. Weight losses resulting from successive extractions of wood with both alcohol and hot water, showed that larger amounts of the extractable material were removed from lime and pine, than spruce and kempas, the latter displayed the lowest weight loss of the four woods examined. In contrast to the results from the hot extractions, pine showed the largest weight loss (~16%) in the extractions in cold water.

Spruce displayed weight losses similar to those achieved in the combined alcohol and hot water extractions. Lime and kempas displayed weight losses broadly similar to those obtained when these woods were extracted in alcohol.

The amounts of cold water soluble materials recovered from the dried extracts resulting from the alcohol, hot water and cold water extractions of spruce, pine, lime and kempas are presented in Figure 3.19, and are expressed as a percentage of the initial dry weight of the unextracted wood. The recovered cold water soluble materials from alcohol extractions constituted 6% of the initial dry weight of unextracted wood in lime, 3% and 4% in spruce and pine, and 2% in kempas. These materials also constituted 60% of the weight losses recorded from the extractions in alcohol in both spruce and pine, 50% of those recorded in lime, and 36% in kempas. The cold water soluble materials recovered from the dried hot water extracts were less than those from the alcohol extracts. In all woods, these materials amounted to less than 3% of the weight of wood and to approximately 50% of the weight losses recorded after extraction in hot water. When the results of the alcohol and hot water extractions were combined, the recovered cold water soluble materials in lime and pine were broadly similar at 7% and those of spruce and kempas were similar at 5%. The combined results also showed that the recovered cold water soluble materials were less than those found when the woods were extracted in cold water alone. In these extractions in cold water, the water soluble material recovered from the dried extract was highest in pine (12%) and lowest in kempas (4%). Lime and spruce displayed broadly similar amounts of these materials (8%). Over 70% of the weight losses recorded after extractions in cold water were recovered as freeze dried material for each of the woods examined.

The results of the sub-surface samples of spruce, pine and lime after extraction in cold water, are presented in Figure 3.20. Figure 3.20(a) displays the weight losses produced in these samples after extractions in cold water, and Figure 3.20(b) displays the recovered cold water soluble materials from the dried extracts of the samples. All these results are expressed as a percentage of the initial dry weight of the unextracted wood.

Extractions in cold water of the sub-surface samples produced weight losses of 7% in lime and 5% in both spruce and pine. Surface samples of these woods, in contrast to the sub-surface samples, produced weight losses two to three times greater after similar extraction procedures. The amount of cold water soluble materials recovered from the dried extracts of the sub-surface samples were broadly similar for the three woods examined. These materials constituted approximately 4% of the weight of the woods. Over 70% of the weight losses recorded after extractions in cold water were recovered as freeze dried material in spruce and pine, and in lime, only 53% of the weight losses recorded were recovered as freeze dried material.

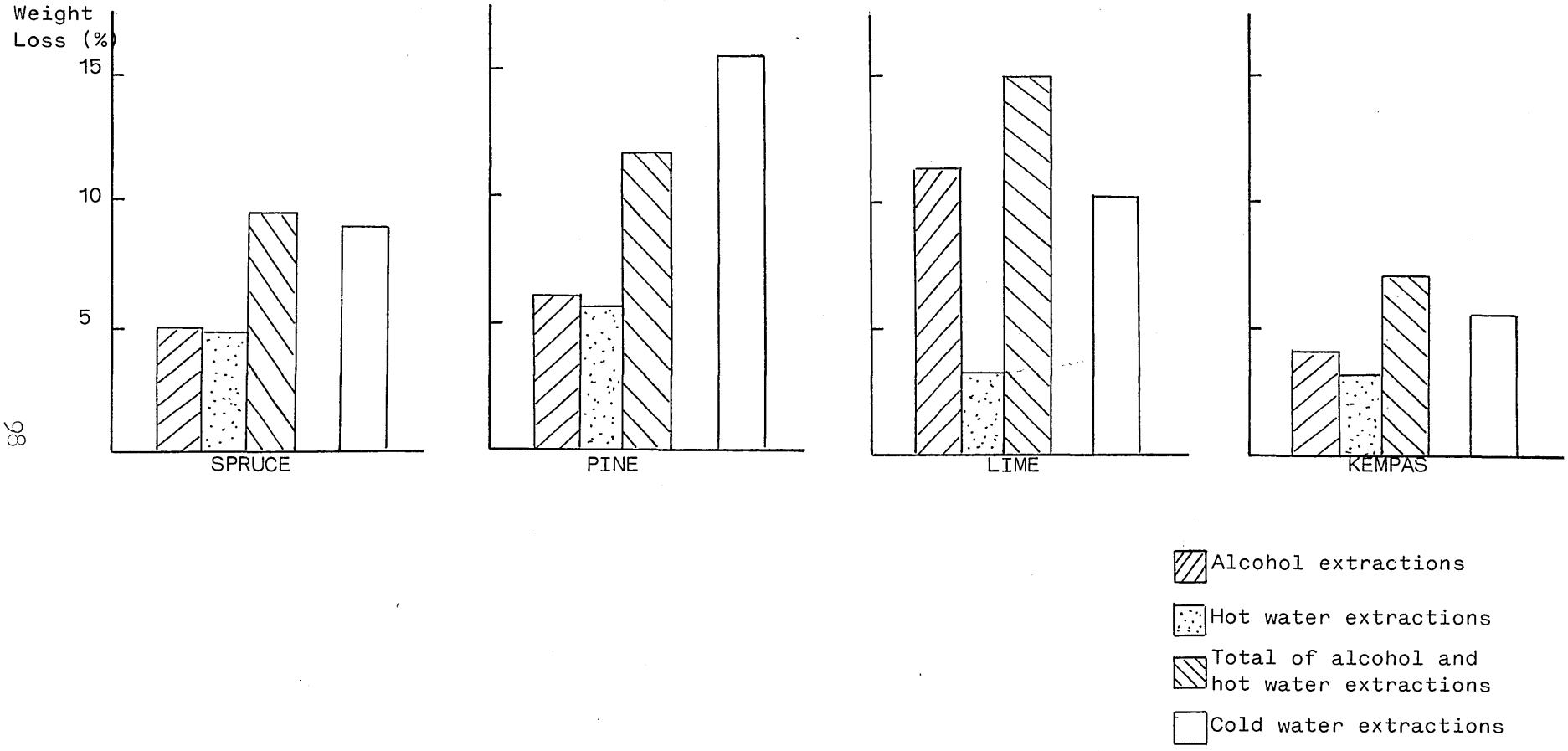


Fig 3.18 Weight losses recorded in surface samples of spruce, pine, lime and kempas when subjected to various extraction procedures. Results are expressed as a percentage of the initial dry weight of the unextracted wood.

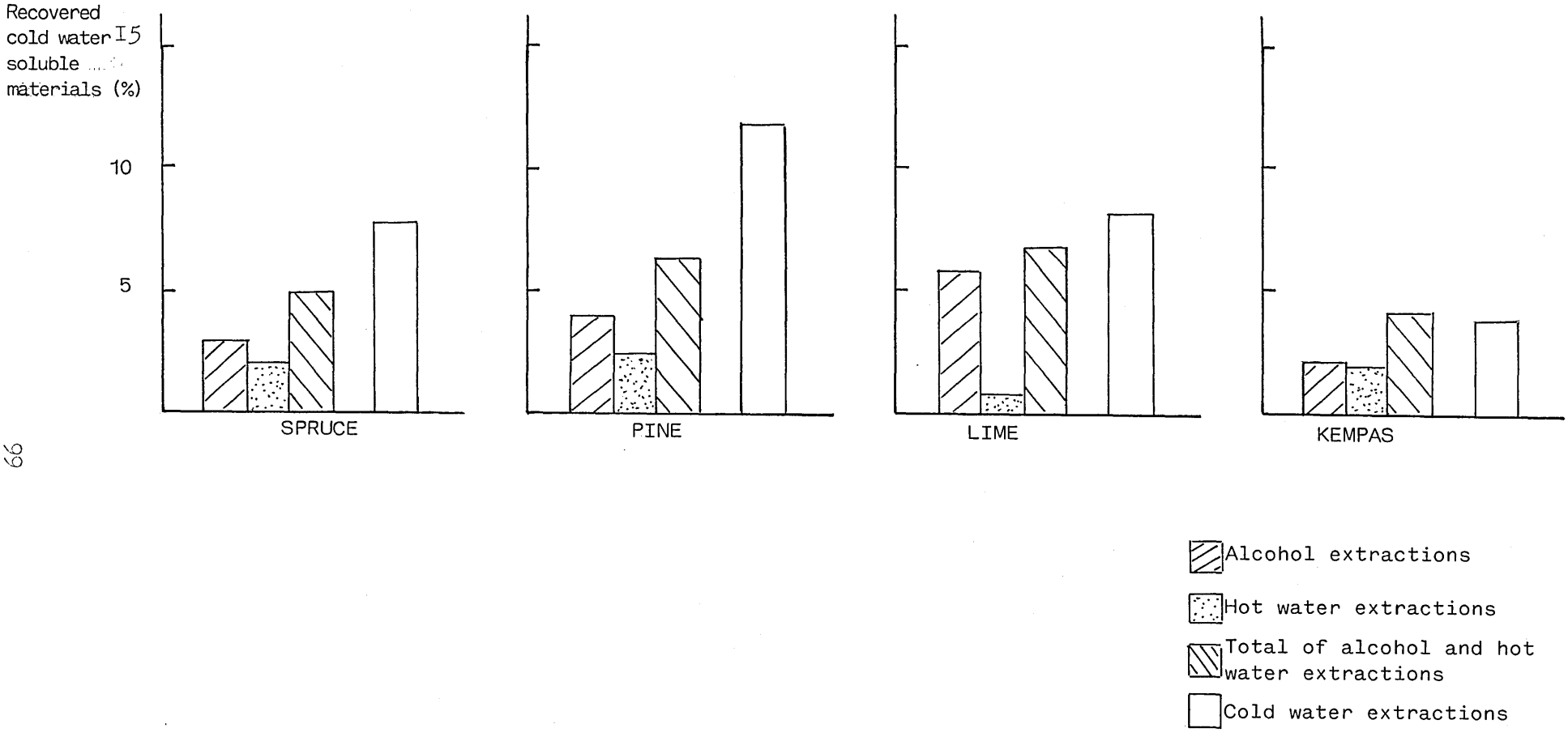
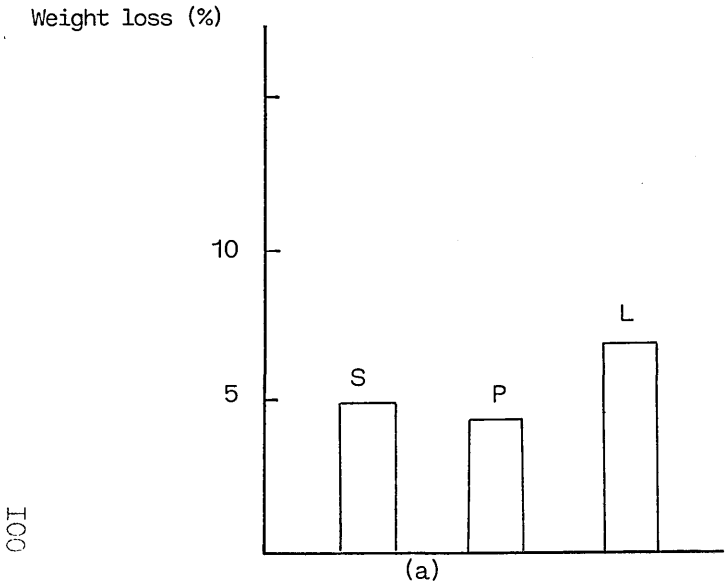


Fig 3.19 Recovered cold water soluble materials obtained from dried extracts of surface samples of spruce, pine, lime and kempas. Results are expressed as a percentage of the initial dry weight of the unextracted wood.



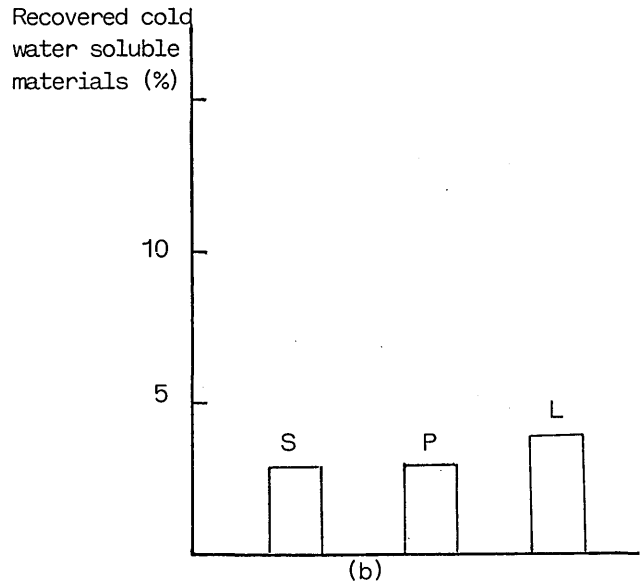


Fig 3.20(a) Weight losses recorded in sub-surface samples of spruce (S), pine (P) and lime (L) after extractions in cold water.

Fig 3.20(b) Recovered cold water soluble materials obtained from the dried aqueous extracts of sub-surface samples of spruce (S), pine (P) and lime (L).

All results are expressed as a percentage of the initial dry weight of the unextracted wood.

3.1.4.1. Soluble carbohydrates in surface samples of spruce, pine, lime and kempas.

The concentrated extracts from the extractions in alcohol, hot water and cold water of surface samples of spruce, pine and lime and samples of kempas, were analysed for total carbohydrate content and total reducing sugar content by the assay methods described in 2.1.5.1. and 2.1.5.2. respectively. The separation of wood sugars in these extracts was determined by HPLC using isocratic conditions. The results from the carbohydrate analysis are presented in Figures 3.22 to 3.24 and in a tabular form in Appendix 12. All the results are expressed as a percentage of the initial dry weight of the unextracted wood. The separation of sugars by HPLC is presented in Figures 3.26 to 3.31. Data obtained from the sugar standards used in the HPLC analysis is presented in Appendix 13.

In instances when wood was successively extracted with alcohol and then hot water, the first procedure removed more carbohydrate material than the second. Alcohol and cold water extracts of spruce, lime and kempas displayed broadly similar concentrations of total carbohydrate content. The total carbohydrate content in pine was higher in cold water extracts than in alcohol extracts. Reducing sugars contributed to a significant proportion of the total carbohydrate content in the softwoods and lime. Glucose and fructose were the predominant sugars in the softwoods, and sucrose was the predominant sugar in lime. Kempas did not show the presence of simple sugars.

a) Spruce

The soluble carbohydrates present in the extracts of surface samples of spruce are presented in Figure 3.21. Extraction in cold water released the greatest amount of soluble carbohydrates. The total carbohydrate content of the cold aqueous extract accounted for approximately 2% of the weight of wood. These soluble carbohydrate materials contributed to a third of the water soluble material recovered from the dried alcohol extracts and to a quarter of the water soluble material recovered from the dried cold water extracts. Reducing sugars contributed to a significant proportion of the total carbohydrate content in the extracts analysed. These sugars constituted 60% of the total carbohydrate content in the alcohol extracts, but a lesser proportion in the cold water extracts (40%).

Glucose and fructose were the predominant reducing sugars and these constituted 70% of the reducing sugar content in the alcohol extracts and 90% in the cold water extracts. Other sugars detected in small quantities (<0.4%) were sucrose and xylose. These sugars along with glucose and fructose were not detected in the hot water extracts.

b) Pine

In the cold water, alcohol and hot water extracts of pine, soluble carbohydrates accounted for 5%, 3% and 1% of the mass of wood respectively (Figure 3.22). Concentrations of these soluble carbohydrates were higher than those in spruce. In the extractions in cold water and in alcohol, carbohydrates accounted for 42% and 50% respectively, of the water soluble material recovered from the dried extracts.

In both the cold water and alcohol extracts, reducing sugars contributed to over 90% of the total carbohydrate content. Glucose and fructose were the predominant sugars and these sugars accounted for 3% and 2% of the mass of wood in the cold water and alcohol extracts respectively. The extraction in cold water also produced small amounts (1% of the mass of wood) of sucrose, xylose and galactose. In the extractions in alcohol, sucrose, xylose, mannose and trace quantities of arabinose were also detected. These sugars collectively contributed to less than 1% of the mass of wood in this extract.

c) Lime

Results of the soluble carbohydrate analysis of lime is presented in Figure 3.23. The results showed that the extractions in cold water and the combined results of the extractions in alcohol and hot water, released similar amounts (3%) of soluble carbohydrates. Soluble carbohydrates in lime accounted for 40% of the water soluble material recovered from both the alcohol and cold water extractions. Unlike spruce and pine, reducing sugars did not account for the majority of the total carbohydrate content in extracts of lime. Reducing sugars contributed to 40% and 33% of the total carbohydrate content in the alcohol and cold water extracts respectively. Glucose and fructose constituted 50% of the reducing sugars in each instance.

Sucrose was detected in lime in concentrations greater than those found in spruce and pine. This non-reducing sugar accounted for 50% of the carbohydrate content in alcohol and cold water extracts. Sucrose and the reducing sugars together contributed to 90% and 80% of the total carbohydrate content in the alcohol and cold water extracts respectively. In the extractions with hot water, reducing sugar concentrations were low, and glucose and fructose were not detected in these extracts.

d) Kempas

Soluble carbohydrate concentration in kempas accounted for less than 1% of the mass of wood (Figure 3.24). Both alcohol and cold water extracts displayed broadly similar carbohydrate contents. Hot water extraction yielded small quantities ($< 0.1\%$) of carbohydrates. Neither reducing sugars, nor sucrose nor other monosaccharides were detected in kempas by the procedures used.

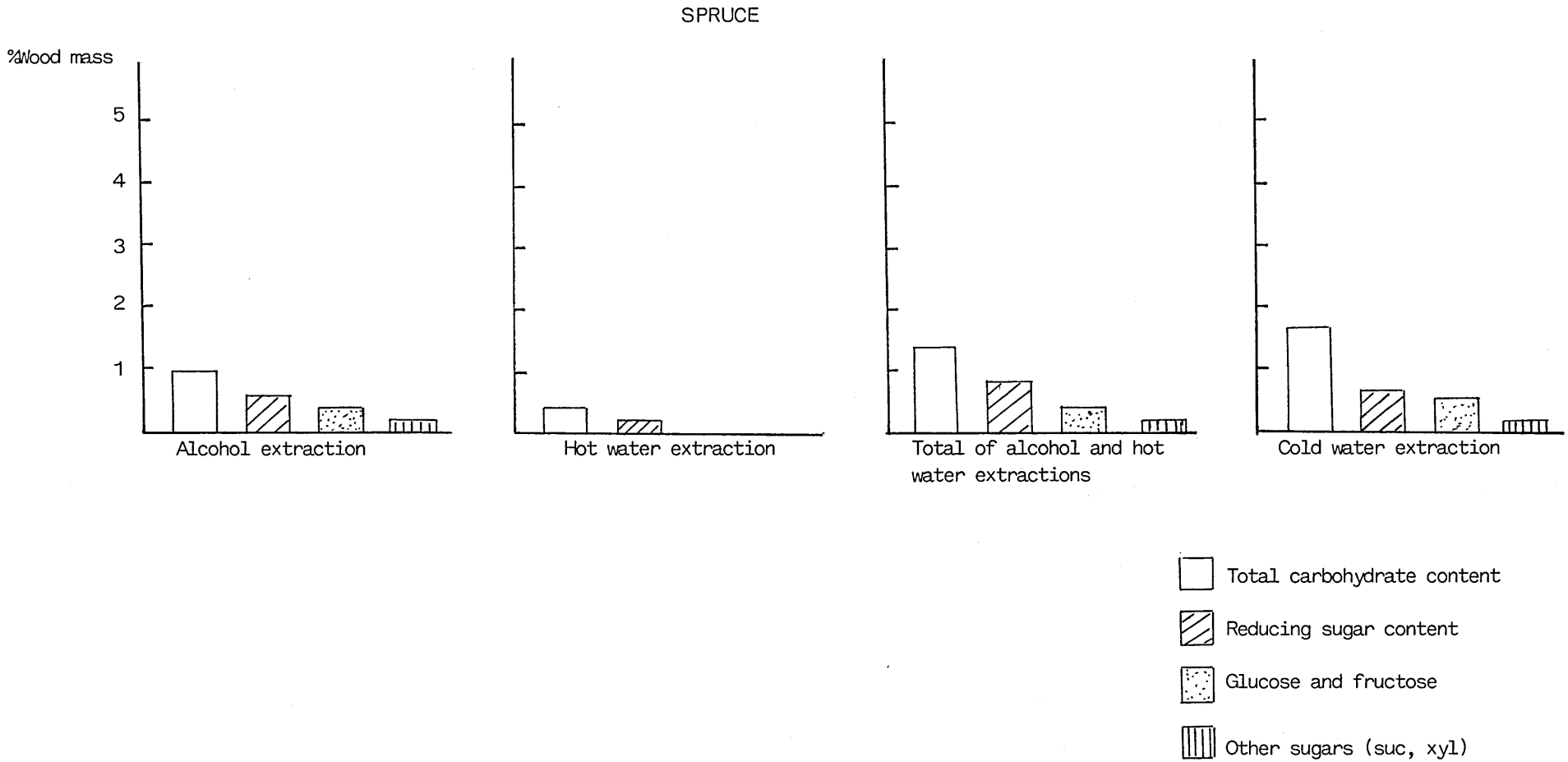


Fig 3.21 Soluble carbohydrate contents in surface samples of spruce after extraction with various solvents. Results are expressed as a percentage of the initial dry weight of the unextracted wood.

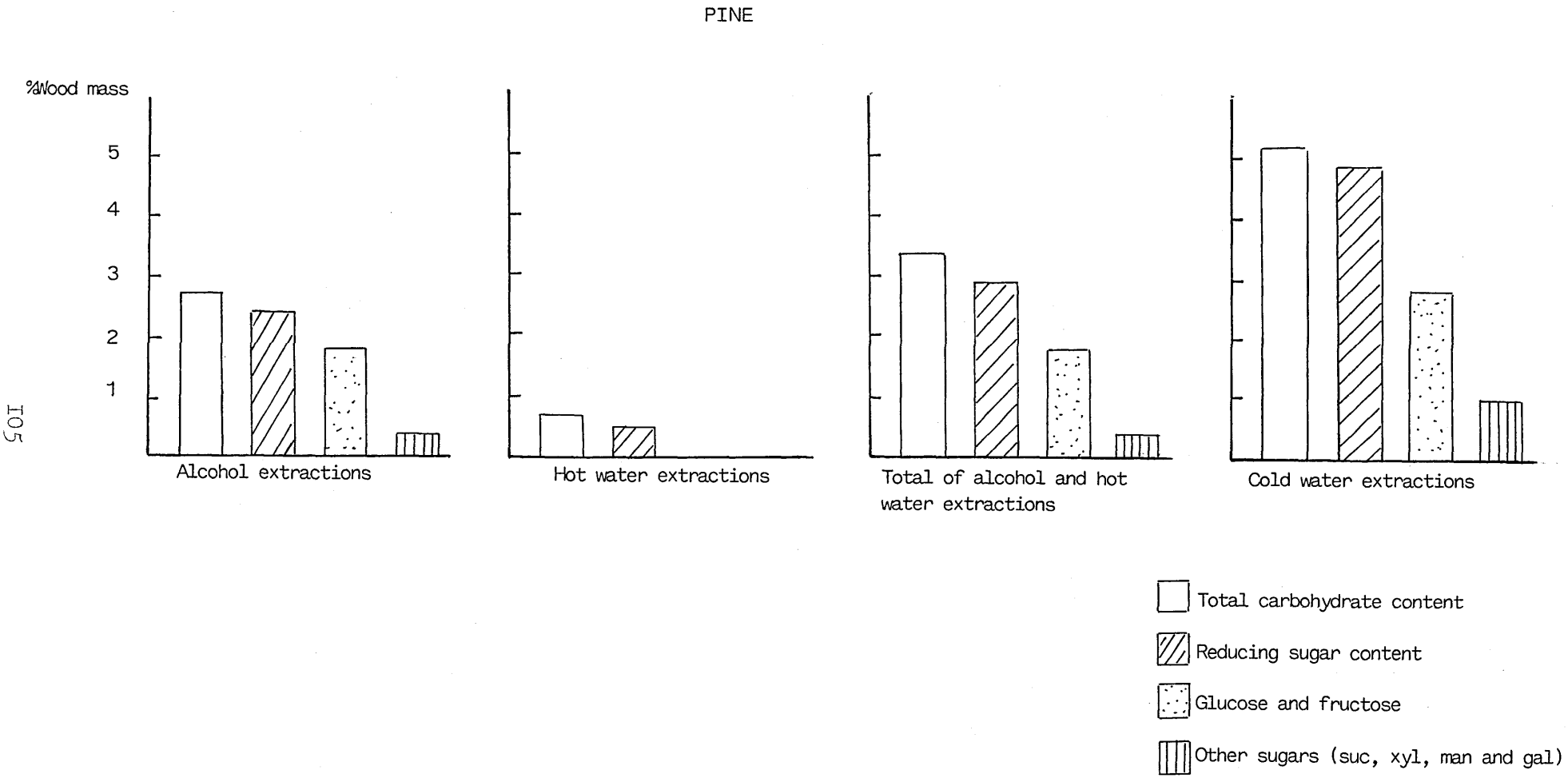


Fig 3.22 Soluble carbohydrate contents in surface samples of pine after extraction with various solvents. Results are expressed as a percentage of the initial dry weight of the unextracted wood.

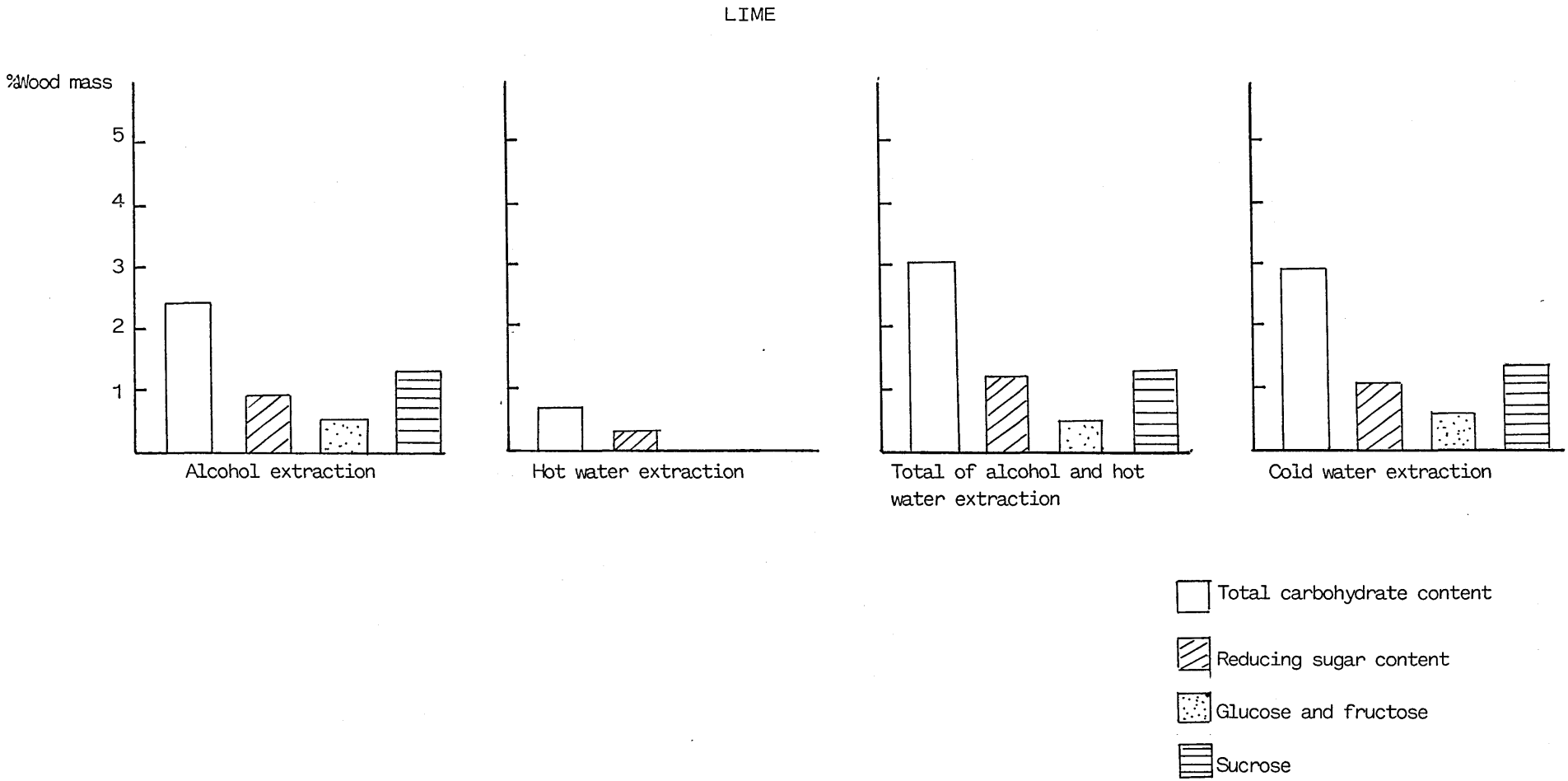


Fig 3.23 Soluble carbohydrate content in surface samples of lime after extraction with various solvents. Results are expressed as a percentage of the initial dry weight of the unextracted wood.

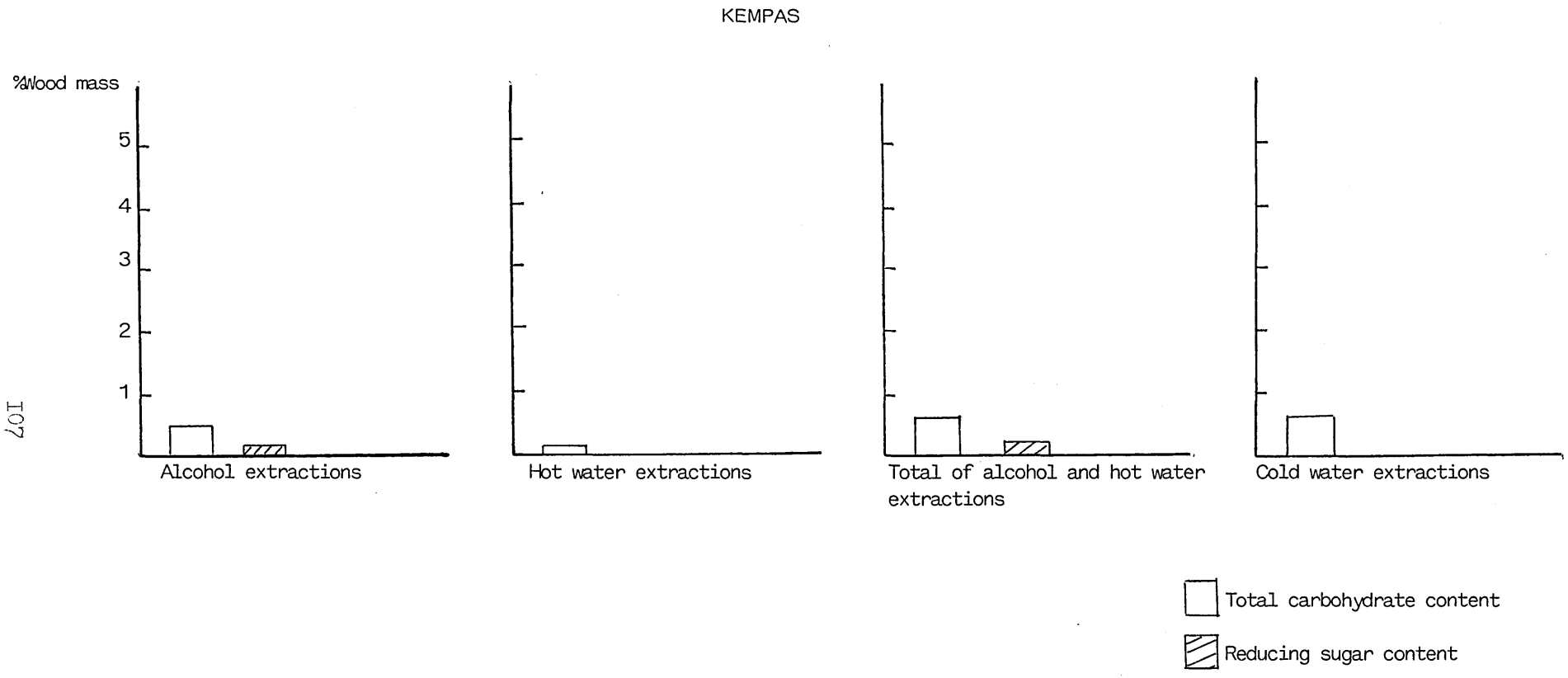


Fig 3.24 Soluble carbohydrate contents in kempas after extraction with various solvents. Results are expressed as a percentage of the initial dry weight of the unextracted wood.

3.1.4.2. Soluble carbohydrates in sub-surface samples of spruce, pine and lime.

Results of the carbohydrate analysis undertaken on sub-surface samples of spruce, pine and lime are presented in Figure 3.25. Concentrations of soluble carbohydrate were higher in surface samples than in sub-surface samples for each wood species examined. In pine, these carbohydrate concentrations were five times and in spruce and lime, twice the concentrations found in sub-surface regions. Soluble carbohydrate constituted 33% of the water soluble material recovered from the dried extract in pine, 25% from lime and 16% from spruce. Reducing sugars accounted for a large proportion (60%) of the soluble carbohydrates in pine; but a lesser proportion in spruce (30%). Glucose and fructose were detected in pine but not in spruce. In lime, sucrose and the reducing sugars accounted for 60% of the total carbohydrate content.

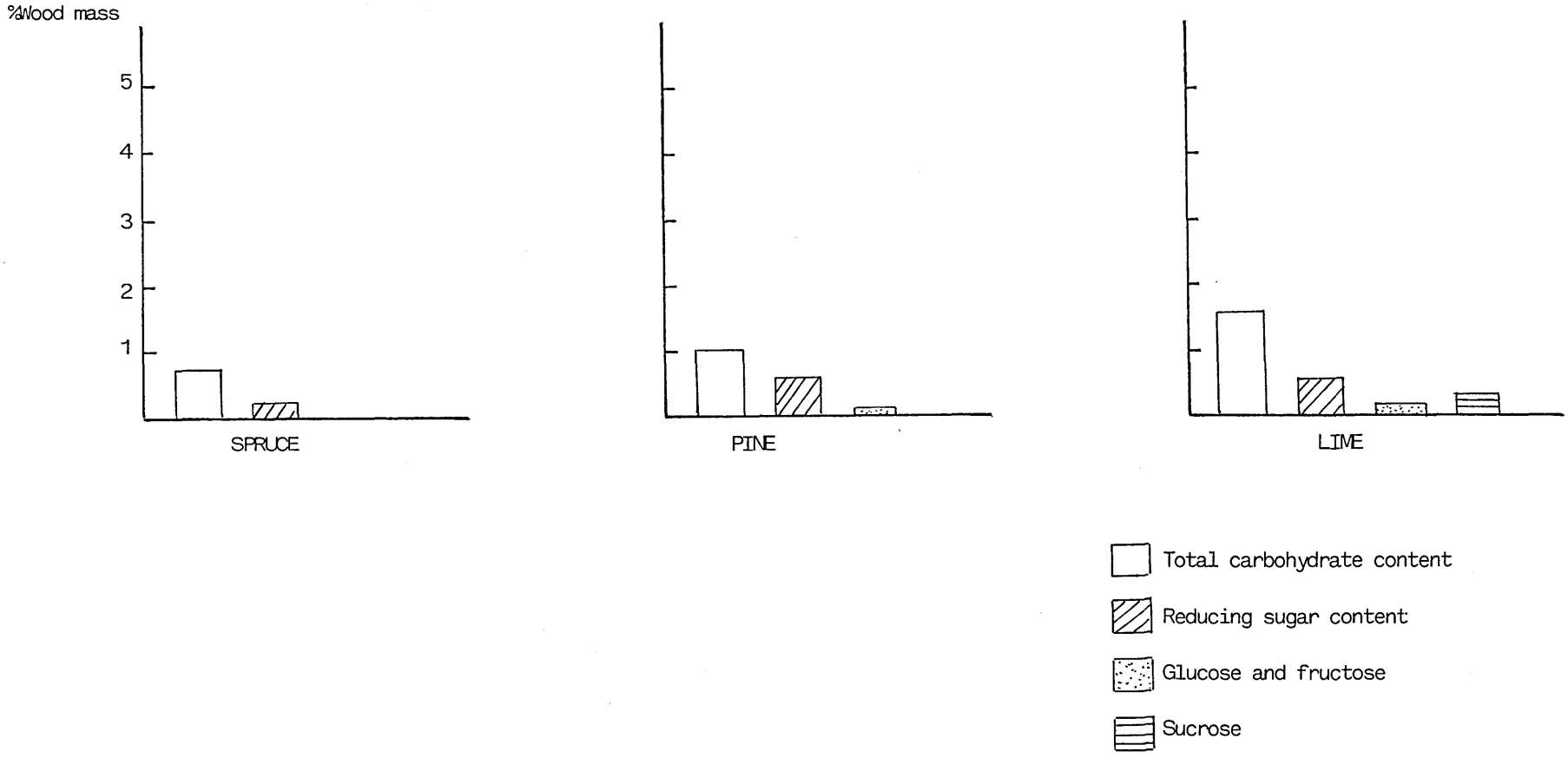


Fig 3.25 Soluble carbohydrates in aqueous extracts of sub-surface samples of spruce, pine and lime. Results are expressed as a percentage of the initial dry weight of the unextracted wood.

Fig. 3.26 Separation of wood sugars in alcohol and aqueous extracts of spruce by HPLC

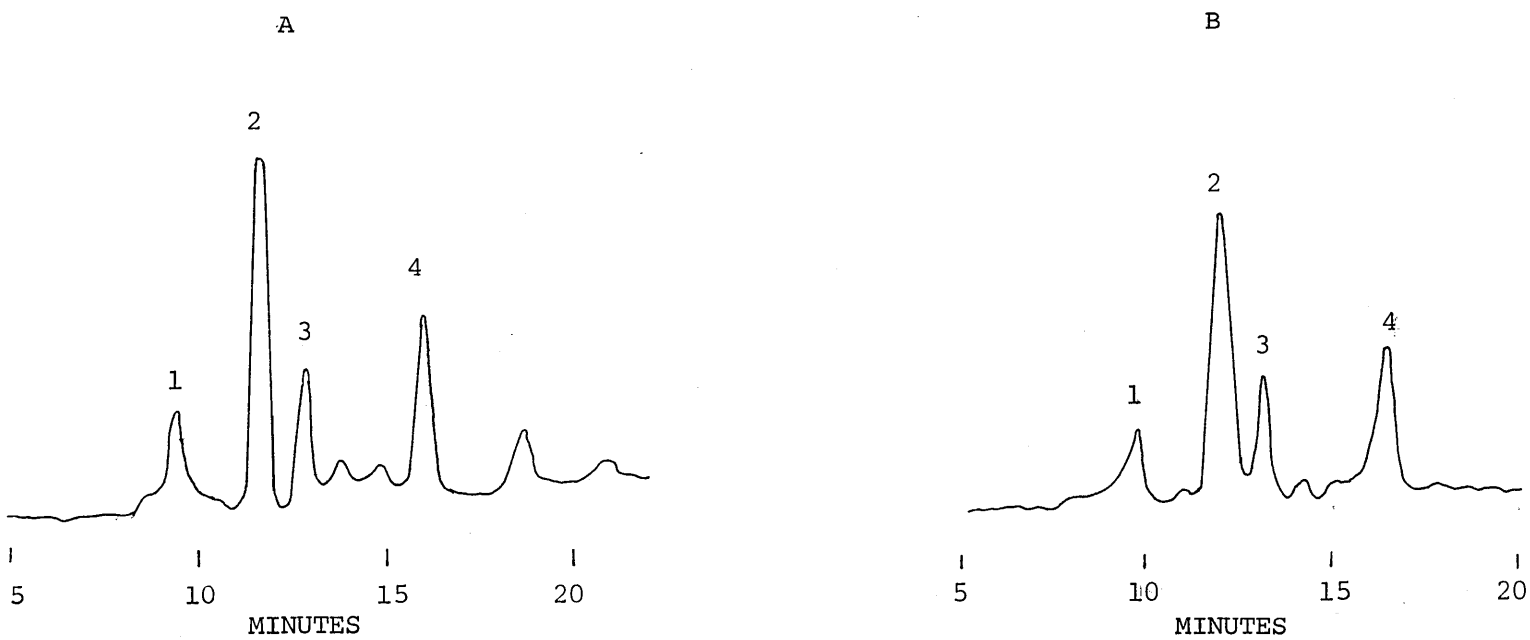
Sample:

A. Alcohol extracts from surface regions of spruce

B. Aqueous extracts from surface regions of spruce

Peaks:

1. Sucrose
2. Glucose
3. Xylose
4. Fructose



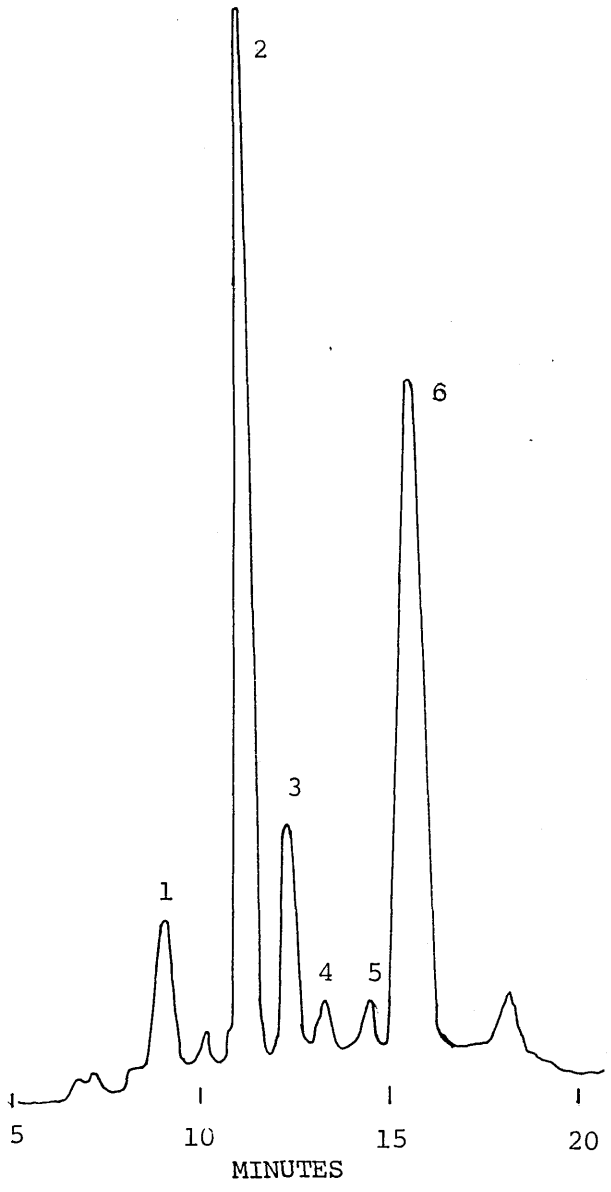


Fig. 3.27 Separation of wood sugars in alcohol extracts of surface samples of pine by HPLC

Peaks:

1. Sucrose
2. Glucose
3. Xylose
4. Galactose
5. Mannose
6. Fructose

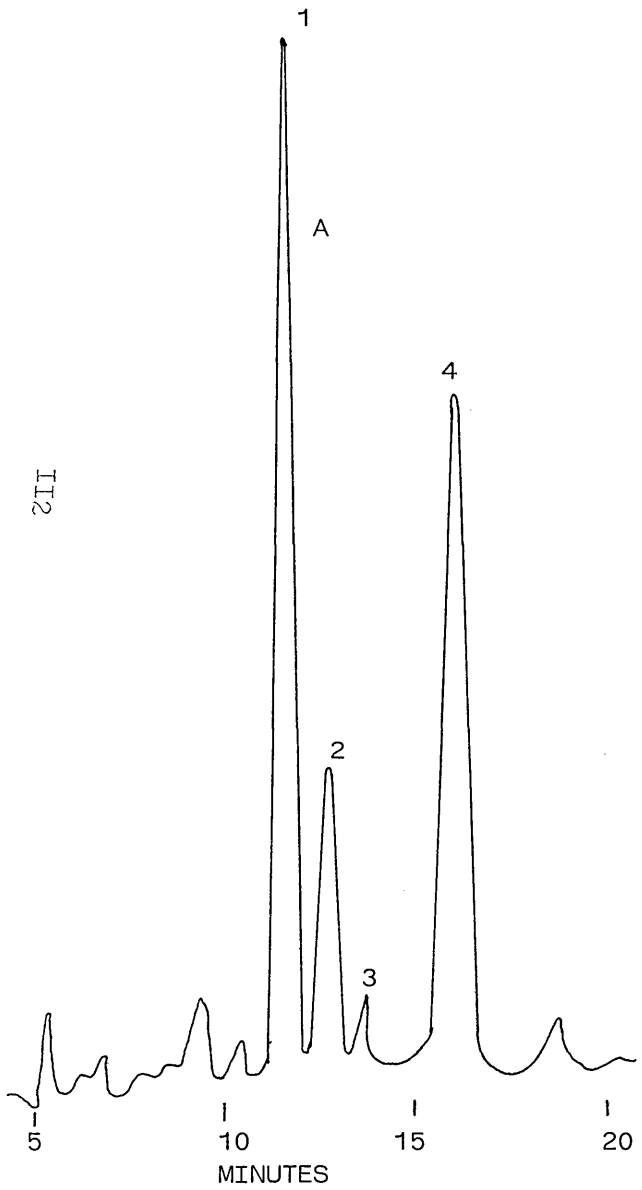


Fig 3.28 Separation of wood sugars in aqueous extracts of pine by HPLC

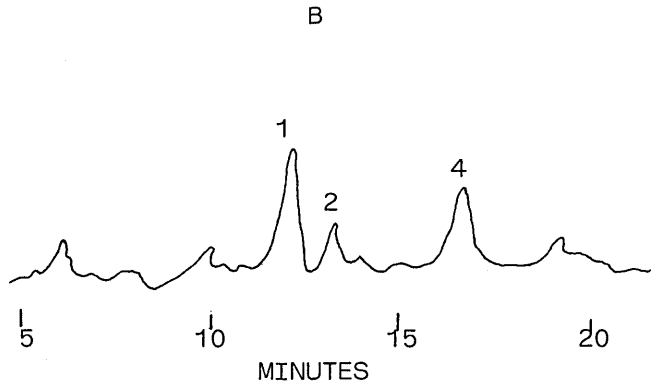
Sample:

A. Extracts from surface regions of pine

B. Extracts from sub-surface regions of pine

Peaks:

1. Glucose
2. Xylose
3. Galactose
4. Fructose



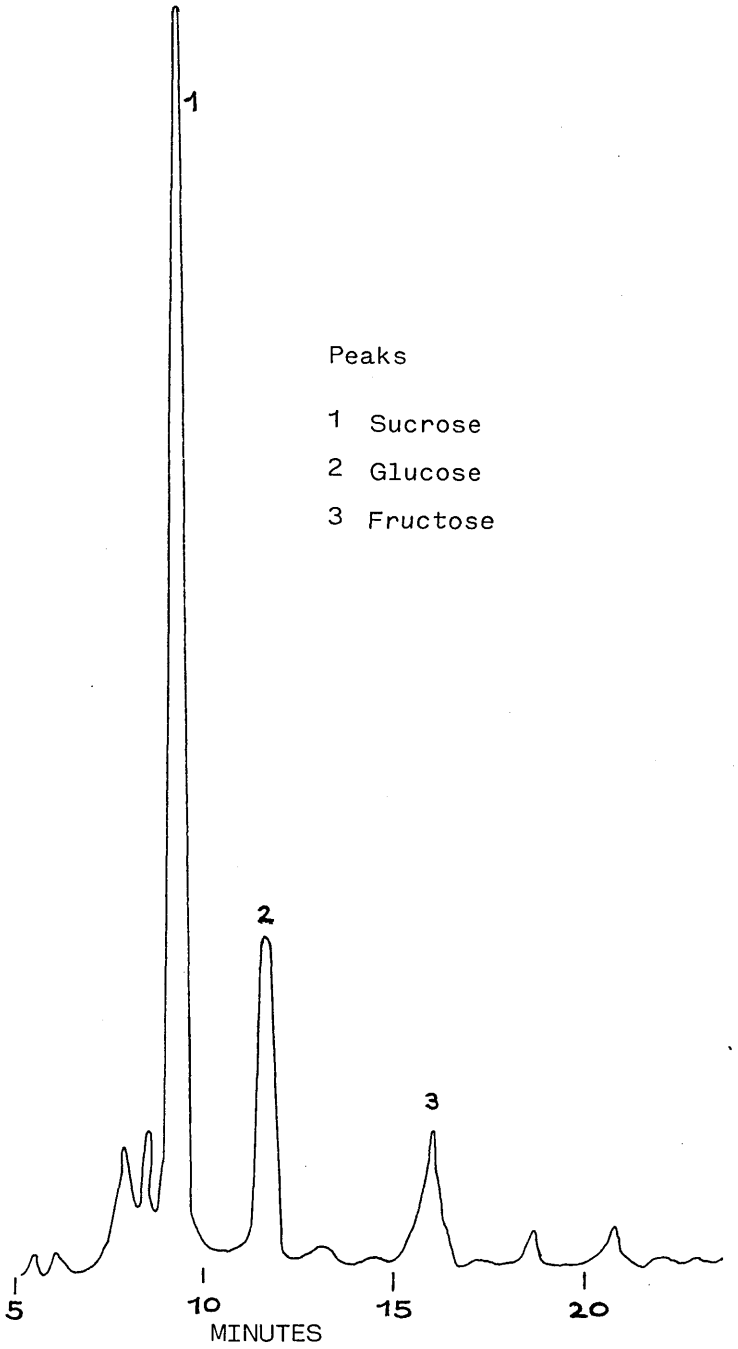


Fig 3.29 Separation of wood sugars in alcohol extracts of surface samples of lime by HPLC

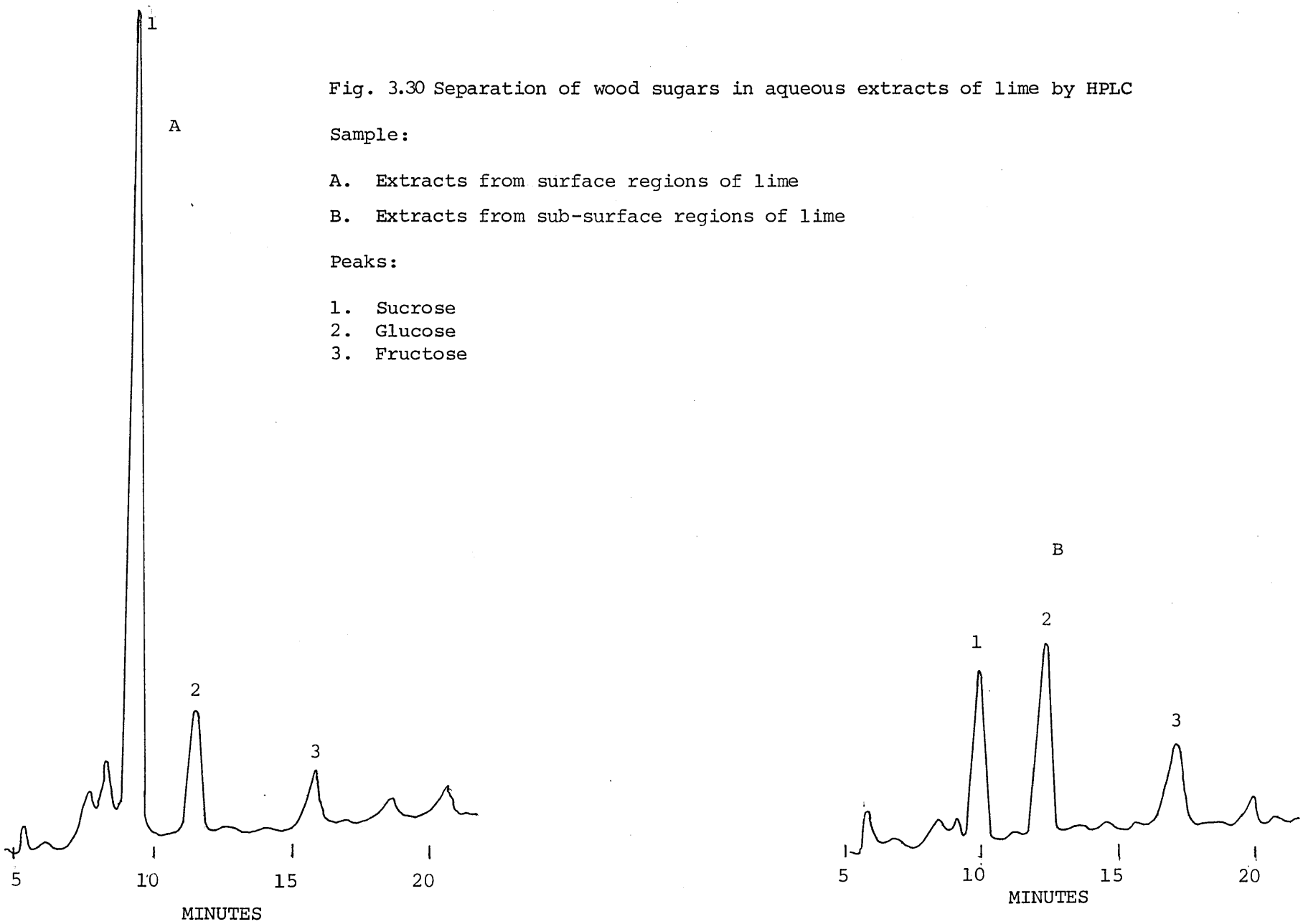


Fig. 3.30 Separation of wood sugars in aqueous extracts of lime by HPLC

Sample:

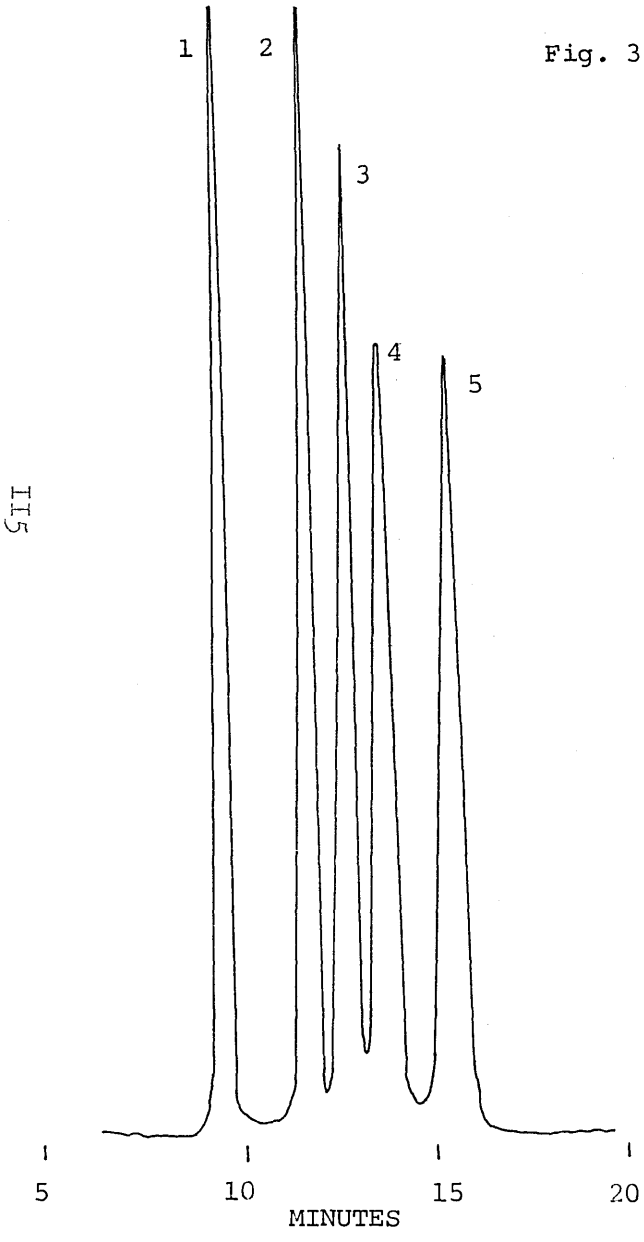
A. Extracts from surface regions of lime

B. Extracts from sub-surface regions of lime

Peaks:

1. Sucrose
2. Glucose
3. Fructose

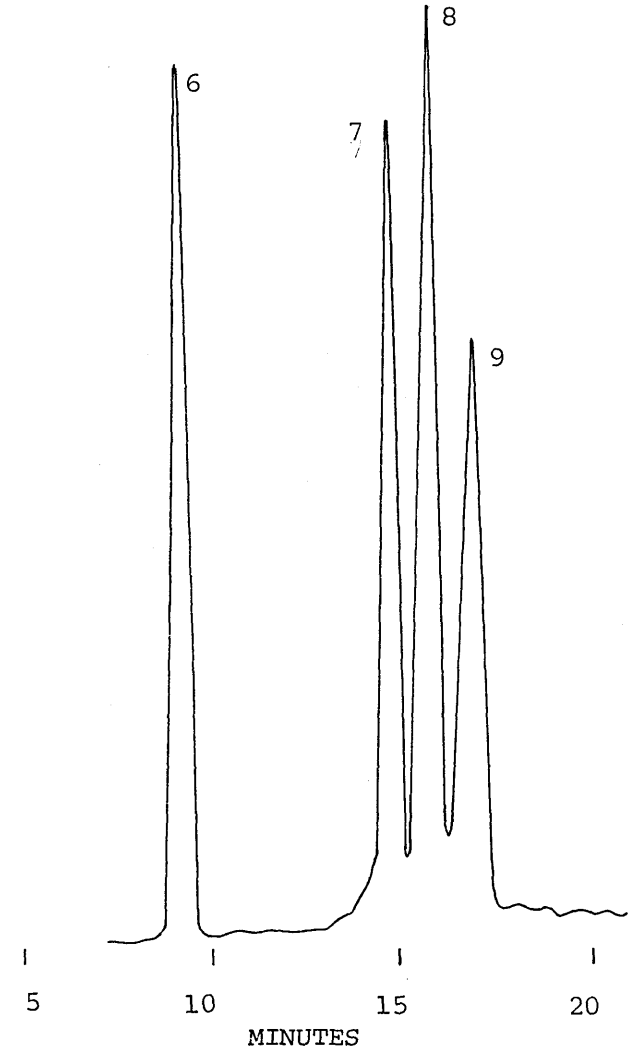
Fig. 3.31 Separation of carbohydrates by HPLC



Sugars at a concentration of 1 mg/ml

Peaks:

1. Sucrose
2. Glucose
3. Xylose
4. Galactose
5. Mannose
6. Raffinose
7. Rhamnose
8. Arabinose
9. Fructose



3.1.4.3. Soluble amino acids in surface samples of spruce, pine lime and kempas.

The results of the amino acid analysis undertaken on the extracts from the surface and sub-surface regions of spruce, pine, lime and kempas are presented in Figures 3.32 and 3.33. These results are expressed as a percentage of the initial dry weight of the unextracted wood. The composition of amino acids in these surface and sub-surface regions are presented in Tables 3.12 and 3.13 respectively.

Soluble amino acids were present in higher concentrations in spruce, pine and lime than in kempas. Kempas displayed minimal concentrations of soluble amino acids. Concentrations of soluble amino acids were broadly similar in alcohol and cold water extracts of spruce, but in pine, concentrations of amino acids were higher in cold water extracts than in alcohol extracts. The total amino acid content in spruce was higher than those in either pine, lime or kempas. In spruce, these amino acids accounted for 0.35% of the weight of wood, and constituted 6% of the water soluble material recovered from the dried alcohol extracts, and 4% from the dried cold water extracts. In pine, extraction in cold water resulted in total amino acid contents twice those found in extractions in alcohol. However, these amino acids contributed to a very small proportion of the recovered cold water soluble material ($\sim$2.5%) in both the dried alcohol and cold water extracts. Amino acid contents in the alcohol and cold water extracts of lime accounted for less than 0.1% of the cold water soluble material recovered from the dried extracts. Kempas displayed minimal amounts of amino acids in the alcohol extracts (0.01%) and trace amounts in the hot water and cold water extracts.

The total amino acid content in aqueous extracts of sub-surface samples of spruce, pine and lime is presented in Figure 3.33. All three woods displayed broadly similar levels of amino acids (0.02%). The results clearly showed that amino acid contents at surface regions were significantly higher than those at sub-surface regions. In spruce, these concentrations were fifteen times those found at sub-surface regions, in pine twelve, and in lime twice the concentrations at sub-surface regions.

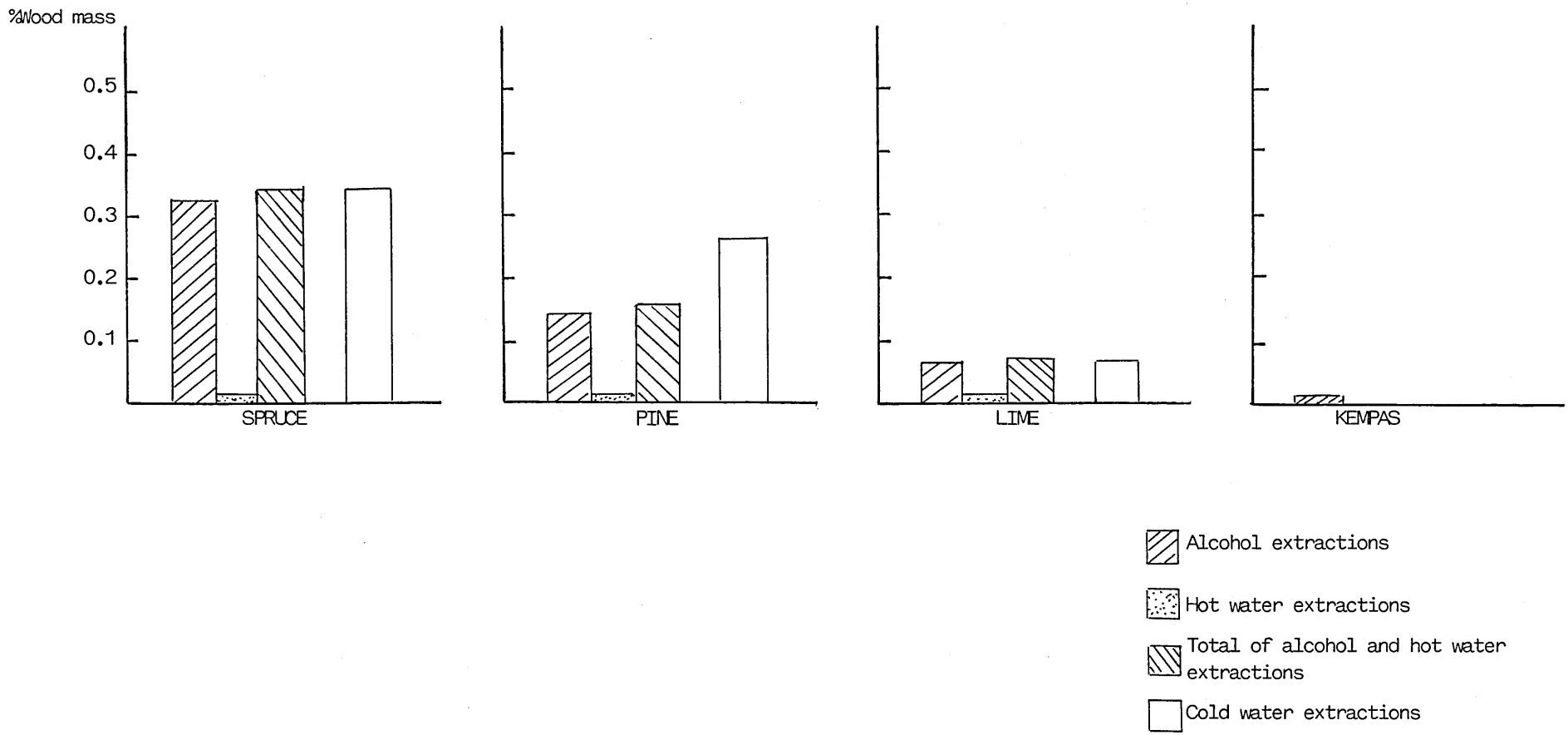


Fig 3.32 Soluble amino acid content in surface samples of spruce, pine, lime and kempas after extraction with various solvents. Results are expressed as a percentage of the initial dry weight of the unextracted wood.

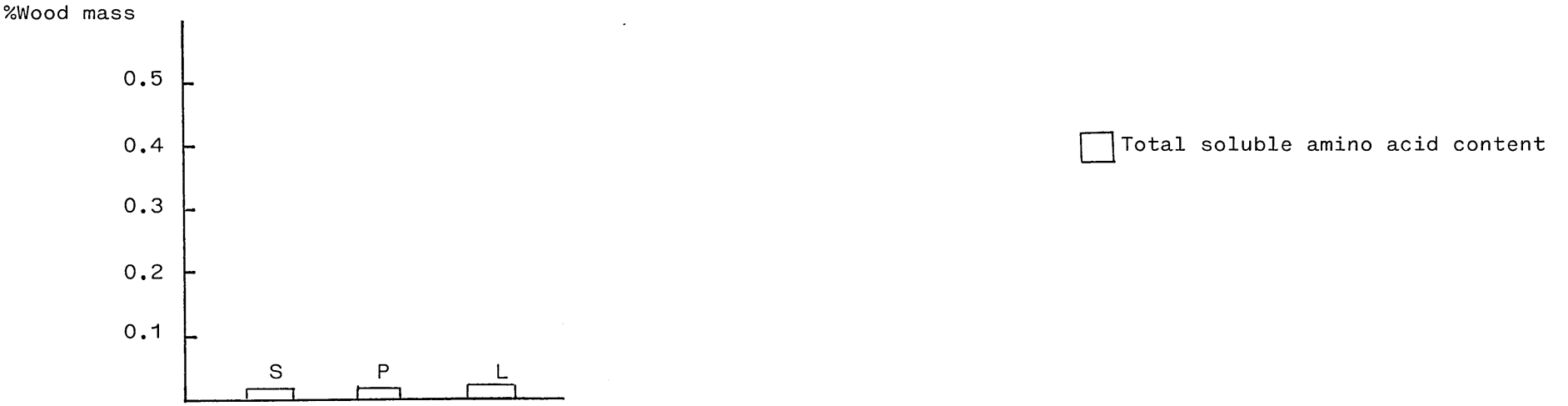


Fig 3.33 Soluble amino acid content in aqueous extracts of sub-surface samples of spruce (S), pine (P) and lime (L). Results are expressed as a percentage of the initial dry weight of the unextracted wood.

The different amino acids found in the extracts of surface samples of spruce, pine, lime and kempas are presented in Table 3.12. These results are expressed as μgrams of amino acid per gram of wood.

In general, amino acids present in the alcohol extracts were similar to those in the cold water extracts. However, concentrations of these amino acids varied with the type of extraction and also with wood type. Cold water extractions removed larger amounts of soluble amino acids, and the softwoods displayed a higher soluble amino acid content.

The major amino acids found in the alcohol extracts of spruce were aspartic acid, alanine, phenylalanine and arginine. These amino acids were also present in high concentrations in the cold water extracts along with serine, glutamine, and tyrosine and histidine. Other amino acids present in the extracts of spruce were glycine, valine, methionine, isoleucine and leucine. Aspartic acid, phenylalanine and arginine were the major amino acids in the alcohol and cold water extracts of pine. In both spruce and pine, concentrations of glutamine were higher in cold water extracts than in alcohol extracts. Tyrosine was detected in the cold water extracts but not in the alcohol extracts in both spruce and pine. The hardwoods lime and kempas displayed lower amino acid contents than the softwoods. In lime, aspartic acid, glutamine and arginine were the major amino acids in both the alcohol and cold water extracts. Kempas displayed trace amounts of aspartic acid, serine, glycine, alanine and arginine. The major amino acids common to spruce, pine and lime therefore were aspartic acid, glutamine and arginine.

The range of amino acids found in the aqueous extracts of sub-surface samples of spruce, pine and lime (Table 3.13) was smaller than that found in the extracts of surface samples. Glutamine and arginine were the major amino acids in all three woods tested. In lime, glutamine concentrations were broadly similar in extracts from both surface and sub-surface regions, but in spruce and pine, concentrations of this amino acid in surface regions were two and eight times the concentrations found in sub-surface regions. In lime and pine, arginine concentrations, at surface regions, were twice those at sub-surface regions, and in spruce, five times those at sub-surface regions.

Table 3.12 Soluble amino acids in surface samples of spruce, pine, lime and kempas after extractions in alcohol, hot water and cold water. Results are expressed as µg amino acid per gram wood.

Amino Acid	SPRUCE				PINE				LIME				KEMPAS			
	Alcohol	Hot Water	Alcohol and hot Water	Cold Water	Alcohol	Hot Water	Alcohol and hot water	Cold Water	Alcohol	Hot Water	Alcohol and hot water	Cold Water	Alcohol	Hot Water	Alcohol and hot water	Cold Water
asp	987	9	996	724	94	6	100	389	227	5	232	67	19	–	19	tr
ser	83	5	88	454	56	8	64	26	–	2	2	tr	11	–	11	tr
gln	29	tr	29	140	16	tr	16	317	83	tr	83	85	tr	tr	tr	tr
gly	27	3	30	96	16	2	18	248	2	tr	2	–	tr	–	tr	tr
ala	126	3	129	85	86	3	89	110	79	4	83	71	4	–	4	tr
val	31	tr	31	131	25	tr	25	53	–	–		–	–	tr	tr	–
met	–	–	–	143	–	–	–	–	–	–		–	–	–	–	–
ile	33	–	33	126	14	tr	14	27	–	–		–	–	–	–	–
leu	36	–	36	126	28	tr	28	21	–	–		–	–	–	–	–
tyr	–	–	–	289	–	–	–	634	–	–		–	–	–	–	–
phe	158	tr	158	211	269	16	285	350	–	–		–	16	–	16	–
his	–	–	–	271	–	–	–	193	–	–	–	–	–	–	–	–
arg	1699	15	1714	557	703	5	708	182	217	–	217	166	tr	–	tr	tr
Total Amino Acid Content	3209	35	3244	3353	1307	40	1347	2550	606	13	619	318	101	tr	101	tr
Soluble Amino Nitrogen	717	7	724	544	312	6	318	371	136	2	96	132	15	tr	15	tr

– not detected
tr trace qualities ($< 0.02\ \mu g g^{-1}$ wood)

Table 3.13 Soluble amino acids in aqueous extracts of sub-surface samples of spruce, pine and lime. Results are expressed in μgram per gram wood.

Amino Acid	Spruce	Pine	Lime
asp	25	11	38
ser	-	-	-
gln	59	36	79
gly	tr	tr	tr
ala	tr	tr	39
val	-	tr	-
met	-	tr	tr
ile	-	tr	tr
leu	-	tr	tr
tyr	-	-	tr
phe	tr	-	tr
his	-	-	-
arg	121	96	99
Total Amino Acid Content	206	143	255
Soluble Amino Acid	53	39	60

\- not detected
tr trace quantities ($< 0.02\ \mu g g^{-1}$ wood)

A comparison of the total nitrogen content (as determined by micro-kjeldahl analysis) of surface and sub-surface samples of unextracted wood and the soluble amino nitrogen content (determined from the amino acid analysis of cold water extracts) is presented in Table 3.14.

Table 3.14 Total nitrogen and soluble nitrogen contents in dried samples of spruce, pine, lime and kempas.

		Wood Type			
		Spruce	Pine	Lime	Kempas
Surface Samples	Total Nitrogen Content (%)	0.12	0.13	0.17	0.26
	Soluble Amino Nitrogen (%)	0.05	0.04	0.01	tr
Sub-Surface Samples	Total Nitrogen Content (%)	0.08	0.07	0.14	NT
	Soluble Amino Nitrogen (%)	0.005	0.004	0.006	NT

NT no trial

tr trace amounts

The results showed that soluble amino nitrogen constituted a significant proportion of the total nitrogen content in the surface samples of the softwoods, but to a lesser proportion in the surface samples of lime. In spruce and pine, soluble amino nitrogen contributed approximately 40% and 30% respectively, of the total nitrogen contents at the surface regions. In lime, soluble amino nitrogen constituted 6% of the total nitrogen content at the surface.

The total nitrogen contents at sub-surface regions were lower than those in surface regions. Concentrations of soluble amino nitrogen were broadly similar in spruce, pine and lime, and these soluble amino nitrogens contributed 6% of the total nitrogen content in the woods. It is clear from the results, that the migration and accumulation of soluble amino acids at the surfaces of wood during drying, account for a significant proportion of the total nitrogen seen at these surfaces.

Conclusions

The conclusions drawn from this experiment were:

1) concentrations of soluble carbohydrates and amino acids were higher at surface regions than at sub-surface regions. Greatest amounts of these soluble nutrients were obtained from the extractions in cold water,

2) softwoods in general, displayed higher concentrations of soluble carbohydrates and amino acids to hardwoods, and temperate woods displayed higher soluble nutrient concentrations to the tropical wood kempas,

3) in spruce and pine, glucose and fructose were the predominant sugars, but in lime, sucrose was the predominant sugar,

4) soluble amino nitrogen constituted a significant proportion of the total nitrogen content at the surface regions of the wood tested,

and

5) the major amino acid common to spruce, pine and lime were aspartic acid, glutamine and arginine.

3.2. Soil Burial Studies

3.2.1. Studies using unpreserved wood

The results of all soil burial studies undertaken on lime, beech, pine and spruce to determine the influence of added soluble carbohydrates and amino acids on the decay and nitrogen transfer to these woods are described in this section. In the soil burial studies with carbohydrates, test blocks were impregnated with sugar solutions to obtain blocks with concentrations of sugar, representative of those found at surface regions of dried wood. Concentrations of 1% w/w and 7% w/w sugar were also included to provide sugar concentrations above and below the levels found in the outer 3mm of dried wood in previous experiments (3.1.4.). Beech blocks, representing a non-durable hardwood commonly used in wood preservative studies was also included in the experiment and were impregnated with sugar to provide test blocks of 1% w/w, 3% w/w, 4% w/w and 6% w/w sugar.

In the soil burial studies with amino acids, test blocks were impregnated with amino acid solutions of 0.45% w/v, 0.90% w/v, 1.80% w/v and 3.60% w/v to obtain increases in nitrogen contents approximating to 0.2%, 0.3%, 0.5% and 0.9%. A supplementary experiment was also undertaken to investigate the effect of amino acids on test blocks impregnated with amino acids (3.60% w/v), and buried in an undried condition at 100% moisture content. Lime was selected as a test species for this experiment. The influence of a mixture of amino acids and sugars were also studied with this species. The losses of soluble nitrogenous materials artificially incorporated into pine blocks and also in pine blocks containing redistributed soluble nutrients were examined.

In all experiments, the parameters monitored were moisture content, weight loss and nitrogen transfer to wood. In the studies with carbohydrates, weight loss and nitrogen transfer were expressed as a percentage of the initial preburial weight of wood and also as a percentage of the initial preburial weight of wood and the weight of added sugar. Weight losses and nitrogen transfer in subsequent studies were expressed only as a percentage of the preburial weight of wood.

3.2.1.1. The influence of added soluble carbohydrates and amino acids on the decay and nitrogen transfer to hardwoods and softwoods.

The influence of added soluble carbohydrates and amino acids on weight losses and nitrogen transfer to unpreserved wood in soil burial studies are presented in Figures 3.34 to 3.48. In general, added soluble nutrients showed little influence on decay in both hardwoods and softwoods, and the levels of decay for varying treatments for each wood species were broadly similar.

In each wood species, significant weight losses were observed over the duration of the soil burial period. In the studies with carbohydrates, evaluation of weight losses as a percentage of the initial weight of wood and the weight of sugar, showed blocks impregnated with sugar to have higher weight losses than control blocks. The differences in weight losses between the blocks impregnated with sugar and control blocks, corresponded approximately to the weight increments in the block as a result of the weight of sugar. Softwoods displayed lower weight losses than hardwoods in all the soil burial experiments, and weight losses were not significant ($<3\%$) in softwoods until after 3 weeks burial in soil.

The nitrogen contents in wood blocks increased over the duration of the soil burial. In the hardwoods, these nitrogen increases were accompanied by weight losses in the early stages (0-3 weeks) of the soil burial. In the softwoods, nitrogen content increases were not accompanied by weight losses during this period. In the studies with carbohydrates, the differences in nitrogen contents of the test blocks after burial, calculated using the methods described for the evaluation of weight losses in 3.2.1., were small. Nitrogen contents calculated on the preburial weight of wood and the weight of added sugar were marginally lower to those calculated on the preburial weight of wood alone. In the studies with amino acids, blocks impregnated with amino acids showed nitrogen losses during the initial stages of burial (weeks 0-3). These nitrogen losses were observed in hardwood blocks impregnated at 1.8% w/v and 3.60% w/v amino acid concentrations, and in all softwood blocks impregnated with amino acids. Control blocks of both hardwoods and softwoods showed no depletion in nitrogen content during these initial stages. During weeks 3 - 12, the nitrogen contents of blocks continued to increase but with a gradual diminution in the rate of increase with time.

Statistical analysis were undertaken on the results obtained from the soil burial experiments.

The analysis were undertaken using the statistical package mounted on a Dec 20 computer (R. Houchard, Statpack Program, Decsystem 20, Western Michigan University, 1974). Two-way analysis of variance were undertaken on most results. However, analysis of variance were not undertaken on the following studies:-

1. Weight losses in the study with carbohydrates in beech, pine and spruce;
2. nitrogen results in the studies with carbohydrates and amino acids in both hardwoods and softwoods.

Weight loss results from these studies showed little difference between treatments and nitrogen results from the amino acid studies also showed little variation between different treatments after 3 weeks soil burial. For these reasons, it was considered unnecessary to undertake statistical analysis.

a) Lime

The influence of added soluble carbohydrates and amino acids on weight losses in lime are presented in Figure 3.34 and Figure 3.36. In the study with carbohydrates, the weight of sugar incorporated into test blocks from the uptake of sugar solution, ranged from 3mg to 18mg for sugar concentrations 1% w/w to 7% w/w. All blocks irrespective of treatment showed similar weight losses initially when calculated on the preburial weight of the block (Fig 3.34a). Control blocks and blocks impregnated with sugar showed a rapid increase in weight loss during the period weeks 3 - 9. Weight losses of over 40% were recorded at week 9. Control blocks and blocks impregnated with 1% w/w and 7% w/w sugar displayed similar weight losses of 55% at the final sampling period. Blocks impregnated with 3% w/w and 5% w/w sugar showed weight losses of 43% and 63% respectively. Two way analysis of variance undertaken on the results showed that the weight losses incurred by the different carbohydrate treatments were not significant at the 5% level. Variations in weight losses of blocks were large within each treatment.

Weight losses calculated as a percentage of the weight of wood and the weight of sugar after impregnation (Fig 3.34b) displayed similar trends to those described for Figure 3.34a.

However, at the first sampling interval, weight losses of control blocks were shown to be lower than weight losses of blocks impregnated with sugar. During this period, test blocks with increasing sugar concentration showed correspondingly higher weight losses to control blocks, but thereafter, weight losses were broadly similar for the different treatments.

In the study with amino acids, all test blocks showed similar weight losses at 3 weeks (Fig 3.36). Control blocks and test blocks impregnated with amino acid concentrations of 0.45% w/v, 0.90% w/v and 1.80% w/v displayed broadly similar weight losses over the duration of the experiment. However, weight losses of test blocks impregnated with amino acid concentrations of 3.60% w/v, were lower than those of control blocks and amino acid blocks of lower concentrations. T-tests undertaken on mean weight losses of control blocks and amino acid blocks of 3.60% w/v, at sampling periods of 6, 9 and 12 weeks, were significant at the 5% level. However 2-way analysis of variance showed that differences in weight losses of all blocks impregnated with amino acids and control blocks, were not significant at the 5% level.

All test blocks achieved weight losses of over 40% after 6 weeks soil burial. Control blocks and test blocks with 0.45% w/v, 0.90% w/v and 1.80% w/v amino acid concentrations achieved weight losses of approximately 65% at the final sampling period. The overall weight losses of test blocks, in the study with amino acids were higher than those observed in the study with carbohydrates. Comparison of the rates of weight loss of control blocks in the amino acid study and control blocks in the carbohydrate study, showed that weight losses occurred at a faster rate in the amino acid study. Mean rates of weight loss of controls in the amino acid study were at 5.5% per week over the 12 week soil burial, and mean rates of weight loss of controls in the carbohydrate study, were at 4.8% per week over a similar period. This difference in rates at which weight loss occurred, accounted for the higher weight losses seen in the amino acid study.

Weight losses of control blocks buried at 100% moisture content (Fig 3.37) were similar to control blocks buried in an air-dried condition, (10% moisture content).

However, weight losses of test blocks impregnated with amino acid concentrations of 3.60 w/v and buried at 100% moisture content (Fig 3.37), were higher than those which had been buried in an air-dried condition (Fig 3.36), and were broadly similar to the amino acid blocks of 0.45% w/v, 0.90% w/v and 1.80% w/v which were buried air-dried.

Control blocks and blocks impregnated with a mixture of sugar and amino acids, and buried at 100% moisture content did not show significant weight losses ($>3\%$) during the first week of the soil burial (Fig 3.38). During the sampling period week 3, test blocks impregnated with a mixture of sugar and amino acids displayed lower weight losses (6%) than control blocks, but weight losses of all test blocks at week 6 were similar. No further analysis of weight losses were undertaken beyond the 6 week period. Results showed that sugars, amino acids and a mixture of these did not influence the decay of wood significantly in soil burial.

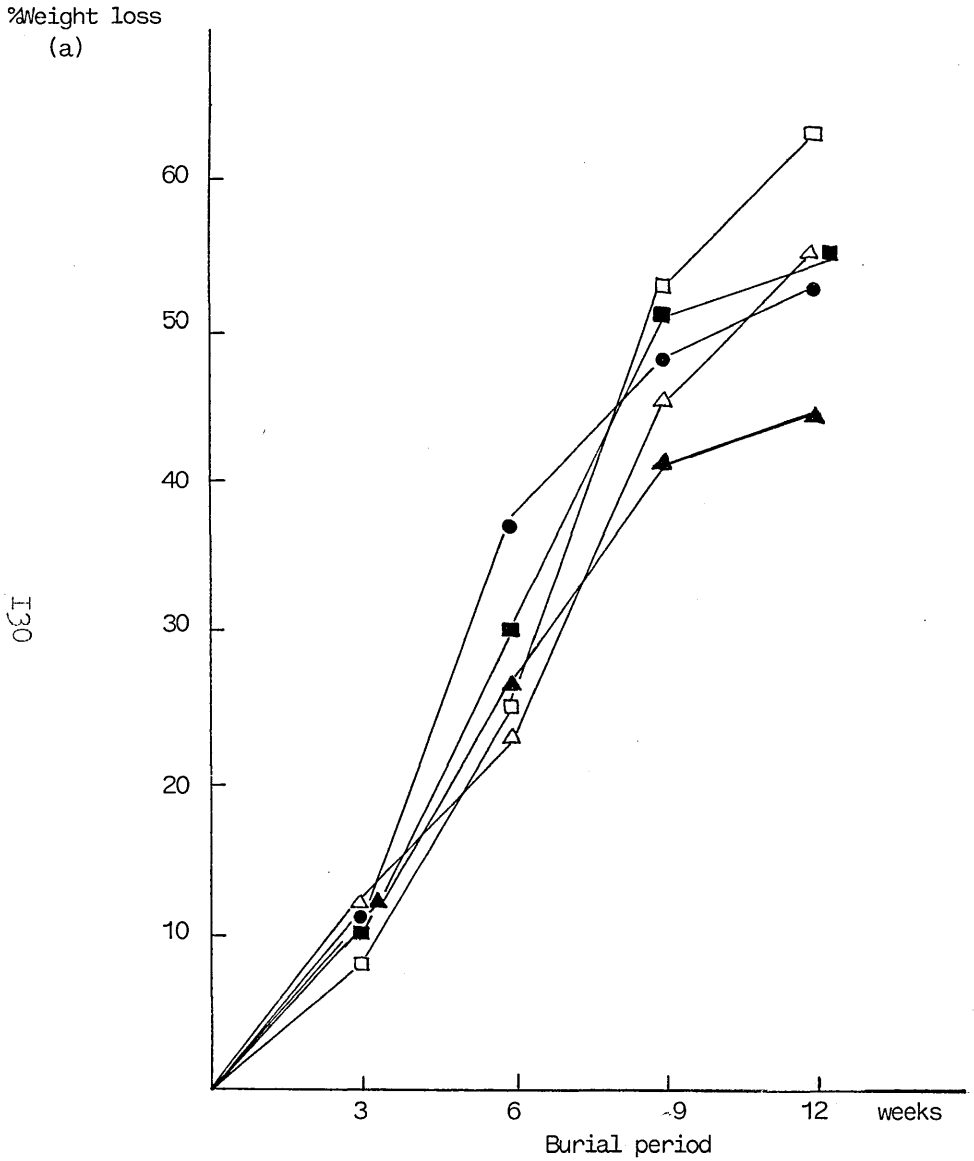
Results of the nitrogen contents of test blocks impregnated with carbohydrates are presented in Figure 3.35. Figure 3.35(a) describes the nitrogen contents of test blocks calculated on the preburial weights of the block, and Figure 3.35(b) the nitrogen contents calculated on the weight of the block plus weight of sugar. Nitrogen contents of the test blocks prior to soil burial were below 0.15% (Fig 3.35). During the period 0-3 weeks, the nitrogen contents of these blocks showed a rate of increase of 0.06% per week. The rate of nitrogen increase showed a gradual diminution thereafter. At each sampling period, the nitrogen contents of controls and blocks impregnated with sugar were broadly similar. The nitrogen contents of blocks impregnated with sugar between the period 6-9 weeks and 9-12 weeks were fairly constant at 0.5%. Control blocks displayed slight increases to 0.55% during the final sampling period.

In the study with amino acids, the initial nitrogen content of the control blocks was 0.17% (Fig 3.36). The initial nitrogen contents of blocks impregnated with amino acid concentrations of 0.45% w/v, 0.90% w/v, 1.80% w/v and 3.60% w/v were 0.26%, 0.36%, 0.54% and 0.95% respectively. During the period 0-3 weeks, control blocks and blocks impregnated with lower amino

acid concentrations showed an increase in nitrogen contents to 0.3%, but test blocks impregnated with higher amino acid concentrations showed a decrease in nitrogen content. All blocks regardless of treatment displayed a rapid rate of nitrogen increase during the period 3-6 weeks. These rates (0.06% per week) were similar to those observed in the carbohydrate studies. The rate of increase of nitrogen content of all blocks during the period 6-9 weeks were less rapid. In the final sampling period, all blocks with the exception of amino acid blocks of 3.60% w/v displayed a decrease in nitrogen content. The overall nitrogen content of the test blocks in this study were higher than those in the carbohydrate study despite the initial decrease in nitrogen content.

The nitrogen contents of control blocks buried at 100% moisture content (Fig 3.37), were comparable to the nitrogen contents seen in control blocks in the previous studies. Blocks impregnated with 3.60% w/v amino acid concentrations and buried at 100% moisture content, showed a decrease in nitrogen content during the period 0-3 weeks. This loss of approximately 60% of the initial nitrogen on emplacement of the blocks in soil, was also observed in the previous amino acid study. The nitrogen contents of control blocks and blocks impregnated with amino acids and buried at 100% moisture content, were similar over the duration of the burial period.

Lime blocks impregnated with a mixture of sugar and amino acids (Fig 3.38) showed a decrease in nitrogen contents during the first week of the soil burial. Control blocks, both wet (100% moisture content) and air-dry (10% moisture content), showed an increase in nitrogen content from 0.18% to 0.2% during this period. Between the period 1-3 weeks, control blocks and blocks with a mixture of sugar and amino acid showed similar nitrogen contents of 0.3%. These high nitrogen contents of the sugar and amino acid blocks were not accompanied by large weight losses during this period. The high nitrogen contents were attributed to residual nitrogen retained in the block. During the period 3 - 6 weeks, control blocks and blocks impregnated with a mixture of sugars and amino acids displayed similar weight losses and nitrogen contents.



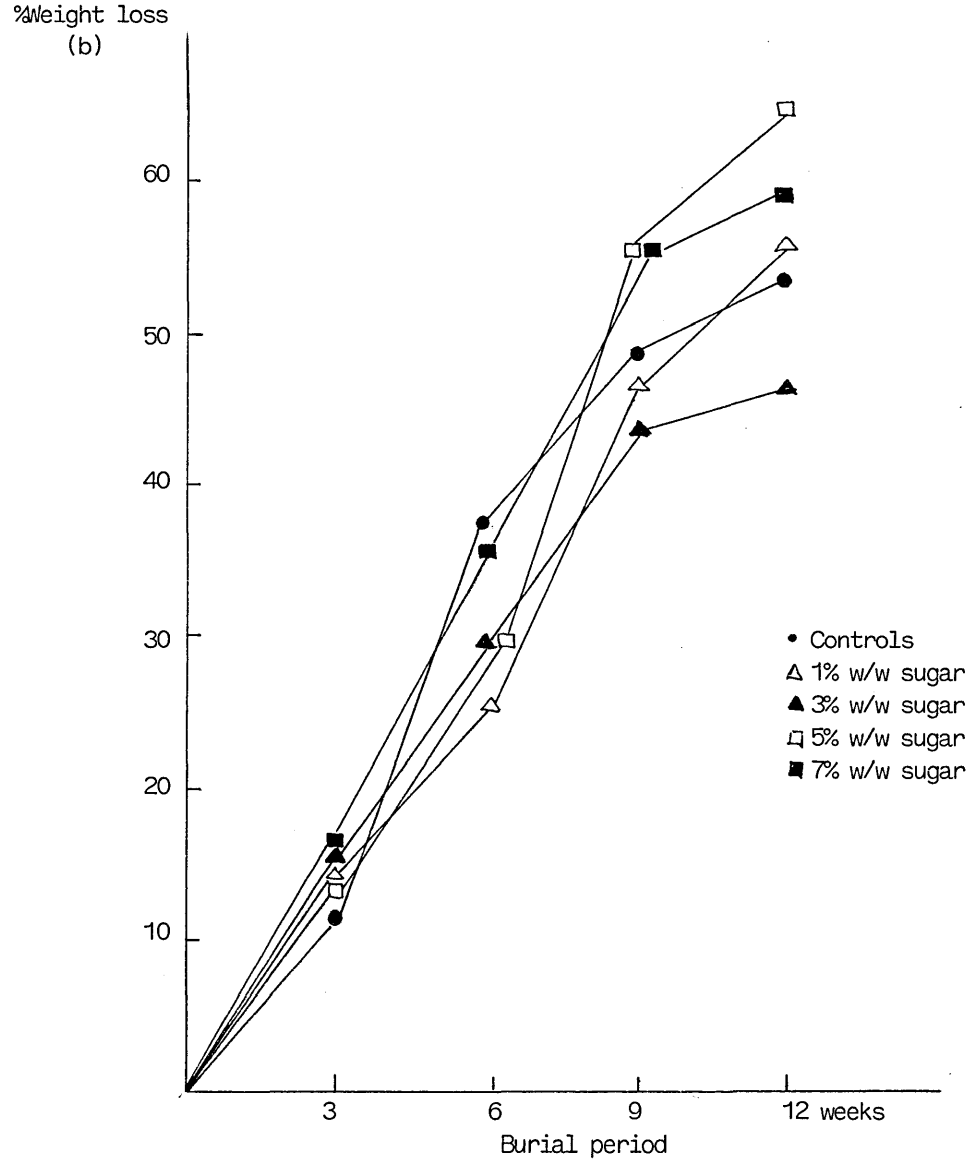


Fig 3.34 Weight losses in lime blocks impregnated with sugars, after burial in soil for the time periods indicated.
(a) %weight losses calculated on the preburial weight of the block.
(b) %weight losses calculated on the preburial weight of block and weight of sugar.

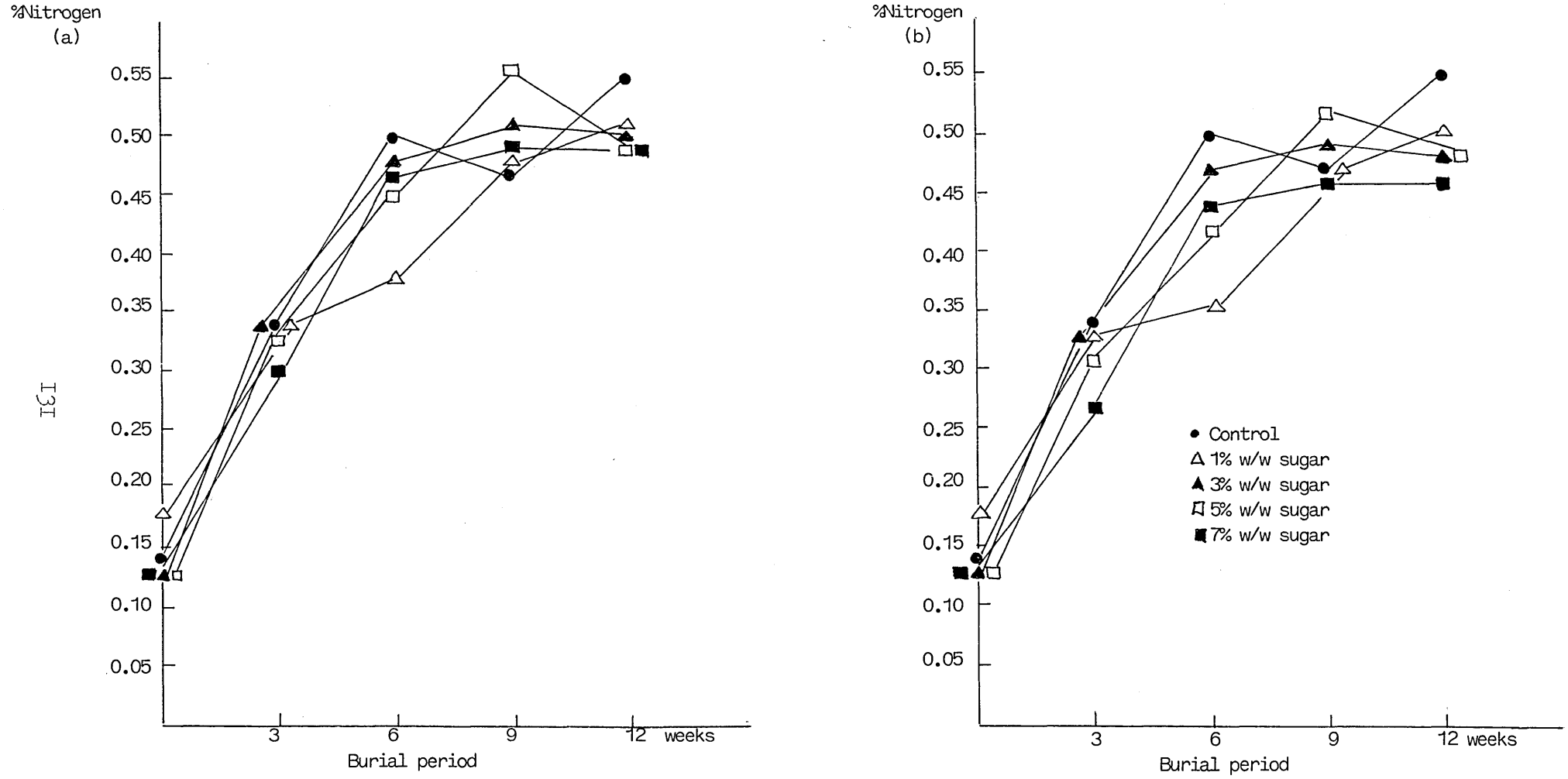
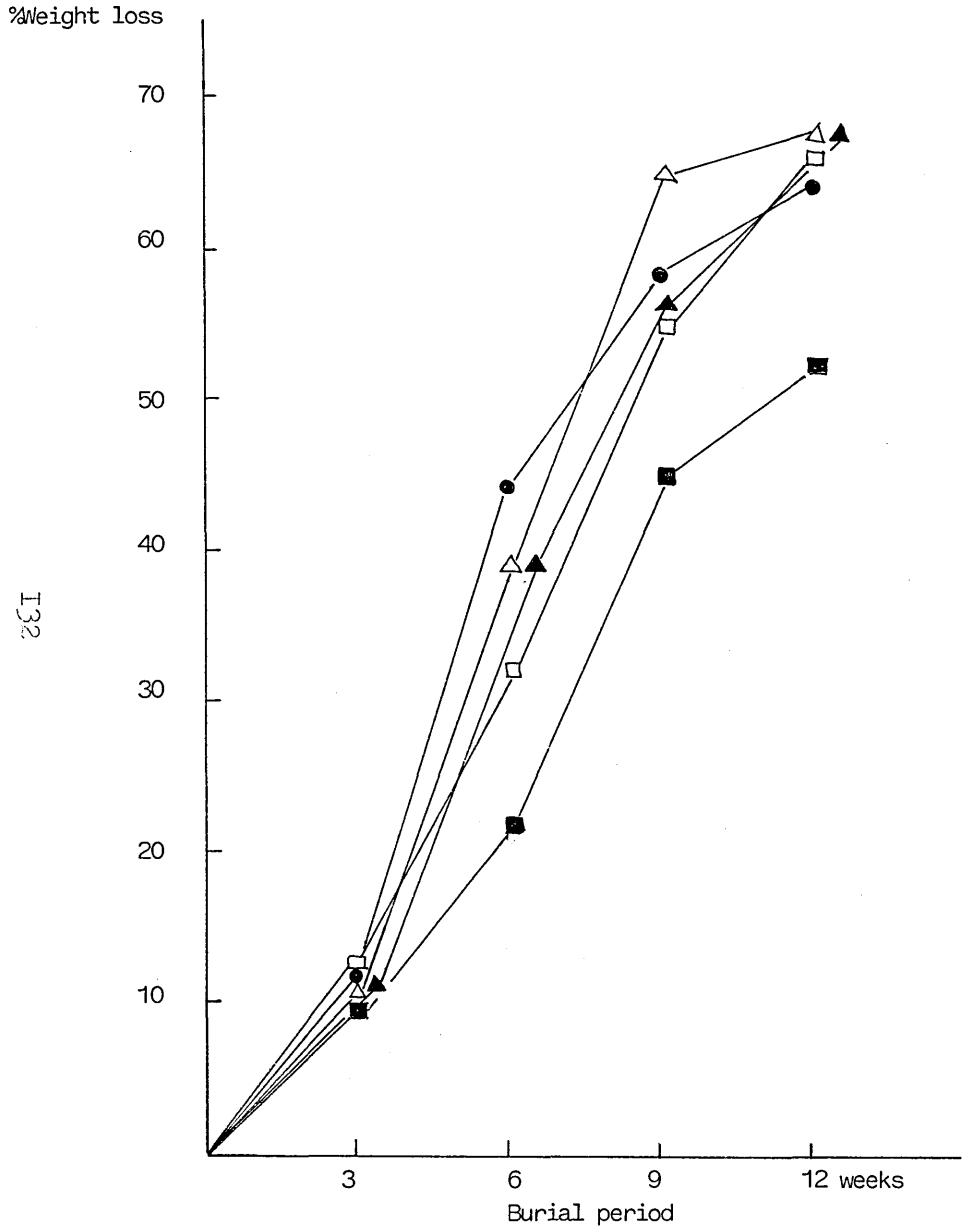


Fig 3.35 Total nitrogen contents in lime impregnated with sugars, after burial in soil for the time periods indicated.

(a) %Nitrogen calculated on the preburial weight of the block.
(b) %Nitrogen calculated on the preburial weight of the block and weight of sugar.



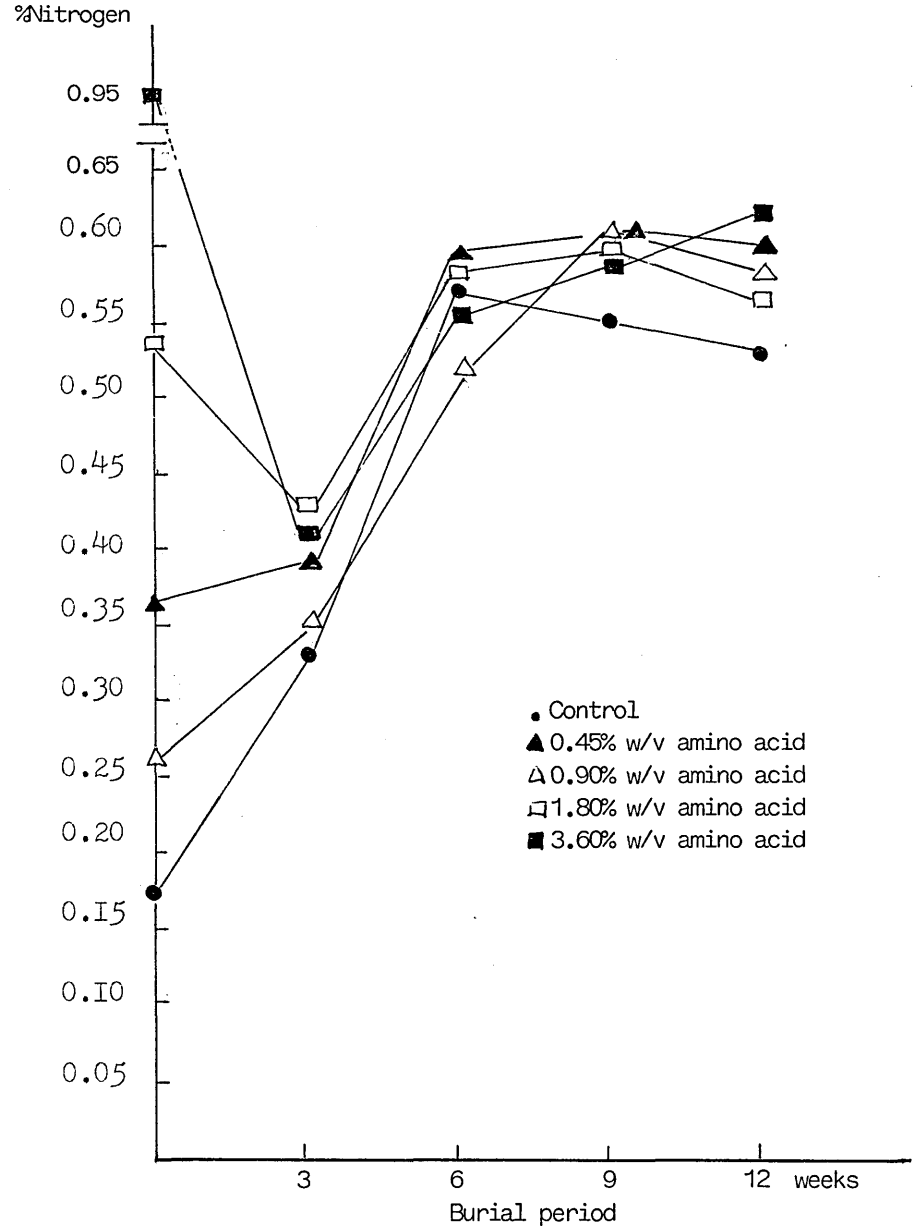
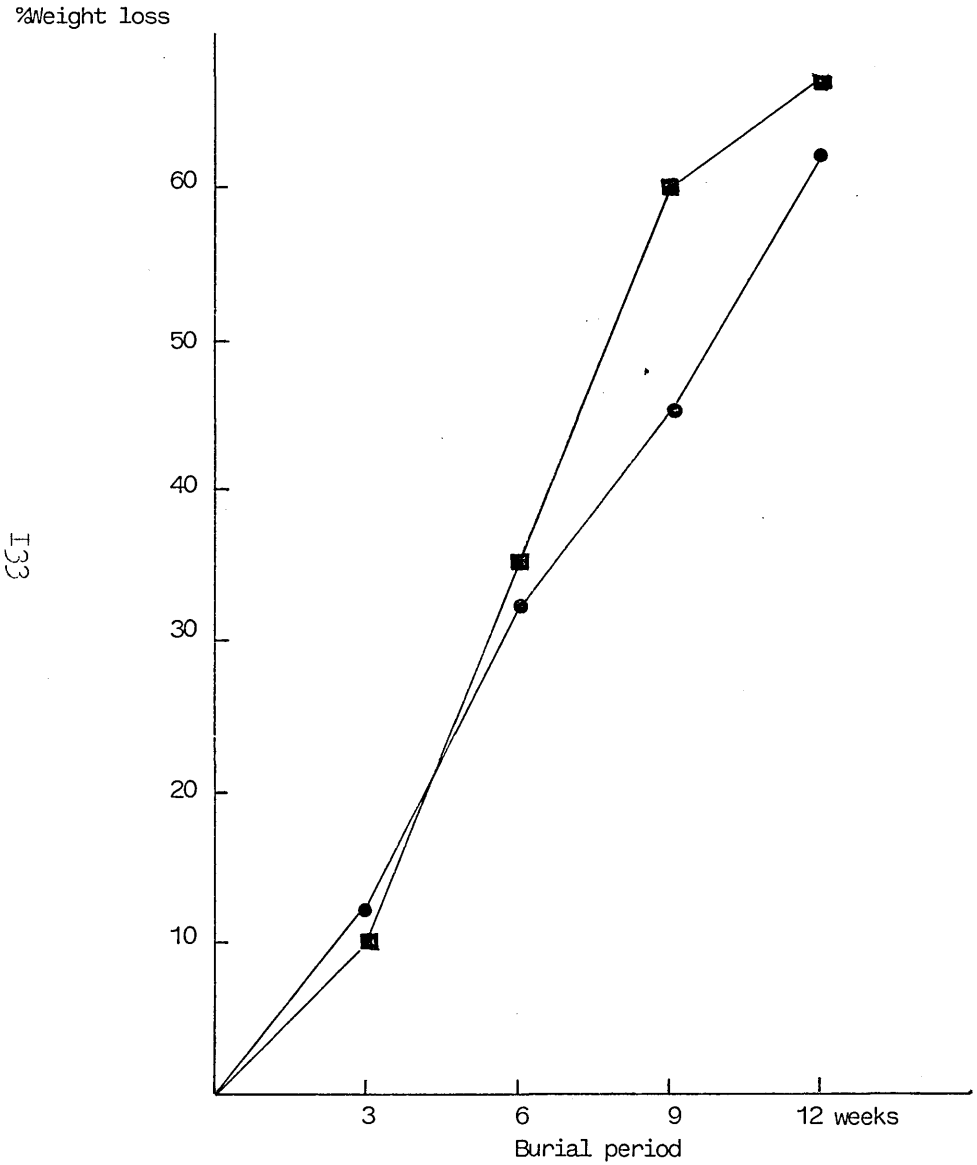


Fig 3.36 Weight losses and total nitrogen contents in lime blocks impregnated with amino acids, after burial in soil for the time periods indicated.



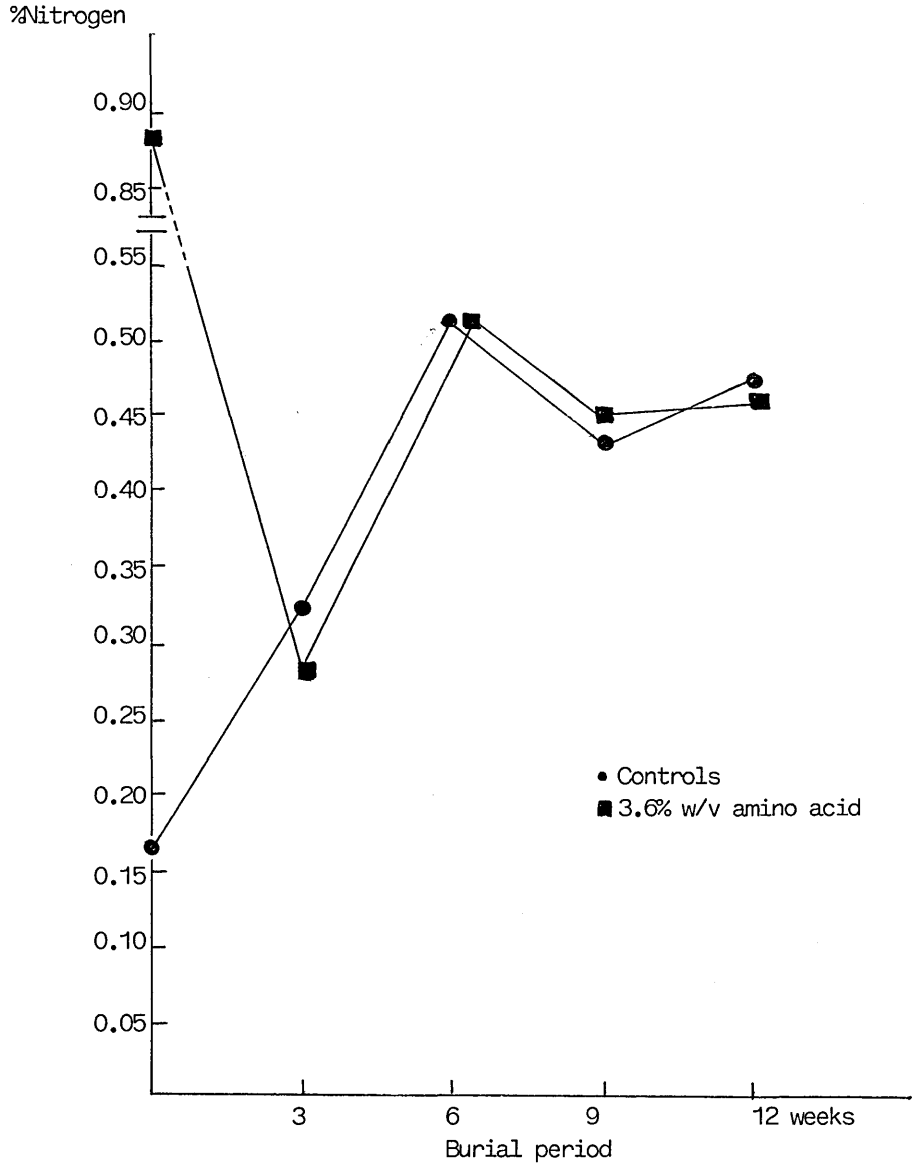
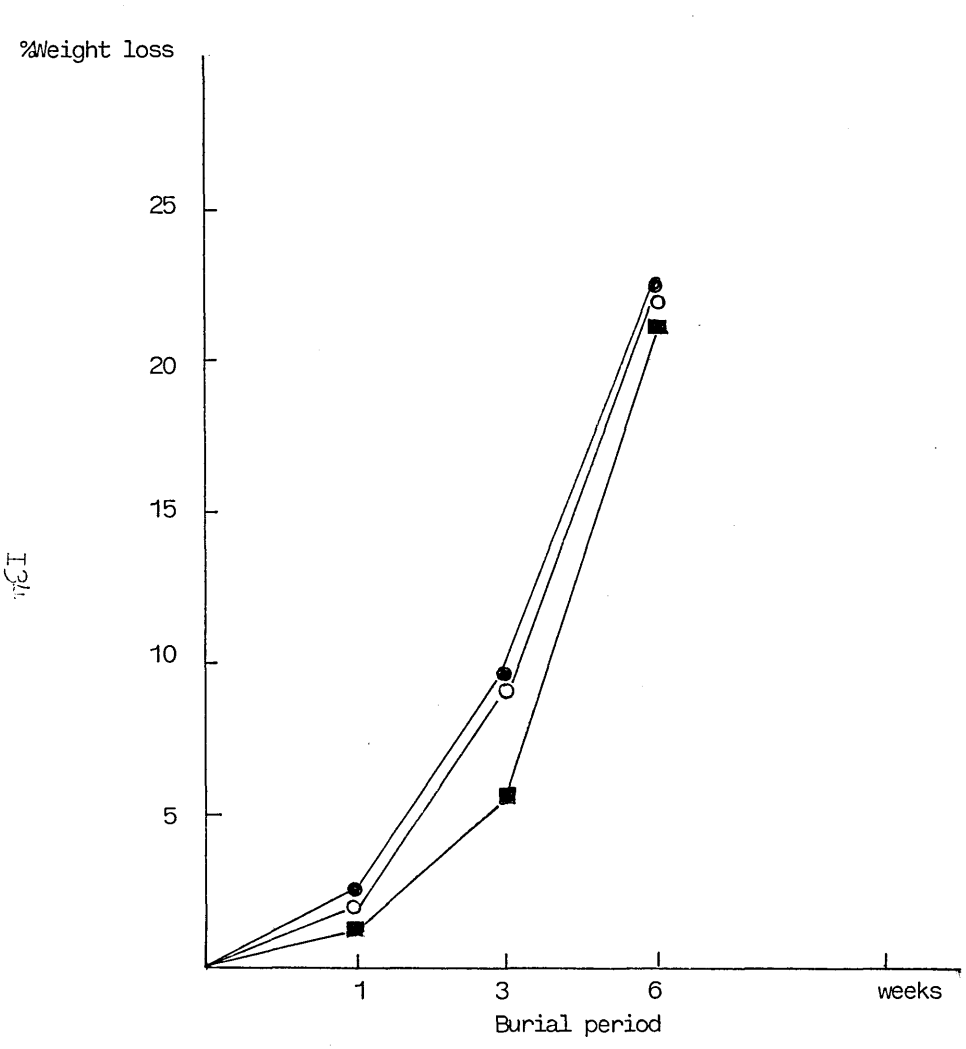


Fig 3.37 Weight losses and total nitrogen contents in lime impregnated with amino acids, and buried in an undried condition (100% moisture content) for the time periods indicated.



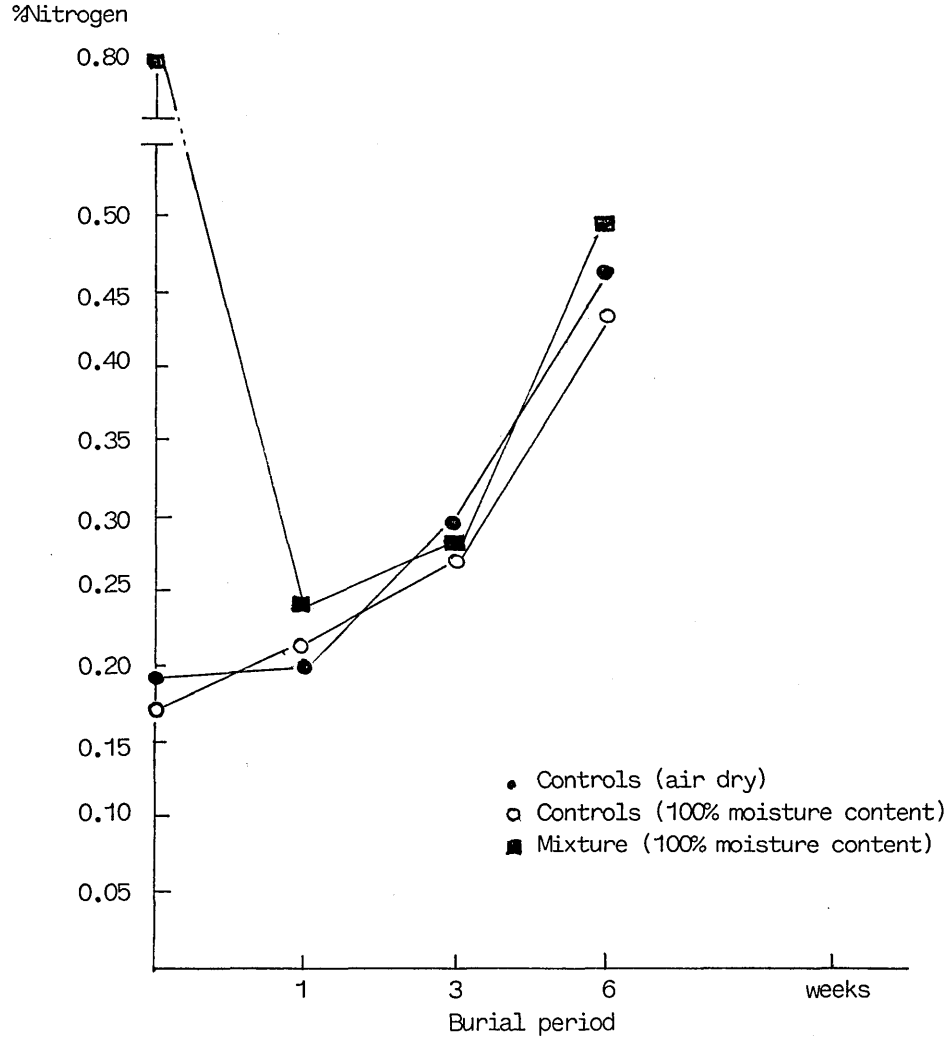


Fig 3.38 Weight losses and total nitrogen contents in lime impregnated with a mixture of sucrose and arginine, and buried at 100% moisture content in soil for the time periods indicated.

b) Beech

The influence of added soluble carbohydrates on weight losses and nitrogen contents in beech are presented in Figure 3.39 and 3.40 respectively. All blocks showed weight losses lower than those observed in lime. Beech blocks recorded weight losses at a rate of 2.3% per week over the duration of the soil burial. Control blocks displayed weight losses similar to those of blocks impregnated with sugar, and weight losses of these blocks of varying sugar concentrations were also similar over the duration of the burial (Fig 3.39a). When weight losses of control blocks and blocks impregnated with sugar were calculated as a percentage of the preburial weight of the block and the weight of sugar after impregnation (Fig 3.39b), a similar result to that in lime was observed, in which difference in weight loss between controls and blocks impregnated with sugar corresponded approximately to the weight of the sugar incorporated in the block. All test blocks showed similar weight losses of 33% at the final sampling.

In the study with amino acids, both controls and blocks impregnated with amino acids displayed weight losses of approximately 7% at week 3 of the soil burial (Fig 3.41). During the subsequent sampling periods, controls blocks showed higher weight losses than those of the blocks impregnated with amino acids. During the sampling periods, weeks 6 and 9, control blocks displayed weight losses of 37% and 39% respectively. Weight losses of blocks impregnated with varying amino acid concentrations were broadly similar over the duration of the experiment. Two-way analysis of variance of these results showed that the differences in weight losses of the varying amino acid treatments were not significant at the 5% level. Rates of increase of weight loss were rapid during the period week 3-6, but diminished during the later sampling periods. Control blocks and amino acid blocks showed weight losses of approximately 50% and 40% respectively during the final sampling period. Weight losses incurred by the test blocks in this study were larger than those in the carbohydrate study. As with lime, rates at which weight loss occurred in the amino acid study were faster than those in the carbohydrate study. In the amino acid study, weight loss occurred at a mean rate of 4% per week, over the 12 week soil burial period.

The nitrogen contents of beech blocks in the carbohydrate study is presented in Figure 3.40. The nitrogen contents of control blocks and blocks impregnated with sugar were broadly similar at each sampling period. Test blocks displayed initial nitrogen contents of 0.1% prior to soil burial. During the period 0-3 weeks and 3-6 weeks, nitrogen contents of the blocks were seen to increase rapidly. However, during the later sampling periods, a gradual diminution in the rate of increase of nitrogen was observed. All blocks displayed a final nitrogen content of approximately 0.38%. The nitrogen contents of test blocks calculated as a percentage of the preburial weight of the block and the weight of sugar after impregnation is presented in Figure 3.40(b). The nitrogen pattern of these blocks were similar to those described for Figure 3.40(a).

In the amino acid study, the nitrogen contents of the test blocks prior to soil burial ranged from 0.13% for control blocks to 0.60% for blocks impregnated with amino acids (Fig 3.41).. During the period 0-3 weeks, blocks impregnated with amino acid concentrations of 3.60% w/v and 1.80% w/v showed decreases in nitrogen contents. These blocks decreased in nitrogen content from 0.6% and 0.34% respectively to 0.23% . The nitrogen contents of control blocks and blocks of lower amino acid concentrations displayed increases in nitrogen during this period. All blocks showed a rapid increase in the rate of nitrogen (0.09% per week) during the period 3-6 weeks. During the period 6-9 and 9-12 weeks, control blocks and blocks impregnated with amino acid concentrations of 0.45% w/v, 0.90% w/v and 1.80% w/v, displayed broadly similar nitrogen values. However, blocks impregnated with amino acid at concentrations of 3.60% w/v, showed large nitrogen increases from 0.23% to 0.55% during the period week 3-6. The nitrogen content of these blocks remained relatively constant over the next two sampling periods. The final nitrogen content of the blocks ranged from 0.49% for controls to 0.58% for blocks impregnated with amino acid.

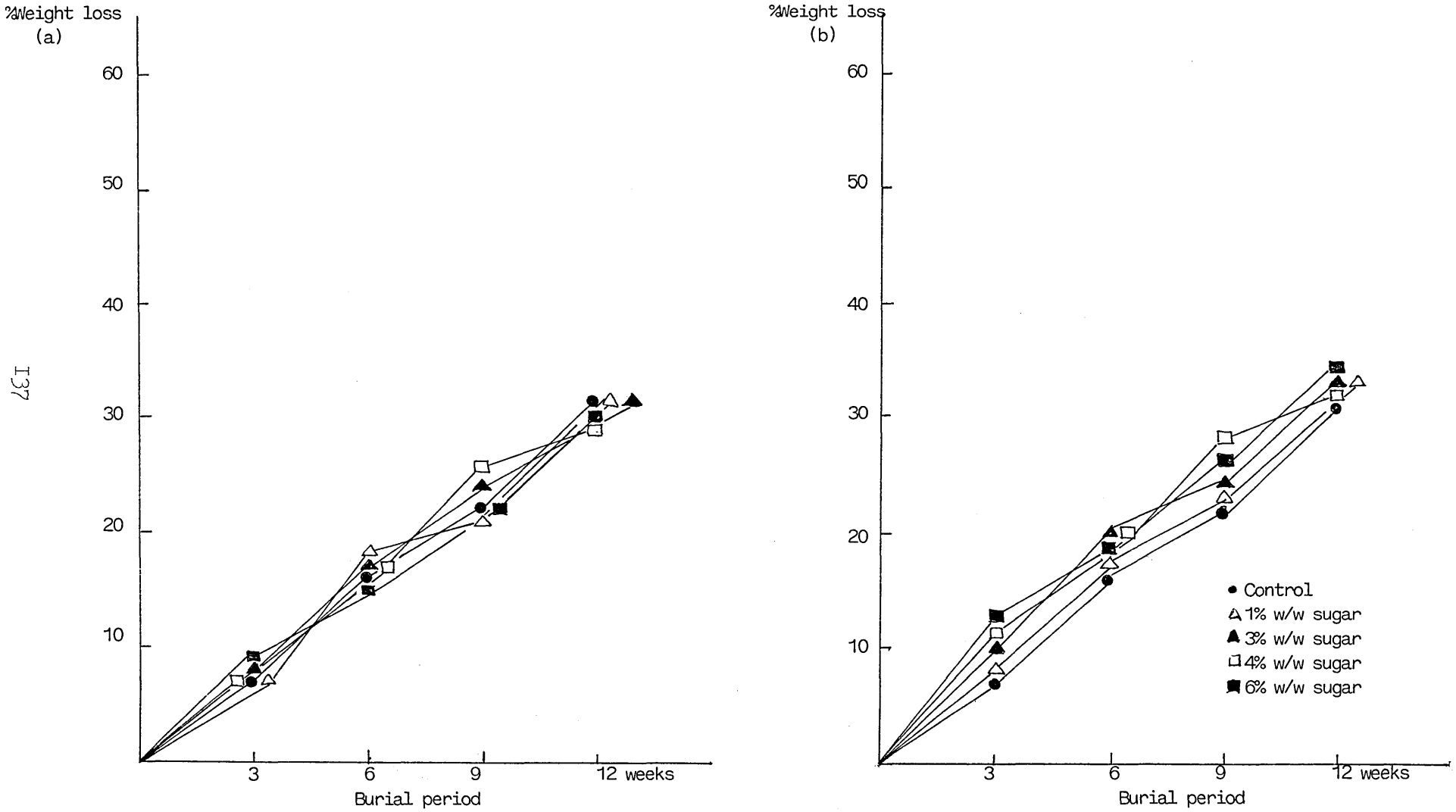


Fig 3.39 Weight losses in beech impregnated with sugars, after burial in soil for the time periods indicated.

(a) %weight losses calculated on the preburial weight of the block.
(b) %weight losses calculated on the preburial weight of the block and weight of sugar.

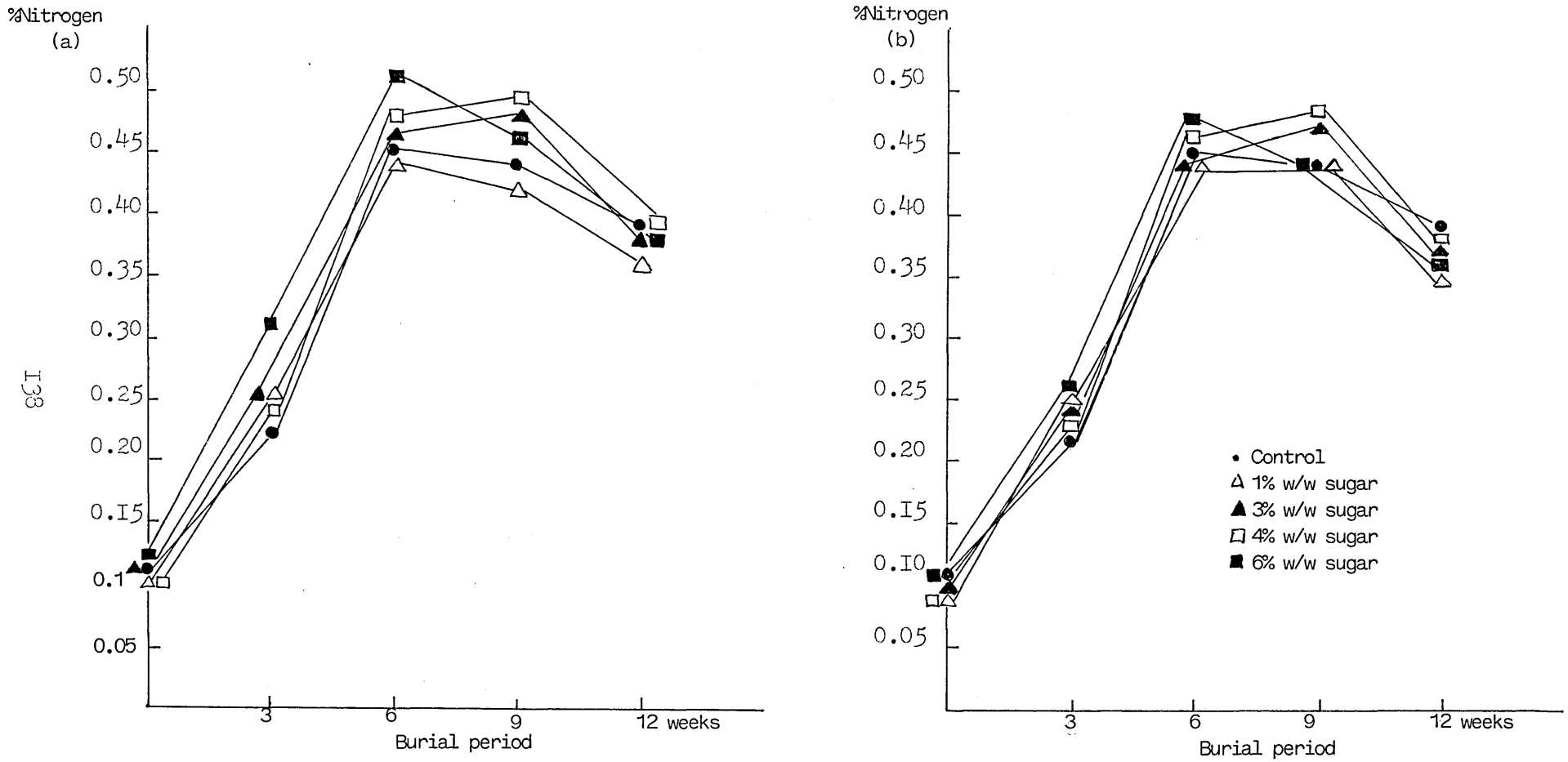
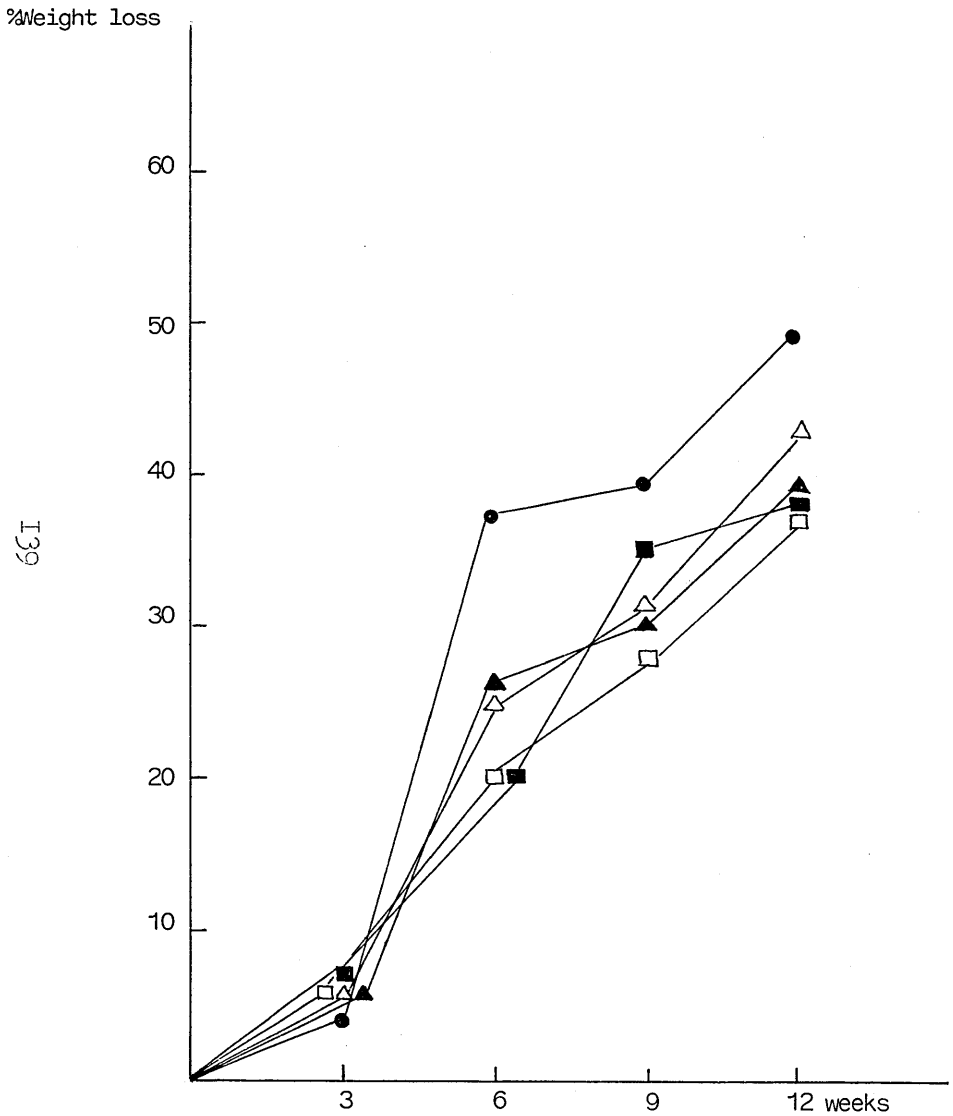


Fig 3.40 Total nitrogen contents in beech impregnated with sugars, after burial in soil for the time periods indicated.

(a) %Nitrogen calculated on the preburial weight of the block.
(b) %Nitrogen calculated on the preburial weight of the block and weight of sugar.



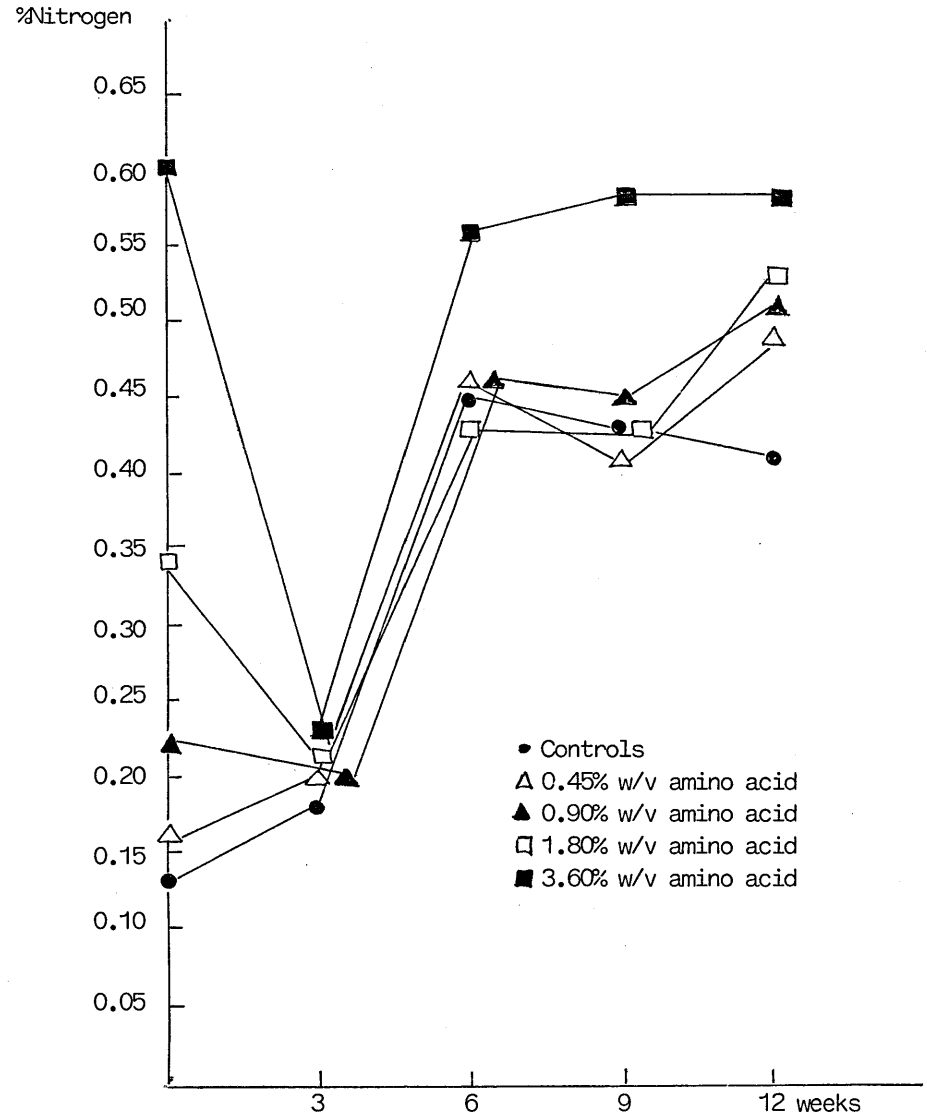


Fig 3.41 Weight losses and total nitrogen contents in beech impregnated with amino acids, after burial in soil for the time periods indicated.

c) Pine

The effects of added soluble carbohydrates and amino acids on weight losses in pine are presented in Figure 3.42 and 3.44. In the study using carbohydrates (Fig 3.42a), all blocks displayed broadly similar weight losses over the duration of the soil burial. Blocks impregnated with varying concentrations of sugar showed little influence on decay in pine. Weight losses of these blocks increased at approximately the same rate (2%) each week between weeks 6-12 of the soil burial. Blocks displayed weight losses of approximately 14% at the final sampling period.

Weight losses evaluated on the preburial weight of the blocks and weight of sugar after impregnation (Fig 3.42b), displayed differences in weight loss between control blocks and blocks impregnated with sugar. These differences in weight losses corresponded approximately to the weight increments as a result of the weight of sugar. Blocks impregnated with sugar displayed large weight losses in the final sampling period, with blocks of 1% w/w sugar displaying a weight loss of 16%, blocks of 3% w/w and 5% w/w sugar displaying weight losses of 17%, and blocks of 7% w/w sugar displayed a weight loss of 19%. Control blocks displayed similar weight losses (14%) to those described for Figure 3.42a.

In the study with amino acids, both control and amino acid blocks displayed weight losses of 4% during week 6 of the soil burial (Fig 3.44). In the period weeks 6-9, blocks showed an increase in weight loss from 4% to between 7-10%. Weight losses continued to increase, with amino acid blocks of 1.80% w/v and 3.60% w/v, displaying weight losses of 12% at week 12. Control blocks and the remaining amino acid blocks (0.45% w/v and 0.90% w/v), displayed broadly similar weight losses of 14% for the same period. 2-way analysis of variance undertaken showed that significant differences did not exist in the weight losses of control blocks and amino acid blocks over the 12 week soil burial.

The influence of added soluble carbohydrates on nitrogen transfer in pine is presented in Figure 3.43. Figure 3.43a describes nitrogen contents in blocks calculated on the preburial weight of the block, and Figure 3.43b describes nitrogen contents calculated on the preburial weight of the block and the weight of sugar after impregnation.

Both methods of evaluation displayed similar trends of nitrogen transfer over the duration of the burial. The overall pattern showed blocks of all treatments displaying similar nitrogen values during each sampling period.

The initial nitrogen contents of blocks prior to soil burial, varied from 0.14% to 0.19%. During the period weeks 0 to 3, nitrogen contents of these blocks increased to 0.2%. These nitrogen increases were not accompanied by weight losses. The rate of nitrogen increases in the blocks continued at the same rate (0.02% per week), over the period weeks 3 to 6. These rates increased further to 0.05% per week during the weeks 6 to 9, with control blocks and blocks impregnated with sugar displaying nitrogen values of around 0.43%. During weeks 9 to 12, nitrogen contents of the blocks decreased to 0.33%. Decreases in nitrogen contents of the blocks during the final sampling interval were also observed in the hardwoods.

The influence of added soluble amino acids on nitrogen transfer in pine is presented in Figure 3.44. Blocks impregnated with amino acids showed decreases in nitrogen contents during the period 0 to 3 weeks. The losses in nitrogen contents in these blocks were largest in the blocks impregnated with the higher amino acid concentrations. The nitrogen contents of these blocks were reduced by 60% and 80% of their nitrogen values obtained after impregnation. Over the same period, i.e. weeks 0-3, control blocks showed small increases from 0.10% to 0.12%. Nitrogen contents of blocks which were below 0.2% during weeks 0 to 3, rose to 0.2% and 0.25% during weeks 3 to 6. During weeks 6 to 9, blocks showed an increase in nitrogen content from 0.2% and 0.25% to 0.43%. During the final sampling period, the nitrogen contents of the blocks decreased to between 0.32% and 0.38%. In general, blocks impregnated with amino acids displayed broadly similar nitrogen contents to blocks impregnated with sugar after soil burial.

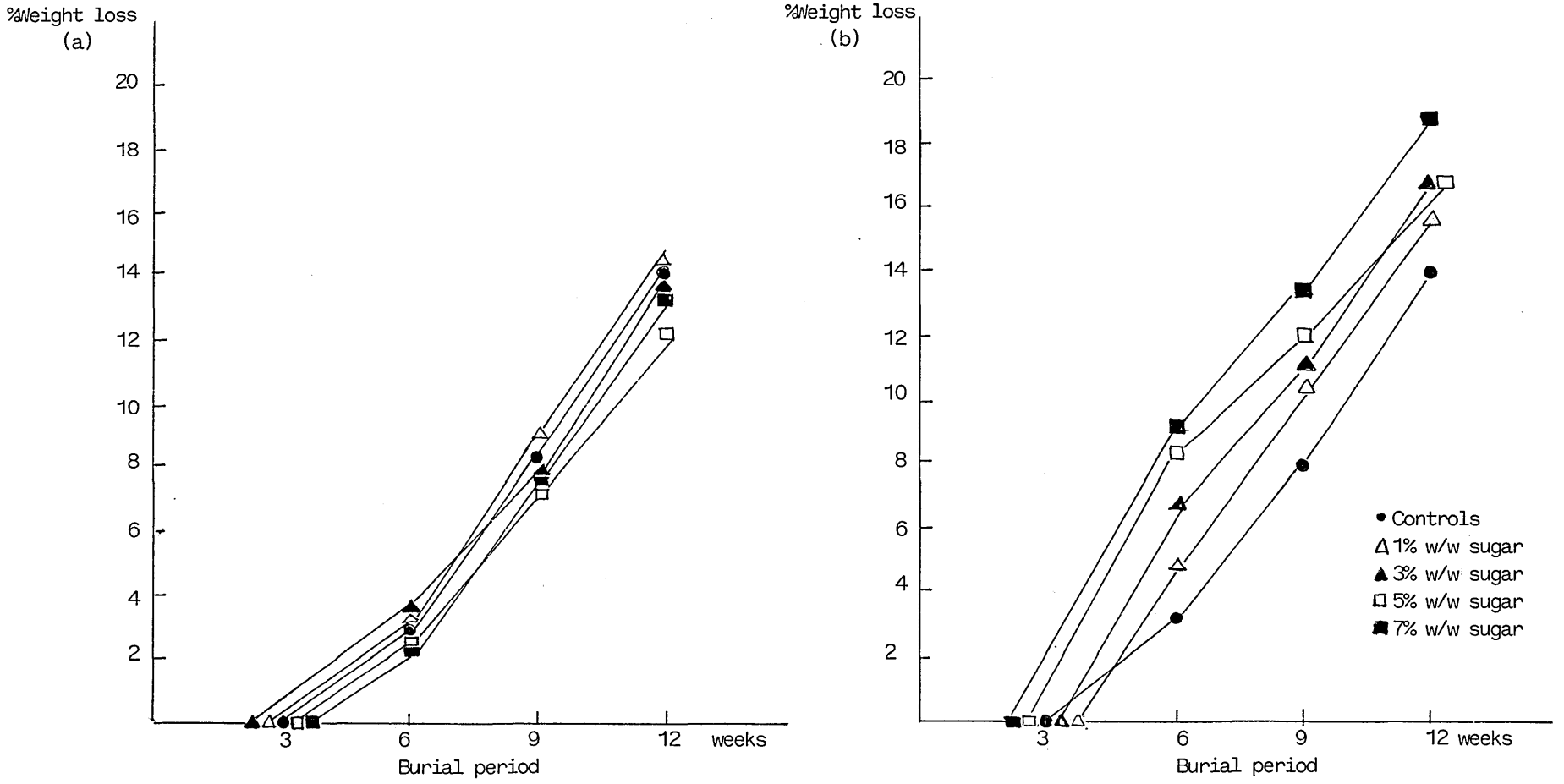


Fig 3.42 Weight losses in pine impregnated with sugars, after burial in soil for the time periods indicated

(a) %Weight losses calculated on the preburial weight of the block.
(b) %Weight losses calculated on the preburial weight of the block and weight of sugar.

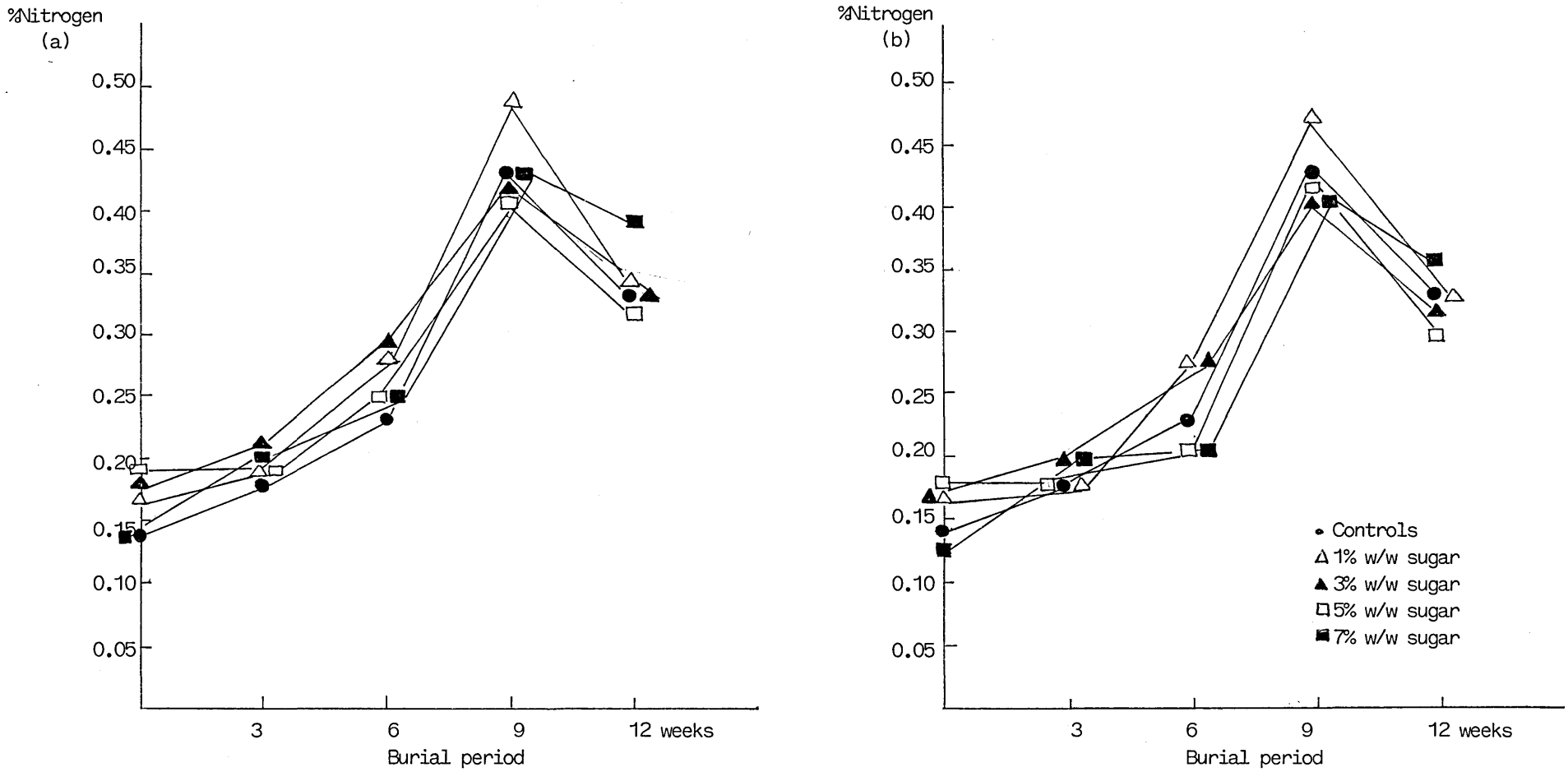


Fig 3.43 Total nitrogen contents in pine impregnated with sugars, after burial in soil for the time periods indicated.

(a) %Nitrogen calculated on the preburial weight of the block.
(b) %Nitrogen calculated on the preburial weight of the block and weight of sugar.

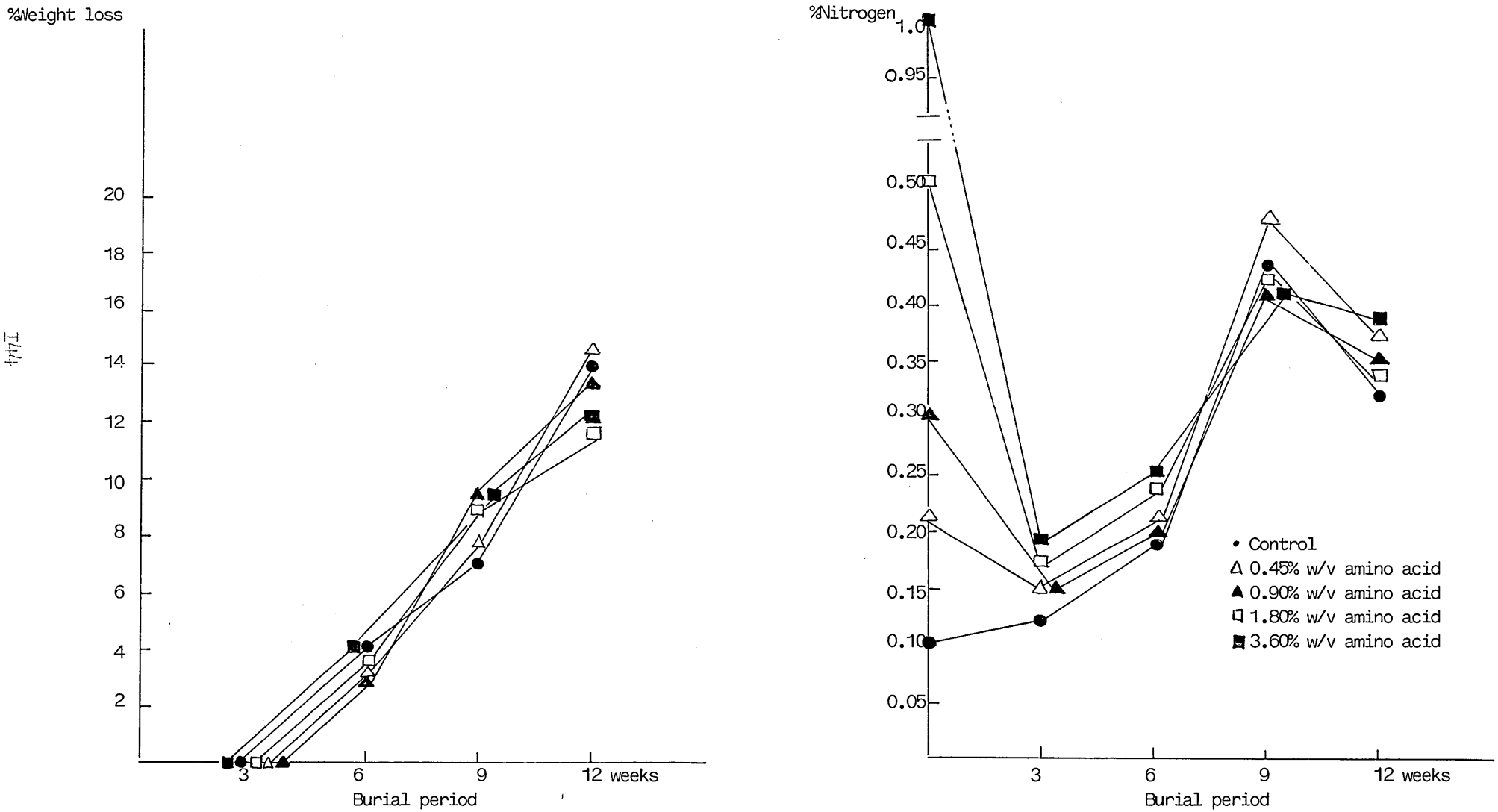


Fig 3.44 Weight losses and total nitrogen contents in pine impregnated with amino acids, after burial in soil for the time periods indicated.

d) Spruce

Results of the effect of added soluble carbohydrates on the weight loss and transfer in spruce is presented in Figure 3.45 and Figure 3.46 respectively. Figure 3.45a describes the weight losses calculated on the preburial weight of the block, and Figure 3.45b, the weight losses calculated on the preburial weight of the block and weight of sugar after impregnation.

Similarities were observed in the results from the carbohydrate study and the amino acid study of both spruce and pine. Both woods displayed similar weight losses in these studies, and weight losses in these woods were not observed until 3 weeks after soil burial. In spruce, as was in pine, the incorporation of sugar of varying concentrations into test blocks showed little influence on weight losses in these blocks. A similar result was also observed with the amino acid blocks in the study with amino acids. During the final sampling periods (week 12), control blocks and blocks impregnated with sugar displayed broadly similar weight losses of 14%, the same as that in pine, when calculated on the preburial weight of the blocks. In the evaluation of weight losses based on the preburial weight of the block and the weight of added sugar, weight losses of control blocks were observed to be lower than those of the blocks impregnated with sugar. The disparity in the weight losses between control blocks and sugar blocks were consistant with the increase in the block weight resulting from the weight of the sugar incorporated into the block.

In the study with amino acids, control blocks and amino acid blocks displayed broadly similar weight losses (3-4%) during week 6 of the soil burial. At weeks 9 and 12, control blocks and amino acid blocks of 0.45% w/v, displayed lower weight losses than those of higher amino acid concentrations. Statistical analysis (2-ANOVA) undertaken to compare differences in weight loss of control blocks and amino acid blocks, showed that significant differences did not exist at the 5% level.

In the study with carbohydrates, the initial nitrogen contents of the blocks were relatively high for blocks removed from the sub-surface regions of the wood (Fig 3.46). The nitrogen contents of these blocks however increased further over the duration of the soil burial.

During the period weeks 0 to 3, and weeks 3 to 6, all blocks showed a small rate of increase in nitrogen content of 0.01% per week. In the period weeks 6 - 9, there was a marked increase in the rate of nitrogen input (0.07% per week), with blocks displaying broadly similar nitrogen contents of around 0.45%.

Nitrogen contents of the control blocks and the sugar blocks during the final sampling period decreased to 0.43%. In general, the overall nitrogen contents in spruce in the carbohydrate study were higher than those in pine in a similar study, though weight losses for both woods in these studies were broadly similar.

In the study with amino acids, nitrogen contents of control blocks increased from 0.08% to 0.16% during the period 0-3 weeks (Fig 3.47). During the same period, blocks impregnated with amino acids showed a decrease in nitrogen content. The decrease in nitrogen was largest in blocks with higher amino acid concentrations. Nitrogen contents of these blocks decreased from 1%, 0.5% and 0.42% to 0.23% during the initial stages of the burial. All blocks displayed a gradual increase in nitrogen contents during the next sampling interval (weeks 3 to 6). During the period weeks 3 - 6, and weeks 6 - 9, increases in nitrogen contents were accompanied by weight losses. Weight losses continued to increase during weeks 9 to 12, but nitrogen contents of the blocks decreased to between 0.33% to 0.43%. Nitrogen contents of spruce in this study were similar to those of pine for a similar study.

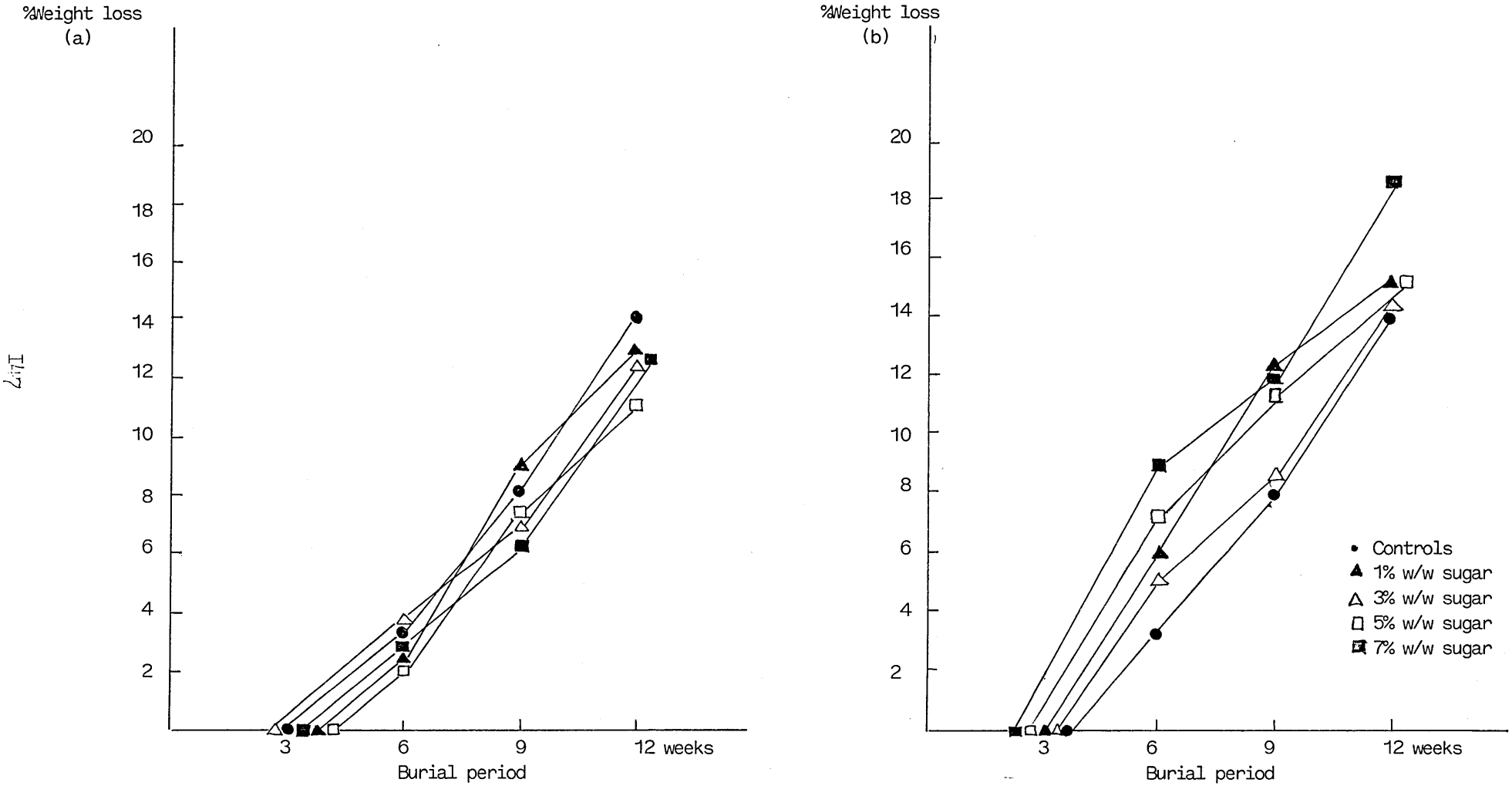


Fig 3.45 Weight losses in spruce impregnated with sugars, after burial in soil for the time periods indicated

(a) %Nitrogen losses calculated on the preburial weight of the block.
(b) %Nitrogen losses calculated on the preburial weight of blocks and weight of sugar.

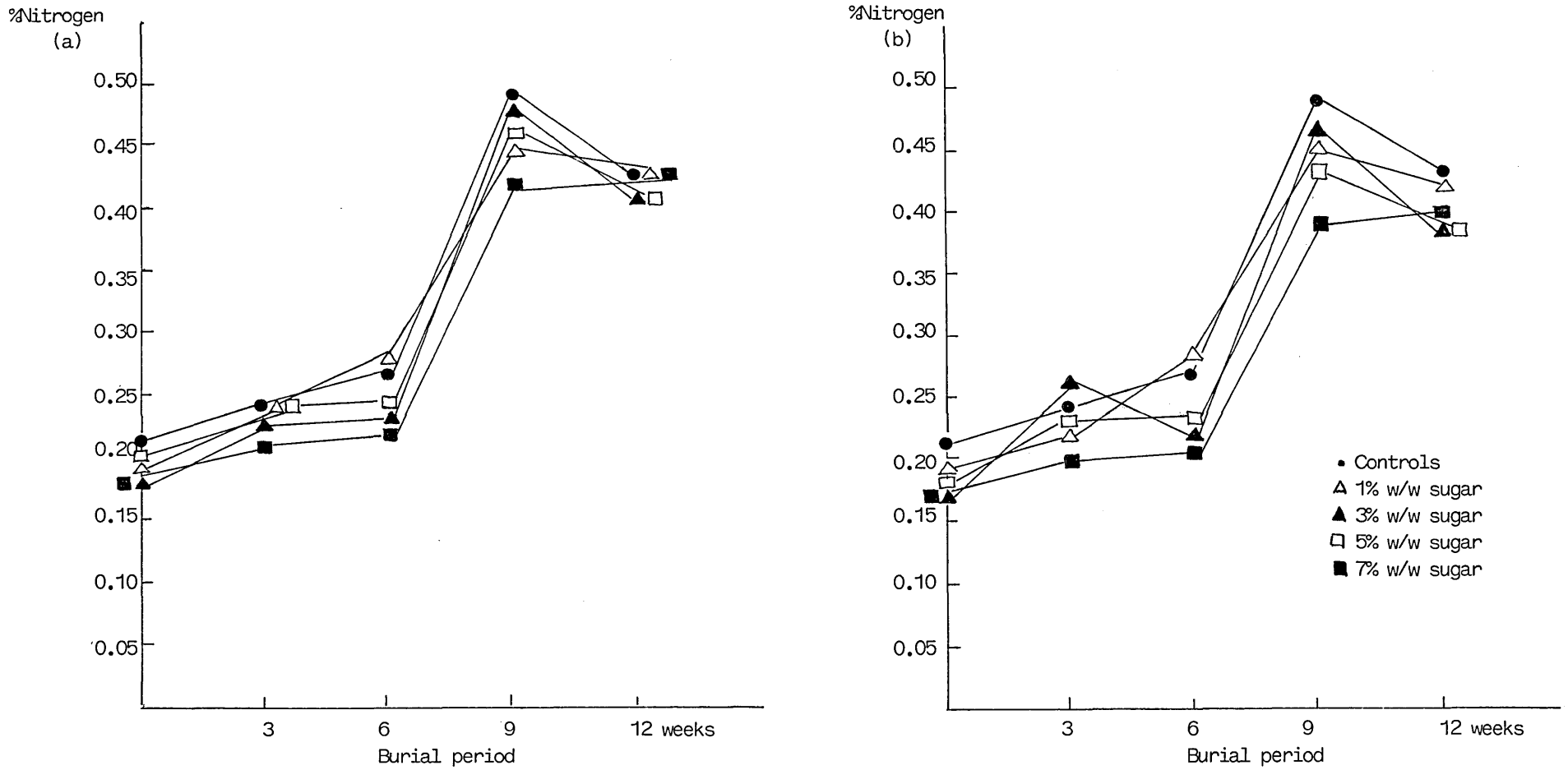


Fig 3.46 Total nitrogen contents in spruce impregnated with sugars, after burial in soil for the time periods indicated.

(a) %Nitrogen calculated on the preburial weight of the block.
(b) %Nitrogen calculated on the preburial weight of the block and weight of sugar.

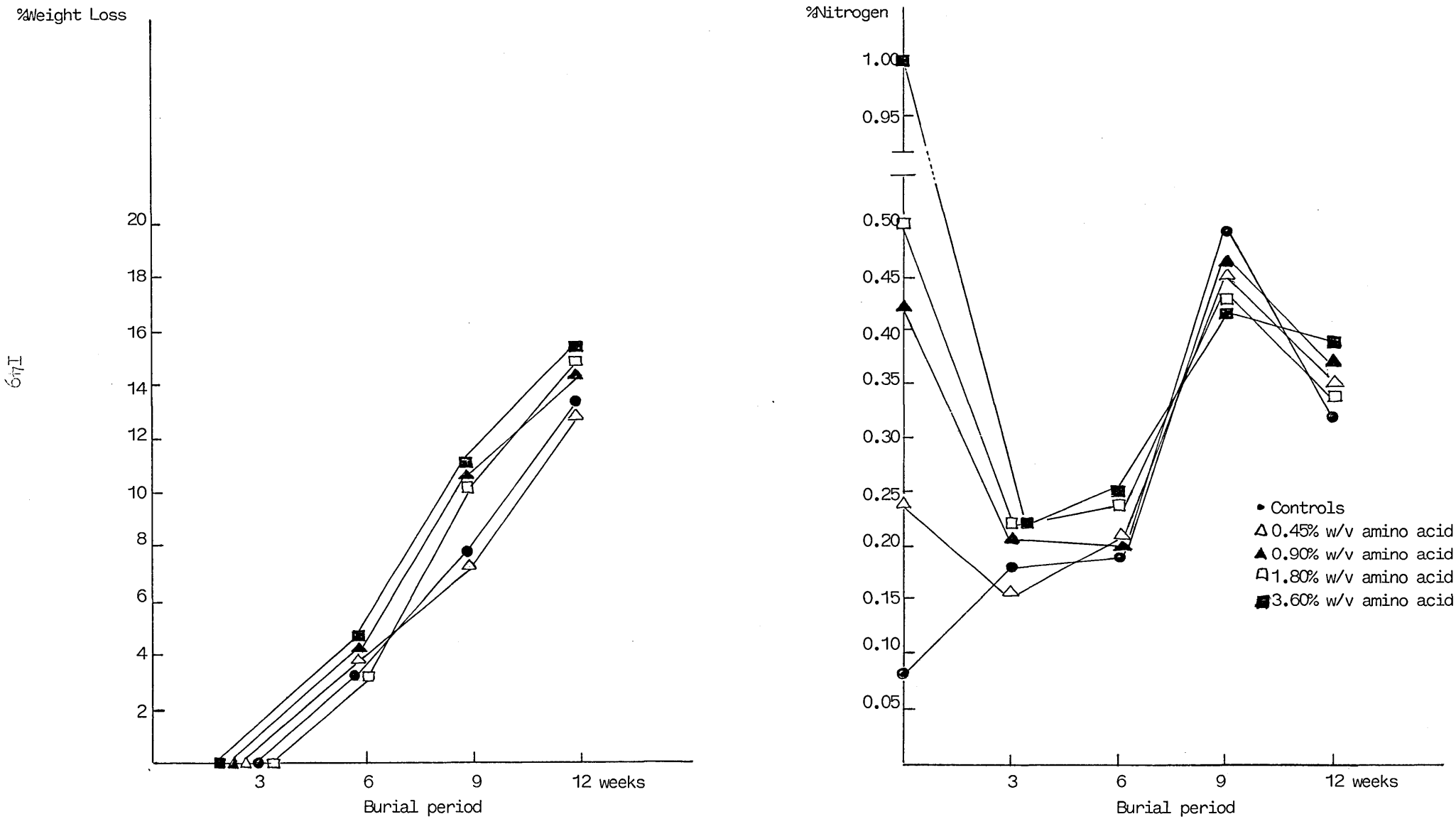


Fig 3.4 Weight losses and total nitrogen contents in spruce impregnated with amino acids, after burial in soil for the time periods indicated.

3.2.1.2. The loss of amino acids from blocks impregnated with amino acids after soil burial.

Results from the previous experiments showed that blocks impregnated with amino acids displayed losses in nitrogen during the initial stages of the soil burial. A supplementary experiment was undertaken to investigate how soon after emplacement of the wood blocks in soil, the added amino acids were lost to the soil system. Pine was selected as a test species, and blocks of pine containing redistributed soluble nutrients, were also included in the experiment as blocks with high initial nitrogen contents, resulting from the redistribution of soluble nutrients during drying. Pine blocks without redistributed soluble nutrients were included as controls. Blocks impregnated with arginine (so as to achieve high nitrogen levels), and blocks containing redistributed soluble nutrients, were buried at 100% moisture content and also in an air-dried condition.

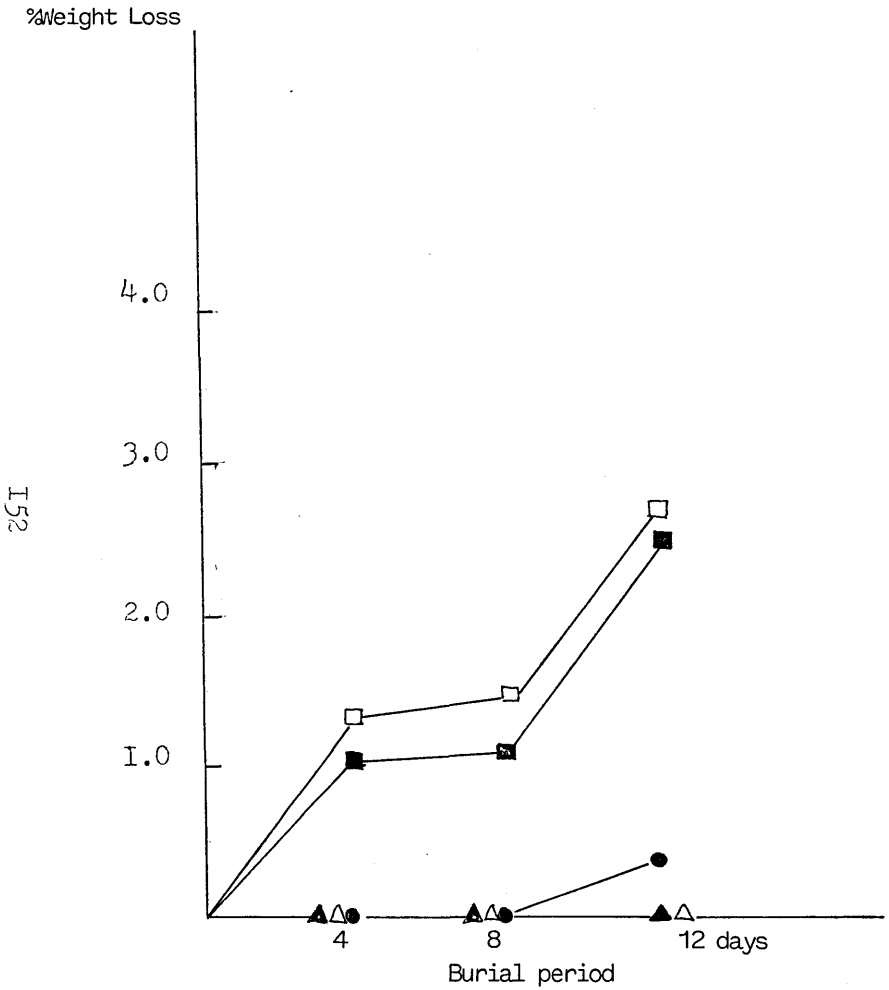
The weight losses and nitrogen contents obtained in this experiment are presented in Figure 3.48. The moisture contents of the blocks were 40% and 70% for blocks buried air dry and at 100% moisture content respectively, over the twelve day burial period. Weight losses of all the blocks were calculated on the preburial weight of the block.

Weight losses evaluated on the preburial weight of the block were not significant ($<3\%$) in any of the test blocks over the duration of the experiment. Control blocks showed weight losses of $<1\%$ and blocks incorporated with arginine showed no weight losses after the final sampling period. Blocks with redistributed soluble nutrients displayed small weight losses over the duration of the experiment though these weight losses did not exceed 3%. Differences in mass loss of blocks buried at 100% moisture content and buried air dried, were small in blocks containing redistributed soluble nutrients.

The nitrogen content of the control blocks showed a gradual increase from 0.08% to 0.12% over the 12 day soil burial experiment. Blocks with redistributed soluble nutrients displayed higher initial nitrogen contents (0.13%) to controls, and the nitrogen contents of these blocks remained relatively constant over the soil burial period. Both control blocks and blocks containing redistributed soluble nutrients displayed similar nitrogen contents at the final sampling period.

However, the nitrogen contents in blocks with redistributed soluble nutrients were accompanied by small mass losses which were not observed in the control blocks. Blocks containing redistributed soluble nutrients buried air dry and at 100% moisture content, showed similar nitrogen levels throughout the experiment.

Blocks impregnated with arginine both at 100% moisture content and air dry displayed similar patterns in nitrogen contents in this experiment. These blocks showed a decrease in nitrogen contents from 0.8% to 0.27% during the initial stages (days 0-4) of this experiment. This decrease continued and nitrogen levels fell to 0.19% after 8 days. The loss of nitrogen from the blocks during this stage was 66% of the initial input at zero time. This loss of nitrogen corresponds to the loss of nitrogen from the blocks impregnated with amino acids (1.80% w/v) in the amino acid study of pine (3.2.1.1b). The nitrogen contents of the blocks remained fairly constant over the next sampling period. The soil burial was only undertaken for 12 days, as results had indicated that the majority of the amino acids incorporated into the blocks had leached out by this stage.



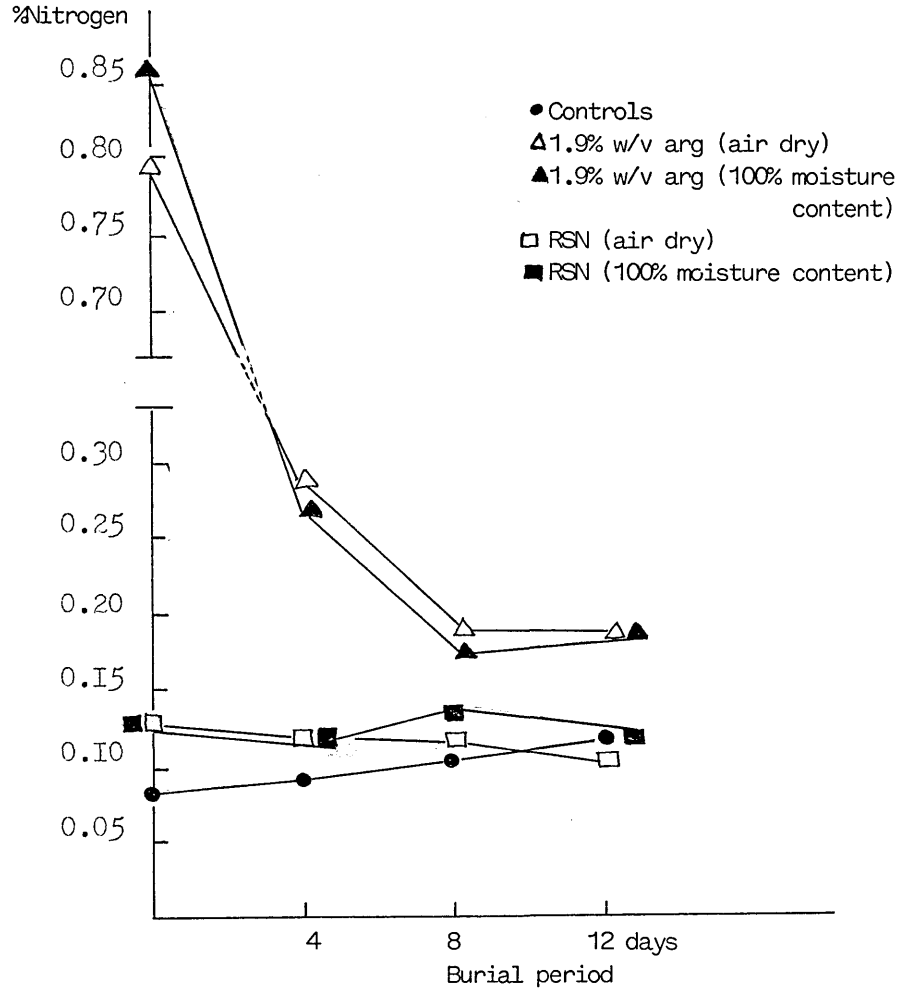


Fig 3.48 Weight losses and total nitrogen contents in pine impregnated with arginine, and pine containing redistributed soluble nutrients (RSN), after burial in soil for the time periods indicated.

Conclusions

The conclusions drawn from the soil burial studies of unpreserved wood were:

1) decay susceptibility of hardwoods and softwoods were not influenced by the incorporation of soluble carbohydrates and amino acids to these woods,

2) mixtures of amino acids and sugars impregnated into lime test blocks did not influence the decay status or nitrogen transfer to these blocks,

3) nitrogen contents of wood increased as mass loss increased, however a level of nitrogen of approximately 0.20% was necessary before significant mass loss occurred,

4) blocks impregnated with amino acids showed losses (as seen by a decrease in total nitrogen content) during the early stages of the soil burial. Losses of carbohydrates in a similar manner are thought to occur, though this was not investigated,

5) blocks containing redistributed soluble nutrients did not show losses of these nutrients during the early stages of the soil burial. This suggests that some form of "binding" of these nutrients to wood surfaces exists.

3.2.2. Studies using preservative treated wood.

The results of the soil burial studies undertaken to determine the influence of amino acids on decay and preservative performance in CCA treated test blocks are described in this section. Previous studies showed that unpreserved wood blocks impregnated with amino acids (3.2.1.1.), displayed losses of amino acids to soil during burial. A preliminary study was therefore undertaken with CCA treated lime blocks impregnated with arginine. Lime was selected as a test species as it was more decay susceptible than beech, pine and spruce, and arginine was selected as it is an amino acid with a higher nitrogen content, and is also a major amino acid component in extracts of lime. Wood blocks in this study were buried at 100% moisture content.

The results of this exploratory study showed that when arginine was incorporated into CCA treated blocks, decay and preservative efficacy in the blocks were influenced. A second soil burial study, similar to the preliminary study, was undertaken for a more detailed investigation into the influence of amino acids in preservative treated wood. For this study, the other major amino acid glutamine, found in the extracts of green and dried wood (3.1.2. and 3.1.4.) was used for comparison. CCA treated lime, beech, pine and spruce were selected as test species for this experiment.

Concurrent with this investigation, a soil burial study was also undertaken to investigate the influence of soluble carbohydrates on CCA treated lime and pine. Lime wood blocks were impregnated with sucrose, and pine with glucose, to obtain in both types of wood, sugar concentrations of 0.5% w/w, 1% w/w, 2% w/w and 4% w/w. All test blocks in both the glutamine and sugar studies were buried in an air-dried condition. This was to allow comparison with previous studies undertaken on CCA treated wood at this laboratory in which blocks were buried air-dry.

In the soil burial studies in which CCA treated blocks were impregnated with amino acids, the following parameters were examined; moisture content, weight loss and nitrogen content. Weight losses and nitrogen contents were calculated on the preburial weight of the block and moisture contents were based on the final dry weight of the block. Analysis of copper and chromium contents were also undertaken in CCA treated blocks impregnated with glutamine.

In the soil burial studies with carbohydrates, only weight losses and moisture contents were measured. The time remaining for the experimental analysis was insufficient for a more detailed investigation into this study. Also, results from this study showed that soluble carbohydrates had no effect on decay of CCA treated lime and pine.

3.2.2.1. The influence of added soluble amino acids and carbohydrates on decay and preservative performances in CCA treated hardwoods and softwoods.

The results showed that both amino acids incorporated into CCA treated blocks influenced decay in lime (Fig 3.52 and 3.53). Clearly, increasing weight losses were observed with increasing amino acid concentrations in preserved lime blocks, but in beech, weight losses were broadly similar in preserved blocks of varying glutamine concentrations. Weight losses did not occur in preserved pine and spruce blocks, both with and without glutamine inclusions, over the duration of the twelve week soil burial experiment.

In the studies with carbohydrates, preserved lime blocks, both with and without sucrose additions, displayed broadly similar weight losses over the duration of the experiment (Fig 3.54). In pine, the addition of glucose to preserved wood blocks failed to influence weight loss in these specimens (Fig 3.57). Preserved blocks of pine were protected at the sub-toxic level of the preservative treatment over the twelve weeks soil burial.

In the studies with CCA treated blocks impregnated with amino acids, loss of both amino acids to the soil after burial was observed for all wood species tested. These losses of amino acids, noted by the decrease in nitrogen contents after 3 weeks soil burial, were apparent in all test blocks. The losses were greater in the softwoods than in the hardwoods, and in each wood species, largest losses of amino acids were observed in blocks at the highest treating concentrations of amino acid. In pine, these losses were 75% of the total nitrogen content prior to soil burial, in spruce 66%, in beech 54%, and in lime 50%. The nitrogen contents in blocks impregnated with amino acid concentrations to achieve total nitrogen contents in the blocks of 0.2%, showed broadly similar nitrogen levels after three weeks soil burial.

In both hardwoods and softwoods, nitrogen contents of unpreserved and preserved control blocks increased during the initial stages of soil burial. In the hardwoods, these nitrogen contents were above 0.2% and were accompanied by weight losses, but in the softwoods, the nitrogen contents were below 0.2% and no weight losses were recorded in these blocks.

All blocks regardless of initial treatment showed increased nitrogen contents after three weeks. Preserved control blocks of hardwoods displayed lower nitrogen contents than both unpreserved blocks and preserved blocks with amino acids. In the softwoods, nitrogen contents of both preserved controls and preserved blocks impregnated with amino acids were broadly similar over the duration of the soil burial. In lime, beech and pine, the nitrogen contents of blocks impregnated with different amino acid concentrations were seen to increase during the final sampling period (weeks 9-12). Spruce however, displayed a result similar to that observed in the study with amino acids described in 3.2.1.1. In these studies, decreases in nitrogen contents were observed during weeks 9-12 for all treatments in the four wood species.

The moisture contents of the blocks measured over the duration of the experiment varied with wood type and sampling time. Unpreserved blocks of both hardwoods and softwoods, and preserved blocks of hardwoods showed increased moisture contents after twelve weeks soil burial. The increases in moisture contents were accompanied by weight losses in these blocks. In lime, moisture contents increased from 10% to 120% during the first three weeks of soil burial. Final moisture contents of 200% were recorded in lime after twelve weeks soil burial. Beech displayed moisture contents of 100% after three weeks, and 154% after twelve weeks soil burial. The moisture contents in preservative treated softwoods remained relatively constant over the sampling periods. Spruce displayed mean moisture contents of 200% and pine mean moisture contents of 170% over the duration of the soil burial.

Interestingly, when preserved blocks impregnated with glutamine were left to dry in the laboratory at ambient temperatures, blocks impregnated with the higher glutamine concentrations, displayed efflorescence at the evaporative surfaces of the blocks. Efflorescence was not observed in preserved blocks with lower glutamine concentrations.

The efflorescence observed in preserved blocks of higher glutamine concentrations is clearly seen in Figure 3.49a. Figure 3.49b showed precipitates in a pine block impregnated with glutamine (2.74% w/v) to achieve an increased nitrogen content in the block of 0.6%. Blue specks were seen in the white precipitates which were thought to be attributed to the copper in the CCA treated blocks chelating with the amino acids. Significant losses of copper were observed in the preserved blocks impregnated with glutamine.

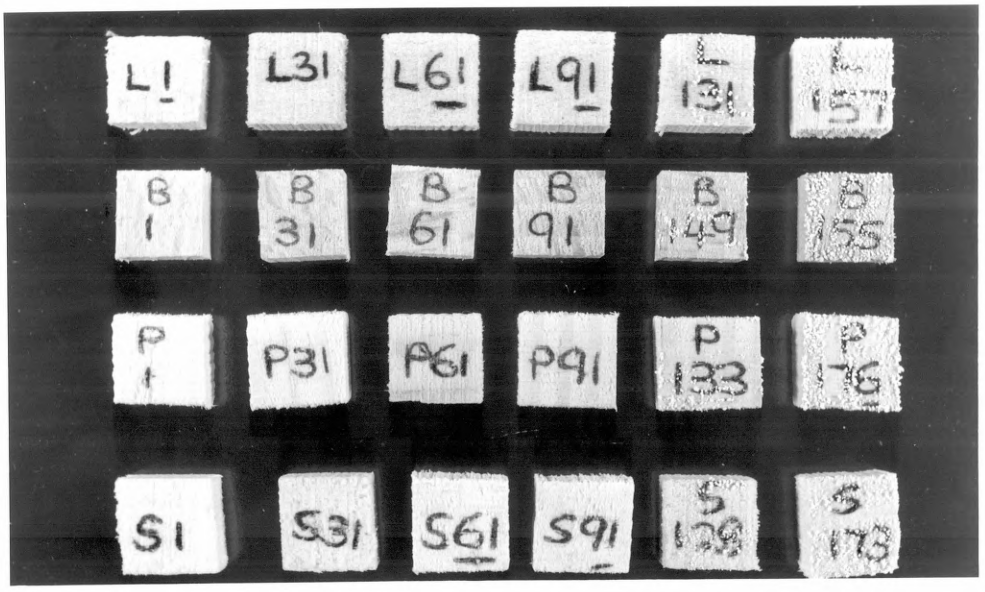


Fig 3.49a Efflorescence in preserved blocks impregnated with high glutamine concentrations. Test blocks are aligned in order of treatment, unpreserved controls, preserved controls and preserved blocks with increasing glutamine concentrations

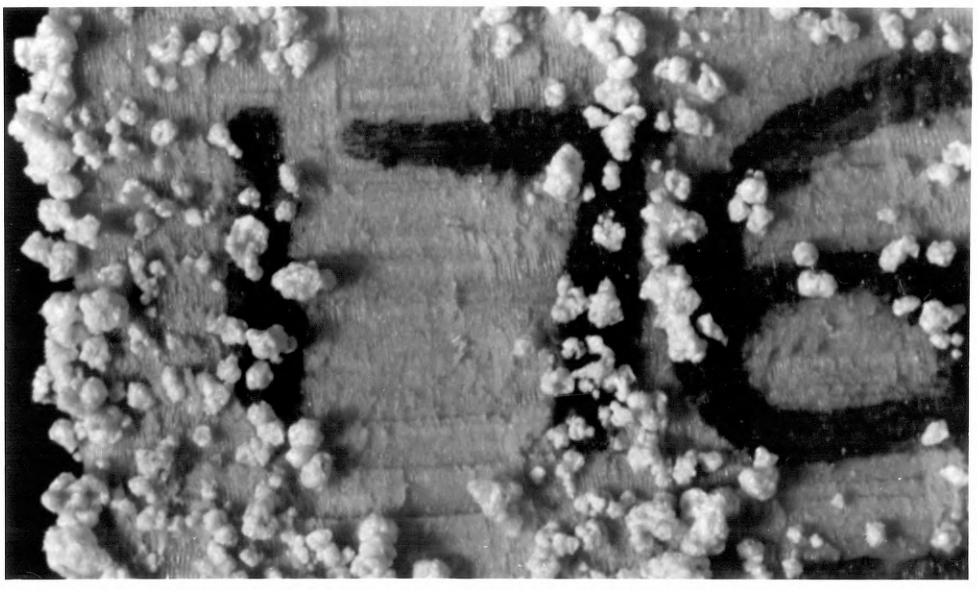


Fig 3.49b Precipitates on the evaporative surface of a pine test block impregnated with 2.74% w/v glutamine.

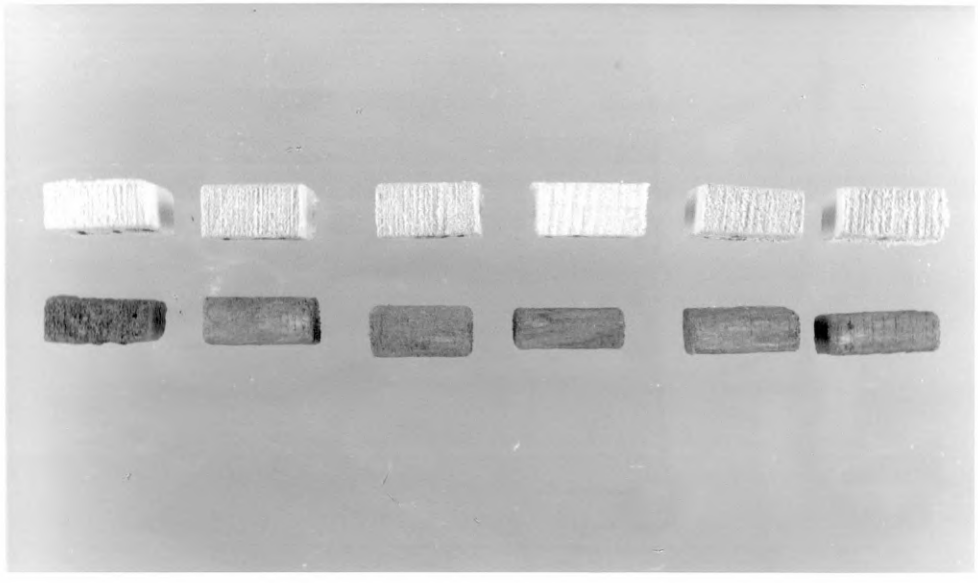


Fig 3.50a Unpreserved and preserved lime blocks of varying glutamine concentrations prior to soil burial (light coloured blocks) and after 12 weeks soil burial (dark coloured blocks). Significant weight losses were recorded in all blocks.

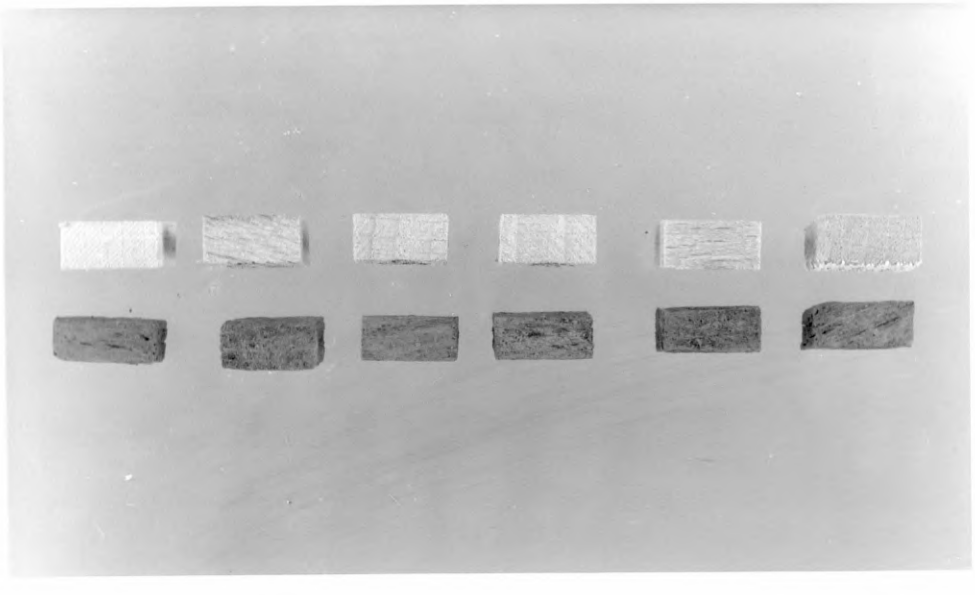


Fig 3.50b Unpreserved and preserved beech blocks of varying glutamine concentrations prior to soil burial (light coloured blocks) and after 12 weeks soil burial (dark coloured blocks). Weight losses were recorded in blocks of all treatments.

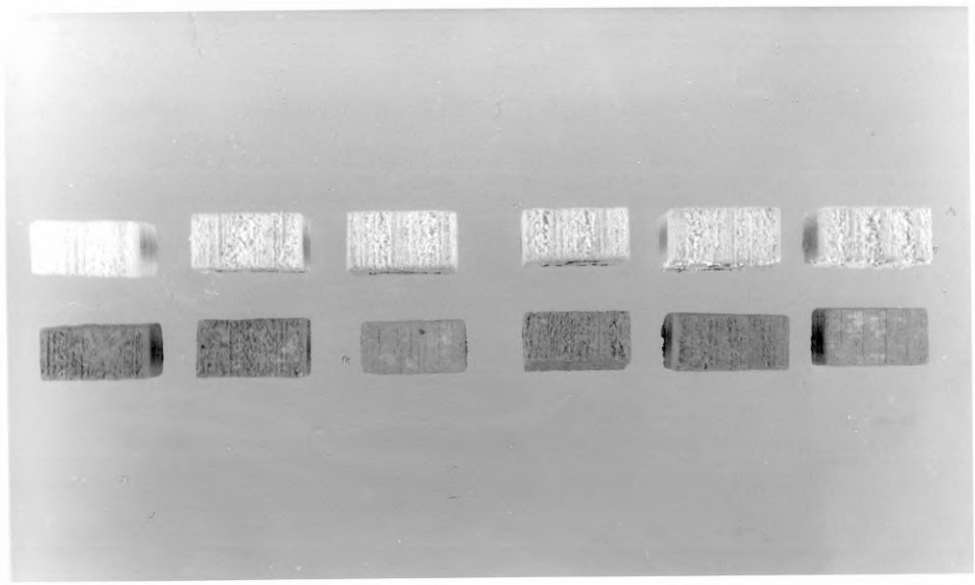


Fig 3.51a Unpreserved and preserved pine blocks of varying glutamine concentrations prior to soil burial (light coloured blocks) and after 12 weeks soil burial (dark coloured block). No weight losses were recorded in preserved blocks after twelve weeks soil burial.

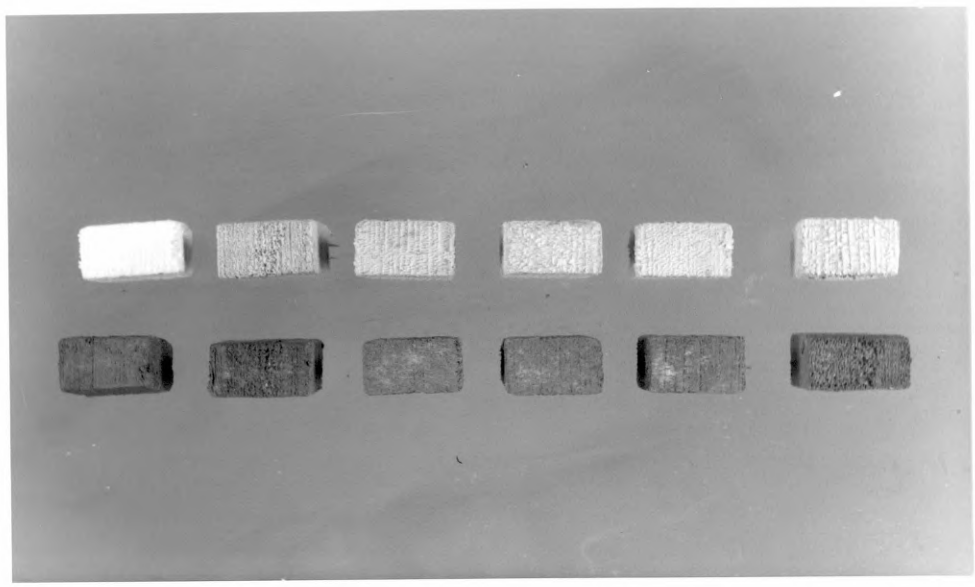


Fig 3.51b Unpreserved and preserved spruce blocks of varying glutamine concentration prior to soil burial (light coloured blocks) and after 12 weeks soil burial (dark coloured blocks). No weight losses were recorded in the preserved blocks over the duration of the soil burial.

a) Lime

The effect of arginine and glutamine on weight losses in CCA treated lime is presented in Figures 3.52 and 3.53 respectively.

In both studies, weight losses in blocks of all treatments increased with time. Unpreserved blocks displayed higher weight losses at each sampling period than preserved blocks. Preserved blocks impregnated with amino acids displayed higher weight losses than preserved control blocks.

In the arginine study, preservative treated control blocks showed significant weight losses (4%) during the initial stages of the soil burial (weeks 0-3). Unpreserved control blocks and preserved blocks impregnated with arginine, displayed weight losses between 4% - 7% during this period. Rates at which weight loss occurred in unpreserved blocks increased from 2% per week to 3% per week between weeks 0-3 and 3-6. Rates of increase of weight loss of preserved blocks remained constant at 1.6% per week during these two sampling periods. Weight losses of preserved blocks impregnated with varying concentrations of arginine, were broadly similar at 10% at week 6. At the final sampling interval, week 12, significant weight losses were observed in all blocks. During this period, unpreserved blocks displayed weight losses of 30%, preserved blocks impregnated with arginine between 17-21% and preservative treated control blocks at 15%.

In the glutamine study, weight losses were not considered to be significant ($<3\%$) in preservative treated control blocks and, in preserved blocks impregnated with lower concentrations of glutamine during the initial stages of the soil burial (weeks 0-3). At weeks 3, 6 and 9, weight losses displayed by preserved blocks impregnated with the lowest concentration of glutamine (0.44% w/v) were broadly similar to preservative treated control blocks. Rates of increase of weight loss were rapid in unpreserved blocks during the period weeks 0-9, but diminished thereafter. Weight losses of approximately 30% were recorded in these blocks at the final sampling period. Preserved blocks both with and without glutamine additions, displayed a gradual increase in the rate of weight loss over the duration of the soil burial.

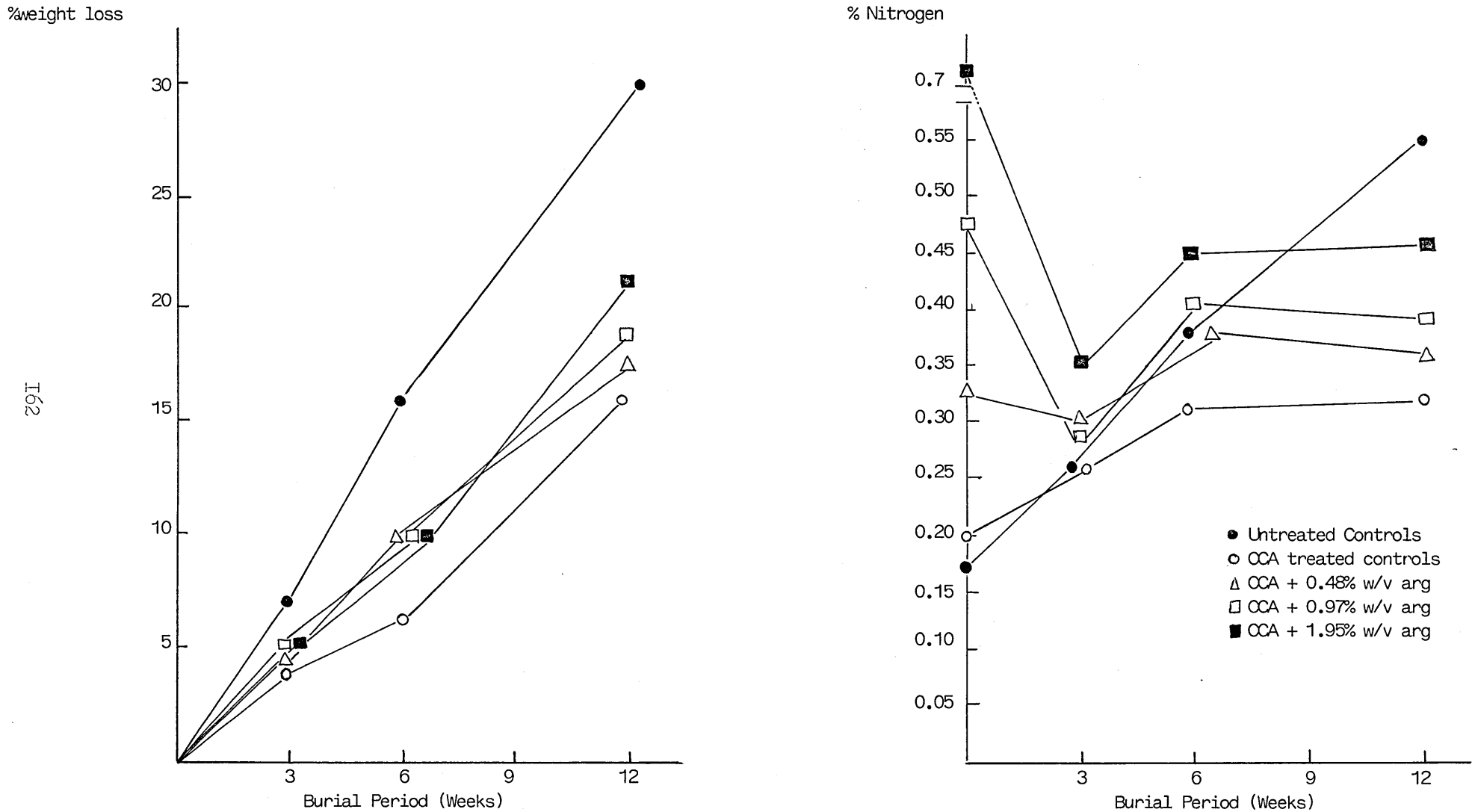


Fig 3.52 Weight losses and total nitrogen contents in CCA treated lime impregnated with arginine, after burial in soil for periods up to twelve weeks.

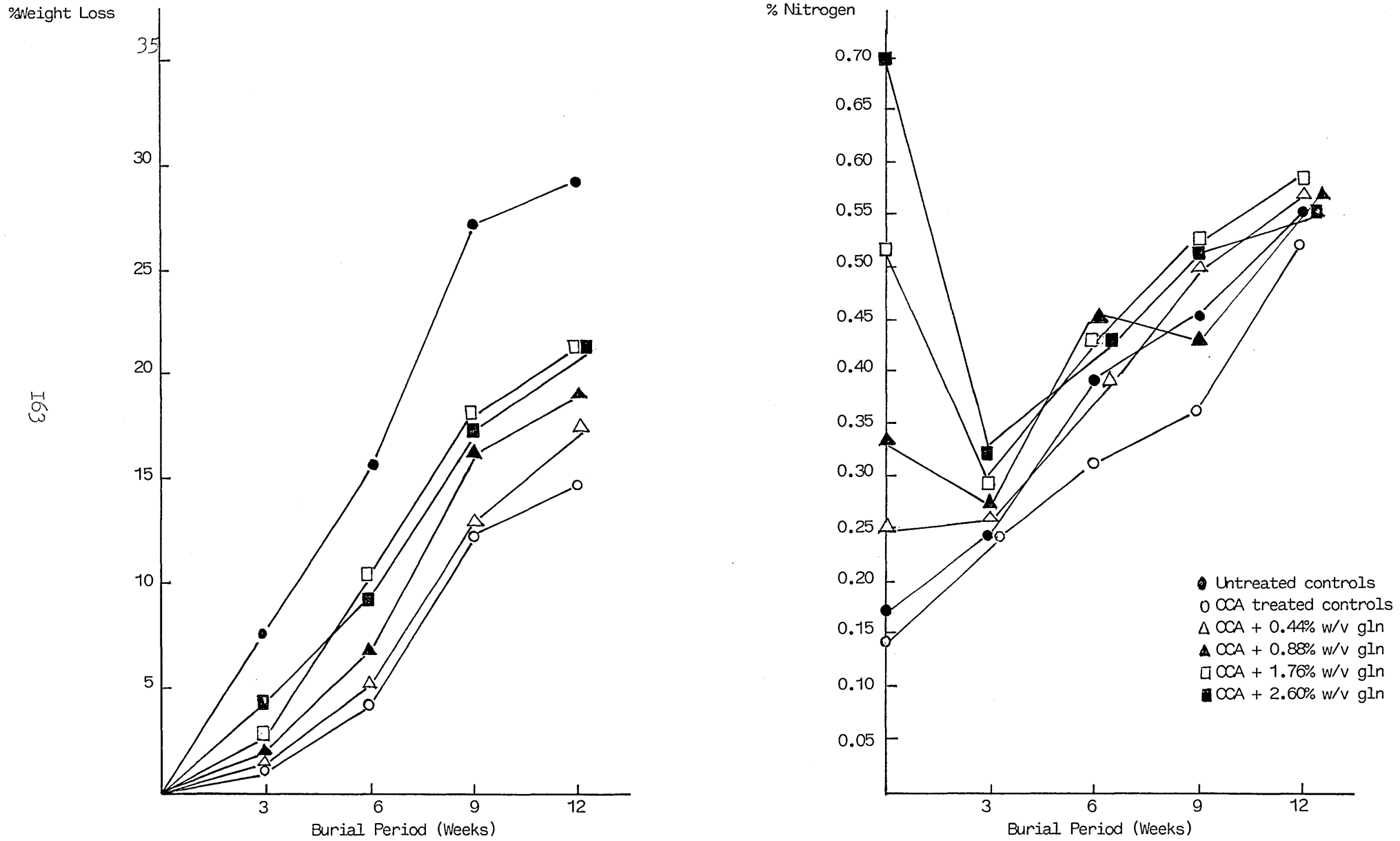


Fig 3.53 Weight losses and total nitrogen contents in CCA treated lime blocks impregnated with glutamine, after burial in soil for periods up to twelve weeks.

At the final sampling period, weight losses recorded in these blocks were at 15% for preserved control blocks, and between 16% - 21% for blocks impregnated with glutamine.

Statistical analysis to compare the differences in weight loss of preserved controls and preserved blocks impregnated with amino acids is presented in Table 3.15 for both the arginine and glutamine studies. 1-way analysis of variance (Column II) of the comparison of weight loss of blocks of different treatments showed a significant difference existed ($p=0.001$) in both the arginine and glutamine studies. 1-way analysis of variance (1-ANOVA) of the comparison of weight losses within each treatment over the duration of the soil burial period (Column III) also showed a significant difference at $p=0.001$. 2-way analysis of variance (2-ANOVA) of comparison of difference in weight loss between treatments over the duration of the soil burial period (Interaction, Column IV) showed that highly significant differences in the rates of decay existed for blocks at each amino acid impregnation level, $p=0.01$, in the arginine study and $p=0.001$ in the glutamine study. These results support the conclusion that amino acids impregnated into CCA treated lime blocks influence the decay susceptibility of the blocks and also the preservative performance.

The results of the nitrogen analysis undertaken on CCA treated blocks impregnated with amino acids are presented in Figure 3.52 for the arginine study and Figure 3.53 for the glutamine study.

Both studies displayed similar patterns in nitrogen contents over the duration of the experiment. At the first sampling interval (week 3) losses in amino acids were observed in preserved blocks impregnated with amino acids. The total nitrogen contents displayed by these blocks after the loss of amino acids were between 0.25% and 0.35%. Also, during this period unpreserved blocks and preserved control blocks displayed increases in nitrogen from 0.17% and 0.14% respectively to 0.25%.

In the arginine study increases in nitrogen contents were observed in preserved blocks after 3 weeks. During this period (3-6 weeks) unpreserved blocks and preserved blocks impregnated with arginine displayed similar rates of increase of nitrogen content (0.03% per week).

Table 3.15 1-ANOVA and 2-ANOVA comparison of weight loss differences between CCA-treated controls and CCA-treated blocks impregnated with amino acids.

Wood Type	I	II	III	IV
Lime	CCA controls	xxx	xxx	xx
	CCA + 0.48% w/v arg	xxx	xxx	xx
	CCA + 0.97% w/v arg	xxx	xxx	xx
	CCA + 1.95% w/v arg	xxx	xxx	xx
Lime	CCA controls	xxx	xxx	xxx
	CCA + 0.44% w/v gln	xxx	xxx	xxx
	CCA + 0.88% w/v gln	xxx	xxx	xxx
	CCA + 1.76% w/v gln	xxx	xxx	xxx
	CCA + 2. % w/v gln	xxx	xxx	xxx

xxx and xx represent probabilities of $< 0.1\%$ and 1.0% respectively, that differences arising from comparisons of each column could arise by chance.

Column I Treatment (%w/v amino acid)

Column II 1-ANOVA to determine whether there is a significant difference in weight loss of blocks of different treatments

Column III 1-ANOVA to determine whether there is a significant difference in weight loss within each treatment over the duration of the burial.

Column IV (Interaction) 2-ANOVA to determine whether any significant difference in weight loss exists between treatments over the duration of the soil burial.

Nitrogen contents of preserved blocks impregnated with 0.97% w/v and 1.95% w/v arginine, were higher than those of the unpreserved blocks, but these high nitrogen contents were not accompanied by high weight losses in the preserved blocks with arginine.

In the period weeks 6 - 12, there was a marked increase in nitrogen content of unpreserved blocks from 0.38% to 0.55%, with a concomitant increase in weight loss from 16% to 30%. During the same period, nitrogen contents of preserved blocks remained fairly constant at 0.30% in preserved control blocks, and between 0.35% and 0.45% in preserved blocks with arginine.

In the study with glutamine, unpreserved blocks and preserved blocks displayed a gradual increase in nitrogen contents after 3 weeks soil burial, unpreserved blocks and preserved blocks impregnated with glutamine showed a rate of increase of nitrogen input of 0.05% per week between weeks 3 - 6. Rates of increase of nitrogen of these blocks diminished thereafter.

During the period weeks 0 - 9, preserved blocks impregnated with glutamine showed higher nitrogen contents to unpreserved blocks. Despite the lower nitrogen contents, weight losses displayed by the unpreserved blocks were significantly higher than those of the preserved glutamine blocks.

At the final sampling period (week 12) unpreserved blocks and preserved blocks impregnated with glutamine displayed similar nitrogen contents at 0.55%. Preservative treated control blocks displayed nitrogen contents of 0.50% during this period. In the glutamine study, nitrogen contents of unpreserved blocks and preserved blocks at week 12, were higher than blocks of similar treatment in the arginine study.

1-ANOVA and 2-ANOVA of comparison of the mean nitrogen contents of preserved controls and preserved blocks impregnated with amino acids is presented in Table 3.16. Statistical analysis were undertaken on nitrogen data obtained during weeks 3-12 of the soil burial. Comparison of differences in nitrogen contents between treatments were made from this period as results showed that losses in nitrogen occurred in blocks impregnated with amino acids during the initial stages of the soil burial. Statistical analysis of comparison of the nitrogen contents in beech, pine and spruce were also undertaken. Using data from weeks 3 - 12, 1-ANOVA of comparison of nitrogen contents in blocks of different treatments (Column II) showed that

a significant difference existed between treatments in both the arginine study and the glutamine study. Significant differences ($p=0.001$) in nitrogen contents were observed within each treatment over the soil burial period (Column III). Highly significant differences also existed ($p=0.001$) between the nitrogen contents of the controls and blocks with amino acids over the duration of the experiment (Interaction, Column IV). These results show that despite the loss of amino acids from the blocks during the early stages of the soil burial, significant differences in nitrogen contents between treatments still existed over the duration of the soil burial.

Table 3.16 1-ANOVA and 2-ANOVA comparison of the differences in nitrogen contents between CCA-treated controls and CCA-treated blocks impregnated with amino acid.

Wood Type	I	II	III	IV
Lime	CCA controls	xxx	xxx	xx
	CCA + 0.48% w/v arg	xxx	xxx	xx
	CCA + 0.97% w/v arg	xxx	xxx	xx
	CCA + 1.95% w/v arg	xxx	xxx	xx
Lime	CCA controls	xxx	xxx	xx
	CCA + 0.44% w/v gln	xxx	xxx	xx
	CCA + 0.88% w/v gln	xxx	xxx	xx
	CCA + 1.76% w/v gln	xxx	xxx	xx
	CCA + 2.60% w/v gln	xxx	xxx	xx

xxx and xx represent probabilities of $< 0.1\%$ and 1% respectively, that differences arising from comparisons of each column could arise by chance.

Column I Treatment (%w/v amino acid)

Column II 1-ANOVA to determine whether there is a significant difference in nitrogen contents in blocks of different treatments.

Column III 1-ANOVA to determine whether there is a significant difference in nitrogen content within the treatment over the burial period.

Column IV (Interaction) 2-ANOVA to determine whether any significant difference in nitrogen contents exist between treatments used over the duration of the soil burial.

The relationship between nitrogen contents of blocks and weight loss over the soil burial period of twelve weeks is shown in Table 3.17. The correlation coefficients at each treatment level were calculated using the Statpack programme (R. Houchard, Statpack Program, Decsystem 20, Western Michigan University, 1974). In preserved blocks impregnated with amino acids, correlation coefficients were calculated using data obtained from weeks 3 to 12. Nitrogen data from week 0 was ommitted, because, the high nitrogen contents from the addition of amino acids of varying concentrations, would mask any significant correlation between the nitrogen contents and weight loss during soil burial. The results showed that a significant correlation exists between nitrogen contents of the blocks, and the decay status of the woods at each treatment.

Table 3.17 Data showing correlation between nitrogen content and weight loss in unpreserved and preserved lime at varying amino acid concentrations.

Amino Acid	Treatment	Correlation coefficient
Arg	Unpreserved controls	0.9701
	Preserved controls	0.7168
	CCA + 0.48% w/v	0.6067
	CCA +0.97% w/v	0.5954
	CCA +1.95% w/v	0.7330
Gln	Unpreserved Controls	0.8130
	Preserved controls	0.7632
	CCA +0.44% w/v gln	0.6901
	CCA +0.88% w/v gln	0.7692
	CCA +1.76% w/v gln	0.7087
	CCA +2.60% w/v gln	0.6197

The effect of sugar on weight losses in CCA treated lime is presented in Figure 3.54. Unpreserved and preserved wood blocks showed increases in weight loss over the duration of the experiment. Weight losses displayed by unpreserved blocks were significantly higher than those of the preserved blocks. During the first sampling period (week 3), unpreserved blocks displayed weight losses of 7%. During this same period, weight losses were not significant ($<3\%$) in preserved control blocks and in preserved blocks with sucrose. Significant weight losses (6%) were observed in these blocks at week 6. Both preserved control blocks and preserved blocks impregnated with sucrose displayed broadly similar weight losses over the duration of the experiment. Preserved blocks displayed a mean weight loss of 20%, and unpreserved blocks a weight loss of 35% during this final sampling period.

In contrast to the preserved blocks impregnated with amino acids, preserved blocks impregnated with sugar displayed lower weight losses during the period weeks 0-9. However, during the final sampling period (week 12), preserved blocks impregnated with amino acids and preserved blocks impregnated with sugar displayed broadly similar weight losses.

Statistical analysis to determine the differences in weight losses between preserved control blocks and preserved blocks impregnated with sugar was considered unnecessary as results showed that sugars did not influence decay and preservative performance in preservative treated lime.

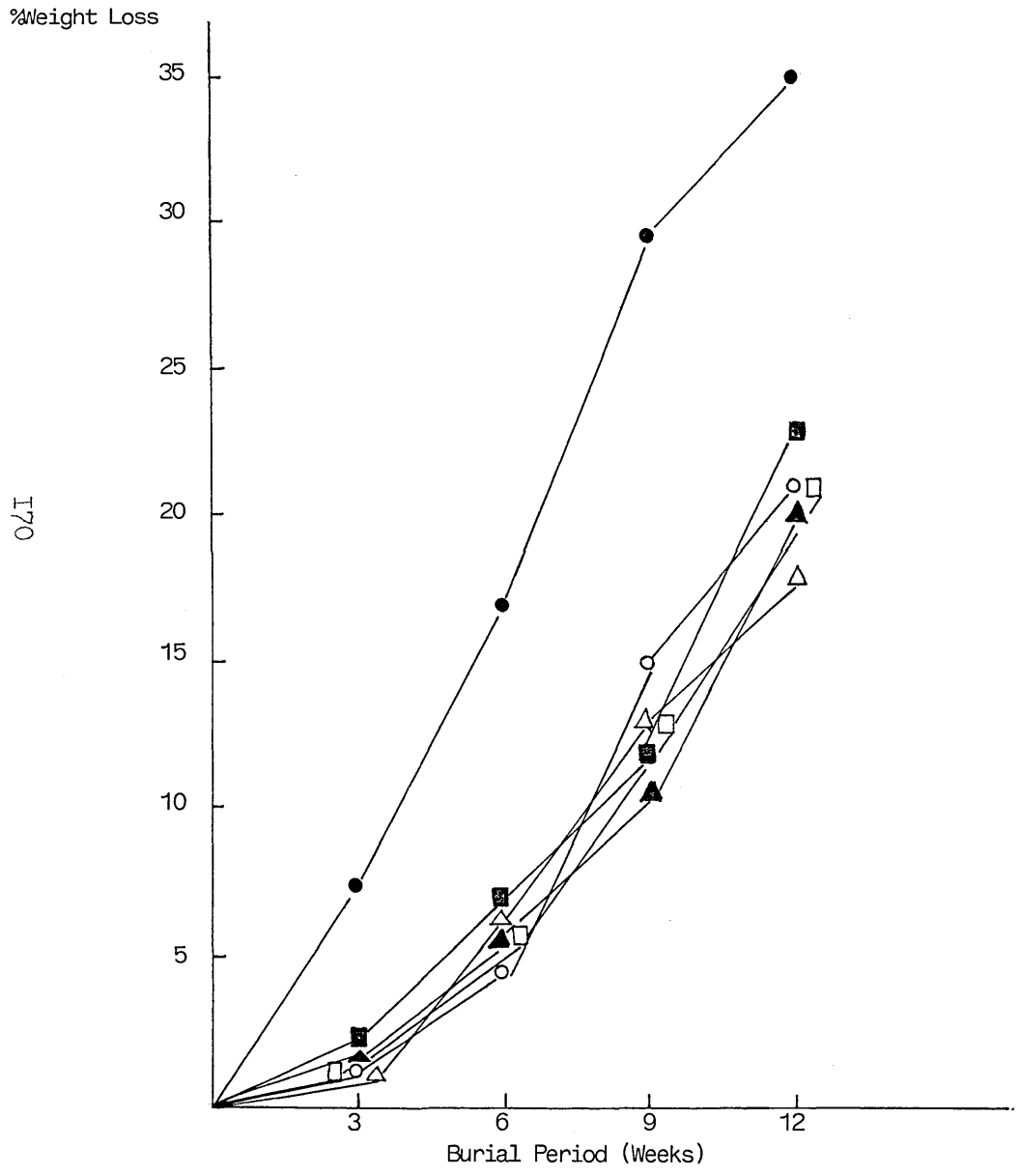


Fig 3.54 Weight losses in CCA treated lime impregnated with sucrose, after burial in soil for period up to twelve weeks.

b) Beech

The effect of glutamine on weight losses and nitrogen transfer to CCA treated beech blocks are presented in Figure 3.55.

In beech, the incorporation of amino acids to preserved blocks appeared to show an influence on decay in these blocks at the initial stages of the soil burial (weeks 0-3). During this period, preserved blocks impregnated with glutamine, displayed larger weight losses than those of the preserved control blocks. In the later sampling periods (in contrast to lime), weight losses were broadly similar in both preserved control blocks and preserved blocks impregnated with glutamine.

During the first sampling period (week 3) weight losses of 3% to 6% were observed in unpreserved blocks and preserved blocks impregnated with glutamine. Weight losses of preserved control blocks did not become significant (>3%) during this period.

In the period weeks 3-6, weight loss occurred at a rate of 2% per week in both unpreserved and preserved blocks. Also, during this period, preserved control blocks displayed broadly similar weight losses to preserved blocks impregnated with glutamine. Weight losses continued to increase in unpreserved and preserved blocks during weeks 6-9, but showed a diminished rate of increase in the period weeks 9-12. At this final sampling period, unpreserved blocks displayed weight losses of 23%, and preserved blocks both with and without glutamine additions, displayed broadly similar weight losses of 20%.

The results of the nitrogen analysis (Fig 3.55), showed that the initial nitrogen contents of preserved control blocks and unpreserved blocks increased from 0.10% and 0.15% respectively to 0.24% after three weeks soil burial. Preserved blocks impregnated with glutamine, displayed higher nitrogen contents to unpreserved blocks at week 6, but these high nitrogen contents in the glutamine blocks were not accompanied by large weight losses. At week 9, preserved blocks of varying glutamine concentrations displayed similar nitrogen contents at 0.40%. Unpreserved and preserved control blocks showed small increases in the nitrogen contents during this period.

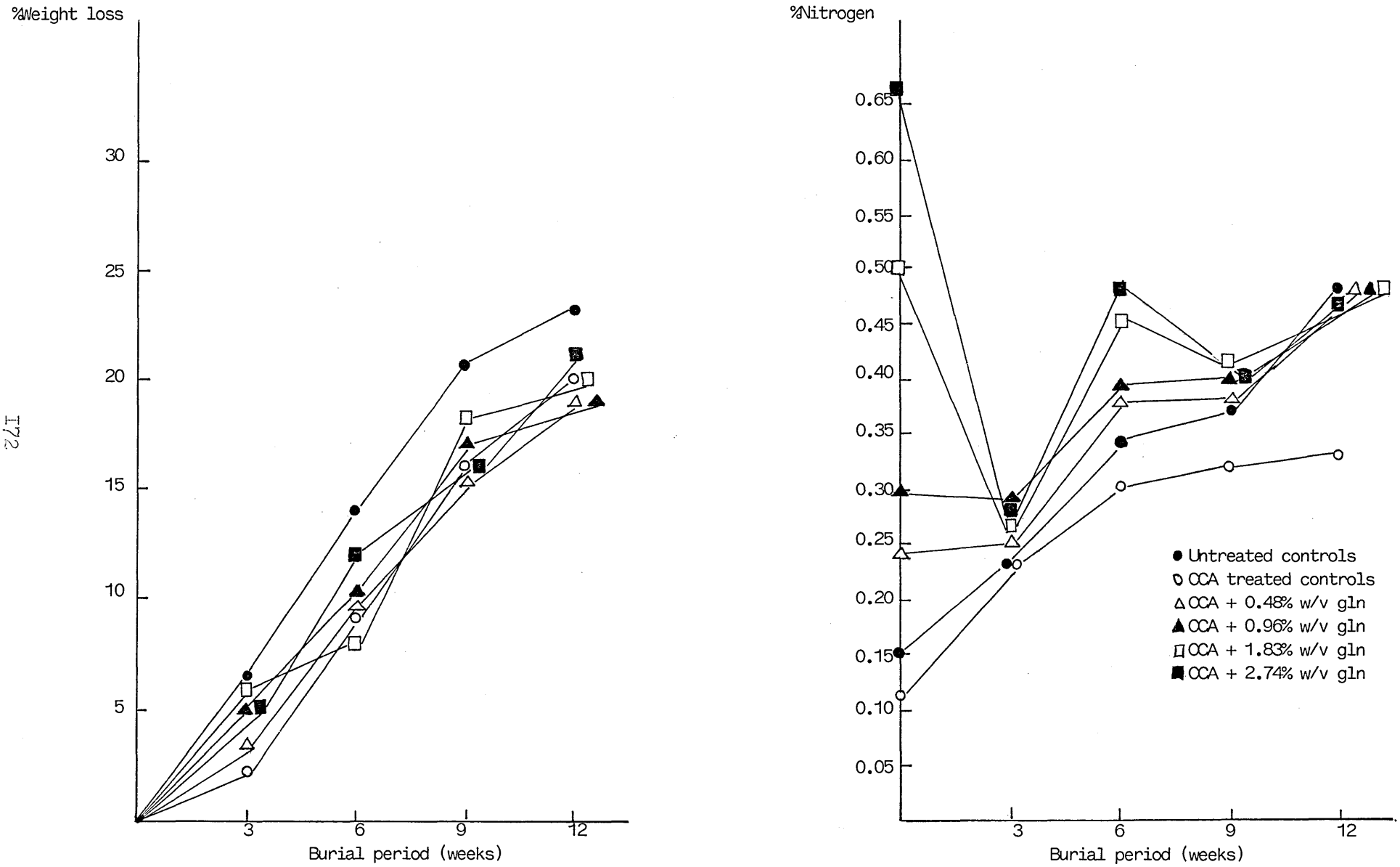


Fig 3.55 Weight losses and total nitrogen contents in CCA treated beech blocks impregnated with glutamine, after burial in soil for periods up to twelve weeks.

At the final sampling period, unpreserved and preserved blocks impregnated with glutamine displayed broadly similar nitrogen contents at 0.49%. Though the nitrogen contents of preserved control blocks at 0.33% were lower than preserved blocks impregnated with glutamine, the weight losses displayed by these preserved blocks of different treatment were broadly similar during this final sampling period.

Statistical analysis of comparison of the differences in weight loss and nitrogen contents between preserved controls and preserved blocks impregnated with amino acids are presented in Table 3.18. Results of 1-ANOVA showed that no significant differences existed in weight loss between treatments used (Column II), but a significant difference ($p=0.001$) existed in the weight losses of blocks within each treatment over the duration of the soil burial (Column III). 2-ANOVA (Interaction) of the differences in weight loss between treatments and the duration of the soil burial showed that in beech, different treatments imposed on the preserved blocks had no significant effect on the weight losses of these blocks over the duration of the experiment. The probability value obtained for comparison of the differences in nitrogen contents between preserved controls and preserved blocks impregnated with glutamine, showed that highly significant differences existed ($p=0.001$) in nitrogen contents of blocks of difference treatment (Column II), and also within each treatment over the duration of the soil burial (Column III). The 'Interaction' Column (Column IV) showed that a significant difference existed ($p=0.001$) in the nitrogen contents of blocks between treatments over the soil burial period.

Table 3.18 1-ANOVA and 2-ANOVA comparison of differences in (i) weight loss and (ii) nitrogen content between CCA treated controls and CCA treated blocks impregnated with glutamine.

		I	II	III	IV
(i)	Weight loss	CCA controls	NS	xxx	NS
		CCA + 0.48% w/v gln	NS	xxx	NS
		CCA + 0.96% w/v gln	NS	xxx	NS
		CCA + 1.83% w/v gln	NS	xxx	NS
		CCA + 2.74% w/v gln	NS	xxx	NS
(ii)	Total Nitrogen Content	CCA controls	xxx	xxx	xxx
		CCA + 0.48% w/v gln	xxx	xxx	xxx
		CCA + 0.96% w/v gln	xxx	xxx	xxx
		CCA + 1.83% w/v gln	xxx	xxx	xxx
		CCA + 2.74% w/v gln	xxx	xxx	xxx

xxx and NS represent probabilities of $< 0.1\%$ and $> 5\%$ respectively, that differences arising from comparisons of each column could arise by chance.

Column I	Treatment (%w/v gln)
Column II	1-ANOVA to determine whether there is a significant difference in weight loss or nitrogen content for the different treatment used.
Column III	1-ANOVA to determine whether there is a significant difference in weight loss or nitrogen content within each treatment over the soil burial period.
Column IV (Interaction)	2-ANOVA to determine any significant difference between weight losses or nitrogen contents for control and blocks with glutamine over the burial period.

The correlation between nitrogen contents of blocks and weight loss over the decay period of twelve weeks is shown in Table 3.19. A procedure similar to that described for lime was used to calculate these correlation coefficients. In beech, a significant correlation exists between nitrogen contents of the blocks and the decay status of the wood at each treatment level.

Table 3.19 Data showing correlation between nitrogen contents and weight loss in unpreserved and preserved beech of varying glutamine concentrations.

Treatment	Correlation coefficient
Unpreserved controls	0.7434
Preserved controls	0.9449
CCA + 0.48% w/v gln	0.8482
CCA + 0.96% w/v gln	0.6263
CCA + 1.83% w/v gln	0.5397
CCA + 2.72% w/v gln	0.6853

c) Pine

The influence of glutamine on the weight loss and nitrogen transfer to preserved pine blocks is presented in Figure 3.56.

In pine, weight losses were observed in unpreserved blocks, but not in preserved blocks over the duration of the experiment. The nitrogen contents of unpreserved blocks and preserved controls increased after week 0, but nitrogen contents of preserved blocks impregnated with glutamine displayed increases in nitrogen only after 3 weeks soil burial.

During the period weeks 0-3, nitrogen contents of blocks impregnated at the higher glutamine concentrations decreased by approximately 70% of its initial total nitrogen content. Blocks at the lower glutamine concentrations also displayed losses but these losses in nitrogen were less than 50% of the initial total nitrogen content.

In the period weeks 3-6, unpreserved and preserved blocks both with and without glutamine additions, displayed similar rates of increase of nitrogen content (0.02% per week). The nitrogen content of these blocks regardless of treatment were broadly similar at 0.2% and 0.25%. In the period weeks 6-9, nitrogen contents of preserved controls and unpreserved blocks increased from 0.20% and 0.25% respectively to 0.30%. At the final sampling period (week 12), nitrogen contents in these blocks decreased to 0.22% in preserved controls, and 0.25% in unpreserved blocks. Preserved blocks impregnated with glutamine displayed nitrogen contents of 0.20% at week 9, and nitrogen contents between 0.22% to 0.27% at week 12.

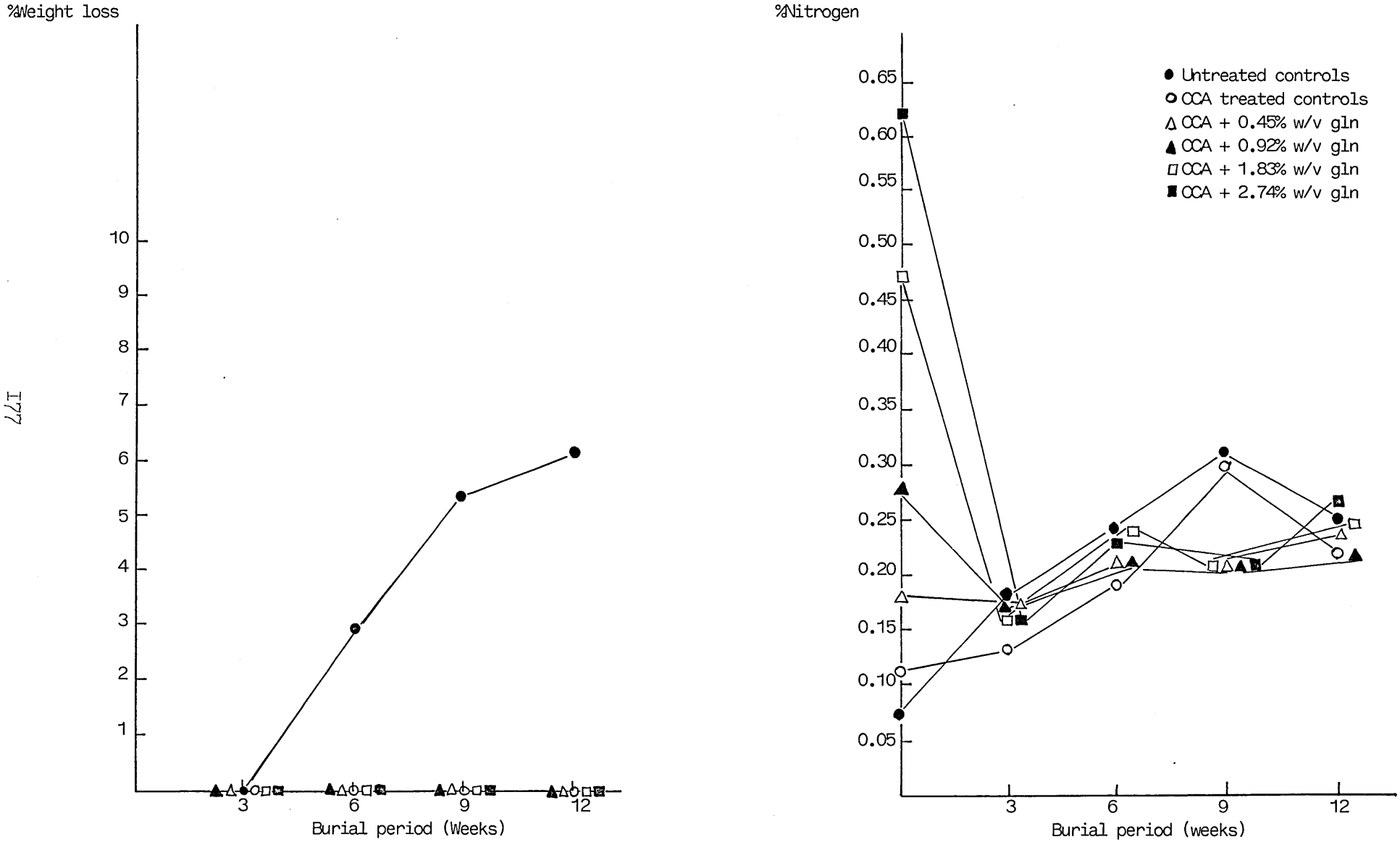


Fig 3.56 Weight losses and total nitrogen contents in CCA treated pine blocks impregnated with glutamine, after burial in soil for periods up to twelve weeks.

Two-way analysis of variance of the differences in nitrogen contents in preserved blocks of varying treatment is presented in Table 3.20. The statistical analysis were undertaken using nitrogen data obtained from weeks 3-12 of the soil burial. The results showed that after the loss of glutamine to soil (weeks 0-3), differences in nitrogen contents in blocks of varying glutamine concentrations did not exist (Column II). However, a significant difference existed ($p=0.001$) in nitrogen contents of the blocks within each treatment over the soil burial period i.e. differences in nitrogen contents were observed in the blocks with time.

The 'Interaction' column i.e. comparison of the effect of different treatments on the nitrogen contents of the blocks over the duration of the soil burial showed that a significant difference existed at $p=0.05$.

Table 3.20 1-ANOVA and 2-ANOVA of comparison of the differences in nitrogen contents between CCA treated controls and CCA treated blocks with glutamine.

Wood Type	I	II	III	IV
Pine	CCA controls	NS	xxx	x
	CCA + 0.45% w/v gln	NS	xxx	x
	CCA + 0.92% w/v gln	NS	xxx	x
	CCA + 1.84% w/v gln	NS	xxx	x
	CCA + 2.74% w/v gln	NS	xxx	x

xxx, x and NS represent probabilities of $<0.1\%$, 5% and $>5\%$ respectively, that differences arising from comparisons of each column could arise by chance.

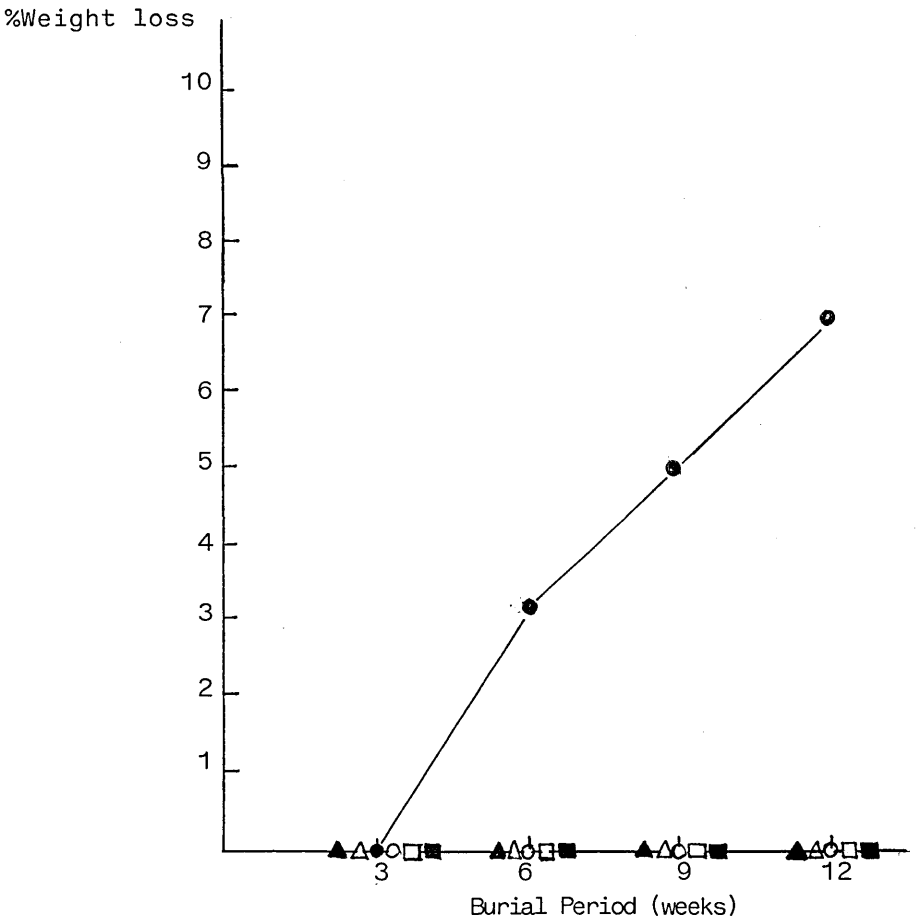
Column I Treatment (%w/v gln)

Column II 1-ANOVA to determine whether there is any significant difference in the nitrogen content in the different treatments employed.

Column III 1-ANOVA to determine whether there is a significant difference in nitrogen content within each treatment over the duration of the soil burial.

Column IV (Interaction) 2-ANOVA to determine whether there is any significant difference between nitrogen contents for controls and blocks with glutamine over the burial period.

In the study with carbohydrates (Fig 3.57), weight losses were not observed in preserved blocks. These blocks were protected at the sub-toxic level (0.05% w/v CCA) over the twelve weeks soil burial. Final weight losses of 7% were recorded in unpreserved blocks. Weight losses displayed by these blocks were broadly similar to the weight losses displayed by unpreserved blocks in the study with amino acids.



● Untreated controls
○ CCA treated controls
Δ CCA + 0.50% w/w glc
▲ CCA + 1.00% w/w glc
□ CCA + 2.00% w/w glc
■ CCA + 4.00% w/w glc

Fig 3.57 Weight losses in CCA treated pine blocks impregnated with glucose, after burial in soil for periods up to twelve weeks.

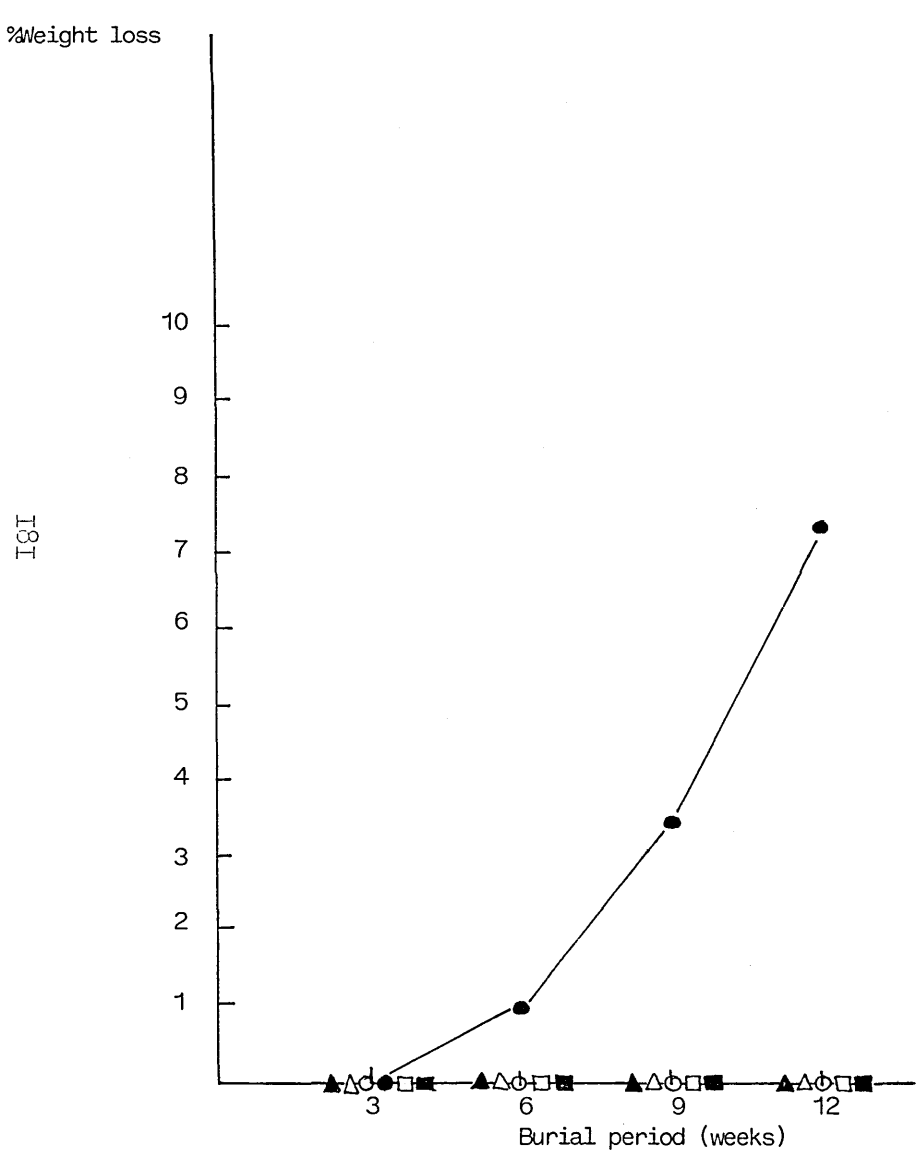
d) Spruce

The effect of glutamine on weight loss and nitrogen transfer to CCA treated spruce is presented in Figure 3.58.

Like pine, weight losses were observed in unpreserved blocks but not in preserved blocks. Unpreserved blocks displayed significant weight losses six weeks after soil burial. At the final sampling period (week 12), weight losses of these blocks were at 7%.

As with the hardwoods and pine, nitrogen contents of preserved blocks impregnated with glutamine also decreased during the initial stages of the soil burial. The decrease in nitrogen contents resulting from the loss of glutamine to the soil, were significantly higher (60%) in blocks at the higher glutamine concentrations. Unpreserved blocks and preserved controls displayed increases in nitrogen content during this period (weeks 0-3). At the final sampling period (week 12), nitrogen contents of unpreserved blocks were at 0.28%, and were accompanied by a significant increase in weight loss from 3% to 7%. The nitrogen contents of preserved blocks both with and without glutamine additions decreased during this period. Final nitrogen contents of these blocks were between 0.15% and 0.20%.

Probability values obtained for the 2-way ANOVA calculations for differences in means of nitrogen contents of preserved blocks of varying treatments is presented in Table 3.21.
The results of these statistical analysis were similar to those seen in pine. Differences in the mean nitrogen contents of preserved controls and preserved blocks with glutamine over the soil burial period were significant at $p=0.05$.



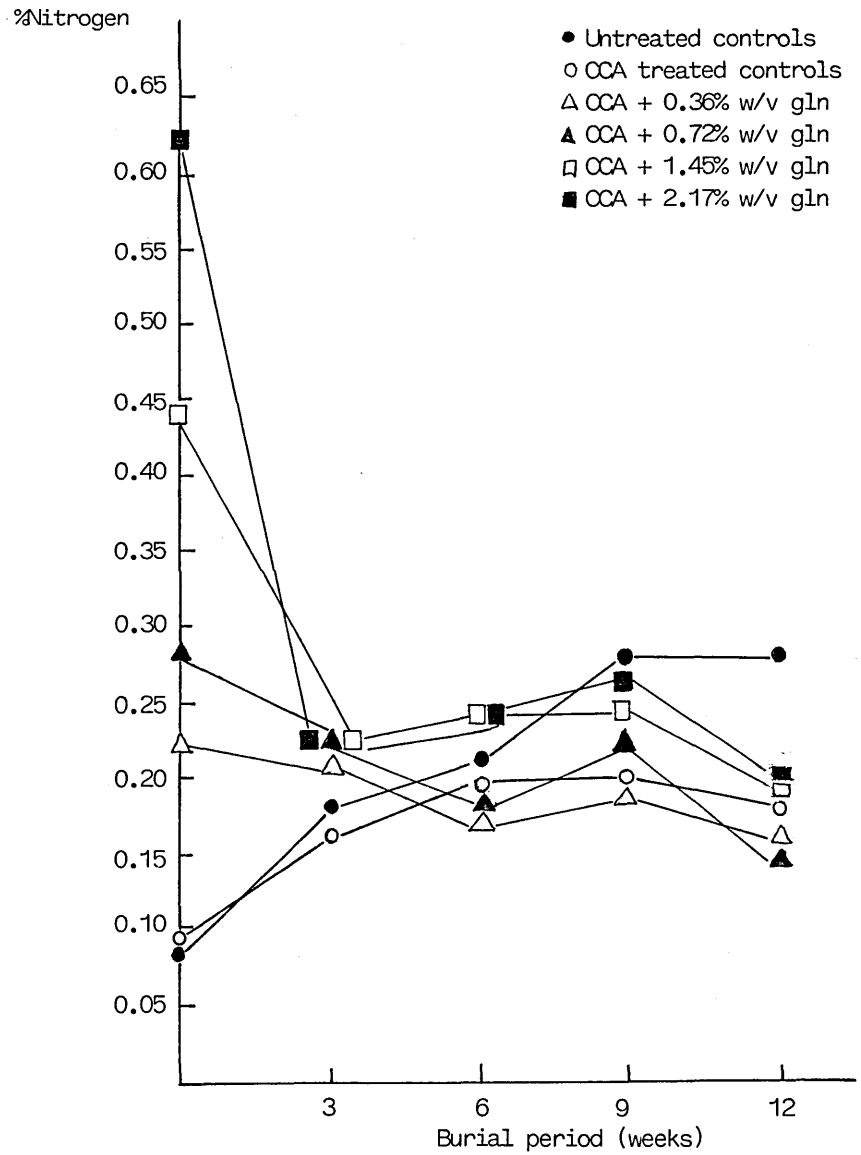


Fig 3.58 Weight losses and total nitrogen contents in CCA treated spruce blocks impregnated with glutamine, after burial in soil for periods up to twelve weeks.

Table 3.21 1-ANOVA and 2-ANOVA. Comparison of the differences in nitrogen contents between CCA treated controls and CCA treated blocks with glutamine.

Wood Type	I	II	III	IV
Spruce	CCA Controls	NS	xxx	x
	CCA + 0.36% w/v gln	NS	xxx	x
	CCA + 0.72% w/v gln	NS	xxx	x
	CCA + 1.45% w/v gln	NS	xxx	x
	CCA + 2.72% w/v gln	NS	xxx	x

xxx, x and NS represent probabilities of $<0.1\%$, 5% and $>5\%$ respectively, that differences arising from comparisons of each column could arise by chance.

Column I Treatment (%w/v gln)

Column II 1-ANOVA to determine whether there is any significant difference in nitrogen content in the different treatments employed.

Column III 1-ANOVA to determine whether there is a significant difference in nitrogen content within each treatment over the duration of the soil burial.

Column IV 2-ANOVA to determine any significant difference between nitrogen contents for controls and blocks with glutamine over the burial period.

3.2.2.2. The effect of glutamine on the copper and chromium contents of CCA-treated hardwoods and softwoods.

The copper and chromium contents of preserved blocks over the duration of the soil burial are presented in Figures 3.59 - 3.62. The copper contents of preserved lime, beech and pine prior to soil burial were broadly similar at each glutamine impregnation level. At this same sampling period, preserved control blocks of spruce i.e. those without glutamine additions, showed higher copper contents than blocks impregnated with glutamine. One-way analysis of variance of mean copper contents of spruce blocks, both with and without glutamine additions, showed copper contents in these blocks not to be significantly different ($F=2.86$, $p=0.05$). Trace amounts of the elements ($<0.01\%$ w/w) were present in unpreserved wood, and these amounts remained relatively constant over the duration of the soil burial.

The mean copper and chromium contents of the blocks prior to soil burial, and the copper and chromium contents in the treating solution, are presented in Table 3.22. The ratios of metals in the blocks and as salts in the treating solution are also presented in this table. The results showed that different levels of absorption of the elements from the treating solution were observed in the four wood species tested. Spruce showed an over absorption of both copper and chromium from the treating solution. Lime and pine displayed broadly similar copper contents in the block as in the treating solution, but beech showed lower absorption of copper. The hardwoods also showed lower chromium contents in the blocks, indicating an under absorption of this element in the wood. Cu:Cr ratio of the hardwoods however, were similar to the ratios of these metals as salts in the treating solution. The softwoods displayed lower Cu:Cr ratios to the hardwoods.

Comparisons of the levels of copper and chromium in the blocks determined by chemical analysis, to levels calculated from liquid uptake data, showed higher contents of the elements in the blocks. The selective adsorption ratios of copper and chromium are presented in Table 3.23. The results showed that the ratios obtained for chromium were higher than those obtained for copper. These results show that chromium is selectively adsorbed to the wood.

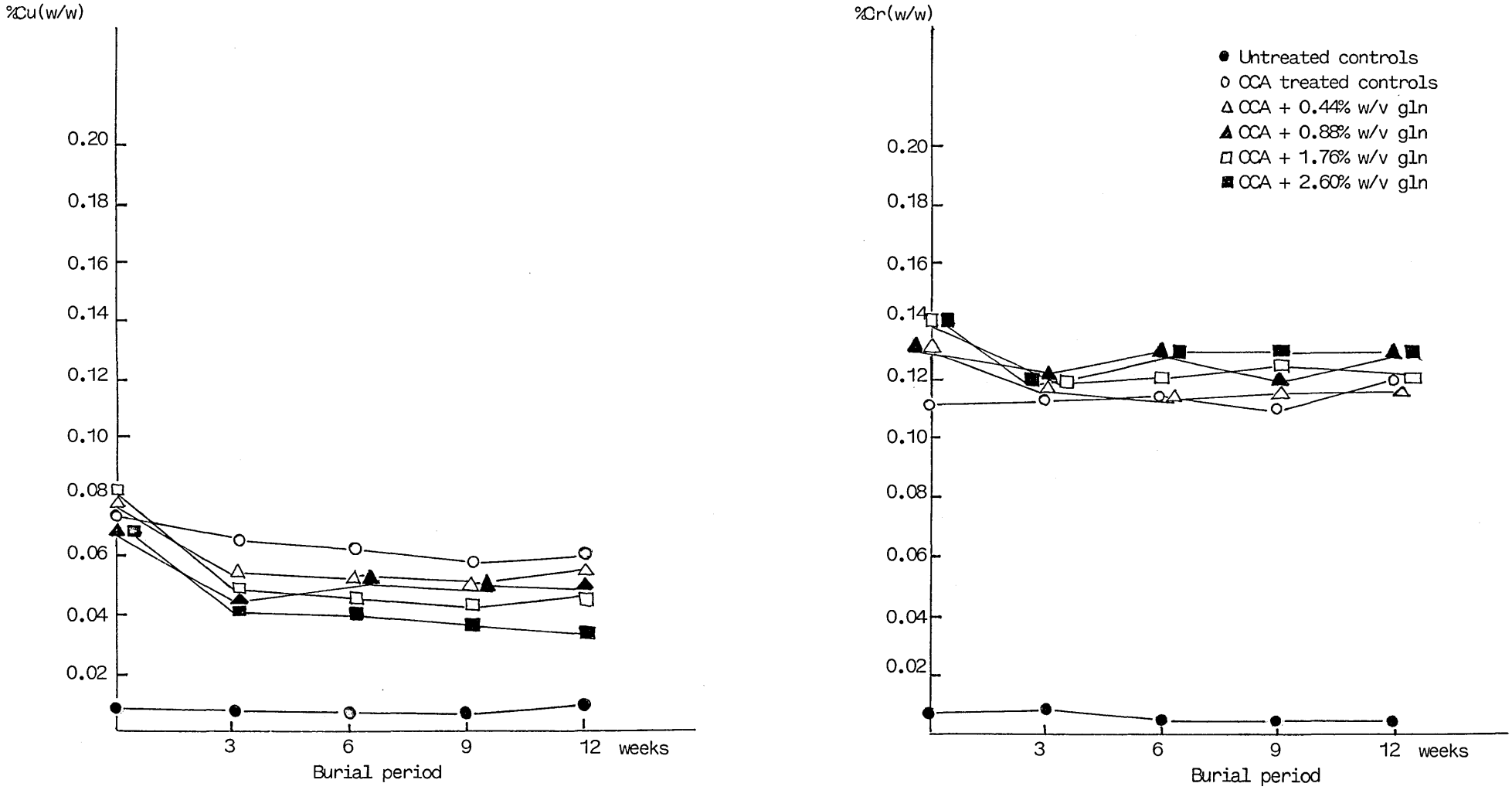


Fig 3.59 %Copper and %Chromium in CCA treated lime impregnated with glutamine, after burial in soil for periods up to twelve weeks.

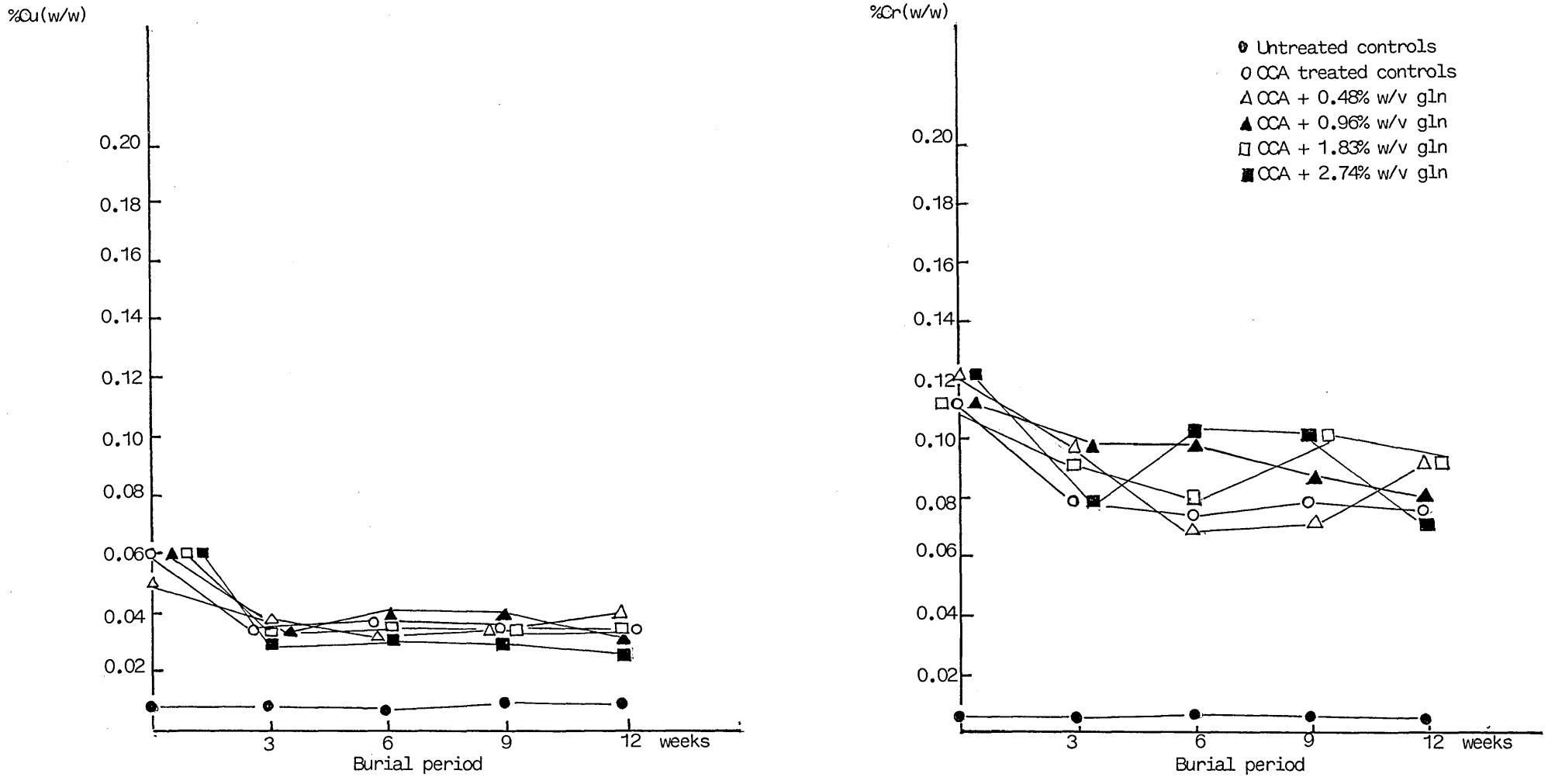


Fig 3.60 %Copper and %Chromium in CCA treated beech impregnated with glutamine, after burial in soil for periods up to twelve weeks.

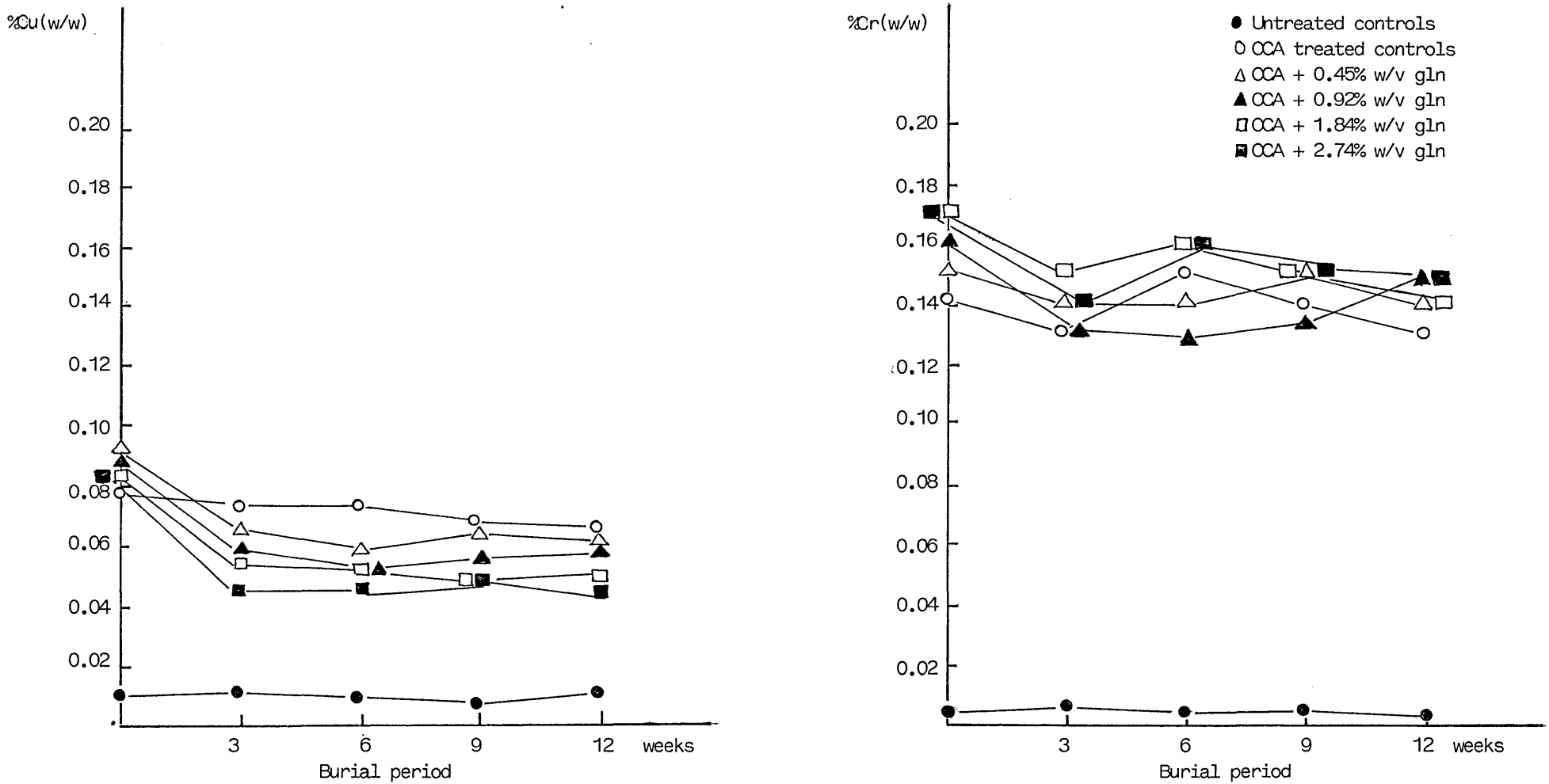
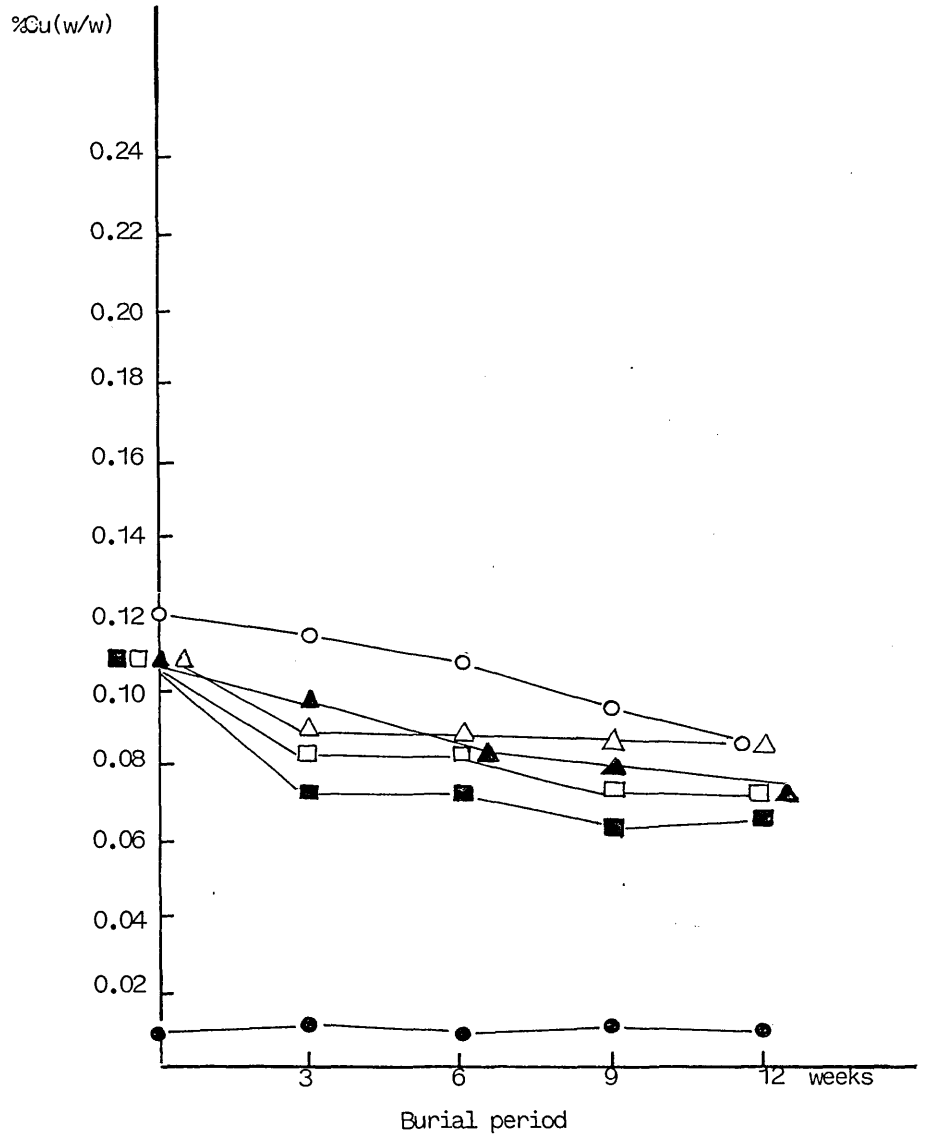


Fig 3.61 %Copper and %Chromium in CAA treated pine impregnated with glutamine, after burial in soil for periods up to twelve weeks.



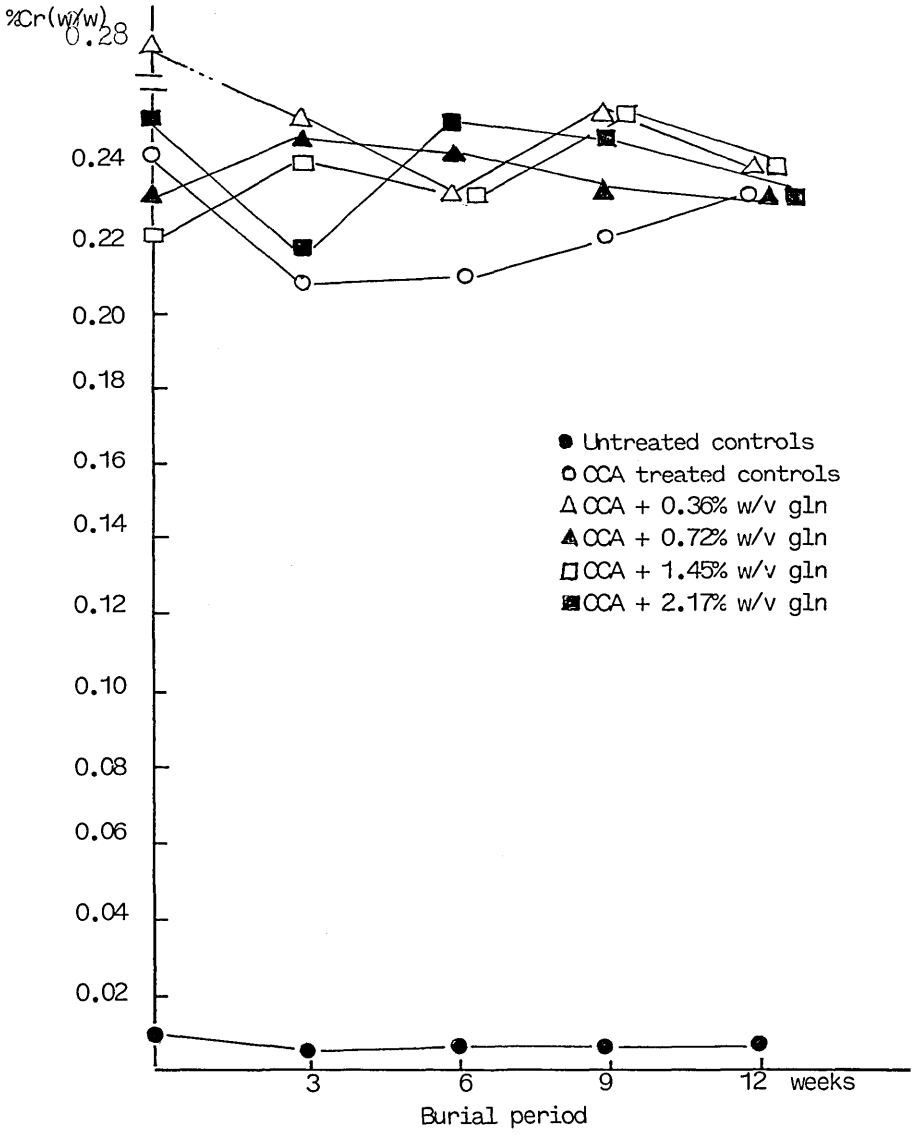


Fig 3.62 %Copper and .Chromium in CCA heated spruce impregnated with glutamine, after burial in soil for periods up to twelve weeks.

Table 3.22 Comparison of metal contents in blocks and as salts in the treating solution.

Wood Type	Copper		Chromium		Cu:Cr	
	% w/w in block	% w/w as salt in treating solutions	% w/w in blocks	%w/w as salt in treating solutions	in block	salt
Lime	0.08	0.09	0.12	0.16	0.57	0.56
Beech	0.06		0.11		0.54	
Pine	0.08		0.16		0.51	
Spruce	0.11		0.24		0.45	

Table 3.23 Selective adsorption ratios in CCA treated lime, beech, pine and spruce at varying glutamine concentrations prior to soil burial.

Wood	Treatment	Selective adsorption ratios	
		Cu	Cr
Lime	CCA controls	1.20	1.30
	CCA + 0.44% w/v gln	1.30	1.40
	CCA + 0.88½ w/v gln	1.20	1.40
	CCA + 1.76% w/v gln	1.30	1.50
	CCA + 2.60% w/v gln	1.20	1.50
Beech	CCA controls	1.50	1.60
	CCA + 0.48% w/v gln	1.30	1.70
	CCA + 0.96% w/v gln	1.50	1.60
	CCA + 1.83% w/c gln	1.50	1.60
	CCA + 2.74% w/v gln	1.50	1.70
Pine	CCA controls	1.10	1.20
	CCA + 0.45% w/v gln	1.20	1.25
	CCA + 0.92% w/v gln	1.20	1.33
	CCA + 1.83% w/v gln	1.10	1.33
	CCA + 2.74% w/v gln	1.10	1.23
Spruce	CCA controls	1.00	1.14
	CCA + 0.36% w/v gln	0.92	1.40
	CCA + 0.72% w/v gln	0.92	1.10
	CCA + 1.45% w/v gln	0.92	1.10
	CCA + 2.17% w/v gln	0.92	1.20

In lime, beech and pine, preserved blocks of varying glutamine treatments displayed broadly similar selective adsorption ratios. However, in spruce, preserved blocks impregnated with glutamine displayed lower selective adsorption ratios to preserved control blocks. In general, hardwoods displayed higher selective adsorption ratios of both copper and chromium than softwoods.

The rates at which copper was lost to the soil, were more rapid in preserved glutamine blocks than in preserved control blocks during the early stages of soil burial. Rates at which copper losses occurred diminished over the remaining sampling intervals. In general, blocks impregnated with higher glutamine concentrations displayed larger copper losses. This pattern was clearly observed in lime, pine and spruce. In beech, copper contents in preserved blocks both with and without glutamine additions, were broadly similar at each sampling period.

The chromium contents recorded in preserved blocks of lime, beech, pine and spruce (Figures 3.59 - 3.62), were higher than the copper contents recorded in these woods at similar sampling periods. Unlike copper which showed greater losses in blocks at higher glutamine concentrations, losses of chromium were broadly similar in blocks of varying glutamine concentrations. During the soil burial, the chromium contents were seen to increase in some preserved blocks. These increases resulted from the variations in the liquid uptakes of the preservative solution in the blocks.

The losses of copper and chromium in preserved blocks of lime, beech, pine and spruce after twelve weeks soil burial are presented in Table 3.24.

Significant losses in copper were seen in the four wood species after twelve weeks burial in soil. Largest losses of copper were seen in beech and smallest in spruce. The copper losses recorded in lime and pine were broadly similar, with preserved controls in these wood displaying copper losses of 17%, and preserved blocks at the highest glutamine concentrations, displaying copper losses of 47%.

Chromium losses from preserved blocks of the four woods tested were less than those observed for copper. Significant losses were observed in beech but in lime, pine and spruce, losses in chromium in these woods after twelve weeks were 20%. In beech, chromium losses up to 42% were observed after a twelve week soil burial.

Table 3.24 Copper and chromium losses recorded in preservative treated lime, beech, pine and spruce blocks of varying glutamine concentrations after 12 weeks soil burial

Wood	Treatment	Cu (%)	Cr (%)
Lime	CCA controls	17.80	0
	CCA + 0.44% w/v gln	28.20	10.76
	CCA + 0.88% w/v gln	25.71	0
	CCA + 1.76% w/v gln	42.50	14.28
	CCA + 2.60% w/v gln	47.14	7.14
Beech	CCA controls	46.60	37.72
	CCA + 0.48% w/v gln	26.00	25.00
	CCA + 0.96% w/v gln	50.00	29.10
	CCA + 1.83% w/v gln	50.00	18.18
	CCA + 2.72% w/v gln	60.00	42.00
Pine	CCA controls	17.10	7.10
	CCA + 0.45% w/v gln	31.75	6.66
	CCA + 0.92% w/v gln	33.75	6.25
	CCA + 1.83% w/v gln	43.70	17.64
	CCA + 2.74% w/v gln	47.50	11.76
Spruce	CCA controls	29.16	4.16
	CCA + 0.36% w/v gln	22.72	11.11
	CCA + 0.72% w/v gln	21.22	0
	CCA + 1.45% w/v gln	33.63	7.27
	CCA + 2.17% w/v gln	39.09	8.00

Statistical analysis of comparison of the mean copper contents in preserved controls and preserved blocks impregnated with glutamine in lime, beech, pine and spruce are presented in Table 3.25.

Results of 1-ANOVA showed that when copper contents were compared, significant differences existed (p=0.001) in blocks of varying glutamine treatments in lime, pine and spruce but not in beech (Column II). At each treatment level, all blocks showed significant difference (p=0.001) in copper contents over the burial period (Column III). Highly significant differences existed in the copper contents for controls and blocks with glutamine, over the burial period in all the wood species tested (Column IV, Interaction).

Statistical analysis to determine the differences in chromium content between preserved control blocks and preserved blocks impregnated with glutamine, was considered unnecessary as results showed little difference between treatments.

Table 3.25 1-ANOVA and 2-ANOVA comparisons of copper contents to detect the significance of losses occuring during soil burial and the effect of added amino acids on rates of loss.

Wood Type	I	II	III	IV
Lime	CCA controls	xxx	xxx	xxx
	CCA + 0.44% w/v gln	xxx	xxx	xxx
	CCA + 0.88% w/v gln	xxx	xxx	xxx
	CCA + 1.76% w/v gln	xxx	xxx	xxx
	CCA + 2.60% w/v gln	xxx	xxx	xxx
Beech	CCA controls	NS	xxx	x
	CCA + 0.48% w/v gln	NS	xxx	x
	CCA + 0.96% w/v gln	NS	xxx	x
	CCA + 1.83% w/v gln	NS	xxx	x
	CCA + 2.74% w/v gln	NS	xxx	x
Pine	CCA controls	xxx	xxx	xxx
	CCA + 0.48% w/v gln	xxx	xxx	xxx
	CCA + 0.92% w/v gln	xxx	xxx	xxx
	CCA + 1.84% w/v gln	xxx	xxx	xxx
	CCA + 2.74% w/v gln	xxx	xxx	xxx
Spruce	CCA controls	xxx	xxx	x
	CCA + 0.36% w/v gln	xxx	xxx	x
	CCA + 0.72% w/v gln	xxx	xxx	x
	CCA + 1.45% w/v gln	xxx	xxx	x
	CCA + 2.17% w/v gln	xxx	xxx	x

xxx, x and NS represent probabilities of $<0.1\%$. 5% and $>5\%$ respectively, that differences arising from the comparisons of each column could arise by chance.

Column I Treatment (% w/v glutamine)

Column II 1-ANOVA to determine whether there is any significant change in copper contents for the different treatment employed.

Column III 1-ANOVA to determine whether there is a significant difference in copper contents within each treatment over the soil burial period.

Column IV 2-ANOVA to determine any significant different between copper contents for controls and blocks impregnated with glutamine over the burial period.

Conclusions

The major conclusions derived from these preservative studies are:

(i) amino acids influence decay susceptibility and preservative efficacy in lime, but not to a significant extent in beech and not at all in pine and spruce

(ii) the amino acids arginine and glutamine, both displayed similar effects on preserved lime blocks; specificity of the amino acid was not a pre-requisite in influencing mass loss and preservative performance in lime

(iii) amino acids incorporated into preserved blocks showed losses during the initial periods of the soil burial. Losses of amino acids were greater in softwoods and this may reflect the fixation mechanisms in these woods. In the hardwoods, although losses were also observed, some retention of the amino acids occurred, which accounted for preserved glutamine blocks and unpreserved blocks displaying broadly similar nitrogen contents

(iv) in unpreserved hardwoods and softwoods, nitrogen contents increased as weight loss increased. However, a level of nitrogen of approximately 0.20% was necessary before significant weight loss occurred. Also, in both hardwoods and softwoods (regardless of treatment), a good correlation existed between weight loss and nitrogen content over the duration of the experiment

(v) elemental analysis showed that chromium was preferentially adsorbed to wood than copper. Copper losses in preserved blocks increased with increasing glutamine concentrations, but chromium losses were not influenced by amino acid concentrations. Blocks at high glutamine concentrations showed efflorescence prior to burial, which suggests that chelation of copper with the amino acid may have occurred. Copper losses (as were amino acid losses) were significantly larger in these blocks than in control blocks

(vi) sugars incorporated into preserved blocks did not influence decay or preservative performance in the hardwood and softwood tested.

CHAPTER 4

DISCUSSION

4 Discussion

The research for this project was undertaken to determine the nature and identity of the soluble carbohydrate and soluble nitrogenous components which migrate and accumulate at evaporative surfaces of dried wood; the distribution of these components on an annual ring basis in green and dried wood; and the role of the individual soluble nutrient components on decay susceptibility and preservative stability.

4.I. Studies on the soluble nutrient components in wood.

Four experiments were undertaken to determine the composition of the soluble nutrients in wood. Experiments I, 2 and 3 of this study were undertaken to determine the distribution of soluble nutrients in green and dried wood. Experiment 4 of this study was undertaken to determine qualitatively and quantitatively the soluble sugars and soluble amino acids that redistribute and accummulate at surface regions of such materials.

Experiment I was a preliminary experiment in which wood samples both green and dried, were milled in a Wiley micro-hammermill prior to extraction. As green wood is difficult to mill, samples in Experiment 2 were chipped to provide small specimens of large surface area. Preliminary work at this laboratory has shown that lower concentrations of soluble carbohydrate were obtained when samples were extracted in block form (Button and McFarlane, pers. comm.). These workers also found that the amount of carbohydrate solubilised from milled wood, was about I0 fold greater than that obtained from wood blocks, when extractions were carried out in water for 30 minutes at 37°C. The 30 minute period was chosen as it was shown that the rate of release of carbohydrate from milled wood decreased markedly after this period. The samples used in Experiments 2 and 3 were divided into 5 and I0 ring groupings. Spruce samples were divided into I0 ring groupings as the annual rings in this wood were situated closer together; and pine samples were divided into 5 ring groupings as annual rings in this wood were positioned further apart. A representative sample of green spruce and pine which had been chipped, was also dried and milled. This provided samples for which the dry weight was known, and the results of which, could be compared directly with samples from surface and sub-surface regions of dried wood. Green samples of lime and kempas were not available and the distribution of soluble nutrients in these woods was not investigated.

A number of extractions were undertaken to determine the soluble nutrients in wood. Aqueous extractions were undertaken in Experiment I, 2 and 3. Aqueous extractions were chosen to remove nutrients that are soluble in water, and which are easily translocated and therefore, readily available to primary colonisers. In the aqueous extractions, the wood to water ratio used was I:I5. This ratio was chosen after preliminary experiments showed that this wood:water ratio gave rapid and reproducible results and was therefore adopted for later experiments.

Experiment 4 involved several sub-experiments in which samples of dried wood were subjected to extraction procedures with different solvents. The procedures adopted for this series of experiments were as those specified in TAPPI standards, T264-om-82 for the preparation of wood for chemical analysis, and T207-om-8I for the water solubility of wood and pulp. In the first sub-experiment, surface samples of dried wood were subjected to successive extractions in 70% aqueous ethanol and hot water. Extractives that are soluble in alcohol include resins, fatty acids and their esters, waxes, tannins, pigments and some phenolic compounds. Materials such as tannins, which are dissolved by both ethanol and water, are removed by the first solvent. Carbohydrates are more soluble in water than in organic solvents. For example, at 20°C, the solubility of glucose in water is Ig/ml, but the solubility of glucose in ethanol, is Ig in 60 mls (Merk Index). The aqueous ethanol used in the extraction has a relatively high polarity, and although it may not be the best solvent for carbohydrates, it is likely to be of sufficient polarity to release mono- or oligosaccharides from wood samples. Extractions in aqueous ethanol were undertaken to provide comparative data with those of Baker, Laidlaw and Smith (I970) and Long (I978). Baker et. al. (I970 op.cit.) extracted wood and frass samples of Anobium punctatum in ethanol to determine the free sugar composition in these samples. Long (I978 op. cit.) in his preliminary experiment on the redistribution of simple sugars during drying of wood, also employed procedures similar to those of Baker et. al.

The results of the first sub-experiment showed that extraction in alcohol removed substantial amounts of soluble material. Weight losses of I2%, 6%, 5% and 4% were recorded for lime, pine, spruce and kempas respectively. Less extractable material was released from the alcohol-extracted wood when it was subjected to the second extraction in hot water.

Most of the weight loss in the milled samples was accounted for in alcohol extract. The larger weight loss displayed by lime is surprising, as hardwoods are known to contain smaller amounts of fats and resins than softwoods.

In the second sub-experiment, surface samples were subjected to a single extraction in cold water. A larger proportion of soluble material was extracted from pine (16%) than from any of the other wood species tested. Spruce and lime displayed similar weight losses to each other (10%) when extracted in cold water. In both sub-experiments, kempas consistently displayed smaller weight losses in comparison to the other woods. It is inferred from these results that kempas has a lower concentration of extractable material.

The extracts from both sub-experiments were dried and redissolved in water. The amount of water soluble material recovered from the dried extracts varied with the type of extraction employed. Recoveries of water soluble fractions of alcohol extracts were low in comparison to those from cold water. In lime, only 50% of the material recovered from the dried alcohol extracts was water soluble. In spruce and pine, the recoveries were 60%, and in kempas 36%. These low recoveries indicate the proportion of polymeric material that is extracted in alcohol which is insoluble in water. Also, prior to solubilisation in water, the dried extracts were weighed in the flasks in which the extractions in alcohol had been previously undertaken. In practice, it was necessary to dry the alcohol extracts in a relatively large flask. The large difference in the weight of the flask and that of the dried residue, is likely to be a source of random error which would therefore limit reliability in the calculation of recoveries.

When alcohol extracted samples were subjected to a second extraction in hot water, and the resulting material freeze dried, it was found that 50% of the freeze dried material was readily soluble in water. These results may indicate the presence of starches which are known to be removed by hot water and are insoluble in cold water. Water soluble material obtained from the cold water extracts of the second sub-experiment, gave higher recoveries than those of the alcohol and hot water extractions. Recoveries of 80% were obtained with each of the wood species tested.

As the results from the sub-experiments showed that the combined extractions in alcohol and hot water removed as much material as that from a single extraction in cold water, the latter method was adopted for sub-surface samples.

A single cold water extraction was chosen as the interest was mainly on soluble components that are readily available to wood colonising organisms. The extraction procedure also has the advantage that it is rapid and easily manageable.

Extractions in cold water of sub-surface samples of spruce, pine and lime produced broadly similar weight losses; in lime these were 7%, and in spruce and pine 5%. Cold water soluble material extracted from surface regions of spruce, pine and lime were 2-3 times those from sub-surface regions. It is apparent from the results that less soluble material is extracted from sub-surface regions, and this corroborates the views of King, Oxley and Long (1974), who showed that redistribution and accumulation of soluble nutrients at wood surfaces occurred during drying.

In experiments undertaken in Section 3.I., all extracts were filtered through membrane filters prior to analyses; therefore, removing any need for clarification by methods used by Laidlaw and Smith (1965) and Long (1978). These authors employed cadmium salts and ion-exchange resins to remove interferring non-sugar material prior to chromatographic separation. As the pore size of the membrane filter was 0.45 μm, most interferring material would have been removed during filtration. Wood extracts were shown to be clear and free from wood debris after filtration. The method used in this study has the advantage that determinations of both sugar and amino acids can be made from the same extracts.

In the analyses of the extracts, group specific assays were employed in the determination of the total carbohydrate content, the reducing sugar content, the amino acid content and the soluble protein content. The composition of the soluble nutrient components were determined using specific enzymatic assays or analytical equipment such as HPLC and the amino acid autoanalyser. Both these items of equipment are capable of resolving a complex mixture into individual components without the need of derivatization.

The results presented in Experiments I, 2 and 3 showed radial distribution patterns of soluble carbohydrates and soluble nitrogenous components in both green and dried samples of spruce and pine. These experiments also showed the migration and accumulation of soluble carbohydrates, reducing sugars, glucose and fructose, soluble protein and amino acids at surface regions of dried wood.

In general, concentrations of the soluble nutrients were highest in the outer sapwood regions but decreased in concentration in the inner sapwood and heartwood regions. This radial distribution of soluble nutrients might be anticipated, since the sapwood region is the physiologically active region of the tree. Radial distribution patterns of soluble carbohydrate and soluble nitrogenous components in surface samples of dried spruce and pine (Experiment 3) displayed steeper concentration gradients than those of sub-surface samples. Concentrations of soluble nutrients in sub-surface samples were relatively low. The gentler gradient observed in radial distribution of soluble materials in these samples are accounted for by smaller differences in nutrient concentrations between the sapwood and heartwood regions.

Interestingly in pine, the total carbohydrate content increased in the heartwood region. This result is in contrast to the general trends observed, where the reducing sugar content, the combined glucose and fructose concentrations, and the soluble amino acid concentrations decreased with increasing distance from the cambium. The increases in total carbohydrate content in pine were observed in the latter ring groupings, ie.ring groups 31-35, 36-40, 41-45 and 46-50. It is postulated that the increases seen in this region are likely to be due to polymeric material reacting with the phenol-sulphuric assay.

The total carbohydrate content recorded for green spruce in Experiment I was three times the concentration recorded for green samples in Experiment 2. However, these two experiments are not directly comparable as ring groups from which the samples were obtained differed, as did the method of preparation of wood prior to extraction. Green pine samples which had been chipped (Experiment 2) displayed higher concentration of soluble carbohydrates than green spruce samples which had been prepared in a similar manner. The total carbohydrate content in green pine was twice that of green spruce. It is deduced from these results that of the two softwood species tested, pine showed greater quantities of soluble carbohydrates.

In both spruce and pine, the reducing sugar content accounted for a significant proportion of the total carbohydrate content of green wood. Marked differences in concentration of total carbohydrate content and reducing sugar content were observed in the outer sapwood region of green spruce, but in the inner sapwood and heartwood regions, concentrations of these materials were broadly similar.

In green wood, reducing sugars constituted 80% of the total carbohydrate content in spruce and pine. Glucose and fructose were the predominant sugars in all ring groupings, and the combined concentration of these sugars constituted 90% of the total reducing sugar content in spruce, and 80% in pine.

Paper chromatography was used initially to determine qualitatively the wood sugars in the extracts. Sugar standards of glucose, fructose, sucrose, xylose, mannose and arabinose were run alongside extracts from the different woods tested. The chromatograms were developed in a descending manner in a solvent of butanol:pyridine:water (6:4:3). The intensities of the spots which appeared in the chromatograms were related to the concentration of the sugars present. Glucose and fructose were shown in the chromatograms to be the major sugars present in the extracts. Sucrose was detected in the chromatograms, as well as traces of a pentose, that was tentatively identified as xylose. A variety of oligosaccharides were also identified in the chromatograms, which had lower mobilities than the monosaccharides, as a result of their larger molecular size. Concentrations of glucose and fructose which were identified as major sugar components in the chromatograms, were quantified using the enzymatic assay. In the later analysis (Experiment 4), separation and quantification of wood sugars were achieved using HPLC.

Samples of spruce and pine that had been chipped, dried and milled displayed higher concentration of soluble carbohydrates than those of green chips. The results of green chips which had been dried and milled are not directly comparable with those of undried chips, as moisture contents of the green chips were not measured. Corrections for moisture in green samples were made at a later date using empirical data available at this laboratory to provide a theoretical comparison. Samples which had been chipped, dried and milled therefore provided samples which allowed the status of green wood to be assessed. The use of milled wood would probably account for the higher concentrations of soluble carbohydrates obtained from the dried chips, as the surface area to volume ratio is considerably higher than that of the green chips. The effect of drying on wood structure is difficult to assess but it would seem that drying does not destroy carbohydrates, at least, at the temperature used.

The results of investigations into the distribution of soluble carbohydrates at surface and sub-surface regions of dried wood also showed that concentration of soluble carbohydrate were higher at the evaporative surfaces.

In spruce, concentration of soluble carbohydrates at surface regions was five times that at sub-surface regions; and in pine, soluble carbohydrate concentration at surface regions was four times that at sub-surface regions. Comparison of the ratio of the concentrations at surface and sub-surface regions showed them to be different in each ring grouping. In general, the ratios of soluble carbohydrates in surface samples to soluble carbohydrate in sub-surface samples decreased with increasing distance from the cambium. In the heartwood regions of surface and sub-surface samples, these ratios were broadly similar. The results have also shown that the concentration of soluble carbohydrates at evaporative surfaces was three times those of the dried chipped samples of spruce, and twice those of similar samples of pine. Reducing sugars constituted a significant proportion of the total carbohydrate content at both surface and sub-surface regions.

The migration and accumulation of carbohydrates to evaporative surfaces was clearly demonstrated in Experiment 4. Soluble carbohydrates accounted for I-5% of the dried wood mass at surface regions of spruce, pine, lime and kempas. The concentration of these materials at surface regions of pine was five times those at sub-surface regions. In spruce and lime, soluble carbohydrate concentrations at surface regions were twice those at sub-surface regions. In the surface samples of the four wood species tested, pine displayed the highest concentration (5% w/w) of soluble carbohydrate. This corroborates the results obtained in the distribution studies in which pine displayed higher concentration of soluble carbohydrates than spruce, in both green and dried wood.

In general, soluble carbohydrate concentrations in alcohol and cold water samples constituted between 25%-50% of the water soluble material recovered from the dried extracts. It is deduced from this finding that a significant proportion of the material extracted from surface samples is non-carbohydrate in nature. Besides the sugars, water soluble materials include inorganic salts, tannins, cycloses and cyclitols, some polysaccharides and nitrogenous materials including the amino acids. The latter has been shown to constitute only 0.3% of the mass of wood. As the aim of this project was centered primarily on the soluble carbohydrate and soluble amino acids present at surface regions of wood, investigations into the other water soluble components were not undertaken, but this remains an interesting area for further study.

The separation and identification of soluble sugars contained in the extracts was achieved by HPLC in Experiment 4. The equipment became available in the department during this period of the project. It was found to be the preferred method of analysis to the colorimetric assays used in Experiments I-3 in this study for several reasons. All monosaccharides and a range of oligosaccharides would be completely separated under isocratic conditions and without the need of derivatization. The operation is automated and samples could be analysed and quantified within 20 minutes.

The main mode of separation of the wood sugars in the extracts in the HPLC column is by ligand exchange (Goulding, 1975). In this mode of separation, ligands are partitioned by virtue of their complexing strength for a metal ion sorbed on the exchange resin. The lead ion in the cation exchange resin used acts as a counter ion, and separations are undertaken with degassed deionised water. When the eluant (deionised water) is passed through the column, a hydration sheath is formed round the exchange site of the lead counter ion. When the sample is introduced into the column, this hydration sheath is displaced by the polar OH^- groups in the sugar molecules. The complexing strength of the sugars at the exchange sites is dependant on the stereochemical arrangement of the sugar molecule; the greater the affinity of the sugar for the column, the higher the retention time. In the analyses, the presence of a small peak at 5 mins in each of the extracts, may be accounted for by the presence of oligosaccharides in the extract, which would be eluted first through the column.

It has been shown that reducing sugars contribute to a significant proportion of the total carbohydrate content at the surface and subsurface regions of spruce, pine and lime. Glucose and fructose were the principal sugars in the extracts of the softwoods. Other sugars present in these woods were xylose, mannose, galactose and trace quantities of arabinose. Extracts of lime differed from that of softwoods, in that sucrose was the predominant sugar and accounted for 50% of the total carbohydrate content. Glucose and fructose however, accounted for only 50% of the total reducing sugar content in this wood. The assay used to detect reducing sugars in the extracts is a group specific assay, and all compounds capable of reducing the dinitrosalicyclic reagent will be quantified by this assay. It is thus postulated that the remaining 50% of the reducing material in the extracts of lime may be attributed to components with carbonyl groups.

Soluble carbohydrates in kempas only constituted I% of the wood mass. Neither the reducing sugars nor sucrose were detected in this wood. To further investigate the composition of soluble sugars in tropical woods, keruing (Dipterocarpus spp.) and a eucalypt (Eucalyptus regnans, Muell) were subjected to cold aqueous extractions and analysed for their wood sugar composition by HPLC. As with kempas, low molecular weight sugars were not detected in these woods. It was considered that if these low molecular weight sugars were present, then they must be present in amounts below the sensitivity of the detection system. It was postulated that since the tropical woods tested represented randomised samples from different geographical locations, it is probable that the concentration of soluble carbohydrates in these woods are lower than those of the temperate woods.

Like the soluble carbohydrates, soluble amino acids also migrate and accumulate at evaporative surfaces of wood during drying. Specific amino acids were shown to migrate to surface regions during the drying process. The principal amino acids detected in the extracts of green wood, were also the principal amino acids detected in extracts of surface samples of dried wood. Concentrations of amino acids in the latter samples were higher than those in the undried wood.

Amino acids constituted a small proportion of the soluble nutrients in the extracts of green spruce and pine (Experiment 2). Concentrations of these soluble amino acids were 0.05% w/w in pine, and 0.03% w/w in spruce. Radial distribution patterns of soluble amino acids were observed in both these woods. In general, concentrations of amino acids decreased from the sapwood to the heartwood regions. However in pine, increases in concentration of amino acids were observed in ring groups 2I-25 and ring groups 26-30. The increase in concentration observed at this sapwood-heartwood boundary, may be attributed to a change from the physiologically active cells in the sapwood region, to the inactive cells in the heartwood region.

Besides the amino acids, the concentration of soluble proteins were also investigated in experiments undertaken in Section 3.I. In the preliminary experiment, analysis of soluble protein in aqueous extracts of green spruce were undertaken using a dye binding micro-assay. Results of these analyses showed that soluble protein constitutes no more than 0.03% of the mass of wood.

In Experiment 2, acid hydrolysis of extracts from outer sapwood regions of green spruce and pine were undertaken to determine the soluble protein concentration in these regions. Proteins consist of long polypeptide chains, with a large number of amino acid units joined by peptide linkages. If significant amounts of soluble protein are present in the extracts, acid hydrolysis will yield a notably greater concentration of amino acids. In the hydrolysed extracts of green spruce, amino acid concentrations were one and a half times those of the unhydrolysed extracts. As increases in amino acid concentrations in the hydrolysates are attributed to the presence of soluble protein, it is clear that in spruce, soluble protein constitutes a small proportion of the soluble nutrients.

In pine, concentrations of amino acids in the hydrolysates were lower than those of the unhydrolysed extracts. The presence of high concentrations of soluble carbohydrates can lead to loss of amino acids during acid hydrolysis (Ambler, 1981). The results of the soluble carbohydrate analyses showed that concentrations of soluble carbohydrates in pine were higher than those of spruce. Therefore, it is possible that losses of amino acids occurred during hydrolysis, and this may explain the lower concentrations of amino acids in the hydrolysates of pine.

The hydrolysates of spruce and pine displayed a similar composition of amino acids. Threonine, serine, glutamic acid and proline were detected in the hydrolysates, but were not detected in the unhydrolysed extracts. Glutamine in the extracts was on hydrolysis, deaminated to its corresponding acid, glutamic acid. The presence of serine, threonine and tyrosine in the hydrolysates suggests that peptide (or peptides) was originally present in the extracts. With the exceptions of serine, threonine and tryptophan, most of the other protein amino acids detected in the extracts are stable to the conditions of hydrolysis used in the experiment. The increases in glycine, alanine, valine, isoleucine and leucine recorded after hydrolysis are presumably due to the presence of peptides. As soluble protein was not a principal concern of this study, only two replicate hydrolysis were undertaken for each sample. This preliminary experiment will need to be extended, to obtain a more complete assessment of the soluble protein concentration in extracts of spruce and pine.

The results of the amino acid analyses showed that concentrations of soluble amino acids were significantly higher at surface regions than at sub-surface regions of dried wood.

It has also been shown that soluble amino acids could constitute up to 0.3% of the mass of wood at surface regions of dried spruce, and to 0.25% at similar regions in dried pine (Experiment 4). In both these woods, soluble amino acid nitrogen accounted for a significant proportion of the total nitrogen content at the surfaces. It is postulated that in softwoods, a large proportion of the soluble nitrogenous components that migrate and accumulate at wood surfaces are amino acids.

In lime, soluble amino acids did not form the bulk of the nitrogenous components that migrate to evaporative surfaces during drying. In this wood species, soluble amino acids constituted less than 0.25% of the mass of wood at surface regions. The soluble amino acid nitrogen at these surfaces accounted for only 6% of the total nitrogen seen at these regions. It might appear that hardwoods contain much lower concentrations of amino acids than softwoods. However, as lime was the only temperate hardwood to be studied in detail, further work on other representative hardwoods of this class will be required to substantiate this suggestion.

The results of the analyses undertaken in Experiment 4, showed that soluble amino acids constituted a small proportion of the soluble nutrients in kempas. Concentrations of these soluble amino acids were 0.01% w/w in the alcohol extracts. In the cold water extracts, only trace amounts of amino acids were detected. It has been shown that a significant amount of the nitrogen in kempas is in a soluble form (King et al , 1981b). As soluble amino acids are present in such small amounts in kempas, the soluble nitrogen content must therefore be accounted for by other substances, such as alkaloids, which are present in larger amounts in tropical woods. Further work is merited in the investigation of soluble nitrogenous components in kempas.

Surface samples of spruce and pine which had been extracted in cold water, released larger quantities of soluble amino acids than samples which had been extracted in alcohol. The amino acids detected in cold water extracts of spruce and pine showed a similar composition to the amino acids detected in the alcohol extracts of these woods. The solubility of amino acids in aqueous solutions is dependant on the polarity of its side chain. Although amino acids have an α-carboxyl and an α-amino group, they differ from each other in their side chains, which vary in structure, size, electric charge and solubility in water.

The polarity of the group on the side chain can vary widely from totally non-polar or hydrophobic groups, to highly polar or hydrophilic groups. In both the alcohol and cold water extractions, the amino acid concentration in wood extracts were well below saturation level in either solvent. Although water is a more polar solvent than aqueous alcohol, it is unlikely that polarity can be used to account for the observed differences in extraction.

Tyrosine and histidine in the wood extracts showed unusual behaviour in that, they were detected in cold water extracts, but were not detected in alcohol extracts. These two amino acids together with phenylalanine are eluted in a region of the chromatogram, at a point when pH and temperature changes are effected. At this stage, peptides and other unknown substances can appear in the chromatogram. As such, there is some uncertainty in the quantitative values of the above amino acids, This is heightened during the course of amino acid analysis, as drifts in retention times, are more pronounced in the latter period of the analytical run. On the other hand, aspartic acid and arginine, appear at the acidic and basic ends of the chromatogram respectively, and therefore, a much greater degree of confidence is attached to their qualitative and quantitative estimations.

Amino acids detected in the extracts of lime and kempas, showed greater solubility in aqueous alcohol than in cold water, and this may indicate the ease of extraction of the wood species. In lime, concentrations of amino acids in the alcohol extracts were twice those of the cold water extracts. In kempas, only trace quantities of amino acids were detected in the cold water extracts. The number of amino acids detected in the hardwoods was less than that of the softwoods. Only five amino acids were detected in the extracts of lime. These were aspartic acid, glutamine, glycine, alanine and arginine. In the extracts of kempas, aspartic acid, serine, alanine and arginine were detected. Clearly, further work is required in the determinations of the composition of soluble amino acids in these hardwoods.

4.2 Soil burial studies

The studies of total nitrogen balances in wood in soils by King et. al. (1983) showed that soluble nutrients present at evaporative surfaces of hardwoods and softwoods, are important as determinants of decay by soft-rot fungi. The presence or absence of these soluble nutrients has been considered by Banerjee and Levy (1971) to be an important factor in determining the extent of colonisation by micro-organisms of wooden fence posts in soil contact. To investigate the role of these soluble nutrients on wood decay, a number of soil burial experiments were conducted to determine the influence of soluble sugars and soluble amino acids on wood decay and nitrogen transfer from soil to wood, and to determine the influence of these materials on the toxic limits of wood preservatives.

4.2.1. Soil burial studies using unpreserved wood.

Investigations into the soluble nutrient composition at surface regions of dried wood (3.1.4.), showed that glucose and fructose were the predominant simple sugars present at these regions, and aspartic acid, glutamine and arginine, the major soluble amino acids present at similar regions, in the three wood species tested. These nutrients were incorporated into wood blocks of low nutrient status as sole sources of additional carbohydrate (Experiment 1), or as sole sources of additional nitrogen (Experiment 2). A combination of soluble sugar and amino acids was also included in the investigation (Experiment 4). Low nutrient wood blocks were impregnated with these soluble nutrients, so as to replicate wood of high nutrient status, i.e. surface regions of dried wood.

The wood species chosen as test material in the soil burial studies were spruce, pine, lime and beech. Spruce, lime and pine were selected as soluble nutrients in these woods had been previously determined in experiments undertaken in Section 3.1.4. Kempas was not used in the soil burial studies, as data obtained from analyses showed concentrations of soluble nutrients to be low in this wood species. Instead beech was selected as the second representative hardwood in the soil burial studies. It is a wood of low nutrient status and along with spruce, pine and lime, has been frequently used in wood decomposition studies at this laboratory and elsewhere, and from which comparative data can be obtained.

In soil studies using unpreserved wood, blocks were impregnated with glucose and fructose solutions to obtain in these blocks concentrations of sugar representative of those found at surface regions of dried wood. In spruce, these were 2% w/w of the mass of wood, in pine 5% w/w and in lime 3% w/w. Concentrations of I% w/w and 7% w/w sugar were included to provide sugar concentrations above and below the levels found at surface regions. Beech was impregnated with sugar solutions to provide test blocks of similar concentrations to those of spruce, pine and lime. However, the uptake of sugar solutions at the higher concentrations was less due to the higher density of this wood. Hence, beech blocks had sugar concentrations of the following: I% w/w, 3% w/w, 4% w/w and 6% w/w.

Soil studies undertaken at this laboratory have shown that weight loss becomes significant only when nitrogen contents had increased to an estimated C:N ratio of 200:I i.e. approximately 0.2% w/w of the wood. Using this criteria, wood blocks in the amino acid study were impregnated with amino acid solutions of 0.45% w/v, 0.90% w/v, I.80% w/v and 3.60% w/v to obtain increases in nitrogen contents approximating to 0.2%, 0.3%, 0.5% and 0.9% respectively. Wood blocks were impregnated with amino acid solutions containing equimole quantities of aspartic acid, glutamine and arginine. Equimole concentrations of each amino acid was used as amino acids, unlike the monosaccharides, differ in molecular weight, and differ to each other in the number of nitrogen atoms in their molecular formula. Nitrogen contents of wood blocks were increased to a threshold value of 0.2% and above, to provide a range of concentration of soluble nitrogen, and to ascertain if these concentrations would stimulate rapid colonisation of wood by fungi, since soluble nitrogen is no longer a limiting factor.

The results of the carbohydrate study showed broad similarities to those of the amino acid study. In general, the added soluble nutrients showed little influence on decay in both hardwoods and softwoods. In each study, and for each wood type, the levels of decay as measured by weight loss, for the varying concentrations of sugar or amino acid were broadly similar. In each wood species, significant weight loss was observed over the duration of the soil burial period. The hardwoods displayed larger weight losses than the softwoods at each sampling period, and also at each similar sugar or amino acid concentration level. The weight losses demonstrated by the hardwoods and softwoods in these studies were in broad agreement with those obtained from other wood decomposition studies at this laboratory.

In the carbohydrate study, two methods of calculation were chosen for the evaluation of weight losses. The first method was based on the calculation normally used at this laboratory, in which weight losses were calculated as a percentage of the preburial dry weight of the block. A second method was required as it was necessary to encompass the weight increments in the block resulting from the uptake of sugar solutions. In this second method, weight losses were calculated as a percentage of the preburial dry weight of the block and the weight of sugar in the block, the weight of sugar in the blocks being probable values determined by the liquid uptake of the sugar solutions.

The two methods of calculation produced two different interpretations of weight loss. When weight losses were calculated as a percentage of the preburial weight of wood, control blocks displayed broadly similar weight losses to blocks impregnated with sugar. However, when weight losses were calculated as a percentage of the initial weight of the block and weight of sugar, a trend was observed in which test blocks with increasing sugar concentrations showed correspondingly higher weight losses than control blocks. The difference in weight loss between control blocks and blocks impregnated with sugar, corresponded to the weight increments in the block, as a result of the weight of sugar. Thus, this method of evaluation would magnify the weight losses recorded in the "sugar" blocks, and the larger weight losses observed by this method of evaluation, may not be related to decay taking place in the blocks. Weight losses calculated as a percentage of the preburial dry weight of the block, however, would give real values of weight loss and not artefacts, and the influence of sugars on wood decay can then be related to the presence or absence of soluble sugars.

In the amino acid study, weight losses of lime test blocks impregnated with amino acid concentrations of 3.60% w/v, were lower than those of the control blocks and blocks impregnated with lower concentrations of amino acids. The lower weight losses seen in these "amino acid" blocks may be a result of the higher amino acid concentrations inhibiting colonisation of wood by wood-inhabiting fungi. Also, drying procedures used after impregnation of the test blocks with amino acids, may have rendered the amino-nitrogen unavailable as a source of nitrogen. Amino acids are known to react with sugars at high temperatures and at high pH to form dark coloured products (Ellis, 1959). The optimum conditions for this reaction of the amino group of amino acids and the glycosidic center of the sugars, occurs at a fairly low water content, a pH of 7 to 10 and at a high temperature (60-100°C).

In the soil experiment with amino acids, blocks after impregnation with amino acid solutions of pH 4.2, were dried at a temperature of 40°C. An obvious conclusion from this is that the conditions used in the amino acid study were not the optimum conditions required for the reaction of amino acids and sugar. Also, blocks after drying did not show any colour changes. To ascertain that drying had no effect on the added amino acids in the wood blocks, a supplementary study was undertaken with control blocks and blocks impregnated with 3.60% w/v amino acid. Test blocks in this study were buried at 100% moisture content. The results of this supplementary study showed that weight losses displayed by the control blocks and "amino acid" blocks were broadly similar. Weight losses of these blocks were also comparable to the control and amino acid blocks of lower concentration in Experiment 2, which had been buried air-dry. It was thus postulated that drying at the temperatures used in Experiment 2, did not affect blocks which had been impregnated with amino acids.

The weight losses displayed by lime and beech in the carbohydrate study (Experiment 1), were lower than those of lime and beech in the amino acid study (Experiment 2). This difference may be attributed to the different rates at which weight loss occurred in these studies, and also to box to box variation in the two experiments. Control blocks in the carbohydrate study displayed an average rate of weight loss of 4.8% per week; and control blocks in the amino acid study displayed rates of weight loss of 5.5% per week. It is probable that minor variations in environmental factors such as moisture, may also influence these slight weight differences. Weight losses recorded by softwoods differed to those of hardwoods, in that, softwood blocks displayed similar weight losses in both the carbohydrate and amino acid study.

It was deduced from the weight losses that the added sugars had little influence on wood decay. This deduction was confirmed when results of the nitrogen analyses of wood blocks after burial showed that control blocks displayed broadly similar nitrogen contents to blocks impregnated with sugar. In each wood species, nitrogen increases were shown to occur in all wood blocks during the first sampling period. In the hardwoods, these nitrogen increases were accompanied by weight loss in the early stages of the soil burial (weeks 0-3). In the softwoods, increases in nitrogen contents of wood blocks were not accompanied by weight loss during this period. In the carbohydrate study, significant weight loss occurred when nitrogen contents in wood blocks reached a threshold value of 0.2%.

This nitrogen value is in close agreement with those of King, Mowe, Bruce and Smith (1983) and Mowe (1983) in their wood decomposition studies undertaken at this laboratory.

The increases in nitrogen contents observed during the earlier sampling periods have been suggested to be a result of microbial translocation from soil to wood (King et al , 1981a). Large increases in nitrogen contents only occurred in blocks undergoing decay. These considerable nitrogen increases in wood after exposure to soil are postulated by King to be a result of an active invasion process occurring in soil regions adjacent to wood interfaces. Interestingly, a decrease in nitrogen content was observed in test blocks in each wood species during the final sampling period in the carbohydrate study. This decrease in nitrogen content may be attributed to a transition from active invasion to colonisation of wood blocks, with all that entails, including the major events of the carbon and nitrogen cycles, nitrification, denitrification and nitrogen losses.

In contrast to the carbohydrate study, nitrogen contents of blocks impregnated with amino acids, decreased during the first sampling interval (weeks 0-3). In the hardwoods, the decrease in nitrogen content occurred only in blocks impregnated with higher amino acid concentrations; but in the softwoods, loss of added amino acids occurred in all test blocks impregnated with amino acids. In general, softwoods are more permeable than hardwoods (Wardrop and Davies 1961; Liese and Bauch, 1967; Greaves, 1974b), and this may explain the losses at all concentrations tested in the softwoods. The loss of amino acids from blocks especially at higher concentrations, suggests that some amino acids are retained in the blocks, possibly by complexing. The residual amino acid in these blocks, however, did not influence wood decay.

In beech, blocks impregnated with amino acids (3.60% w/v), showed a rapid increase in nitrogen content during weeks 3-6; the nitrogen contents of these blocks remaining relatively constant at 0.5% thereafter. The large increase in nitrogen contents were not accompanied by large weight losses. The increase in nitrogen contents observed during this period was attributed to the active microbial invasion process occurring in soil regions adjacent to the wood block.

Mixtures of sugar and amino acid incorporated into lime blocks (Experiment 4) did not show any influence on wood decay. Wood blocks containing these mixtures displayed similar weight losses to control blocks.

Similar weight losses and nitrogen contents were displayed by control blocks which had been buried at two different moisture contents i.e. wet (100% moisture content) and air-dry (10% moisture content). Blocks impregnated with the mixture showed a decrease in nitrogen content during the first sampling period. The overall weight loss and nitrogen contents recorded in this study were lower than those of the carbohydrate and amino acid studies. These lower weight losses may be attributed to the higher concentration of soluble nutrients available in the form of sucrose and arginine. The soil burial study using a mixture of amino acid and sugar was not a detailed investigation (only one concentration of the mixture was used). as earlier studies (Experiments I and 2), showed loss of added nutrients from test blocks on emplacement in soil. The loss of these added nutrients from the blocks (especially those at higher soluble sugar/amino acid concentrations), would create a region of high nutrient concentration in soil regions surrounding the block. In such cases, wood inhabiting fungi may for a time develop at the expense of the sugars and amino acids present, and only to a limited degree at the expense of the wood substance. This would explain the similar weight losses and nitrogen contents observed in these soil studies.

As losses of added soluble sugars and soluble amino acids from wood blocks were shown to occur, a short term soil burial study was undertaken to investigate how soon after emplacement of blocks in soil, the added nutrients were lost. The results showed that the majority of the added amino acids were leached from the blocks, twelve days after emplacement in soil. Blocks containing redistributed soluble nutrients were also included in this study. These blocks showed small increases in nitrogen contents which were accompanied by small weight losses. It would therefore appear that the complex of redistributed nutrients that accumulate at surfaces of dried wood has a greater degree of permanence. Control blocks and blocks with redistributed nutrients displayed similar nitrogen contents after twelve days burial. Weight losses of 2.8% were recorded in blocks with redistributed nutrients during this period, and weight loss of 1% were recorded in control blocks for the same period. It is inferred from this result that failure occurs at an earlier period in blocks with redistributed nutrients. Despite the higher nitrogen contents exhibited by blocks impregnated with arginine, no weight loss were recorded in these blocks. It is postulated that micro-organisms in soil regions adjacent to these blocks, would first utilise the leached nutrients, before utilising the wood substrate.

The investigations described in this section demonstrated the difficulty in trying to 'replicate' a wood surface region with a high soluble nutrient concentration. The results of these soil burial experiments failed to show conclusively, the influence of soluble sugars and soluble amino acids on wood decomposition. The loss of the added soluble nutrients from wood blocks after emplacement in soil, made it difficult to assess the effect of each of the added nutrients. Further work is necessary to determine accurate methods for evaluating the influence of soluble sugars and amino acids on wood decomposition.

4.2.2. Soil burial studies using preserved wood

Previous soil burial studies showed that unpreserved blocks impregnated with soluble amino acids displayed losses of amino acids to soil during burial. Consequently, the investigation with unpreserved wood blocks was unable to demonstrate the effect of soluble sugars and soluble amino acids on wood decomposition. In spite of this, investigations into the influence of added soluble nutrients to preserved wood were undertaken. These investigations were pursued for a number of reasons. The major decay agents of preserved wood in soil, soft-rot fungi and insects, require more nitrogen than is usually found in low nitrogen content woods to maximise wood decomposition. During drying of wood, soluble nitrogenous and carbohydrate material accummulate at evaporative surfaces,and these solubles not only enhance decay rates of both hardwoods and softwoods in soil due to soft-rot, but also reduce the toxic limits of preservative treated wood (King, Smith and Bruce, I980). It is hypothesised that complexes may be formed between added soluble nutrients and heavy metals which make them more stable and less leachable in wood. Earlier studies by Henningsson (I976), showed that microbial tolerance of CCA components in laboratory media and in wood was enhanced by the availability of nitrogenous materials. For these reasons, a further series of soil burial experiments were undertaken to investigate if the addition of soluble amino acids and carbohydrates influenced decomposition of preserved wood.

The results of the preliminary study showed that decay and preservative efficacy were influenced by amino acids. Wood blocks in the preliminary study were impregnated with arginine, and buried at I00% moisture content. In the second study, wood blocks were impregnated with glutamine to allow comparison with the arginine blocks in the preliminary study.

In addition, wood blocks impregnated with glutamine were also buried in an air-dry condition to allow comparison with previous soil studies undertaken with CCA treated wood at this laboratory.

Both arginine and glutamine influenced decay in preservative treated lime. The effect of these amino acids on weight loss was very pronounced. Increasing weight losses were observed with increasing soluble amino acid concentrations. Statistical analyses showed a higher significance in the differences in weight loss between varying amino acid treatments, in the glutamine study (p=0.00I) than in the arginine study (p=0.0I). This difference in the level of significance is a probable result of the fewer number of replicate blocks used, a smaller range of amino acid concentrations and the fewer sampling periods used in the arginine study.

In beech, the incorporation of glutamine into CCA treated blocks did not influence wood decay. Weight losses recorded in preserved control blocks and preserved blocks impregnated with glutamine were broadly similar over the duration of the soil burial. These results may be accounted for by beech being a denser wood with more wood substance and therefore, weight losses recorded in these woods appear smaller.

Unlike the hardwoods, weight losses were not observed in preserved softwood blocks over the duration of the soil burial. Softwoods were protected from soft-rot decay at sub-toxic levels of CCA. In their investigations, Butcher and Nilsson (I982) found a good correlation between soft-rot susceptibility of CCA treated wood and its lignin content. These authors suggested that if CCA complexes with lignin high lignin content wood such as the softwoods should be able to complex sufficient CCA to mask all T branch initiation sites, whereas a low lignin content wood like the hardwoods, could not complex enough CCA to permanently mask such sites. Variations in the macro- and micro-distribution of the preservative in wood and the low natural susceptibility to decay of softwoods (Hulme and Butcher, I977a) may also be factors contributing to CCA treated softwood being highly resistant to decay.

Preserved test blocks impregnated with amino acids also showed loss of amino acids to soil. Losses of amino acids were greater in the softwoods than in the hardwoods and in each wood species, largest loss of amino acids was recorded in test blocks impregnated with the higher amino acid concentration.

It is noted from the results that the effect of amino acids on preserved wood may not have been observed if the procedure of impregnation had been different. If amino acids had been incorporated into wood blocks prior to preservative treatment, then all of the amino acids may have been lost during the second impregnation. As amino acids were impregnated into wood blocks after preservative treatment, it was postulated that some complexes may have been formed between the amino acids and the heavy metals, thereby making these components more stable and less leachable in wood. This study has illustrated the difficulty in replicating natural situations in simplistic laboratory studies.

In both hardwoods and softwoods, nitrogen contents of unpreserved and preserved control blocks displayed increases in nitrogen input during soil burial. Increases in nitrogen contents of preserved control blocks occurred prior to any significant weight loss. This suggests that the preservative depresses decay but not nitrogen increases. The nitrogen increases observed in preserved softwood blocks were not accompanied by weight losses. It is postulated that these increases are a result of sacrificial colonisation by micro-organisms. The nature of these sacrificial invaders may need further investigation.

Rates of weight loss and nitrogen input to blocks in the glutamine study were closely correlated in all four wood types tested, whether untreated or treated with CCA. However, these rates of increase in nitrogen content of wood and weight loss from wood were dependant on the wood species and preservative presence. Untreated hardwoods demonstrated larger weight loss and higher nitrogen contents than untreated softwoods. Similarly, untreated wood blocks showed rates of weight loss and nitrogen input greater than CCA treated blocks of the same wood type. As weight losses and nitrogen contents were greater in the hardwoods than in the softwoods; this result confirms other information (Butcher and Nilsson, I982) on the susceptibility of hardwoods to decay.

It was noted that when preserved blocks impregnated with glutamine were left to dry in the laboratory at ambient temperatures, blocks impregnated with high glutamine retentions displayed efflorescence at the evaporative surfaces. Preservative treated wood blocks impregnated with lower concentrations of glutamine did not display this effect. The precipitates seen at the evaporative surfaces are indicative of a reaction of the amino acids with the unfixed soluble preservative; the blue specks observed in the precipitates being attributed to the copper in CCA, chelating with the amino acids.

It is known that most metal ions chelate readily with carboxylic and amino groups. These amino groups have been shown in studies described in 4.I., to concentrate in large amounts at surface regions during drying of wood. As the incorporation of glutamine into preserved wood blocks was undertaken after the blocks had been cured and fixed, the precipitates formed at the higher glutamine retentions, suggests that a significant proportion of the soluble preservative may remain unfixed. It has been demonstrated at this laboratory (Briscoe, I987), that CCA leaches to soil from treated wood. Elemental analyses of preservative treated test blocks after soil burial (Figures 3.59 to 3.62) in this thesis, showed greater loss of copper from blocks treated with amino acids than from controls, which indicates that some chelation with amino acids had taken place, and would thus account for the losses seen. Microbial transfer to wood from soil will, on senescence release protein and amino acids which may equally form complexes with heavy metals.

Copper and chromium analyses were undertaken using atomic absorption spectrophotometer. Generally, consistent results were obtained for copper and chromium analyses. Arsenic analyses were not undertaken due to technical problems encountered with the arsenic lamp. The results of arsenic analyses by other workers at this laboratory, has shown that arsenic determinations on atomic absorption spectrophotometers by direct analysis of the solution produced variability in results. This method has been employed in Dundee, as it was the only suitable method, since copper and chromium also had to be analysed within the same samples. Furthermore, as the main aim of this project was to investigate the influence of added soluble nutrients on decay in preserved wood in soil, arsenic analyses were not considered essential since this element is primarily a toxicant to insect attack.

Results of the copper analyses showed little variability between replicate wood blocks at each glutamine treatment level, although variability was shown with the chromium analyses. The few anomalies which occurred here were associated with slight variation in block size and associated variations in solution uptake by the wood blocks and selective adsorption of individual elements.

Analysis of unburied preservative treated control blocks showed that in spruce, copper and chromium contents were larger than the copper and chromium contents present as salts, in the treating solution (Table 3.22).

Beech displayed results which were in contrast to that of spruce. Copper and chromium contents in beech blocks were lower than the copper and chromium contents present as salts in the treating solution. The over-absorption and under-absorption shown by spruce and beech respectively, may be attributed to the different anatomical features in these two wood species. Spruce was less dense than pine, lime and beech. This feature at least in theory may have permitted greater moisture and preservative uptake by spruce in contrast with pine, despite its known lesser permeability. Furthermore in practice, very little work has been undertaken on the selective adsorption of metallic elements to wood. In pine, lime and beech, selective adsorption of preservatives in wood were broadly similar irrespective of the glutamine retentions. However, spruce displayed results which differed to those of pine, lime and beech. Test blocks impregnated with glutamine displayed lower selective adsorption ratios of copper to preserved control blocks. It is difficult to interpret the results obtained for the selective adsorption ratios in spruce.

Losses of copper and chromium from the preserved blocks occurred during soil burial. The results showed that quite a large percentage (up to 50%) of the preservative was leached from the amino acid treated wood blocks during burial in soil. The main losses in preservative occurred during the first three weeks of burial, before decay had started. This last portion may represent chelated, soluble preservative which was readily leached from the blocks due to diffusion in soil water. It is presumed that the preservative remaining in wood was in an insoluble form, either as a precipitate or complexed to the wood structure. The presence of glutamine in test blocks was clearly associated with greater losses of copper; metal loss increased with increasing glutamine retentions in the block. Chromium losses were also apparent, but did not show trends relating to glutamine concentrations. After the initial losses of CCA, further losses were small in all preserved wood blocks, both with and without glutamine inclusions. It was apparent that despite copper losses, softwoods were still protected at preservative concentrations below the toxic threshold, as hypothesised by Butcher and Nilsson, (1982).

Concurrent with the investigation on the influence of glutamine on preservative treated wood, a soil burial study was undertaken to investigate the influence of soluble carbohydrates on CCA treated lime and pine. Lime test blocks were impregnated with sucrose and pine blocks were impregnated with glucose.

Results of these experiments showed that wood decomposition and preservative performance in lime and pine were not influenced by soluble sugars. In lime, weight losses occurred in both unpreserved and preserved test blocks. However, weight losses recorded by preserved control blocks and preserved blocks impregnated with sucrose were broadly similar over the duration of the soil burial. In pine, weight losses were only recorded in unpreserved control blocks. The preservative treated softwood blocks were protected at sub-toxic concentrations over the twelve week soil burial. Nitrogen data was not available for this study with soluble sugars as the time remaining for experimental analyses was insufficient for nitrogen analyses to be undertaken.

4.3 General Discussion

It has been shown at this laboratory that soluble nutrients that migrate and accumulate at evaporative surfaces of wood during drying, enhance wood decay, reduce toxic limits and decrease preservative stability in preserved and unpreserved forms of hardwoods and softwoods (King et al, I98Ia). The precise chemical formulation of the soluble nutrient components at the surfaces of dried wood however, had not been investigated. As such, this formed the working objectives for this project. This project examined qualitatively and quantitatively, the soluble carbohydrate and nitrogenous components which redistribute to wood surfaces during drying. The role of these nutrients on wood decay in soil contact and their influence on the performance of CCA preservatives was also evaluated.

The investigation undertaken for this thesis has shown for the first time, the migration and accumulation of specific soluble amino acids at evaporative surfaces of dried wood, and also provided extensive quantitative information on the migration of soluble carbohydrates to these surfaces. In the softwoods, soluble sugars that redistributed to the evaporative surfaces were glucose, fructose, sucrose, xylose, mannose, galactose, and trace quantities of arabinose. Of these sugars, glucose and fructose were the predominant sugars and accounted for approximately 60% of the total carbohydrate content found at surface regions. In lime, sucrose, glucose and fructose were detected at the evaporative surfaces and sucrose was the predominant sugar in this wood. Sucrose and the combined concentrations of glucose and fructose, accounted for approximately 90% of the total carbohydrate content at the surfaces of lime. Several oligosaccharides were also detected in the softwoods and lime, but these were not examined further in the study.

The composition of soluble sugars at surface and sub-surface regions of spruce, pine, and lime were similar to those found in other studies. Sucrose, glucose and fructose were detected in the sapwood of beech and birch (Dietrichs, I963), in the increment cores from sapwood of spruce, lime, sycamore and birch (Holl, I98I) and in the needles of Scots pine and Norway spruce (Theander, I98I). Sucrose, shown to be a predominant sugar in extracts of lime, was also a predominant sugar in the wood species tested by Holl (I98I, op.cit.). The work of Baker et.al. (I970) on the nutrition of Anobium punctatum larvae also showed the presence of simple sugars and oligosaccharides in extracts of wood and frass samples.

Sucrose, galactose, glucose, fructose, arabinose and a series of oligosaccharides were detected in these samples. Though concentrations of these soluble materials were small, they were readily utilised by the insect larvae.

The high concentrations of glucose seen at surface regions of pine (Section 3.I.4.) complement the finding of Long (I978). In the latter investigation, glucose concentrations at surface regions were shown to be ten times those at sub-surface regions; and these concentrations constituted 0.6% of the dry weight of wood. The concentration of glucose in experiments undertaken for this thesis showed higher concentrations than that of Long's. In the alcohol extracts of pine, glucose concentrations constituted 0.9% of the mass of wood, at the surface regions, and in the cold water extracts, glucose concentrations constituted I.3% of the mass of wood at similar regions. Cold water extractions were also shown to remove larger quantities of soluble sugar from all the wood species tested in experiments undertaken for this thesis.

The quantitative differences observed in Long's investigation and in this thesis may be attributed to tree to tree variation and also to the different analytical techniques used. In his investigation, Long used paper chromatography for the separation, identification and quantification of simple sugars. The technique employed involved an elution time of several hours after which the spots on thechromatogram were developed, cut out and eluted with acidified aqueous ethanol. The absorbance of the eluant was measured and the sugars were quantified by comparison to standards. The above method employed is relatively insensitive, involves several manual steps, and is limited by resolution of the paper chromatography.

Soluble sugars in the extracts of the wood samples tested in this project were detected by HPLC. This analytical method is an improvement to that used by Long. Errors in experimentation are minimised, as after the sample has been loaded, the analysis is automated. The carbohydrate analysis column used also provides good resolution when disproportionate carbohydrate quantities are present; sugars with similar retention times on paper
matography are effectively separated by this column. In the chromatography solvent system used by Long, arabinose, fructose and mannose were situated too closely in the chromatogram to be effectively separated. Thus the techniques employed in experiments undertaken for this thesis, provide a much clearer qualitative and quantitative assessment of the sugars that redistribute to the surfaces of wood during drying.

The soluble amino acids that migrate and accumulate at surface regions of wood during drying have been shown to contribute to a significant proportion of the nitrogen content in spruce and pine, but to lesser proportions in lime. Aspartic acid, glutamine and arginine were the predominant amino acids in the extracts of these woods. These amino acids were also shown in other investigations to be present in significant concentrations in the sap of apple trees (Bollard, I957a), in the xylem sap of loblolly pine (Barnes, I963) and in white spruce needles (Durzan, I968a,b). Durzan (I968 op.cit.) considered arginine to be a main nitrogenous storage compound in spruce, which reflected a process by which reduced nitrogen levels in wood were concentrated in a nitrogen rich storage compound. Glutamine was shown to be a major amino acid in the xylem saps of Pinus radiata D. Don (Bollard, I957c) and Pinus taeda L. (Barnes, I963). It was suggested by these authors that glutamine served as a translocatory form of nitrogen. The presence of this amino acid in the sap would suggest that glutamine will move with the transpiration stream in the xylem. As glutamine can be translocated, it can therefore be readily available as a source of nitrogen for micro-organisms.

The soluble nutrients shown to accumulate at evaporative surfaces during drying would stimulate rapid colonisation of wood by a range of micro-fungi. Though these soluble components constitute a small proportion of the wood mass, they are nevertheless, very important as they are present in a form that can be easily utilised by micro-organisms. The presence of other soluble nutrient components, such as the sugar alcohols and water soluble polysaccharides, such as the galactomannans and arabinogalactans, which were not investigated as part of this project, are also thought to migrate to the evaporative surfaces during drying. It is probable that these solubles too, may have a contributory role to wood decay.

As wood itself has a low nitrogen content (Cowling and Merrill, I966), the supply of nutrients and particularly, those containing nitrogen, is a major factor affecting the decay of wood by micro-organisms. It has been shown in Experiment 4 in Section 3.I., that the bulk of the nitrogenous materials that redistribute to wood surfaces during drying are in the form of amino acids. These soluble nutrients have been found to be readily utilised by wood attacking fungi. Henningsson (I967) showed that the component amino acids of soluble nitrogen in wood supported growth of a number of wood destroying fungi.

Nitrogen given in the form of amino and amide nitrogen was utilised by all the basidiomycete fungi tested. Ammonium compounds were also good sources of nitrogen, but nitrate proved to be a poor source of nitrogen. Baker et. al. (1970) in their investigation on the nitrogen utilisation by Anobium punctatum of pine sapwood, showed that a considerable amount of cell wall protein passed through the larval gut of the insects, and emerged in frass without any change in its composition. These authors showed that the soluble protein, a minor constituent in wood, was utilised more completely than the insoluble material. The soluble sugars found at surface regions of dried wood can also be readily utilised by micro-organisms. Henningsson (1967, op.cit.) showed that several wood destroying fungi were able to utilise low molecular weight sugars as sources of carbon. Glucose and fructose both promoted rapid growth in the fungi tested, and sucrose was also a good carbon source. However, some of the fungi tested were unable to utilise sucrose, and this was probably due to the lack of invertase.

Work described in Section 4.I., showed that concentration of soluble carbohydrates and soluble amino acids were significantly higher at surface regions of dried wood than at sub-surface regions. Though the concentration of these nutrients varied among the wood species, all the wood species tested were consistent in displaying higher concentrations of soluble carbohydrates and amino acids at surface regions. Soluble carbohydrate concentrations at surface regions of pine were shown to be five times those at sub-surface regions in the outer sapwood. In spruce and lime, these concentrations were twice those at sub-surface regions. Soluble amino acids also showed large differences in concentrations between surface and sub-surface regions. In spruce and pine, concentrations of soluble amino acids at surface regions were fifteen times, and twelve times respectively, the concentrations found at sub-surface regions. In lime, surface concentrations were twice those of sub-surface areas.

The concentration of soluble sugars and amino acids that migrate and accumulate at wood surfaces during drying may be associated with seasonal felling. Concentrations of simple sugars have been shown to be higher during the winter months in Scots pine needles (Ericsson, 1979), and in increment cores obtained from sycamore, birch, lime and spruce tree trunks (Holl, 1981). Levi and Cowling (1968) showed that nitrogen was present in wood in greater quantities before foliation than after foliation.

Seasonal variation in soluble nitrogen has also been shown to occur in the cambial area (Clark and Mills, 1970), in the sap of wood (Bollard, 1957b) and in roots, buds and leaves of white spruce saplings (Durzan, 1968b). Aphid populations were also shown to correlate directly with seasonal variation of certain amino acids in Sitka spruce needles (Parry, 1974). Therefore, timber felled during seasons of high nutrient concentration and subsequently dried, by reason of redistribution, may be more susceptible to decay by micro-organisms.

It is well known that sapwood is more susceptible to decay by wood inhabiting micro-organisms. The distribution studies of Section 3.I., showed the concentrations of soluble sugar and amino acids to be higher in the outer sapwood regions, than the inner sapwood and heartwood regions. In the sapwood, the longitudinal and ray parenchyma cells function as storage tissue. However, as sapwood ages, the parenchyma cells lose their cytoplasm, and these changes are usually coincident with a general decrease in nutrient concentration across the sapwood. Thus wood prepared from outer sapwood regions and felled during seasons of high nutrient concentrations, may have a higher decay susceptibility to wood destroying micro-organisms.

The seasonal variation of soluble nutrients in wood, and the large difference shown in the nutritional status at surface regions and subsurface regions of planks dried under laboratory conditions, may reflect the nutritional state of timber and wooden fence posts in soil contact. Logs and wooden posts are dried before insertion into soil. Consequently, soluble nutrients contained by them will have been redistributed to the evaporative surfaces. These high nutrient profiles at the wood-soil exposure regions are likely to provide a stimulus for organisms from outside the wood to the interior. The presence of the soluble sugars and amino acids shown to accumulate at surfaces of dried wood may predetermine the succession of organisms that colonise wood. Successional events in the colonisation of both untreated and preservative treated wood have shown bacteria to be the primary colonisers, and basidiomycetes to be the climax microflora (Banerjee and Levy, 1971; Clubbe and Levy, 1982). However, for the fungal species which are capable of acting as 'sugar fungi' in the presence of simple nutrients and are also capable of acting as soft-rot fungi, enough soluble nitrogen would be accumulated at dried surfaces of wood, to support soft rot production, when the soluble carbohydrates are depleted.

Also, with decreasing soluble nutrient status after prolonged exposure to soil, the colonisation of wood becomes selective and ultimately, with only traces of soluble nutrients present, the wood substrate is inhibiting to most organisms other than the basidiomycetes.

The soluble sugars and amino acids shown to redistribute to evaporative surfaces in Section 4.I., were presumed to have a role in wood decay. Experiments I-5 of the soil burial studies were designed to examine the influence of these nutrients on decay susceptibility of unpreserved woods; and experiments 6-8 were undertaken to investigate the effect of added sugars and amino acids on wood decay and preservative stability in preserved hardwoods and softwoods. Soil burial experiments were selected as soil provides the most aggressive environment to which wood may be exposed. Savory and Bravery (I97I) considered that the major advantage of soil burial systems was that preserved wood was subjected to the mixed microflora naturally present in soil. These authors identified such environmental factors as temperature, moisture availability, duration of incubation, and specimen size as important determinants of decay rates in laboratory testing regimes.

The experiments undertaken in the soil burial studies highlighted the problems encountered when trying to replicate wood of high nutrient status. The addition of soluble sugars and amino acids to unpreserved wood blocks did not influence decay or nitrogen transfer from soil to wood. Wood blocks impregnated with individual soluble nutrients displayed loss of these added nutrients on emplacement of the blocks in soil. Consequently, nitrogen contents and weight losses in these blocks displayed similar patterns to those of the controls. However, blocks containing redistributed soluble nutrients were shown to influence both wood decay and nitrogen transfer from soil to wood. These blocks did not show loss of nutrients during soil burial. It is likely that the accumulation of soluble nutrients, and materials such as waxes, gums and resins at wood surfaces during drying, results in a reaction between these materials which may accord some degree of permanence to the soluble nutrients at the wood surfaces. It is known that soluble amino acids react with sugars during drying to form coloured compounds in a browning reaction. Planks of wood in experiments undertaken in Section 3.I., showed a colour change from pale yellow when planks were in a green condition, to a darker yellow when planks were dried under laboratory conditions.

This colour change is indicative of a change in condition of the wood, resulting from the migration and accumulation of soluble materials at evaporative surfaces of dried planks. It is therefore possible that soluble nutrients forming complexes at wood surfaces, are then released slowly to the soil, thereby providing a constant stimulus for the colonisation of the wood substrate by soil inhabiting micro-organisms.

The insertion of wood in soil, especially when containing redistributed soluble nutrients, may alter soil conditions at the periphery of the block. Fungi and bacteria in soil are considered to exist mainly as inactive or dormant forms. Microbiostasis in soil has been suggested to be due to the depletion of nutrients (Lynch, 1982). Furthermore, it has been postulated (Smith, 1980) that when wood is emplaced in soil, small traces of nutrients diffuse from the wood, counteracting fungistasis and stimulating the germination of dormant fungal species. The specific soluble sugars and amino acids found at the evaporative surfaces of wood in this study, have been shown to ellicit behavourial responses by bacteria (Howe, 1983). In his investigations, Howe also showed that wood decay fungi displayed hyphal extensions towards wood during growth, and such responses were demonstrated by fungal colonies developing towards wood baits. In the same study, bacteria both Gram positive and Gram negative, and representative of wood and soil inhabiting genera, displayed positive chemotactic responses to cold aqueous wood extracts. Responses of these soil inhabiting bacteria to arginine were significantly greater than responses towards glucose. In this thesis, soil burial experiments using preserved wood showed responses of soil inhabiting microflora to be greater towards arginine and glutamine than to glucose or sucrose, as indicated by weight loss.

Work described in this thesis has shown for the first time that the soluble amino acids found at the evaporative surfaces of wood, influenced the decay of wood in soil and preservative stability therein. CCA stability in both hardwoods and softwoods was reduced by the presence of soluble nitrogen compounds. The presence of CCA in wood blocks depressed the rate of weight loss (decay) but did not depress nitrogen transfer from the soil to the block. Arginine and glutamine incorporated into CCA treated lime blocks influenced both wood decay and preservative efficacy in this wood species. In beech, the effect of added amino acids on preservative stability was not observed, and it is possible that in this wood species that the effect of these nutrients are difficult to see in a short term soil burial.

Spruce and pine were protected from wood decay at the sub-toxic concentration of preservative used. The mechanisms of fixation of CCA in these woods may explain their resistance to decay despite the presence of added amino acids. It has been suggested that lignin is the major fixation site for copper (Butcher and Nilsson, 1982), and that copper can be fixed to retention levels in the S2 layers of high lignin wood species (softwoods), which are in excess of toxic thresholds. In wood species with a low lignin content (hardwoods), copper can be fixed only to a retention level which is below the toxic threshold for soft-rot fungi. Thus, spruce and pine are protected at levels which are sub-toxic for preventing growth, whereas the hardwoods require higher retention of CCA to reduce the growth of soft-rot fungi. These high levels will temporarily prevent soft-rot attacks, but when CCA tolerant soft-rot fungi have colonised the wood, they will be able to cause soft-rot but at a reduced rate.

The loss of CCA components by leaching has been demonstrated by other workers (Evans, 1978; Eriscoe, 1987). It has been shown in soil burial experiments undertaken in Section 3.2., that substantial copper losses were recorded in hardwoods and softwoods treated at sub-toxic levels with CCA and which had also been treated with glutamine. Metal ions are known to chelate very readily with amino groups. It is postulated that the amino acids shown to redistribute to wood surfaces during drying in Section 3.1., may act to detoxify the wood by complexing and chelating with fixed CCA components by ion-exchange mechanism. As chelated forms may be more mobile than fixed materials, this could result in leaching of CCA thus leaving the wood unprotected. This has been demonstrated in soil burial experiments in Section 3.2., in which CCA-treated lime impregnated with high glutamine concentrations, showed large weight losses. The complexing of amino acids with preservative components may also explain the early failure of CCA-treated wood containing redistributed soluble nutrients shown in wood decomposition at this laboratory.

Heavy metals leached to the soil from treated wood may also inhibit bacterial motility by complexing with the flagella. Bacteria demonstrating positive chemotaxis to nutrients leaching from wood may be rendered non-motile by CCA components in wood resulting in a biomass build up in soil regions adjacent to wood surfaces. Microbial biomass in wood has been demonstrated to be capable of causing solubilisation of CCA components (Levi, 1969).

The solubilised preservative may complex with micro-organisms, killing them but rendering the preservative susceptible to further leaching as a function of further microbial activity. It is probable that the continued input of microbial biomass to preserved wood causes the gradual loss of preservative to the soils, thus reducing the effective toxicity of the wood.

Simple nutrients in soil have been suggested to cause release of microbiostasis (Lynch, 1982) and also to direct micro-organisms to wood resources (Howe, 1983). The loss of added nutrients from wood shown by impregnated wood blocks during soil burial experiments described in this thesis, should have resulted in increased concentrations of these nutrients at the wood-soil interface, and it would be anticipated that these nutrients would provide a stimulus for microbial accumulation at wood-soil regions. Accordingly, it would be expected that there would be an enhanced rate of decay in the blocks. However, this effect was not observed in unpreserved blocks which had been impregnated with soluble nutrients. In unpreserved material, it is possible that soil conditions influence the localisation of soluble nutrients leached for wood by water in soil which had been maintained at 80% and 100% water holding capacity. Furthermore, with smaller block volumes, compared to the large volume of soil used in soil bed trials, and the easy mobility of the leached nutrients, the concentration gradients set up were diluted to such an extent in a short time period, that there would have been no opportunity for real stimulation to take place. In preserved wood however, amino acids retained in lime influenced wood decay and preservative stability in this wood species. It is possible that in the preserved blocks, the complexes provided the nutrient stimulation of amino acids and inactivated the biocide by metal complex formation. Furthermore, metal losses took place which has implications for the stability of metal elements in the presence of amino acids.

Work described in this thesis has shown that substrates such as glucose and sucrose do not influence decay and preservative stability in preserved forms of hardwoods and softwoods. While it is possible in the experiment, the soluble sugars incorporated into the wood blocks were not retained in the block, it is also possible that not all soluble nutrients that redistribute to evaporative surfaces during drying influence preservative efficacy.

Nitrogen sources of which wood is deficient, and a combination of nitrogenous and carbohydrate components may well influence preservative stability. Further work is required to investigate if combinations of amino acids and sugars influence the preservative performance of treated timber.

The concentration of preservative components at wood surfaces may have an important role in determining the durability of timber in service. Preservative gradients have been shown in distribution poles of four wood species which had been treated by pressurised sap displacement using CCA (Evans, et. al., 1986). The results of the investigation showed that all four wood species displayed higher CCA content in the outer sapwood which declined radially towards the centre of the pole. The concentration of individual CCA components also varied in a radial direction. Chrome and arsenic concentrations were at a maximum in the outer sapwood but copper concentrations were at a maximum in the inner sapwood. It is possible therefore that in large pieces of timber, unfixed CCA along with soluble nutrients may redistribute to the surface of wood after treatment. If the preservative is susceptible to leaching, and if biodeterioration takes place as shown in this thesis by soluble amino acid presence, then to accord better protection to timber in service, it is essential that the preservative retention be high enough in the outer zones to take account of any leached losses.

The redistribution of soluble nutrients in wood may have implications for timber drying with particular regard to its use in building materials. With the advent of central heating, localised areas of high temperature could be experienced in the vicinity of heating ducts, and surrounding materials could be exposed by high temperatures for prolonged periods. Recent reports from Scandinavia (Bjurman, 1986) have suggested that odour in houses are associated with fungal growth which develop on wood surfaces as a result of nutrient accumulation. The microclimates produced in these houses enable microbial communities to grow thereby causing both aesthetic and health problems. Materials provided to this laboratory by Swedish sources have shown high surface nutrient profiles (King, pers.comm.). In Sweden, wood which showed some surface redistribution of nutrients included specimens which had similar growth rates to those of British species. It is possible that the presence of surface nutrients may be important for surface growth of organisms in dried timber.

Thus the use of carefully dried material with little surface nutrients in well insulated houses may reduce the optimum conditions for growth of fungi, thereby minimising health risks.

The work described in this thesis has established the composition of the soluble nitrogenous components that migrate and accumulate at the evaporative surfaces of dried wood. It has also revealed a strong relationship between specific nitrogenous components and preservative performance. The studies described in this thesis have been laboratory studies using small wood specimens which have been dried under laboratory conditions. Further work is required on large dimension material which have been commercially dried and also treated with a range of biocides.

APPENDICES

APPENDIX I MOISTURE CONTENTS (%)

a. Green Wood

Wood Type	Outer Sapwood	Inner Sapwood	Heartwood
Spruce	160	90	49
Pine	190	100	50

b. Dried Wood

Wood Type	Moisture Content (%)
Spruce	7
Pine	8
Lime	5
Kempas	7

APPENDIX 2 TREATING CONCENTRATIONS OF SUGAR SOLUTIONS USED IN THE CARBOHYDRATE SOIL BURIAL STUDY

Wood Type	Treating Solution Concentration (% w/v)	Mean percentage weight increment in blocks from liquid uptake (%w/v)
Spruce	1.35	1.38
	2.70	3.27
	4.00	5.26
	5.40	7.11
Pine	1.35	1.38
	2.70	3.27
	4.00	5.26
	5.40	7.11
Lime	1.50	1.40
	3.00	3.40
	4.50	5.37
	6.00	7.32
Beech	1.75	1.38
	3.50	2.76
	5.25	4.14
	7.00	5.52

APPENDIX 3 SAMPLE CALCULATION FOR THE PREPARATION OF EQUIMOLE CONCENTRATIONS OF A MIXTURE OF AMINO ACID

Amino Acid	Molecular weight	Total No of N atoms
asp	133	1
gln	146	2
arg	174	4

For 100ml of solution to contain 1×10^{-3} moles of each of the above amino acid,

Amino Acid	Wt. amino acid used/100mls	molN ($\times 10^{-3}$)
asp	0.133	1
gln	0.146	2
arg	0.174	4
Total	0.453	7

wt of N in 100ml of the above solution

$= 7 \times 10^{-3} \times 14g$

$= 98 \times 10^{-3}$ gN/100ml

Assuming the liquid uptake of 1g of wood is 1ml, then the nitrogen incorporated is 98×10^{-5} gN/gwood.

This is approximately equal to 1mgN/gwood or 0.1% w/w N.

Equimole amino acid solutions for 0.2%, 0.4% and 0.8% were calculated in a similar manner.

APPENDIX 4 TREATING CONCENTRATIONS OF AMINO ACID SOLUTIONS USED IN THE SOIL BURIAL STUDY

Concentrations of each amino acid ($x10^{-3}$ mole/100 ml)	Weight of amino acid used (g/100ml)			Total amino acid concentration (% w/v)
	asp	gln	arg	
1	0.133	0.146	0.174	0.45
2	0.266	0.292	0.348	0.91
4	0.532	0.584	0.696	1.81
8	1.064	1.168	1.392	3.62

APPENDIX 5 TREATING CONCENTRATIONS OF AMINO ACID AND SUGAR SOLUTIONS USED IN THE PRESERVATIVE STUDIES

a. Arginine Study

Wood Type	Arginine concentration (% w/v)		
	0.3%N	0.5%N	0.7%N
Lime	0.50	1.00	2.00

b. Glutamine Study

Wood Type	Glutamine concentration (% w/v)			
	0.2%N	0.3%N	0.5%N	0.7%N
Lime	0.43	0.86	1.72	2.58
Beech	0.83	1.63	2.35	3.52
Pine	0.34	0.68	1.35	2.03
Spruce	0.20	0.40	0.80	1.20

c. Carbohydrate Study

Wood Type	Sugar concentrations (% w/v)			
	0.5%w/w	1%w/w	2%w/w	4%w/w
Lime	0.41	0.83	1.65	3.30
Pine	0.32	0.65	1.38	2.60

Sample	Green Wood				Dried Wood (Surface Samples)				Dried Wood (Sub-surface Samples)			
	Total Soluble Carbohydrate content	Total Reducing Sugar Content	Glucose and fructose content	Soluble Protein	Total Soluble Carbohydrate Content	Total Reducing Sugar Content	Glucose and Fructose content	Soluble Protein	Total Soluble Carbohydrate Content	Total Reducing Sugar Content	Glucose and Fructose Content	Soluble Protein
Outer Sapwood	0.55 ± 0.02	0.42 ± 0.03	0.23 ± 0.04	0.06 ± 0.02	0.99 ± 0.05	0.86 ± 0.10	0.62 ± 0.03	0.17 ± 0.02	0.47 ± 0.02	0.23 ± 0.01	0.21 ± 0.02	0.06
Inner Sapwood	0.46 ±0.02	0.08 ±0.01	0.04 ± 0.03	0.01 ± 0.003	0.55 ± 0.01	0.41 ± 0.04	0.13 ± 0.05	0.08 ± 0.01	0.40 ± 0.02	0.08 ± 0.01	0.04 ± 0.01	0.03 ± 0.02
Heartwood	0.15 ± 0.02	0.02 ± 0.03	0.02 ± 0.01	0.01 ± 0.002	0.42 ± 0.06	0.09 ± 0.04	0.04 ± 0.02	0.03 ± 0.01	0.30 ± 0.01	0.06 ± 0.04	0.03 ± 0.03	0.01 ± 0.004
Average	0.39	0.17	0.09	0.03	0.65	0.45	0.26	0.09	0.39	0.12	0.09	0.03

APPENDIX 6 SOLUBLE CARBOHYDRATE AND NITROGENOUS COMPONENTS IN THE OUTER SAPWOOD, INNER SAPWOOD AND HEARTWOOD REGIONS OF GREEN AND DRIED SPRUCE. RESULTS ARE EXPRESSED AS A PERCENTAGE OF THE INITIAL DRY WEIGHT OF WOOD.

APPENDIX 7 SOLUBLE AMINO ACID IN THE OUTER SAPWOOD, INNER SAPWOOD AND HEARTWOOD REGIONS OF GREEN AND DRIED SPRUCE.

Sample	umoles amino acid per gram wood		
	Green Wood	Dried Wood	
		Surface Sample	Sub-Surface Sample
Outer Sapwood	1.55 ± 0.06	2.97 ± 0.05	1.43 ± 0.13
Inner Sapwood	1.37 ± 0.12	1.85 ± 0.01	0.21 ± 0.01
Heartwood	0.33 ± 0.005	0.13 ± 0.004	0.03 ± 0.006
Average	1.08	1.65	0.56

APPENDIX 8 DISTRIBUTION OF SOLUBLE CARBOHYDRATES IN GREEN SPRUCE AND GREEN SPRUCE DRIED IN CHIP FORM. RESULTS ARE EXPRESSED AS A PERCENTAGE OF THE DRY WEIGHT OF WOOD GREENWOOD SAMPLES WERE CORRECTED FOR MOISTURE.

Ring Groups	Green Spruce				Green Spruce dried in Chip Form			
	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose
1 - 10	0.30 ±0.14	0.22 ±0.37	0.10 ±0.11	0.10 ±0.12	0.58 ±0.19	0.49 ±0.10	0.15 ±0.11	0.16 ±0.09
11 - 20	0.11 ±0.07	0.12 ±0.02	0.04 ±0.07	0.05 ±0.04	0.56 ±0.09	0.29 ±0.17	0.12 ±0.06	0.11 ±0.04
21 - 30	0.10 ±0.06	0.12 ±0.32	0.05 ±0.04	0.05 ±0.02	0.39 ±0.11	0.26 ±0.55	0.06 ±0.03	0.05 ±0.004
31 - 40	0.10 ±0.01	0.03 ±0.02	0.01 ±0.01	0.01 ±0.07	0.32 ±0.07	0.08 ±0.11	0.02 ±0.002	0.01 ±0.007
41 - 50	0.05 ±0.04	0.01 ±0.03	0.007 ±0.008	0.001 ±0.006	0.29 ±0.06	0.07 ±0.02	0.03 ±0.002	0.02 ±0.002
Average	0.13	0.10	0.04	0.04	0.43	0.24	0.08	0.07

APPENDIX 9 DISTRIBUTION OF SOLUBLE CARBOHYDRATES IN GREEN PINE AND IN GREEN PINE DRIED IN CHIP FORM. RESULTS ARE EXPRESSED AS A PERCENTAGE OF DRY WEIGHT OF WOOD GREENWOOD SAMPLES WERE CORRECTED FOR MOISTURE.

Ring Groups	Green Pine				Green Pine Dried in Chip Form			
	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose
1-5	1.10 ±0.10	1.06 ±0.24	0.74 ±0.20	0.75 ±0.10	1.30 ±0.38	1.77 ±0.22	0.60 ±0.20	0.50 ±0.06
6-10	0.50 ±0.02	0.49 ±0.12	0.24 ±0.03	0.22 ±0.06	1.10 ±0.23	1.44 ±0.28	0.53 ±0.06	0.50 ±0.03
11-15	0.30 ±0.12	0.24 ±0.01	0.11 ±0.02	0.11 ±0.05	NT	NT	NT	NT
16-20	0.30 ±0.08	0.29 ±0.02	0.16 ±0.06	0.15 ±0.05	0.62 ±0.11	0.74 ±0.12	0.30 ±0.14	0.30 ±0.10
21-25	0.40 ±0.16	0.40 ±0.15	0.19 ±0.03	0.21 ±0.01	0.51 ±0.17	0.59 ±0.77	0.21 ±0.11	0.22 ±0.09
26-30	0.20 ±0.07	0.22 ±0.03	0.08 ±0.01	0.08 ±0.01	0.36 ±0.32	0.35 ±0.05	0.15 ±0.03	0.16 ±0.01
31-35	0.18 ±0.07	0.13 ±0.01	0.06 ±0.02	0.05 ±0.01	0.32 ±0.08	0.14 ±0.05	0.13 ±0.11	0.14 ±0.12
36-40	0.26 ±0.16	0.16 ±0.01	0.07 ±0.03	0.06 ±0.01	0.92 ±0.76	0.16 ±0.03	0.17 ±0.04	0.12 ±0.08
41-45	0.30 ±0.08	0.08 ±0.07	0.02 ±0.03	0.01 ±0.01	1.22 ±0.13	0.08 ±0.07	0.04 ±0.01	0.03 ±0.007
46-50	0.39 ±0.21	0.07 ±0.15	0.01 ±0.001	0.003 ±0.001	0.75 ±0.14	0.07 ±0.02	0.03 ±0.01	0.01 ±0.03
Average	0.39	0.31	0.17	0.16	0.79	0.59	0.24	0.22

NT No Trial

APPENDIX 10 DISTRIBUTION OF SOLUBLE CARBOHYDRATES IN SURFACE AND SUB-SURFACE SAMPLES OF DRIED SPRUCE. RESULTS ARE EXPRESSED AS PERCENTAGE OF THE DRY WOOD WEIGHT.

Ring Groups	Surface samples				Sub-surface samples			
	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose
1-10	3.04 ±0.99	3.03 ±0.35	1.10 ±0.04	1.10 ±0.03	0.33 ±0.37	0.32 ±0.06	0.16 ±0.08	0.16 ±0.06
11-20	1.73 ±0.51	1.89 ±0.42	0.55 ±0.05	0.56 ±0.03	0.26 ±0.11	0.22 ±0.10	0.05 ±0.05	0.06 ±0.05
21-30	0.97 ±0.29	0.83 ±0.28	0.19 ±0.22	0.20 ±0.21	0.21 ±0.13	0.20 ±0.04	0.04 ±0.03	0.03 ±0.02
31-40	0.44 ±0.38	0.47 ±0.06	0.03 ±0.09	0.04 ±0.08	0.24 ±0.11	0.17 ±0.10	0.04 ±0.03	0.04 ±0.03
41-50	0.31 ±0.31	0.08 ±0.04	0.01 ±0.004	0.01 ±0.003	0.32 ±0.14	0.11 ±0.08	0.02 ±0.01	0.02 ±0.02
Average	1.34	1.26	0.37	0.38	0.27	0.20	0.06	0.06

APPENDIX 11 DISTRIBUTION OF SOLUBLE CARBOHYDRATES IN SURFACE AND SUB-SURFACE SAMPLES OF DRIED PINE. RESULTS ARE EXPRESSED AS A PERCENTAGE OF THE DRY WOOD WEIGHT.

Ring Groups	Surface Samples				Sub-surface samples			
	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose	Total Carbohydrate Content	Total Reducing Sugar Content	Glucose	Fructose
1-5	5.82 ±2.3	4.85 ±1.55	2.29 ±0.30	2.29 ±0.20	0.83 ±0.49	0.73 ±0.35	0.32 ±0.11	0.31 ±0.12
6-10	1.87 ±0.08	1.43 ±0.59	0.61 ±0.40	0.65 ±0.20	0.45 ±0.49	0.44 ±0.17	0.18 ±0.06	0.18 ±0.04
11-15	1.63 ±0.75	1.73 ±0.52	0.83 ±0.30	0.83 ±0.20	0.40 ±0.05	0.32 ±0.04	0.15 ±0.01	0.14 ±0.01
16-20	1.78 ±0.47	1.26 ±0.30	0.65 ±0.57	0.64 ±0.40	0.35 ±0.06	0.28 ±0.12	0.14 ±0.20	0.13 ±0.10
21-25	1.74 ±0.006	1.36 ±0.44	0.61 ±0.81	0.61 ±0.70	0.32 ±0.16	0.29 ±0.04	0.13 ±0.12	0.13 ±0.12
26-30	1.31 ±0.01	1.32 ±0.57	0.55 ±0.16	0.55 ±0.16	0.25 ±0.16	0.24 ±0.03	0.04 ±0.15	0.05 ±0.13
31-35	1.13 ±0.03	0.84 ±0.22	0.28 ±0.26	0.28 ±0.25	0.62 ±0.24	0.23 ±0.04	0.09 ±0.04	0.08 ±0.04
36-40	0.99 ±0.01	0.59 ±0.12	0.18 ±0.20	0.19 ±0.10	0.45 ±0.14	0.18 ±0.02	0.05 ±0.03	0.05 ±0.04
41-45	1.08 ±0.03	0.27 ±0.02	0.09 ±0.18	0.10 ±0.20	0.65 ±0.11	0.15 ±0.17	0.01 ±0.05	0.01 ±0.05
46-50	1.58 ±0.40	0.13 ±0.07	0.04 ±0.03	0.04 ±0.03	1.19 ±1.06	0.20 ±0.01	0.02 ±0.03	0.03 ±0.02
Average	1.89	1.37	0.61	0.62	0.55	0.31	0.11	0.12

APPENDIX 12 COMPOSITION AND CONCENTRATIONS OF SOLUBLE CARBOHYDRATES PRESENT IN SURFACE AND SUB-SURFACE REGIONS OF DRIED WOOD. CARBOHYDRATE CONCENTRATIONS ARE EXPRESSED AS A PERCENTAGE OF THE DRY WEIGHT OF WOOD.

Sample	Extraction	Total Carbohydrate Content	Reducing Sugar Content	Glucose	Fructose	Sucrose	Xylose	Galactose	Mannose
Spruce Surface	Alcohol	1.01 ±0.04	0.61 ±0.01	0.30 ±0.002	0.24 ±0.001	0.04 ±0.009	0.10 ±0.004	–	–
"	Hot Water	0.39 ±0.03	0.52 ±0.003	–	–	–	–	–	–
"	Cold Water	1.68 ±0.06	0.64 ±0.03	0.35 ±0.06	0.77 ±0.05	0.10 ±0.001	0.14 ±0.01	–	–
Spruce Sub Surface	Cold Water	0.72 ±0.03	0.19 ±0.02	–	–	–	–	–	–
Pine Surface	Alcohol	2.73 ±0.06	2.39 ±0.06	0.90 ±0.05	0.90±0.07	0.13 ±0.04	0.21 ±0.002	0.15 ±0.04	0.05 ±0.03
"	Hot Water	0.69 ±0.05	0.53 ±0.40	–	–	–	–	–	–
"	Cold Water	5.21 ±0.11	4.91 ±0.96	1.39 ±0.07	1.40±0.11	0.15 ±0.01	0.47 ±0.05	0.36±0.33	–
Pine Sub Surface	Cold Water	0.96 ±0.04	0.57 ±0.01	0.13 ±0.005	0.10±0.01	–	–	–	–
Lime Surface	Alcohol	2.35 ±0.25	0.89 ±0.06	0.27 ±0.01	0.14±0.04	1.25 ±0.08	–	–	–
"	Hot Water	0.66 ±0.07	0.26 ±0.03	–	–	–	–	–	–
"	Cold Water	2.90 ±0.13	1.10 ±0.02	0.35 ±0.003	0.21±0.004	1.44 ±0.03	–	–	–
Lime Sub Surface	Cold Water	1.62 ±0.16	0.56 ±0.04	0.20 ±0.01	0.08±0.004	0.33 ±0.00	–	–	–
Kempas	Alcohol	0.42 ±0.016	–	–	–	–	–	–	–
"	Hot Water	0.12 ±0.014	–	–	–	–	–	–	–
"	Cold Water	0.64 ±0.03	–	–	–	–	–	–	–

– Not Detected

APPENDIX 13 DATA OBTAINED FROM CARBOHYDRATE STANDARDS USED IN HPLC ANALYSIS

Carbohydrate	Concentration ($mgml^{-1}$)	Retention Time (Mins)	Area (Area units)	Response Factor (Area units/ $mgml^{-1}$)	Response Factor Relative to Glucose
STACHYOSE	1.006	8.70	59132	58779	0.848
SUCROSE	1.034	9.85	67247	65036	0.939
RAFFINOSE	1.014	9.27	55659	54890	0.792
GLUCOSE	1.020	12.07	70677	69291	1.000
XYLOSE	1.022	13.15	66341	64913	0.937
GALACTOSE	1.006	14.13	55049	54721	0.789
RHAMNOSE	1.030	14.74	55864	54237	0.783
MANNOSE	1.012	15.98	77265	76349	1.102
ARABINOSE	1.012	15.31	70801	69961	1.010
FRUCTOSE	1.006	16.45	70319	69899	1.009

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