SOME ECOLOGICAL ASPECTS OF THE EARLY MICROBIAL COLONISATION OF WOOD

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SUMMARY

The distribution of nitrogen in freshly-felled pine and spruce, and in these woods after drying, is described. Nitrogen is shown to exist in two forms, a soluble and an insoluble form, in the freshly-felled material. During drying the soluble nitrogen and other soluble materials are found to be transported with moisture to exposed surfaces where they accumulate on evaporation of water. The consequences of this redistribution of soluble nutrients are discussed with reference to susceptibility of wood to both microbial and insect decay.

Soil-burial experiments in Princes Risborough Laboratory test soil using wood blocks, the soil-contact faces of which either contained or did not contain soluble nutrient accumulations, were undertaken. The results of these experiments show a direct correlation between the amount of soluble nitrogen at soil-contact faces (measured as total nitrogen content) and the amount of soft rot produced there. These experiments also showed that soluble nitrogen does not move into wood from soil within which it is buried, and that such nitrogen movement into wood as does occur from soil is in a microbial form.

Microbial activity is greatest at the soil-contact surface. Measurement of respiration confirmed this observation. Moisture movement into wood produces a pH gradient which is highest at the soil-contact surface, the point of highest moisture content, and decreases with distance from the soil-exposure face. The overall effect of soil burial is to increase the pH of the wood.

The implications of soluble nutrient distribution in wood both to enzymatic activity of microfungi and to the successions of organisms colonising wood are discussed.

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CHAPTER I

INTRODUCTION

1.1. INTRODUCTION

Wood and woody materials have been used for structural purposes in most parts of the world since the earliest times. In comparison with other structural materials e.g. steel, of which there are finite reserves available for use, a demand for wood for structural purposes may consistently be satisfied if appropriate reafforestation policies are practised.

Woods generally have high strength-to-weight ratios; are reasonably resistant to weathering, but many species are susceptible to biological deterioration. Borgin (1971) has shown that in situations where the biological decay factor is precluded, wooden structures may have life spans in excess of 1000 years. From the viewpoint of gaining maximal working life from wood, it is necessary to preserve it to inhibit deterioration either by insects, the main agents of deterioration in the tropics, or by fungi, the main agents of deterioration in more temperate climates (Dhanarajan, 1974).

Hueck (1968) classified biological interaction with materials into two groupings, respectively termed "biodegradation" and "biodeterioration". Biodegradation refers to an enhanced economic value of materials as a result of organism interaction, whereas biodeterioration refers to a loss in economic value after such activity. Fungal colonisation of wood may result in biodegradation of the wood (Lindgren, 1952; Ricard and Bollen, 1967; Hulme and Shields, 1970, 1972; Henningsson, Henningsson and Nilsson, 1972; King and Eggins, 1973); but more

generally produces a loss in economic value (biodeterioration). Wood deterioration is defined for the purposes of this study as a loss of the physical or structural characteristics of the wood when these qualities are desirable from the viewpoint of wood utilisation.

As wood is of increasing economic importance, considerable research is being undertaken to provide satisfactory systems to preserve it against decay. This research includes designing systems to evaluate the performance of wood preservatives in short time periods. One system commonly used is the soil-burial system which provides test conditions of considerable severity, and which is presently being examined by the Biodeterioration Section of the Building Research Establishment, Princes Risborough Laboratory, England. The project described in this thesis was sponsored by the latter organisation to provide data which might explain results obtained from soil-burial studies with particular reference to nutritional factors contributing to the biodeterioration by micro-organisms of wood in soil contact.

1.1.1. Fungi in Wood

The fungi which colonise and decay wood may be divided into two major groupings, namely, the wood-rotting basidiomycetes which are capable of producing a total decay of wood; and the microfungi many of which apparently only passively colonise wood but some of which have been shown to be capable of producing a decay form, generally localised to the surface of wood, called "soft rot".

Many data are available on the activities of the wood rotting basidiomycetes as a grouping, and assessment of efficiency of wood preservatives has generally been governed by the ability of preservatives to inhibit these organisms. On the other hand, fewer data are available on the activities of wood-colonising microfungi as a grouping, although data are available on the activities of individual species, or groups of species, e.g. "mould fungi", "staining fungi", "soft-rot fungi", "primary colonising fungi", "secondarily-colonising fungi," etc. In terms of assessment of efficiency of wood preservatives, little attention has been paid to their efficiency against wood-colonising microfungi until relatively recently (Savory, 1954(a); Greaves and Savory, 1965; Gorshin and Krapivina, 1969).

The work of King and Eggins (1973) showed that many microfungi, noted for early colonisation of wood after felling, possessed the ability to produce a spectrum of enzymes which could enable them to deteriorate wood. These enzymes, suggested to be both the C_i and C_x components of cellulase along with polygalacturonase and amylase, were associated with production of an enhanced permeability of wood, frequently observed after wood was colonised by these latter organisms. King (1972) considered that many microfungi which colonise wood posses a potential wood-decay ability. This was confirmed by Nilsson (1973), who showed that the majority of the 169 commonly-found wood-colonising microfungi, some of which were used by King (1972), produced soft rot in wood under laboratory conditions.

A survey of the literature on wood colonisation by microfungi (King, 1972), showed that many microfungi, commonly considered only to colonise wood passively when in the green state, were also capable of producing soft rot in wood. Fungal species concerned in passive colonisation are frequently described in the literature as "mould" or "staining" fungi whereas if producing the undoubted soft rot, particularly in a soil-contact situation, they are described as "soft rot" fungi. This anomalous nomenclature provides an area in which a proper understanding of their role in wood decay might be inhibited. Gorshin and Krapivina (1969), considering this phenomenon, suggested that the use of terms such as "mould", "staining" and "soft rot", used to describe microfungi colonising wood on the basis of the gross effects produced by them in wood, should be discontinued. They suggested that all microfungi colonising wood should be referred to as "micromyces" thus overcoming the ecological connotations (and the lack of differentiation, Eslyn, 1967) associated with the use of descriptive terms.

In comparison with the amount of decay which can be produced by basidiomycetes, the amounts of decay which can be produced by microfungi in large-dimensioned timbers is slight in the short term (Savory, 1954(b)). However, whereas preserved wood is generally resistant to basidiomycete colonisation, wood treated with a wide range of preservatives have been shown to be susceptible to microorganism colonisation and decay (Gorshin and Krapivina, 1969). This colonisation and decay may occur extremely rapidly (Butcher, 1971) and may occur even in wood treated

with copper chrome arsenic solutions (Greaves, 1972).

In view of the indeterminate behaviour of microfungi in apparently both passively colonising wood and in acting as soft-rot fungi, and of the evidence of their colonisation and decay of preserved wood, particularly when in soil contact, an understanding of the ecology of these microfungi is obviously desirable, particularly from the viewpoint of developing rapid methods of assessing the efficiency of wood preservation against them.

1.1.2. Preservative Assessment: the soil-burial method

Savory and Bravery (1971) in reviewing the literature on methods of determining the effects of wood preservatives on microfungi which cause soft rot, have pointed out that with reference to laboratory studies, unlike the basidiomycetes which can decay wood when only water is added, the microfungi in general require further nutrition before decay can be produced by them within short time periods. They have also pointed out that, as yet observation of "sufficiently rapid attack of softwoods by single organisms" to allow assessment of preservative efficiency within short time periods has not been made.

Comparing agar culture with soil-burial methods, Savory and Bravery (1971) considered that the advantage of soil-burial tests was that test specimens were subject to the influence of a natural soil microflora, and that softwoods were attacked more rapidly than by pure culture methods. It was also considered that soil could be remoistened without introducing contaminating micro-

organisms, and nutrients could also be added to facilitate the development of particular organism groupings. Chemical reaction between Abrams salts (frequently used in wood microbiology, Savory, 1954(a); Butcher, 1968), resulting in detoxification of some preservatives, does not occur in the soil block test situation.

The wood soil complex used for assessing the efficiency of wood preservatives provides an ecological niche of severe decay potential activity (Allsopp, 1973), in which soil micro-organisms, which may range from basidiomycetes to bacteria, may penetrate the wood and, if the colonising organisms posses the necessary wood-degrading enzymes, utilise it. This utilisation produces a deterioration in quality of the wood which results in a loss of weight or strength. The degree of weight or strength loss is a measure of liability to decay in a practical situation and hence may be used to assess preservative efficiency.

Microfungal colonisation of dried wood in soil contact tends to be limited to the outer layers of the wood adjacent to the soil-exposure surface (though "staining fungi" may penetrate somewhat deeper), whereas basidiomycetes apparently successfully colonise wood in depth (Banerjee and Levy, 1971). Decay of small wood blocks in a soil-contact situation, where decay in depth is inhibited by the dimensions of the sample, may generally be attributed to the activity of soft-rot fungi. Hence, it is possible to assess the efficiency of wood preservatives against microfungi by treating

small wood blocks with the required preservative and then subjecting them to soil burial for defined time periods before determination of weight or strength loss.

It is postulated that, during soil burial, the soil and wood may be considered as a single ecological unit with the wood acting essentially as a carbohydrate reservoir, and the soil acting as a reservoir for micro-organisms, moisture and nutrients. One of the most important of the latter for the development of soft-rot fungi is nitrogen (Savory, 1954(a), 1954(b); Cartwright and Findlay, 1958; Duncan, 1960(a); Levi and Cowling, 1969; Levy, 1973) in which most woods, particularly softwoods, are deficient (Cowling and Merrill, 1966). Since it is assumed that the wood soil complex may be regarded as a single ecological unit, it is reasonable to expect that a cross-flow of materials between the wood and the soil would occur. This crossflow should facilitate the development of the indigenous soil population, for some of the individuals of which, the wood-rotting basidiomycetes, the wood would be another easily-available nutritional substrate, whereas for some of the others, the microfungi and bacteria, the wood might be totally nutritionally unavailable, but might be passively colonised as an inert substrate (Levy, 1973).

1.2. Purpose of Study

Microfungal colonisation of wood has been related both to high moisture levels in wood and also to the presence in sapwood of simple sugars (Savory, 1954(a),

1966; Cartwright and Findlay, 1958; Scheffer, 1973). Similarly Levy (1969) and Banerjee and Levy (1971) have suggested that nutrient distribution in wood might influence the extent of wood colonisation and the succession of organisms colonising wood in soil contact. As yet, however, it has not been explained why microfungi colonise green wood in depth, sometimes causing staining but generally without producing soft rot, and fail to colonise dry wood in depth but may superficially produce soft rot.

Considering the work of Corbett and Levy (1963(a), 1963(b)), Levy (1969) and Banerjee and Levy (1971) on the successions of organisms colonising wood, that of Savory (1954(a), 1954(b)) and Duncan (1960(a), 1960(b)) on soft-rot production by microfungi, that of King (1972) on the potential decay abilities of blue-staining and mould fungi, and that of Cowling (1970) on the role of nitrogen in wood deterioration, it was postulated that the apparently indeterminate behaviour of microfungi in sometimes passively colonising wood and at other times producing soft rot might be related to nitrogen deficiencies.

The purpose of this study was therefore to examine the phenomenon of soft-rot production by micro-organisms in wood in soil contact with particular reference to nitrogen nutrition.

1.3. Working Hypotheses and Initial Experiments

Two relevant observations are apparent from the literature:

(i) that the quantities of nitrogen in wood, particularly softwoods, are insufficient to support significant amounts of soft-rot production (Armstrong and Savory, 1959) and cellulase production (Levi and Cowling, 1969) in short time periods;

(ii) that soft-rot cavities are produced by microfungi, sometimes after colonisation of green wood (Umezurike, 1969) but particularly after wood has been buried in soil e.g. in fence-post material (Savory, 1954(b), Duncan, 1960(b)) or has been in a water-logged condition.

A working hypothesis was developed from these observations as follows:

(a) If wood is deficient in nitrogen particularly for microfungi, then microfungal production of soft-rot cavities in wood cannot take place unless ancillary nitrogenous nutrients are available to the wood-colonising organisms.

(b) In a soil-contact situation, where soft rot is typically produced, these ancillary nitrogenous nutrients will be provided

by the soil and transferred in some form into the wood.

It was considered that this nitrogen transfer might occur in two possible ways:

(i) by the diffusion of nitrogenous salts into wood; and

(ii) by translocation of nitrogen from soil in the form of colonising organisms including nitrogen-fixing organisms, the mycelium or cells of which, upon autolysis, would supply a nitrogen source to secondary colonising cellulolytic microfungi.

(i) Garrett (1963) suggested that little "free" nitrogen would be available in soil as there would be considerable competition for the small amounts of nitrogen present there by the spectrum of soil-inhabiting organisms. Similarly, Nikolskin (1959) suggested that up to 75% of the nitrogen in soil was in the form of its component micro-organisms. It was therefore considered that the possibility of significant nitrogen diffusion into wood in the form of salts might not be great (unless the soils were recently fertilised).

(ii) Simple sugars including the cell contents of green wood stimulate rapid fungal colonisation of wood (Findlay, 1941) and support the growth of most fungal species (Garrett, 1963). Initial colonisers of wood might generally be assumed to

be sugar fungi which appear to be particularly adapted to rapid growth and sporulation on materials containing simple sugars (Garrett, 1963). Similarly, however, many cellulolytic wood-colonising microfungi also grow extremely rapidly on simple sugars (King and Eggins, 1973), and hence these might also be numbered among the primary colonisers. As the small quantities of sugars present in wood are depleted by these organisms, but without nitrogen beyond that present in wood, the initial microfungal colonisers, whether cellulolytic or non-cellolytic, would senesce and autolyse, and thus provide a nitrogen source to activate the cellulolytic activity of passively-colonising cellulolytic micro-organisms. As a result of the cellulolytic activity of these species, further carbon sources to supply the needs of the passively colonising non-cellulolytic micro-organisms would then be provided. (This does not imply a sequential colonisation of wood does not occur, but it may be that as many microfungi have been shown to be potentially cellulolytic. The consistent re-occurrence of certain cellulolytic species, e.g. Trichoderma sp. in the different stages of colonisation of wood (Butcher, 1968; Sharp and Eggins, 1970a; Sharp and Levy, 1974) might be as much related to nitrogen nutrition as to carbon nutrition).

An exploratory experiment was therefore designed to determine

(a) whether nitrogen migrated to wood in soil contact;

(b) the means by which this migration occurred, and

(c) whether nitrogen migration was related to soft-rot production.

The wood of Sitka spruce (Picea sitchensis Carr.) possesses a non-durable inner sapwood region and is greatly deficient in nitrogen (Cowling and Merrill, 1966). Consequently, it might be considered that test blocks of Sitka spruce would require extra nitrogen before they could be decayed by soft-rot fungi. If sapwood was buried in soil in a green condition, a situation would be provided in which the simple sugars present in the sapwood might stimulate colonisation by sugar fungi. It was considered that this colonisation would produce an increase in the total nitrogen content of the wood.

The inner sapwood being non durable and not containing simple sugars if buried in similar fashion might enable nitrogen diffusion from soil into wood to be measured because it was assumed that intensive fungal colonisation would not occur. Comparison of the results of these experiments with uninoculated control blocks of wood maintained at the laboratory would indicate the extent to which nitrogen moved from soil into both sapwood and inner sapwood either by biological translocation or by diffusion. Similarly, it would be possible, by

microscopic examination of both sapwood and inner sapwood, to determine whether nitrogen transfer from soil to wood was correlated with soft-rot production.

The test blocks used for this experiment were designed in such fashion that each block could be converted into a number of matching sections for various analytical purposes, and also into layers at predesignated distances from the soil-contact surface. (A full description of the test blocks used is presented in Chapter 3, Expt. 3.2: "Materials and Methods".) The blocks were prepared in a green condition, sterilised using the reciprocal tyndallisation technique of Ricard (1971), and placed in contact with soil for a period of three days before incubation over water for a period of 12 weeks. It was envisaged that a series of such experiments would be carried out, increasing the period of soil contact and decreasing the period of incubation over water for each further experiment. It was hoped that this procedure would permit a minimal pick-up of nitrogen from the soil in initial experiments but allow inoculation with micro-organisms. By increasing the initial period of soil contact, it was hoped to determine the threshold level of nitrogen pick-up at which decay could begin. It was considered that it might also be possible to carry out isolations from the wood during these experiments to determine those species of the microfungi colonising the wood which occurred at the various nitrogen pick-up levels.

The results of the first experiment, however,

showed that after the three-day soil-contact period and a 12-week period of incubation over water, the nitrogen levels in the test blocks were far higher than matching unburied control blocks maintained in the laboratory and allowed to dry slowly. This was extremely puzzling as it was considered that the period of soil contact was insufficient to account for the startling differences apparent between control blocks and the soil-inoculated blocks (Table 1.1.). As the blocks both sapwood and inner

TABLE I.I.

Nitrogen Content of Spruce Blocks before and after Burial

	Control Blocks	Inoculated Samples
Sapwood	0.057%	0.156%
Inner sapwood	0.054%	0.079%

sapwood were not heavily colonised as assessed by microscopic examination, it seemed improbable that the increased nitrogen of inoculated blocks was attributable to nitrogen translocation from soil. Biological introduction of nitrogen was further disproved by the fact that inner layers of blocks, which were not apparently colonised and were not in contact with the soil, showed levels of nitrogen as high as the colonised soil-contact layer.

For the reasons stated above and also because

(a) it was considered that the amounts of soluble materials capable of being diffused from soil into saturated green

wood would be slight in a three-day soil-contact period;

(b) of the differences in nitrogen levels evident between inoculated sapwood and inner sapwood samples,

it was considered that diffusion of soluble nitrogen from soil into wood, and nitrogen-fixation activities of colonising organisms, could also be discounted as explanations for the differences in nitrogen contents evident between green and dried wood.

It therefore appeared that the most practical working hypothesis to explain this phenomenon was that spruce contained higher levels of nitrogen when in the green state than when it was dried. A review of the literature (presented in Chapter 2) showed that most previous analyses of nitrogen in wood had been undertaken on green wood alone, or on dried wood alone and that data were not available on the nitrogen status of wood specimens both before and after drying; i.e. the nitrogen content of wood had not been monitored in the transition of wood from a plant, to wood as a structural material. There was therefore no information in the literature to suggest why the differences in nitrogen content observed between the green and dried material should have occurred.

It was apparent that, if differences in nitrogen content existed between green and dried wood, the levels of these differences might have a considerable influence on the indeterminate behaviour of microfungi in some-

times decaying and at other times not decaying wood. Similarly, as the general working hypothesis of this thesis was envisaged to be that nitrogen movement from soil into wood enabled soft-rot microfungi to produce soft-rot cavities, it was obvious that if the nitrogen content of wood could change during an incubation period, measurement of movement of nitrogen from soil into wood would be impossible without further information on the nitrogen content of the wood itself.

It was therefore decided to investigate the nitrogen status of wood with reference to its distribution in green wood and any changes in such distribution on drying. This work forms the first part of this thesis. Changes in nitrogen content of wood in soil contact, initially envisaged to provide the major theme of this thesis, in fact provided a second theme which was related to, and dependent upon, the nitrogen distribution in the green material. These themes were concurrently investigated.

CHAPTER II

STUDIES ON THE DISTRIBUTION OF NITROGEN IN WOOD

2.1. INTRODUCTION

2.1.1. General

It is well established that nitrogen plays a critical role in the decay of wood both by fungi and insects. As early as 1934, Findlay showed that if nitrogenous materials were added to home-grown Sitka spruce, the rate of decay produced by basidiomycetes in laboratory culture would be increased. Hungate (1940) examined decayed wood with sporophores attached and found that nitrogen other than that which had intially been in the wood had taken part in the decay process, a finding confirmed by Cowling and Merrill (1965a). These workers presumed that in a natural situation, nitrogen was obtained from sources outside the wood probably from soil.

Hungate (1941) also showed the importance of nitrogen for termites, and showed that the rate of growth of termites increased if nitrogen, particularly in a yeast form, was added to wood. He also showed that slightly decayed wood apparently suffered greater termite decomposition because as wood rotted, the carbohydrate content decreased and the nitrogen content increased. Fisher (1941) proposed a similar hypothesis and suggested that this was the reason that decayed wood was more susceptible to Xestobium rufovillosum Bletchly (1959) suggested that the outer growth rings of Fagus sylvatica were most suitable for colonisation by Anobium spp. (Becker, 1942, in Bletchly , 1959) because of their high nitrogen content. Becker (1963), suggested that older wood needed additional nitrogen before it could be colonised by Hylotrupes spp.

The nitrogen requirements for basidiomycete fungi would seem to differ from those of the microfungi: Findlay's (1934) findings showed that additional nitrogen added to wood in laboratory culture produced increased decay rates. Further work by Hungate (1940), however, showed that addition of nitrogen to wood blocks in laboratory culture was not necessary for the decay process to occur. An analysis of the nitrogen content of wooden blocks and their fungal inocula both before and after the incubation period showed that the total nitrogen contents were substantially the same thus indicating that nitrogen other than that available in the combined wood blocks and inocula was not necessary for decay to proceed. Experiments carried out with Merulius lacrymans and other basidiomycetes (Klingstrom and Oksbjorg, 1963; Starfinger, 1967) corroborated these results indicating that basidiomycetes could decay wood without addition of extraneous nitrogen materials.

Savory (1954b) showed that soft-rot cavities could be produced in wood by Ascomycetes and Fungi Imperfecti, a finding corroborated by Duncan (1960b) and Krapivina (1960). Siu (1951) showed that relatively large quantities of nitrogen were required by cellulolytic soil microfungi before they became actively cellulolytic. Cartwright and Findlay (1958) considered that the extent of decay produced by Chaetomium globosum could be directly related to the nitrogen content of the support medium, and Armstrong and Savory (1959) found that 5-mm beech veneers had to be soaked in triple-strength Abrams salt solution before significant tensile strength losses could be produced by Chaetomium globosum. Kaune (1970) found that the effect of different nitrogen sources on the

decay rate of six fungal species was so similar as between one fungus and another that he could calculate mean values of increased decay for each nitrogen source.

Although it is evident that the production of soft-rot cavities in wood has been greatly associated with added nitrogen from support media or from soil, it has also been shown that soft-rot cavities may be produced in wood after depletion of cell contents by certain blue-staining fungi, e.g. Botriodiplodia theobromae (Campbell, 1959; Umezurike, 1969). Umezurike considered that starches and simple sugars were utilised before soft-rot cavities were produced, and if these materials were not present before colonisation, then soft-rot cavities would not be produced. An explanation of this soft-rot activity was not proposed from the viewpoint of nitrogen nutrition.

2.1.2. Nitrogen in Wood

Cowling et al (summarised in Cowling, 1970) postulated a theoretical model for the role of nitrogen in wood decay. Cowling and Merrill (1966) considered that herbaceous plants typically contained 1-5% nitrogen whereas wood normally contained 0.03-0.1% nitrogen. At the cambial layer of wood, nitrogen levels might be similar to higher plants in general (1-5%), but as a result of thickening of the wood element with cellulose and lignin during the dilution phase (that period of time, about 30 days, from formation of a wood cell to its maturity), this overall percentage would be reduced. They also considered that a parenchymal death phase and an elution phase occurred in the life of a tree. They considered that if tissue formed at the cambium was to perform a mechanical function in the tree (the vertical tissue), nitrogen remaining

in the lumina of cells would be solubilised and eluted upwards in the transpiration stream of the huge volume of tracheal fluid (the elution phase). If the tissue formed at the cambium was to perform a storage function (the parenchymal or ray tissue), this tissue might remain living for some considerable time after senescence of vertical tissue had occurred. The parenchymal death phase refers to that time in which parenchymal cells used for storage begin to die off.

Merrill and Cowling (1966) considered that the sapwood of wood was not totally comprised of dead tissue, that much of it was living, and consequently nitrogen would be found in the lumina of those living cells (Cowling and Merrill, 1966). Merrill and Cowling (1966) showed that a close correlation existed between total nitrogen and rate of senescence of parenchymal tissue and also between total nitrogen content and the number of parenchyma cells in wood tissue. They also found that nitrogen apart from that in the lumina of cells was mainly present in wood cell primary walls in a proteinaceous form. They also showed that nitrogen could also be present in wood in the form of free amino acids, peptides and nucleic acids confirming the work of Bletchly and Farmer (1959), who showed that the greater proportion of nitrogen in wood was in a non-soluble form. Laidlaw and Smith (1965) and Baker Laidlaw and Smith (1970) produced analyses of the proteins of Scots Pine (Pinus Sylvestris) and confirmed that the major portion of nitrogen in wood was in the form of protein in the cell wall, with less significant amounts of free soluble amino acids also present.

It has been frequently suggested (Gaumann 1936 in

Findlay 1931) that wood has greater susceptibility to decay if felled in summer rather than in winter. Bletchly (1966a, 1969a) showed that wood contains a higher nitrogen content when felled in spring than when felled in Autumn and Levi and Cowling (1968) confirmed this observation for oak. It has also been shown that certain wood tissues have higher nitrogen contents than others. Merrill and Cowling (1966) showed that greater proportions of nitrogen were found in the bark of gymnosperms than augiosperms; in the latter nitrogen was found to be distributed more evenly throughout the trunk. It has similarly been shown that rootwood has higher nitrogen contents than stemwood for eight wood species (Platt, Cowling and Hodges, 1965).

2.1.3. Physiological importance of Nitrogen in Wood to Fungi

Merrill and Cowling (1965a) considered that the limited amounts of nitrogen in wood inhibited its decay by micro-organisms, and Cowling and Merrill (1965a) showed that most wood species in temperate zones had carbon (C) to nitrogen (N) ratios of 350-1250: 1. It was considered that these high C : N ratios might inhibit the production of cellulases by microfungi and hence an excess of nitrogen would have to be added to wood before decay could occur. Merrill and Cowling (1965b) carried out studies with *Polyporus versicolor* and *Lenzites trabea* on wood samples removed from rings 1 (at cambium), 2, 3, and 16 of *Populus grandidentata*, the samples from the different rings being of different nitrogen content. After incubation of these samples for six weeks a positive straight line correlation was obtained between the nitrogen content of the wood and the rates of decay by both fungi. It was found that threefold differences in nitrogen content

resulted in twofold differences in decay rate. It was also suggested that the high nitrogen content of the pith regions (Merrill and Cowling, 1965a) might contribute towards the incidence of heart-rot in wood.

Cowling and Merrill (1965a; 1965b) analysed the vegetative mycelium of Fomes annosus, Fomes laricus and Polyporus betulinus and found that the nitrogen content varied between 0.23% and 3.27%. Mycelial nitrogen levels were found to vary in direct proportion to the nitrogen content of the substrate on which the organisms grew. If the organisms were grown on laboratory media, the mycelial nitrogen content could be as high as 5.02%, whereas if grown on wood could be as low as 0.18%. Analyses of hymenial layers, spores and sporophores, along with decayed wood adjacent to those fruiting bodies revealed that the older portions of fruiting bodies contained less nitrogen than the younger portions which suggested that the organisms in question were capable of some form of nitrogen retrieval. Analyses of the wood adjacent to such fruiting bodies showed that not all nitrogen was removed from the wood which implied that the nitrogen required for sporulation by wood-destroying fungi came either from large amounts of wood or from sources outside the wood itself.

Experiments with the autoclaved mycelium of F. annosus, P. betulinus and F. laricus showed these test species were capable of utilisation of their own autoclaved mycelium as sole source of nitrogen, and that a linear relationship existed between the nitrogen content of the growing mycelium and the log of the C : N ratios in the autoclaved material over the range 8000 : 1 to 82 : 1.

In further experiments on the growth of P. versicolor on various types of nitrogen compounds present in wood, and in its own mycelium, Merrill, Levi and Cowling (1966) showed that asparagine, aspartic acid and glycine were used equally well. Proteins, RNA and DNA were also utilised, although the nucleic acids did not support equally good growth. It was found that ammonium nitrogen sources supported an intermediate growth to the organic nitrogen sources, and that nitrates and nitrites did not support any growth of the organism. Li, Lu, Trappe and Bollen (1967) corroborated the latter fidings and showed that Armillaria melia and Poria weirii, and many other basidiomycetes could not utilise nitfate nitrogen because of a lack of ability to produce nitrate reductase. They also suggested that streptomycetes, which generally utilise nitrates, might preferentially grow in soils containing nitrates, and consequently produce an inhibition of butt rot produced by basidiomycetes by their competitive growth in the soil.

In further experiments on the suitability of various fractions of fungal mycelium as nitrogen sources, Levi, Merrill, and Cowling (1968) showed that mycelium could be used as the sole source of nitrogen but not of carbon for Polystictus versicolor, Fomes applanatus, Lenzites trabea, Poria monticola, Chaetomium globosom, Ceratocystis coerulescens, and Trichoderma viride. It was shown that some of the organisms could use the mycelium of others as sole source of nitrogen, and in the presence of glucose, P. versicolor and L. trabea used their own autoclaved mycelium to support growth. It was also found that free amino acids in mycelium supported better growth than cell wall protein or nucleotides

and that the ascomycetes were less able to survive on their own mycelium than the basidiomycetes; e.g. C. coerulescens and C. globosum were unable to live off their own cell wall material but both P. versicolor and L. trabea were able to use their own extracted mycelium as sole source of nitrogen. The results from these experiments also showed that generally, the white rot fungi produced more growth on all the nitrogen sources used than either the brown rot fungi or the microfungi.

Levi and Cowling (1966) conducted a series of experiments on the relationship of C : N ratios to cellulolytic activity for a number of microfungi and basidiomycetes using the techniques of Rautella & Cowling (1965). The C : N ratios used ranged from 10 : 1 to 1000 : 1 and the results showed that only white-rot fungi produced clearing at all C : N ratios. Soft-rot fungi grew at all C : N ratios but only produced clearing at the lower levels. Staining and mould fungi behaved in similar fashion to the soft-rot fungi. It was therefore concluded that the microfungi in general required more nitrogen than wood possessed to rot wood but not to colonise it. Further studies (Levi and Cowling, 1969) showed that if nitrogen was added to wood blocks, the weight losses produced by microfungi were much increased in comparison with those produced in wood blocks without nitrogen addition thus corroborating the evidence of Cartwright and Findlay (1958).

The work of Levi and Cowling (1969) also showed that preferential allocation of nitrogen could occur when fungi were grown on a range of media containing different C : N ratios. If organisms were grown on media with high C : N

ratios, the organisms might have low mycelial nitrogen content whereas when grown on media with low C : N ratios the organisms might have high mycelial nitrogen content. At the very low C : N ratios, e.g. 4 : 1, a decreased growth of P. versicolor was recorded with an increase in pH of the medium. These results confirm the results of Szegi (1968) who showed that at C : N ratios of less than 20 : 1, cellulolytic activity of many microfungi decreases and ammonia liberation from the medium increases. A further study of the inter-relationship of C : N ratio with decay rate (Darbyshire, Wade, and Marshall, 1969) is in agreement with the work of Levi and Cowling (1969). This study showed that if sugars were added to media supporting the growth of Trametes versicolor (a major cause of "dieback" of apple trees in Australia), a decrease in decay of the wood of Malus sylvestris was noted. If additional ammonia but no additional glucose was added to the medium the decay rate of Malus sylvestris was seen to increase. If both ammonia and glucose were added, little effect was seen on the decay rate, but the amount of mycelial growth was seen to increase considerably.

2.1.4. Discussion and evolution of Project

Summarising the literature presented above, it can be seen that wood is deficient in nitrogen to most fungi from basidiomycetes to mould fungi, and that the nitrogen which is present in wood is primarily in the form of protein bound in the cell walls, and therefore in an unavailable form to fungi which cannot synthesise cellulases to break down those walls.

It is apparent from the literature that the importance of nitrogen in wood is best expressed in terms of its ratio to the carbon present, and that generally C : N ratios in wood range from 350-1250 : 1. C : N ratios at these levels will support the growth and cellulase production of basidiomycetes and will support growth but not cellulolytic activity of the ascomycetes, the ratios being too high to support cellulolytic activity of the less highly evolved and less selective (Gorshin and Krapivina, 1969) microfungi. Consequently, it was considered that the C : N ratios commonly found in wood were too great to support the soft-rot activity of micro-organisms which colonise wood in ground contact.

The literature also shows that in the "natural" situation a greater amount of nitrogen than is contained in wood takes part in the decay process. This "extra" nitrogen is not necessary for the basidiomycetes to produce decay but considerably influences the rate at which decay is produced by them. Analyses of sporophores attached to rotten wood showed that their total nitrogen content was greater than that theoretically present in the wood at the initiation of decay.

Garrett (1963) suggested that the successions of primary

and secondary colonisers colonising cellulosic materials in soil might be related to the nitrogen requirements of the colonising micro-organisms. This organism succession also occurs in wood. As the limited nitrogen content of wood is mainly located in the cell walls, it will not be available to non-cellulolytic microfungi, and the cellulolytic microfungi require a reserve of available nitrogen before they can produce cellulases. It would therefore seem that decay of wood by these fungi without excess nitrogen is theoretically extremely difficult. The only apparent explanations for the satisfaction of the nitrogen need of cellulolytic microfungi in wood in soil contact are one or more of the following:

(a) Nitrogen is microbiologically "fixed" in wood (Sharp and Millbank 1973) ;

(b) Nitrogen diffuses from soil into wood in the form of soluble salts; or

(c) The small quantities of simple sugars in wood stimulate colonisation by micro-organisms which passively penetrate; upon depletion of these simple carbohydrates, the organisms senesce and autolyse thus providing the nitrogen (in a highly amenable amino form, Griffin, 1972) necessary for the cellulolytic microfungi to produce cellulases as suggested in Chapter 1.3.

The results of initial experiments designed to test these hypotheses showed, as stated in Chapter 1, that considerable differences existed between the nitrogen levels of green spruce-wood blocks and those of matching control blocks allowed to dry in the laboratory. For the reasons stated in the

Introduction, viz. lack of colonisation in depth etc., it was considered that these differences were real, and were not due to any artefact produced by experimental conditions.

The literature did not indicate that such results were to be expected. Previous work (Bletchly and Farmer, 1959; Becker, 1962; Bletchly and Taylor, 1964; Laidlaw and Smith, 1965; Baker, Laidlaw and Smith, 1970) had been carried out on wood which had been dried by different methods. The work of Cowling (1970) did not indicate that nitrogen values for dried wood would be different from wood in the green condition.

Other workers have shown considerably lower nitrogen values for dried spruce sapwood than the levels for green sapwood shown by analyses conducted at this laboratory. Nitrogen levels given in the literature for spruce sapwood range from 0.04% and 0.07% for Picea sitchensis (Anon., 1960; 1962 ; Bletchly and Taylor, 1964); 0.06% for Picea excelsa (Becker, 1962); 0.09% for an un-named species of Picea (Merrill and Cowling, 1966); to 0.118% for Picea engelmanni (Cowling and Merrill, 1966).

The above results for P. excelsa, P. engelmanni, and the Picea sp. (i.e. the higher nitrogen values), were obtained from material grown in Germany and the U.S.A., while the lower results for P. sitchensis were obtained for material grown in the U.K. These latter results are some 50%-75% lower than those found for green spruce sapwood (0.156%)but approximately equal to those found for matching dried spruce specimens (0.056%) slowly dried at this laboratory and removed from stock also grown in the U.K.

The differences in nitrogen content between green and dried wood, if genuine, would indicate that the C : N ratio in wood in the green state is considerably less than that of dried wood. This lower C : N ratio might explain from a nutritional viewpoint the greater susceptibility of wood in the green state to colonisation by microfungi, and contribute towards an understanding of the ecology of microfungi colonising, and producing soft rot in wood.

It was therefore decided to investigate the apparent differences between nitrogen levels in wood in the green and in the dried condition im an experiment running concurrently with experiments to determine nitrogen movement from soil into wood.

2.2. EXPERIMENTAL

To determine changes in nitrogen content in spruce during drying, a series of experiments was carried out over an 18-month period between November 1972 and April 1974. Wood samples were removed from different forest sites and from sites in different forests to confirm observations made. The detail and results of these experiments are related in this section, and the results of all experiments are collectively discussed in 2.3. (Discussion and Conclusions).

2.2.1. Nitrogen Distribution in Green Spruce (Experiment 2.1.).

It was apparent from the literature that few data were available for the nitrogen content of green spruce grown in the U.K., and apparent decreases in wood nitrogen content on drying had not been recorded to the author's knowledge. As a first step towards determination of such losses, an experiment in which conditions replicating the initial experiment outlined in the Introduction (1.3) was designed, except on this occasion the purpose of the experiment was to determine any loss in wood nitrogen on drying.

A. Materials and Methods.

Three spruce trees two of which were Picea sitchensis Carr. and one Picea abies Karst, all planted in 1923, and growing in a mixed stand in the Forest of Dean, Gloucs., England, were felled in November 1972. Discs of 3 in. depth were removed at breast height from the two P. sitchensis trees and a disc of similar depth was removed from 5m above

breast height from the P. abies tree (the latter disc was removed at the higher point because of suspected butt-rot in the lower end of the tree). Each disc was marked at a point corresponding to the point on the trunk of the tree which had faced in the northern direction, the discs were then wrapped in polythene and returned to the laboratory where they were stored in a deep-freeze unit at -18°C until analysed.

At the laboratory, the distance between each fifth ring (measured from the cambium to the pith) was plotted on the northern, southern, eastern and western area of each disc. The discs were macroscopically examined, and it was seen that the northern and eastern portions of each disc contained significant amounts of compression wood. The discs were then converted and four sections extending from the cambium to the pith and representing the faces corresponding to the four cardinal points of the compass, were removed. The sections from the northern and eastern portions of each disc were discarded because of the presence of compression wood, and the sections from the western and southern areas were retained for analysis.

The western sections from each disc were selected for the preliminary experiment, the purpose of which was to see if nitrogen was lost on drying of wood. These sections were subdivided in a radial longitudinal direction into three subsections. One subsection was reserved for immediate nitrogen determination while a second subsection was reserved for immediate moisture determination. The remaining subsection was incubated at 20°C and at 95% R.H. (approximately) for seven days. It was hoped that this process might permit continued slow movement of water and possibly cell contents within these sections and inhibit the loss of moisture from

the exposed surfaces. By prolonging in this way the period of drying it was thought that the slow drying of control samples in the initial experiments might be replicated, and the possible loss by cellular respiration of materials other than water andperhaps including nitrogen would be increased. These subsections are referred to below as the "respired" samples.

The subsections for immediate nitrogen determinations for Trees 1 and 2 (Norway and Sitka spruce respectively) were converted into individual growth ring or two growth-ring groupings from the cambium to the pith and analyses were carried out on these individual units. Respired subsections, moisture subsections and all subsections from Tree 3 (the second Sitka spruce tree) were converted into groupings of five growth rings for analysis. The change from single-ring to multiple-ring analysis was decided upon to facilitate more rapid progress of the project and to even out ring-to-ring variation apparent in initial results.

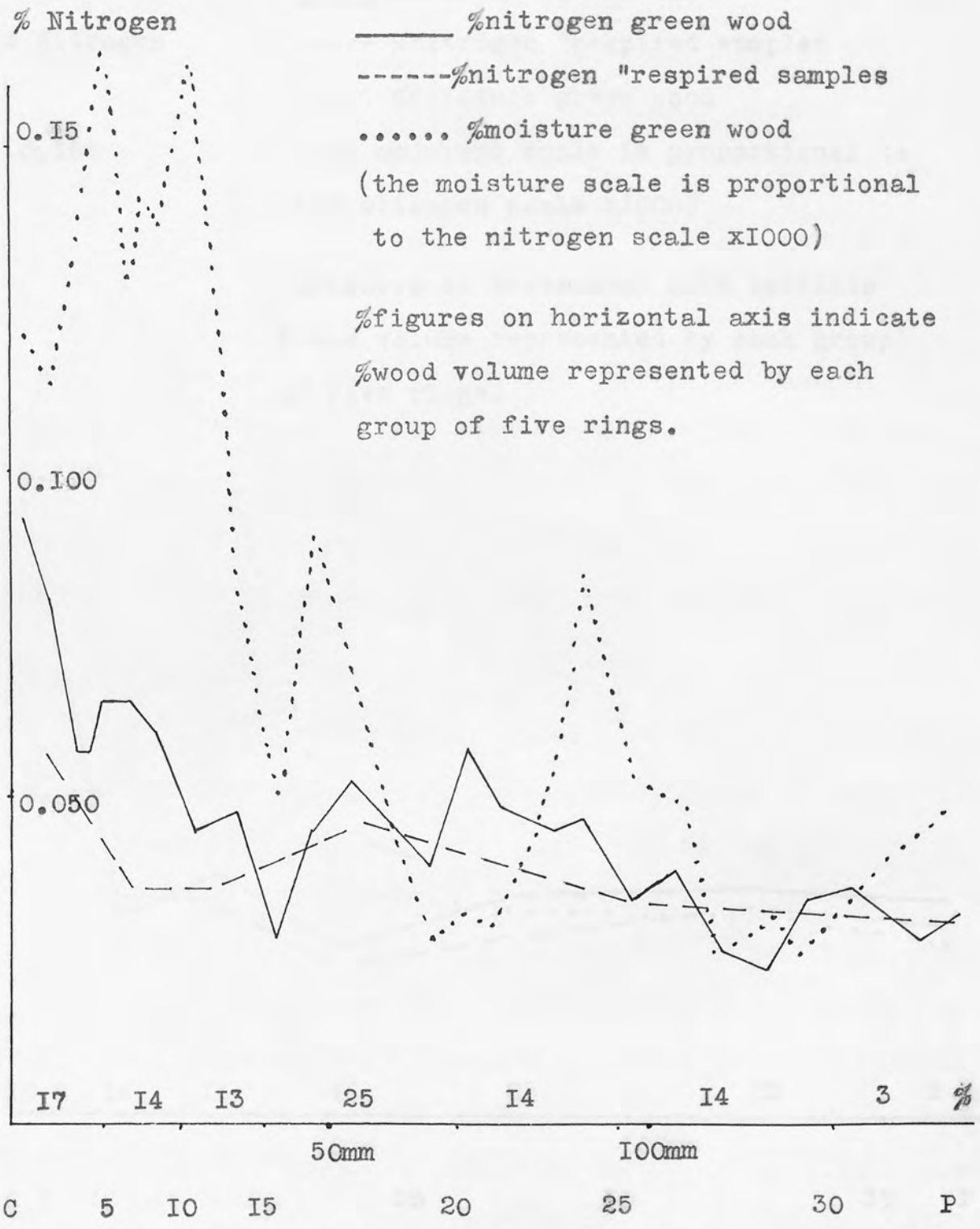
Moisture contents were determined on a dry-weight basis (Cartwright and Findlay, 1958) from the cambium to the pith. Material used for nitrogen determination was prepared after the manner of Laidlaw and Smith (1965) and nitrogen determinations, which were carried out in duplicate, were undertaken using the microkjeldahl method of Humphries (1956).

B. Results

Data for nitrogen content of green spruce,respired spruce, and moisture content area presented in Figs. 2.1, 2.2., and 2.3., which represent data from Trees 1 (*P. abies*) 2 and 3 (*P. sitchensis*) respectively.

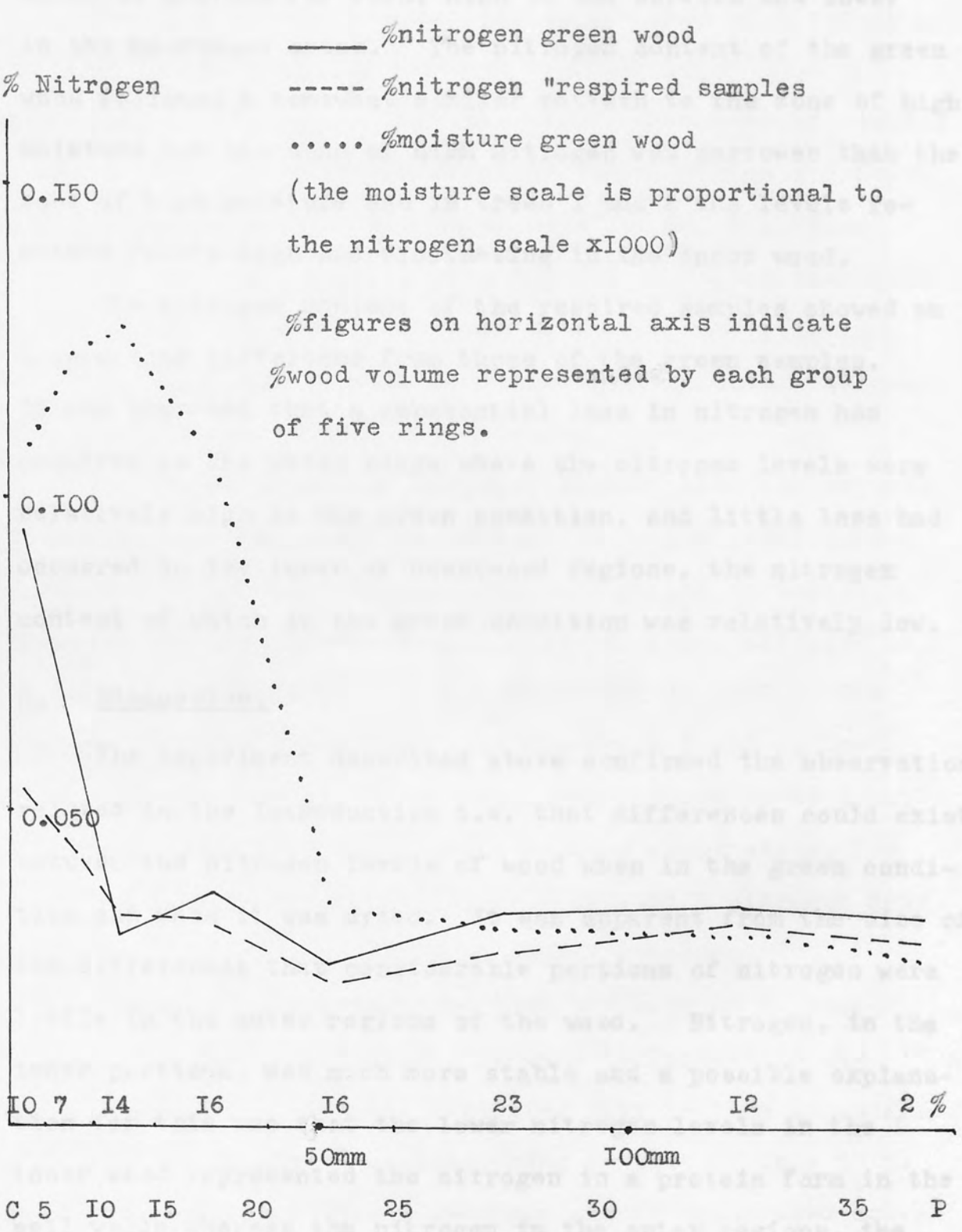
FIGURE 2.I Tree no. I western direction

FIGURE 2.2 Tree no. 2 western direction



Growth rings measured in mm from cambium (C) to pith (p)

FIGURE 2.3 Tree no. 3 western direction



Growth rings measured in mm from cambium (C) to pith (P)

The data for moisture content follows the usually accepted pattern for wood: high in the sapwood and lower in the heartwood areas. The nitrogen content of the green wood followed a somewhat similar pattern to the zone of high moisture but the zone of high nitrogen was narrower than the zone of high moisture and in trees 1 and 2 the levels remained fairly high and fluctuating in the inner wood.

The nitrogen content of the respired samples showed an interesting difference from those of the green samples. It was apparent that a substantial loss in nitrogen had occurred in the outer rings where the nitrogen levels were relatively high in the green condition, and little loss had occurred in the inner or heartwood regions, the nitrogen content of which in the green condition was relatively low.

C. Discussion.

The experiment described above confirmed the observation related in the Introduction i.e. that differences could exist between the nitrogen levels of wood when in the green condition and when it was dried. It was apparent from the size of the differences that considerable portions of nitrogen were labile in the outer regions of the wood. Nitrogen, in the inner portions, was much more stable and a possible explanation for this was that the lower nitrogen levels in the inner wood represented the nitrogen in a protein form in the cell walls whereas the nitrogen in the outer regions, the sapwood, contained nitrogen in two forms, a labile and a non-labile form. This latter is hereafter frequently referred to as "structural" nitrogen since it is contained within the structural material of the wood.

This conclusion was consistent with the published

literature. Cowling and Merrill (1966) and Merrill and Cowling (1966) had shown that some of the nitrogen in wood was in a soluble form thus confirming the work of Bletchly and Farmer (1959) who showed that "traces" of soluble nitrogen were present in Corsican pine. The work of Cowling et al. was subsequently confirmed by Baker Laidlaw and Smith (1970) who showed that "slight amounts" of soluble amino acids were present in Scots pine sapwood. It was therefore presumed that the labile nitrogen in the sapwood might correspond with the soluble or "elutable" nitrogen mentioned in the literature.

Most previous work on nitrogen in wood has been related to the nitrogen requirements of basidiomycetes rotting wood. Consequently, greater emphasis has been placed on total nitrogen content and the distribution of total nitrogen in wood than on soluble nitrogenous materials in wood. The results presented above show that for trees 2 and 3, the nitrogen content of the outer rings had reduced by at least 40% after one week of drying with reduced evaporation, and indicated that in green wood, levels of labile nitrogen were nearly as great as the levels of stable nitrogen.

It is generally considered that the bulk of nitrogen available to fungal colonisers of wood is located in the cell walls and consequently available only to thos organisms capable of synthesis of wood-decaying enzymes. Thus it is generally eonsidered that microfungi cannot decay wood unless ancillary nitrogen sources outside the wood are available to these fungal colonisers.

Few data are available on soluble nitrogen in wood, and the results presented above indicated that significant amounts

of nitrogen existed in a labile form. If the labile nitrogen now demonstrated corresponds with soluble nitrogen, then soluble nitrogen levels in wood at these magnitudes may considerably influence the extent of colonisation and decay provided by microfungi in wood.

As the purpose of this project was to examine wood colonisation and decay by microfungi (the early colonisers of wood) and since soluble nitrogen sources might considerably influence the extent of such colonisation, it was decided to investigate further the amounts and distribution of soluble nitrogen in wood.

2.2.2. Soluble Nitrogen in Green Spruce (Experiment 2.2.).

A. Materials and Methods.

The materials used for this experiment were the southern sections and the remaining material of the western sections from Trees 1, 2 and 3 used in Expt. 2.1.

Nitrogen determinations were carried out on both green wood, and wood which had been extracted with cold water. Determinations were carried out from pith to cambium of each section using single growth rings where possible, and in groups of growth rings where individual rings were too narrow, for green nitrogen determinations for trees 1 and 2. Water extracted samples for all trees were tested in five-ring groupings extending from the cambium to the pith, as were green nitrogen determinations for Tree 3. All nitrogen determinations were carried out in duplicate. The general detail of samples preparation and analysis, unless otherwise stated, is as is presented in Expt. 2.1.

Water extraction of wood sections was carried out according to TAPPI Standard No. TI m 59 but without the prior alcohol benzene, or alcohol water extraction.

B. Results.

Data for nitrogen content of green and water-extracted spruce are presented in Figs. 2.4, 2.5, and 2.6. The data for green spruce, western direction is reproduced from the results of Expt. 1 (2.2.1).

These results show that extraction with cold water produced a fall in nitrogen content. This fall was particularly obvious in the outer sapwood regions (the first 10 to 20 rings from the cambium or 40mm). Heartwood regions showed little nitrogen loss in relation to outer sapwood regions.

FIGURE 2.4 Tree no. I Nitrogen Content

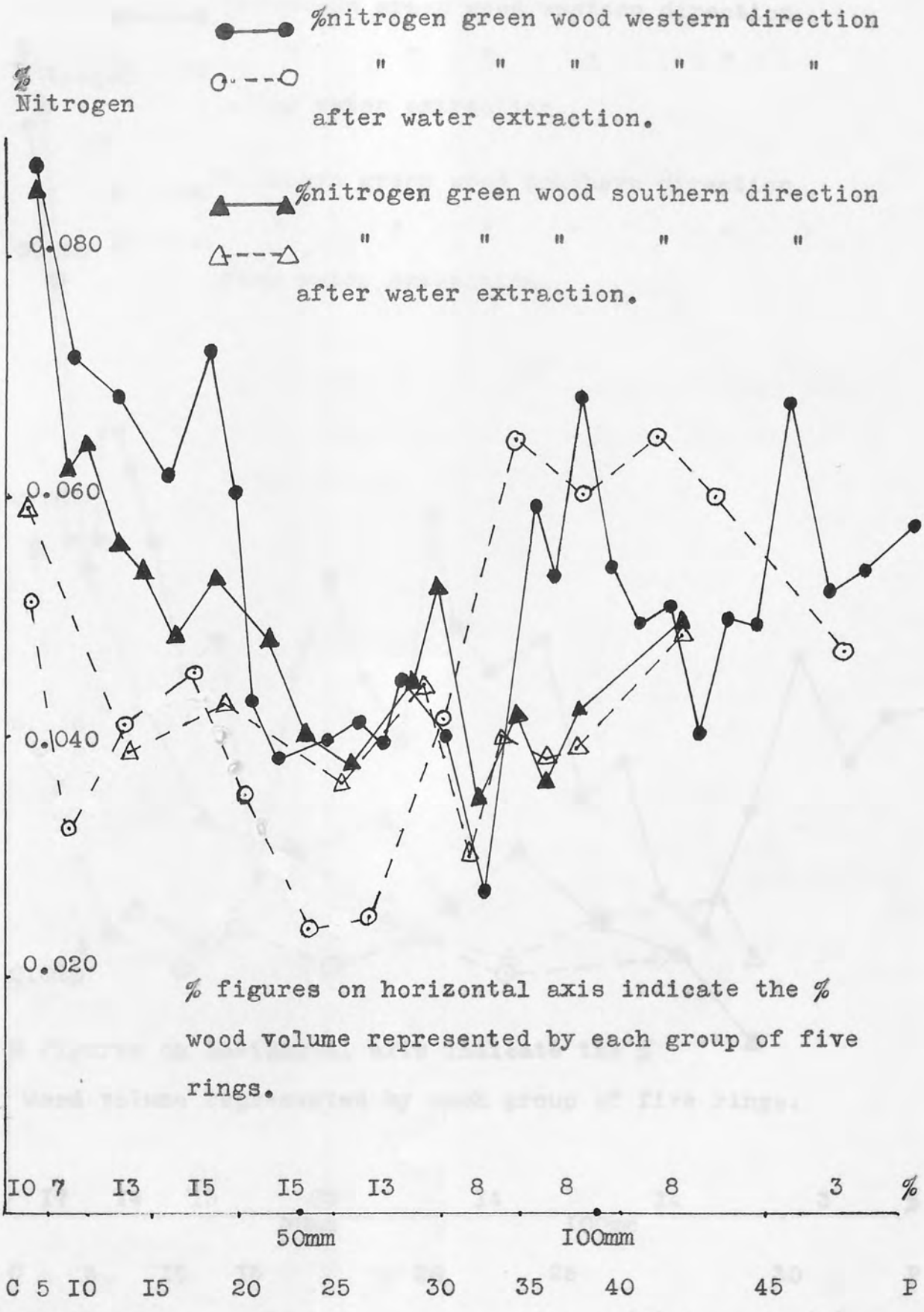


FIGURE 2.5 Tree no. 2 Nitrogen Content

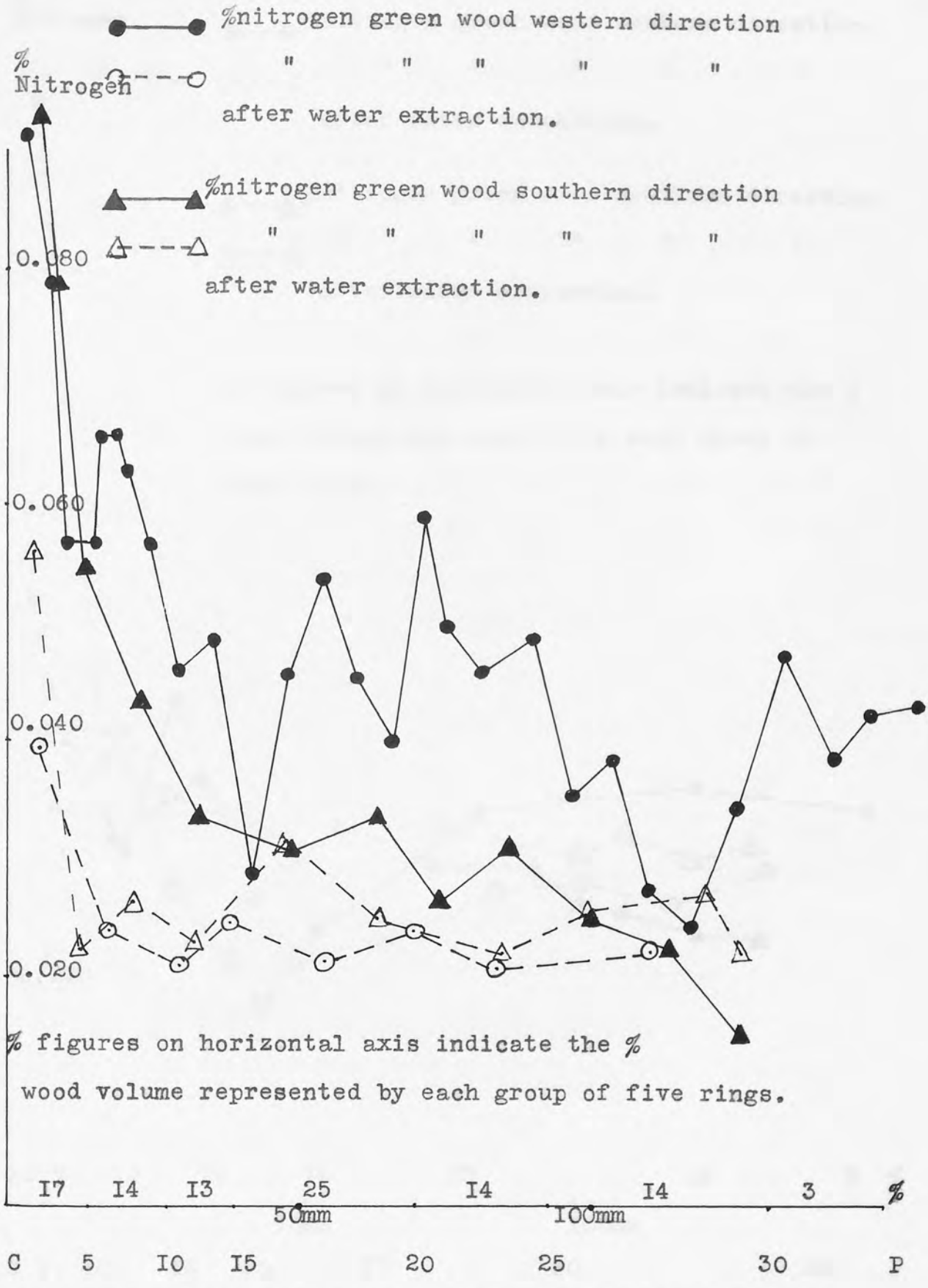
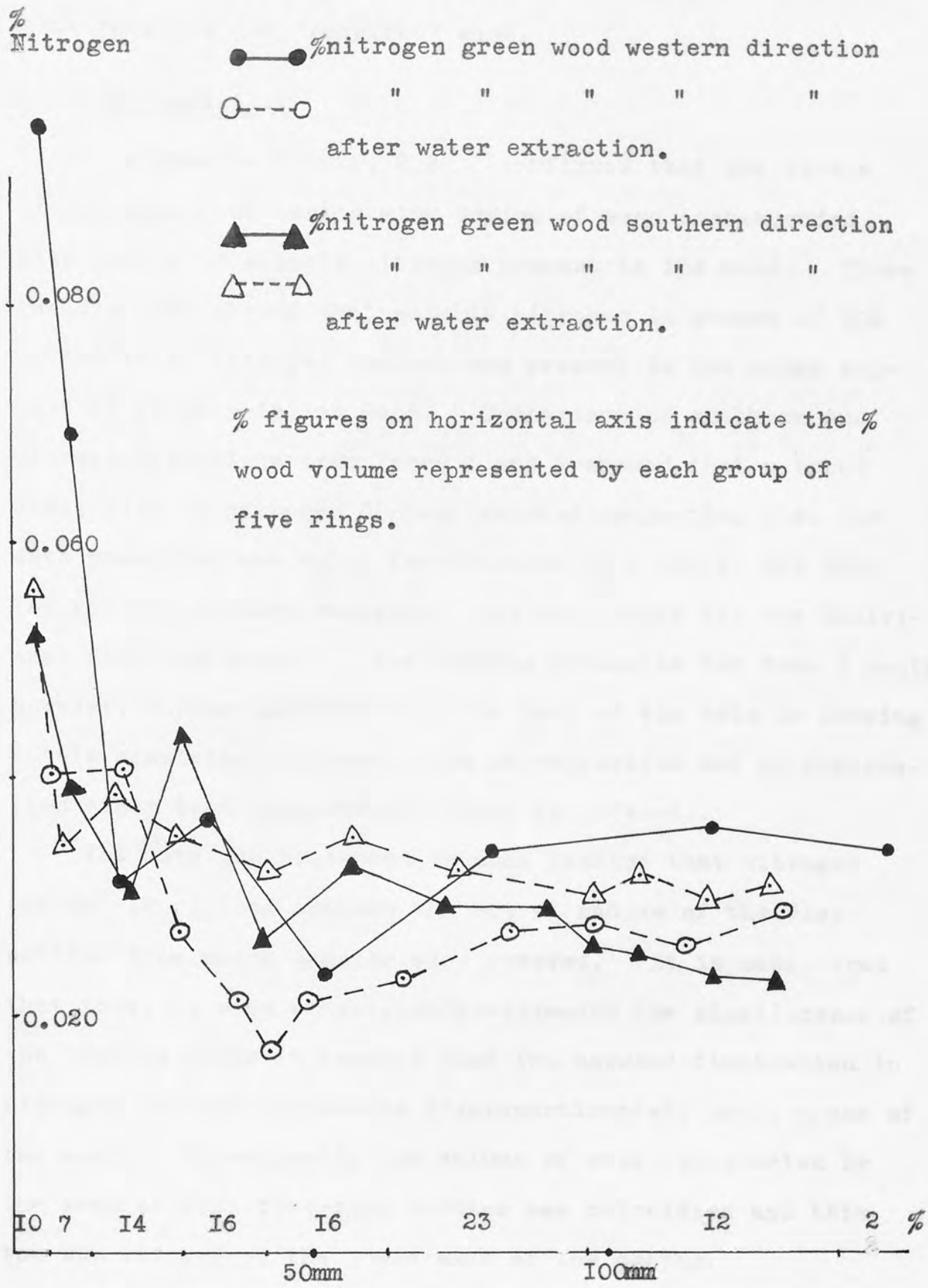


FIGURE 2.6 Tree no. 3 Nitrogen Content



Generally, the drop in nitrogen content was greater than that recorded for "respired" wood.

C. Discussion.

The results of Extp. 2.2 confirmed that the levels of nitrogen lost during slow drying of wood corresponded with levels of soluble nitrogen present in the wood. These results also showed that soluble nitrogen in excess of 40% of the total nitrogen content was present in the outer sapwood of freshly-felled wood. Comparison of southern and western directions from Trees 1 and 2 showed that a broad similarity in nitrogen content existed indicating that the data presented was valid for the disc as a whole, and that the general pattern emerging was not unique for the individual sampling points. The results presented for Tree 3 south, however, differ somewhat from the rest of the data in showing little consistent nitrogen loss on extraction and no explanation other than experimental error is offered.

The data are presented in such fashion that nitrogen content is plotted against the actual radius of the disc section from which samples were removed. It is considered that this, to some extent, underestimated the significance of the results since it appears that the sapwood fluctuation in nitrogen content represents disproportionately small areas of the wood. Consequently the volume of wood represented by the area of each five-ring section was calculated and this too was entered on the lower axis of the graphs.

The results therefore can be shown to indicate a somewhat more significant picture in respect of the amount of wood containing soluble nitrogen. The apparent steep decrease in soluble nitrogen content of the wood is a much more

gradual process related to volume than the graphs indicate. For example, in Tree No. 1 the amount of soluble nitrogen is a significant proportion of the total in the outer 20 rings (i.e. approximately 25% of the radius) but this in fact represents about 45% of the volume of the wood.

It can therefore be seen that in the three trees examined in one forest site in the U.K. nearly half of the volume of wood in the green condition contained significant amounts of soluble nitrogen. From the viewpoint of wood colonisation by microfungi, the significance of this is obvious. However, as the soluble nitrogen content of wood has been stressed little in the past in relation to wood colonisation, it was considered that evidence other than that provided by three discs was required before any further hypotheses were developed. Consequently, it was decided that further material should be examined to confirm the obvervations made above.

2.2.3. Distribution of Soluble and Structural Nitrogen in Green Spruce (Experiment 2.3.).

A. Materials and Methods

Three further Sitka spruce trees were felled, two in April 1973 at the Forest of Dean Gloucs. and one in June 1973 at Radnor Forest Radnorshire. The trees were all approximately 50 years old. Discs of 3" depth were removed at breast height from each tree, packed in polythene and were returned to the laboratory where they were stored in a deep freeze (-18 C) until analysed. (It was later shown (Expt. 2.4.)that storage in a deep-freeze unit did not significantly influence nitrogen distribution in the wood.)

Radial strips 2" wide extending from cambium to pith were removed from the two cardinal points not containing compression wood in each disc. Only one strip was removed

from Tree No. 4. Nitrogen content was determined on green and extracted wood to indicate total and structural nitrogen. Extracted wood was prepared after the manner of Laidlaw and Smith (1965). The manner of examination of strips and their conversion was as described in 2.2.1.A., the exception being that the strips were in all cases converted into five-ring groupings rather than individual rings, and five replicates of each five-ring grouping were tested rather than duplicate replicates of individual rings.

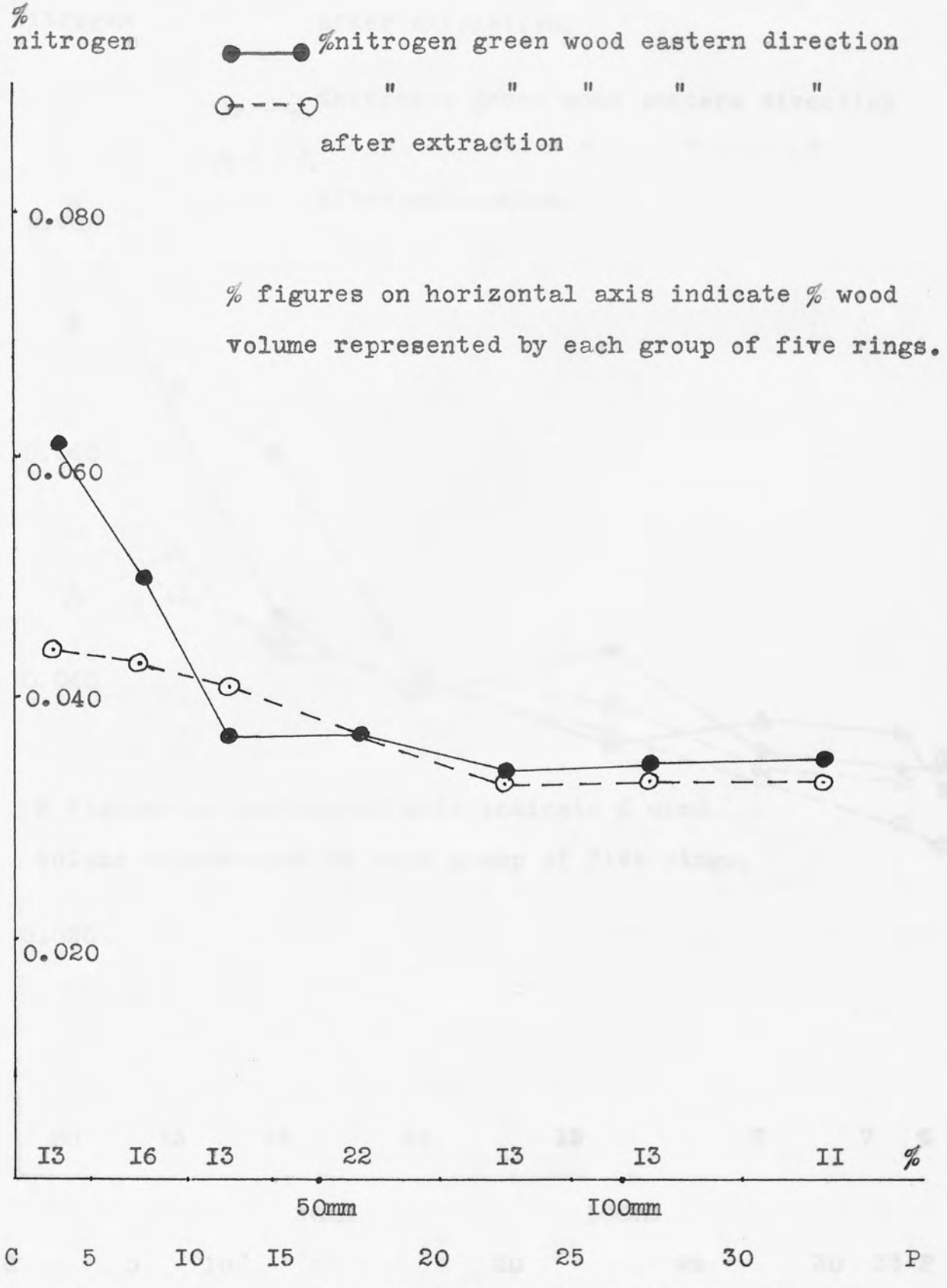
B. Results.

Results of these determinations are presented in Figs. 2.7, 2.8 and 2.9 respectively, indicating data for Trees 4 and 5 felled at the Forest of Dean and Tree 6, felled in Radnor Forest.

C. Discussion.

These data confirmed the results presented in 2.2.2. However, it became more obvious that the combined data were very varied even though a general pattern was indicated, which suggested that considerable amounts of soluble nitrogen were present in spruce sapwood. These levels were particularly high in the outer rings and became gradually smaller until nearly negligible towards the inner sapwood-outer heartwood junction. Comparison of data was made particularly difficult by the variation in size of the material. Not only did nitrogen content vary between trees and between directions as indicated by the cardinal point, but the width of the sample units also varied e.g. whereas Rings 1-5 (measured from the

FIGURE 2.7 Tree no. 4 Nitrogen Content



Growth rings measured in mm from cambium (C) to pith (P)

FIGURE 2.8 Tree no. 5 Nitrogen Content

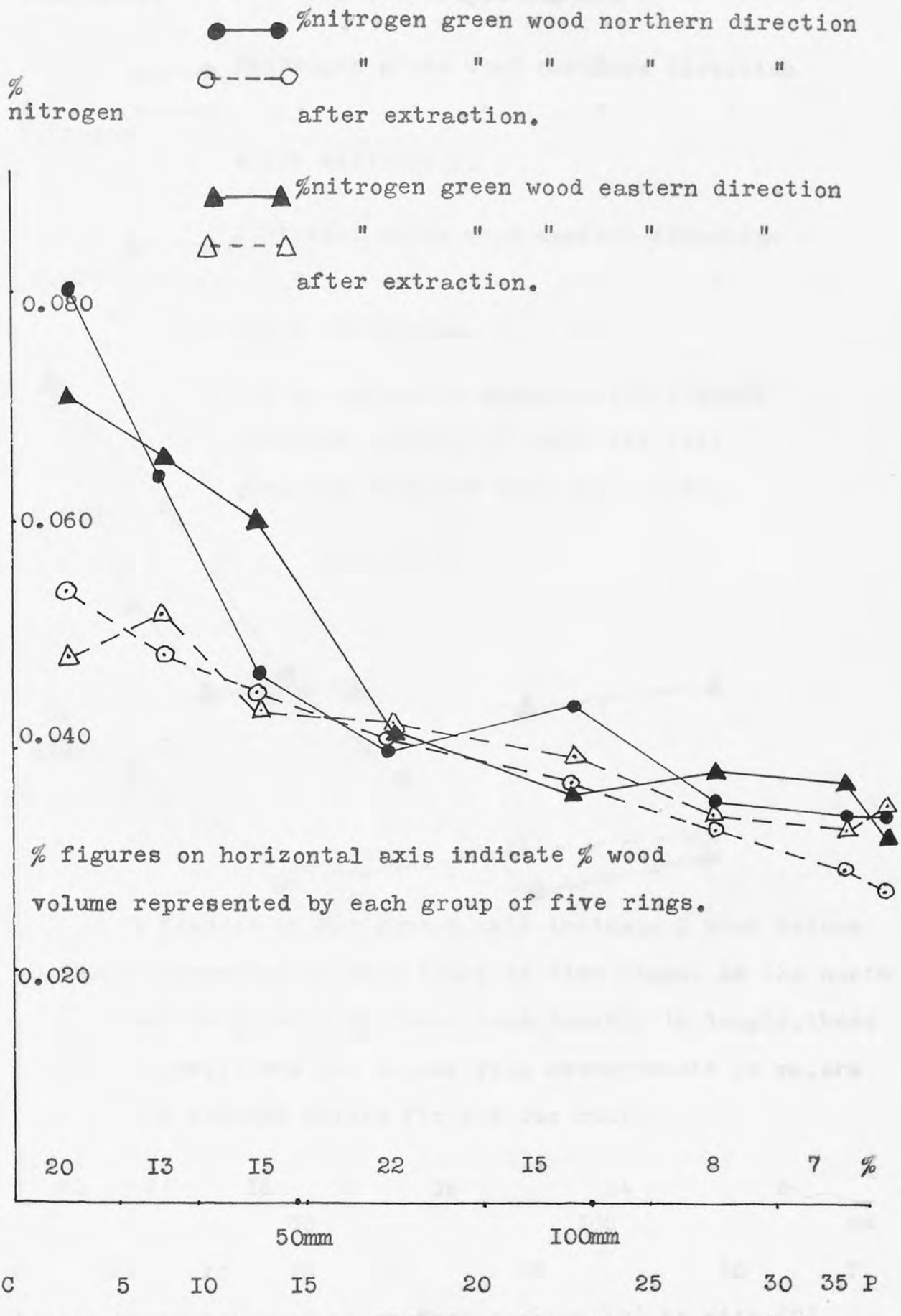
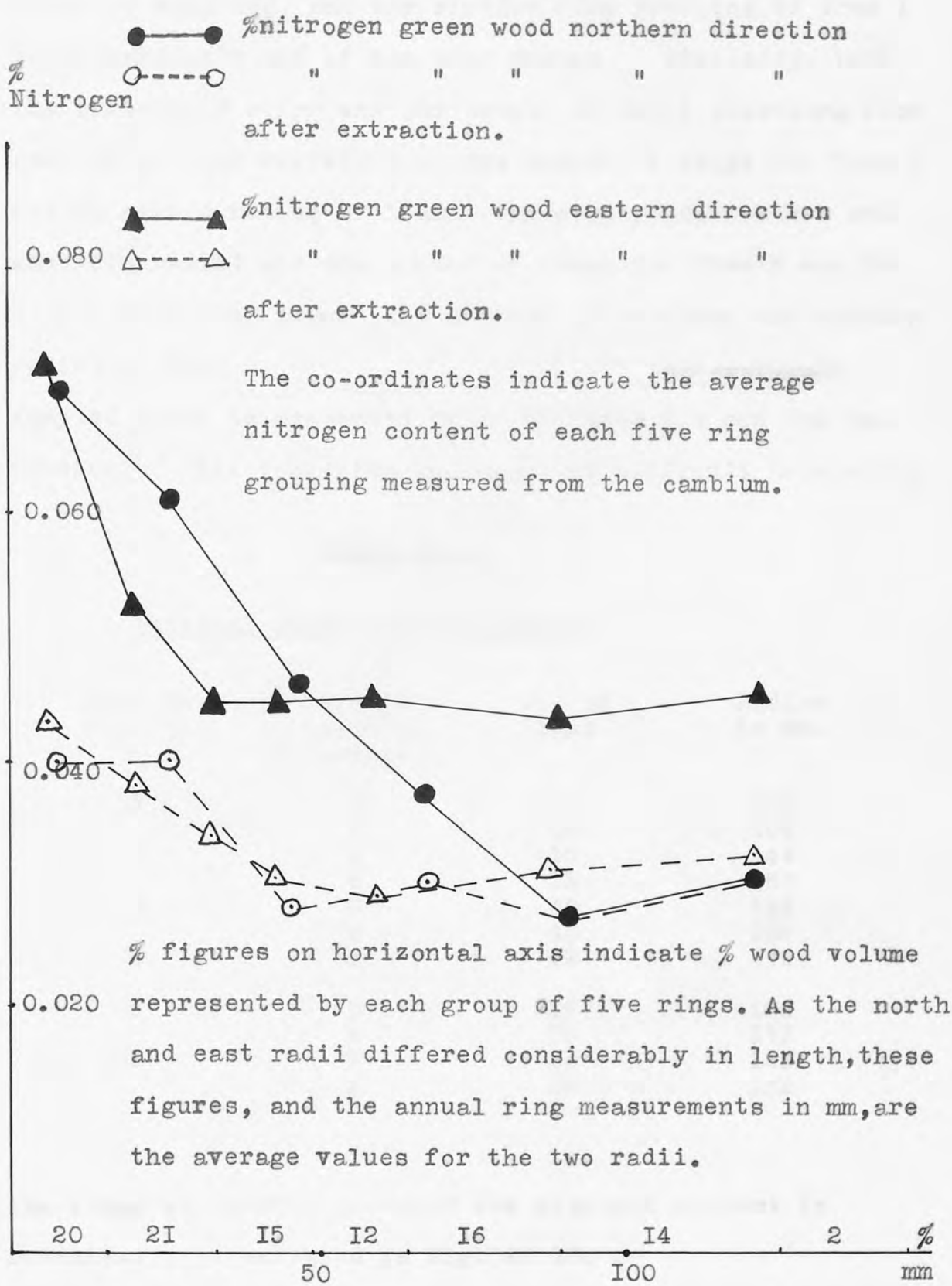


FIGURE 2.9 Tree no. 6 Nitrogen Content



cambium) of Tree 5 represented 20% of the tree volume at the point of sampling, and the similar ring grouping of Tree 1 represented only 10% of the tree volume. Similarly, both the numbers of rings and the length of radii extending from cambium to pith varied, e.g. the number of rings for Tree 1 was 50 over a radius of 156mm (the average of western and southern radii) and the number of rings for Tree 5 was 38 over a radius of 180mm (the average of northern and eastern radii). The data relating to growth variation in the sampled trees is presented fully in Table 2.1 and the influence of this variation in making it difficult to clarify

TABLE 2.1.

Physical Dimensions of Samples

Tree No.	Cardinal point of sample	No. of Rings	Radius in mm.
I	S	50	158
	W	50	154
2	S	35	144
	W	35	157
3	S	40	149
	W	40	158
4	E	42	179
5	N	38	186
	E	38	173
6	N	35	135
	E	35	152

the range of results produced for nitrogen content is graphically illustrated in Fig. 2. IO.

It was therefore evident that a unification procedure was necessary if data from all radii in the different trees were

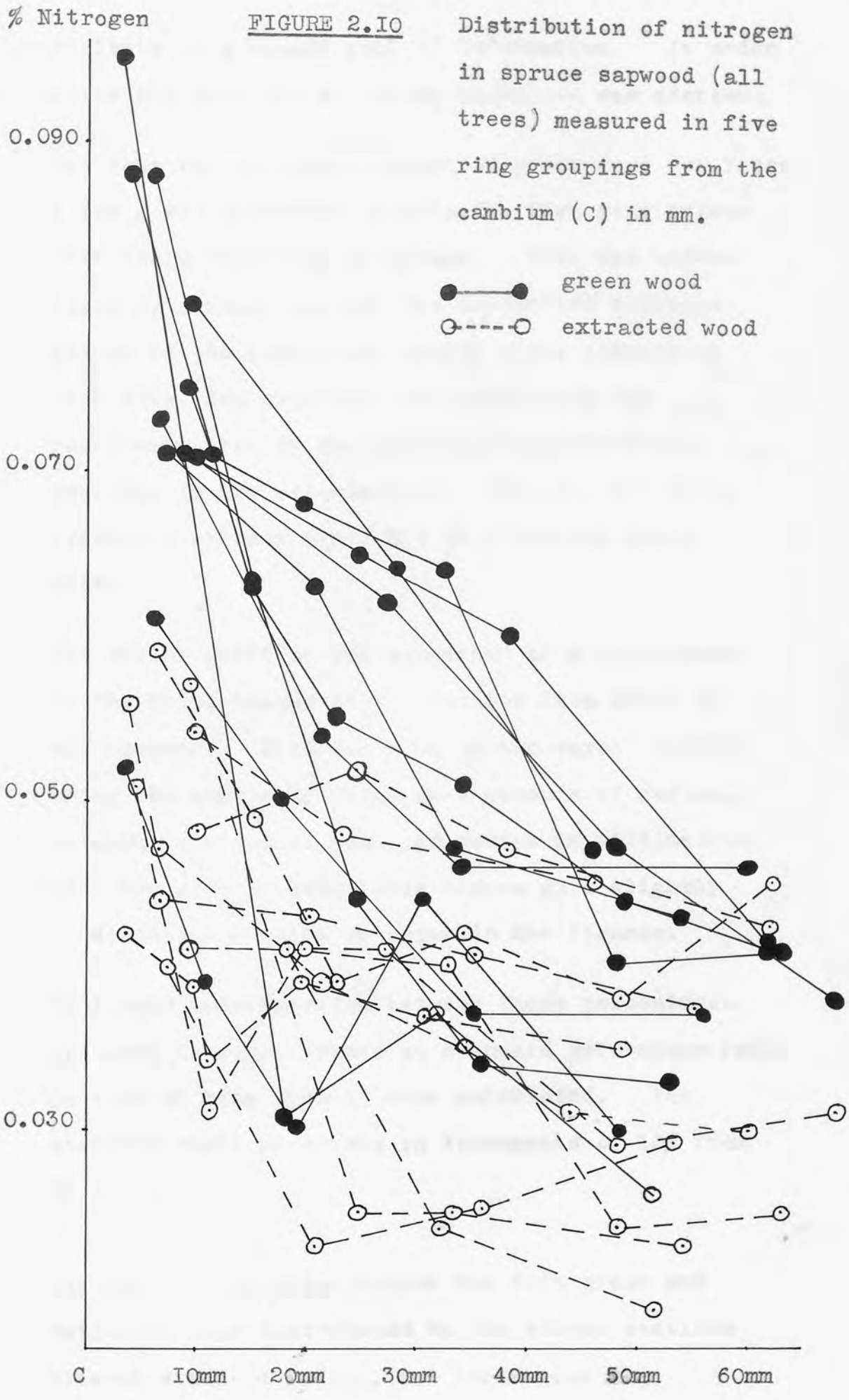


FIGURE 2.10 Distribution of nitrogen in spruce sapwood (all trees) measured in five ring groupings from the cambium (C) in mm.

to contribute to a common pool of information. In order to combine all data the following technique was adopted:

1. The data for nitrogen content of green wood for Trees 1 and 2 was converted to data for five-ring rather than individual-ring groupings. This was undertaken by adding together the duplicated nitrogen values of the individual growth rings comprising each five-ring grouping and by dividing the resultant total by the number of determinations included in the calculation. The data for other trees were already available in five-ring group means.

2. The radial position was expressed as a percentage of the total radius of the section from which it was removed. This was done in two ways: either using the number of rings as a measure of radius, or using the actual measured radius in millimetres. These two ways of expressing radius gave slightly different results as shown in the figures.

3. By linear interpolation between these percentages, presumed nitrogen levels at standard percentage radii (actual or ring number) were calculated. Ten standard radii were used in increments of 10% from 5% to 95%.

4. The total of nitrogen values for both green and extracted wood contributed by the eleven sections at each standard radius, and their mean were calculated.

FIGURE 2.II Mean distribution of nitrogen in the six spruce trees analysed related to % ring number.

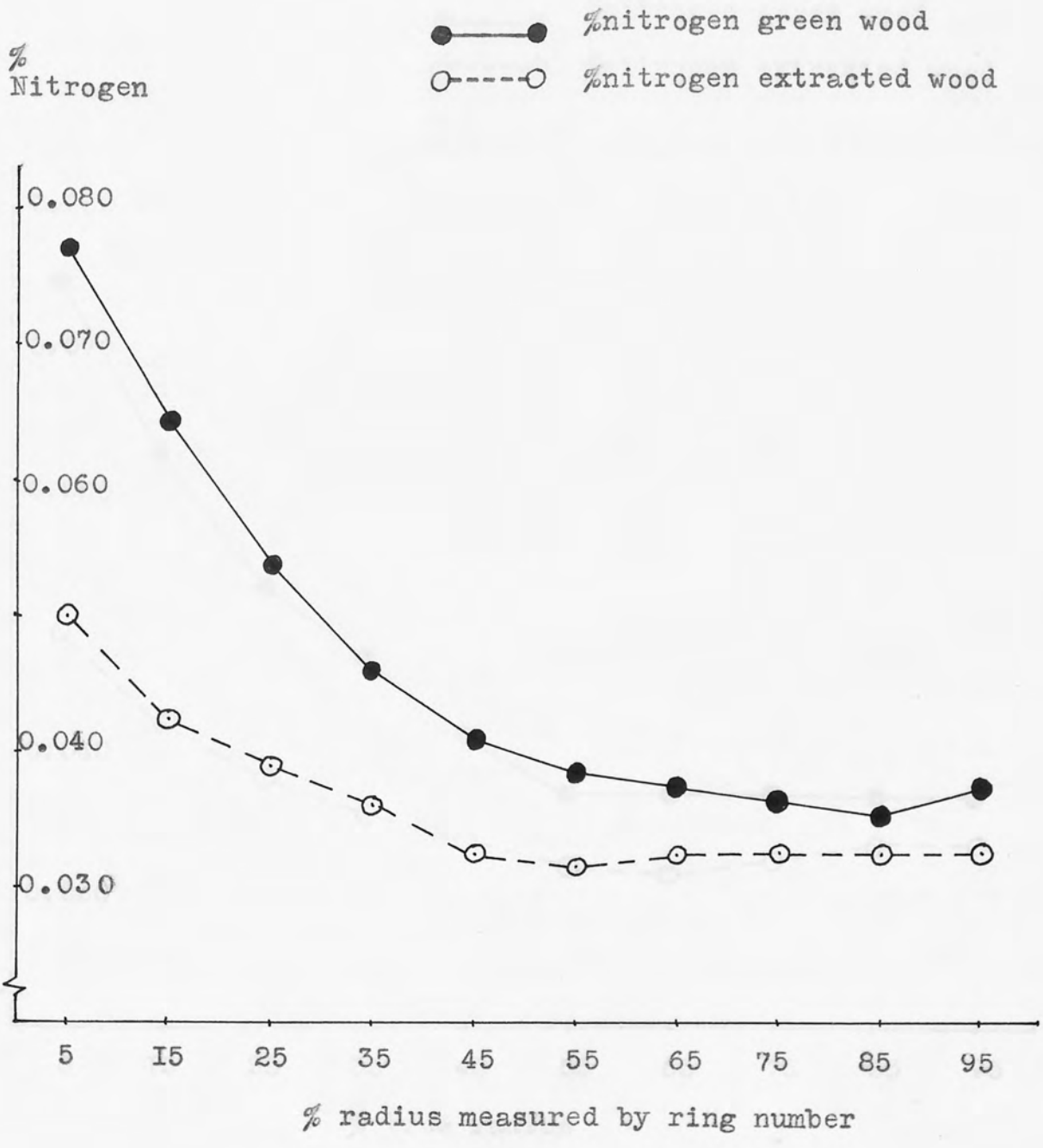


FIGURE 2.I2 Mean distribution of nitrogen in the six spruce trees analysed related to % true radius.

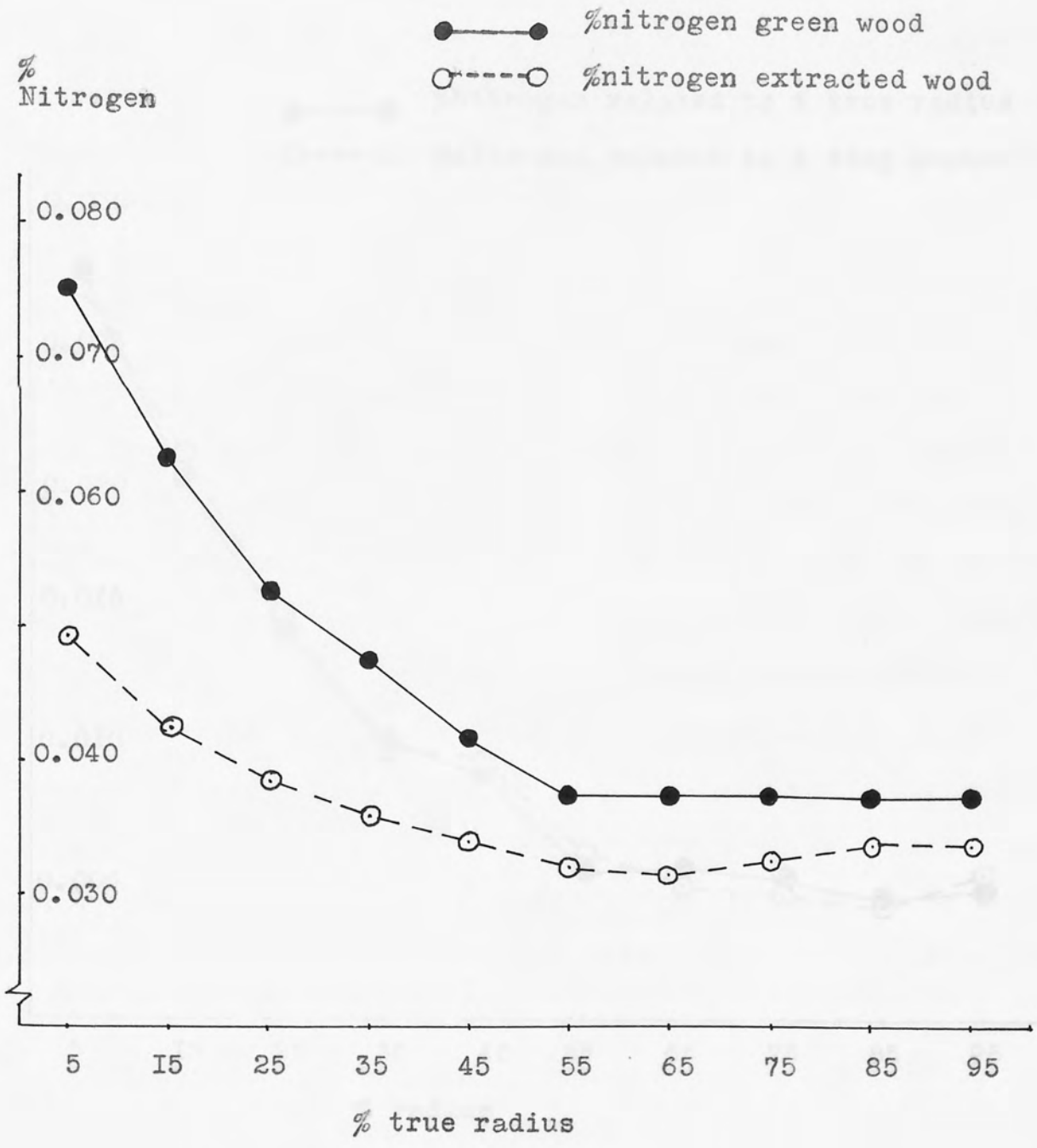


FIGURE 2.I3 Mean % loss in nitrogen content as a result of extraction in the six spruce trees examined

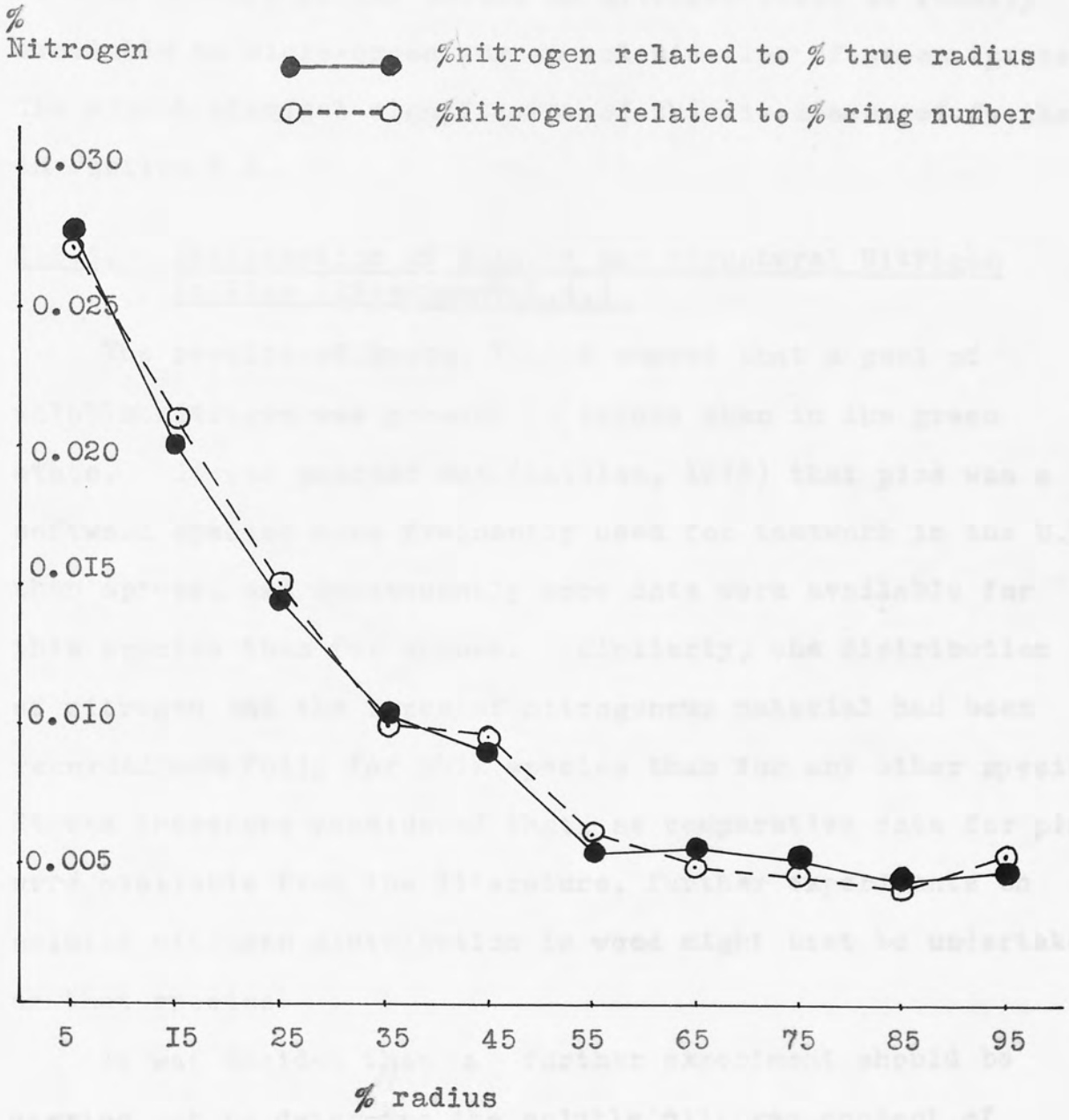


Fig. 2.11 shows the mean distribution of nitrogen arrived at by the above unification procedure using ring number hence age as a measure of radius, while Fig. 2.12 shows the similar distribution when true radius in millimetres was used. Fig. 2.13 shows, on an enlarged vertical scale, the loss of nitrogen as a result of the extraction procedure, based on both ring number and radius. It is suggested these levels of soluble nitrogen might be regarded as some measure of the amount of nitrogen which is readily available to micro-organisms on colonisation of green spruce. The microbiological significance of this is discussed further in Section 2.3.

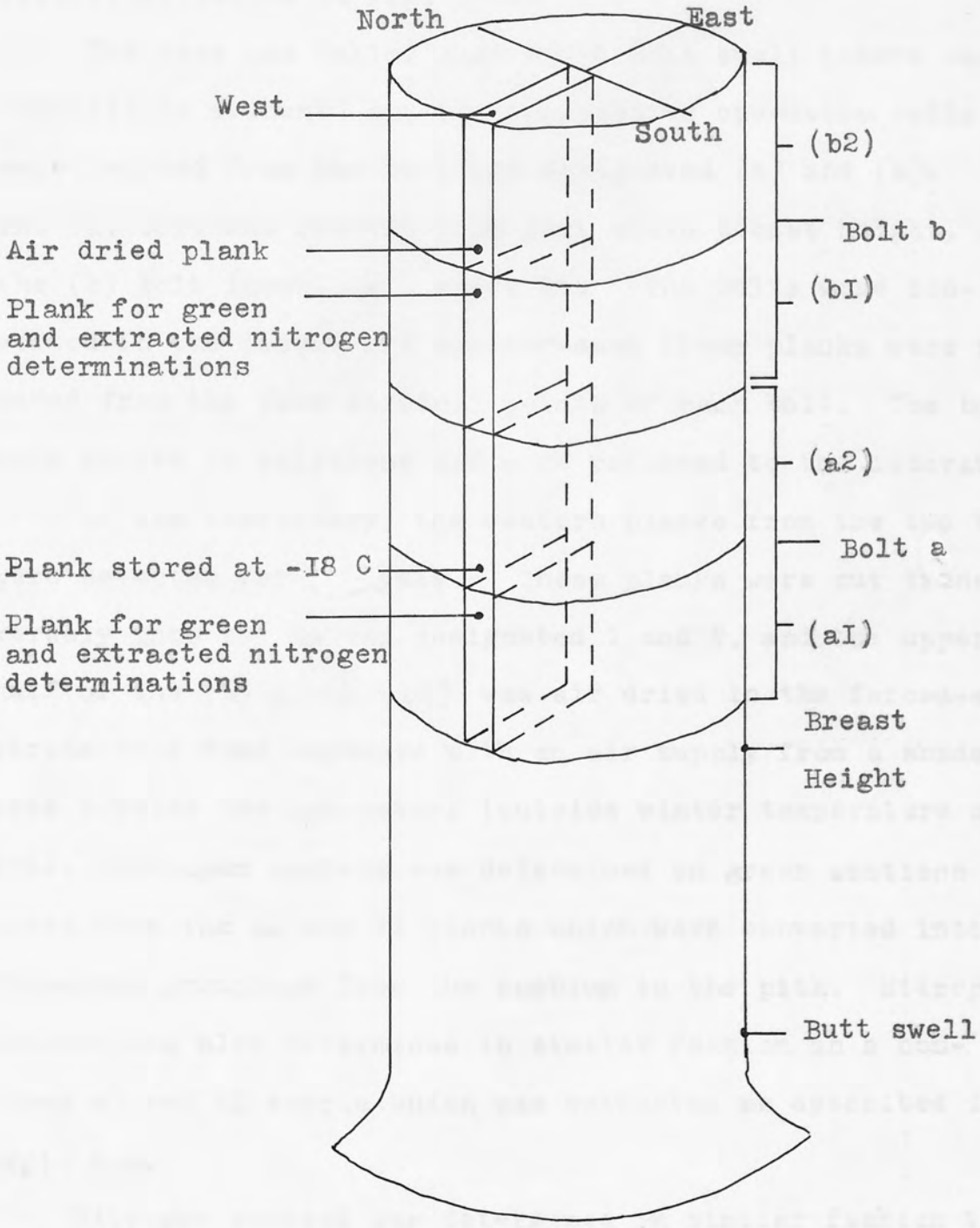
2.2.4. Distribution of Soluble and Structural Nitrogen in Pine (Experiment 2.4.).

The results of Expts. 1 to 3 showed that a pool of soluble nitrogen was present in spruce when in the green state. It was pointed out (Laidlaw, 1973) that pine was a softwood species more frequently used for testwork in the U.K. than spruce, and consequently more data were available for this species than for spruce. Similarly, the distribution of nitrogen and the types of nitrogenous material had been recorded more fully for this species than for any other species. It was therefore considered that, as comparative data for pine were available from the literature, further experiments on soluble nitrogen distribution in wood might best be undertaken on that species.

It was decided that a further experiment should be carried out to determine the soluble nitrogen content of green pine, and to see if the total nitrogen content was affected by drying. It was further decided to determine the nitrogen content of pine after storage in the deep freezer,

FIGURE 2.I4

Selection of material for analysis from pine



as it was considered that storage of green wood in a deep freezer might have influenced the distribution of soluble nitrogen in the spruce.

A. Materials and Methods.

One Scots pine (Pinus sylvestris) tree was felled at the New Forest in December, 1973. This tree was 50 years old, was straight boled, possessed a good crown and was sheltered from wind. The felling and conversion of this tree is illustred in Fig. 2.14.

The tree was felled just above butt swell (there was very little evident) and two consecutive one-metre bolts were removed from the butt and designated (a) and (b). The (a) bolt was removed from just above breast height, and the (b) bolt immediately above it. The bolts were converted in the forest and quarter-sawn 55-mm planks were removed from the four cardinal points of each bolt. The bolts were packed in polythene and were returned to the laboratory.

At the laboratory, the western planks from the two bolts were selected for analysis. These planks were cut transversely into two halves designated 1 and 2, and the upper half of the (b) plank (b2) was air dried in the forced-air stream of a fume cupboard with an air supply from a shaded area outside the laboratory (outside winter temperature about 4°C). Nitrogen content was determined on green sections removed from the a1 and b1 planks which were converted into five-ring groupings from the cambium to the pith. Nitrogen content was also determined in similar fashion on a combined a1 and b1 sample which was extracted as described in Expt. 2.3.

Nitrogen content was determined in similar fashion to above on a section removed from the lower end of the sapwood

of the air-dried plank (to match the sample removed from the b1 plank for green nitrogen content) after drying for one month, also on a section of the a2 plank after storage in the deep-freeze for a two-month period after felling.

Five replicate determinations were carried out on each five-ring grouping tested. Unless otherwise stated, the general experimental detail for these experiments was as outlined in Expt. 2.1.

B. Results.

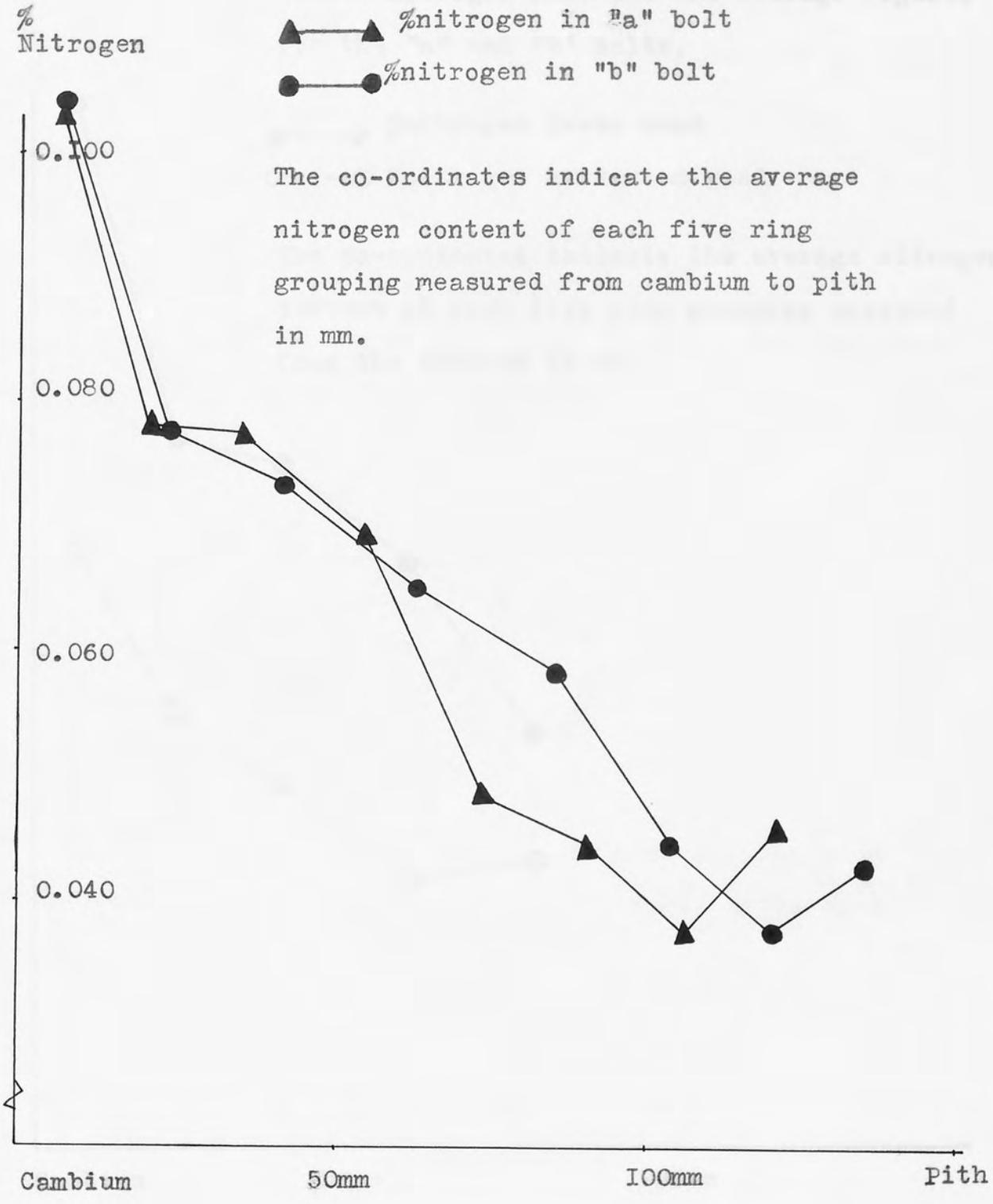
The results of these experiments are presented in Figs. 2.15., 2.16., 2.17, and 2.18. Fig. 2.15. shows the pattern of nitrogen distribution in Scots pine in the green condition, the samples being taken at points 1m apart in the same cardinal area of the trunk. Fig. 2.16. shows the loss of nitrogen on extraction of the combined a and b green samples. Fig. 2.17. shows the changes in nitrogen content on drying of the wood, and Fig. 2.18. shows the lack of influence of freeze drying on nitrogen levels in pine.

C. Discussion.

The foregoing results show that the nitrogen distribution in the pine tree examined corresponded generally to that outlined by Cowling (1970) and to the results for spruce presented in Expts. 2.1-2.3. The pattern for dried wood was similar to data previously presented insofar as nitrogen levels were highest in the sapwood and tended to plateau out in the heartwood area. Little difference was evident between the nitrogen contents of a and b sections (Fig. 2.15), indicating that the results were consistent for more than one height within this pine tree in the green condition.

The results presented in Fig. 2.16. showed that about

FIGURE 2.I5 Nitrogen distribution in green pine, sampled at two points in the trunk.



The co-ordinates indicate the average nitrogen content of each five ring grouping measured from cambium to pith in mm.

FIGURE 2.I6 Nitrogen distribution in green* and extracted pine sapwood.

*Green nitrogen data are the average figures for the "a" and "b" bolts.

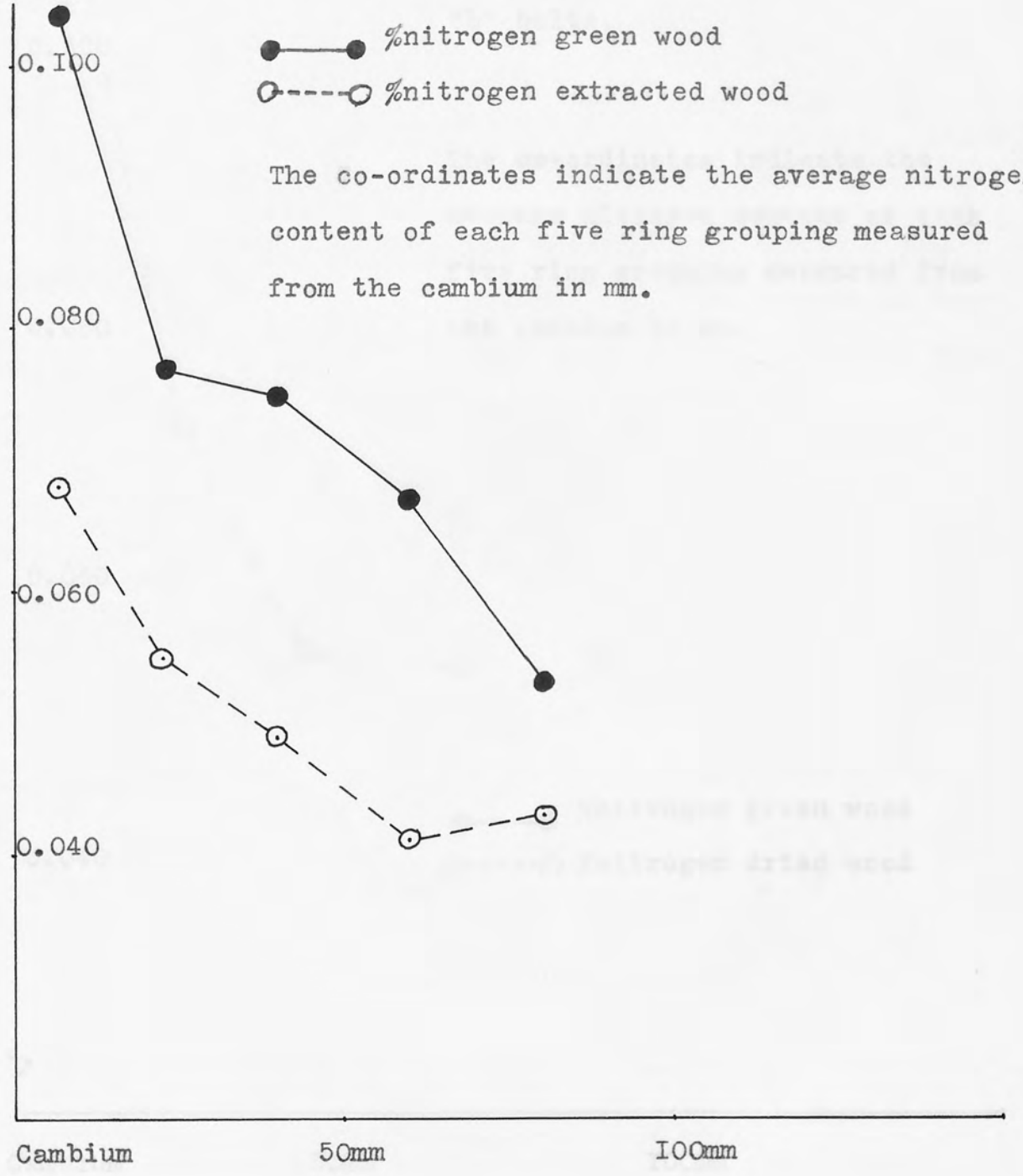


FIGURE 2.17 Nitrogen distribution in green* and dried pine sapwood.

*Green nitrogen data are the average figures for the "a" and "b" bolts.

The co-ordinates indicate the average nitrogen content of each five ring grouping measured from the cambium in mm.

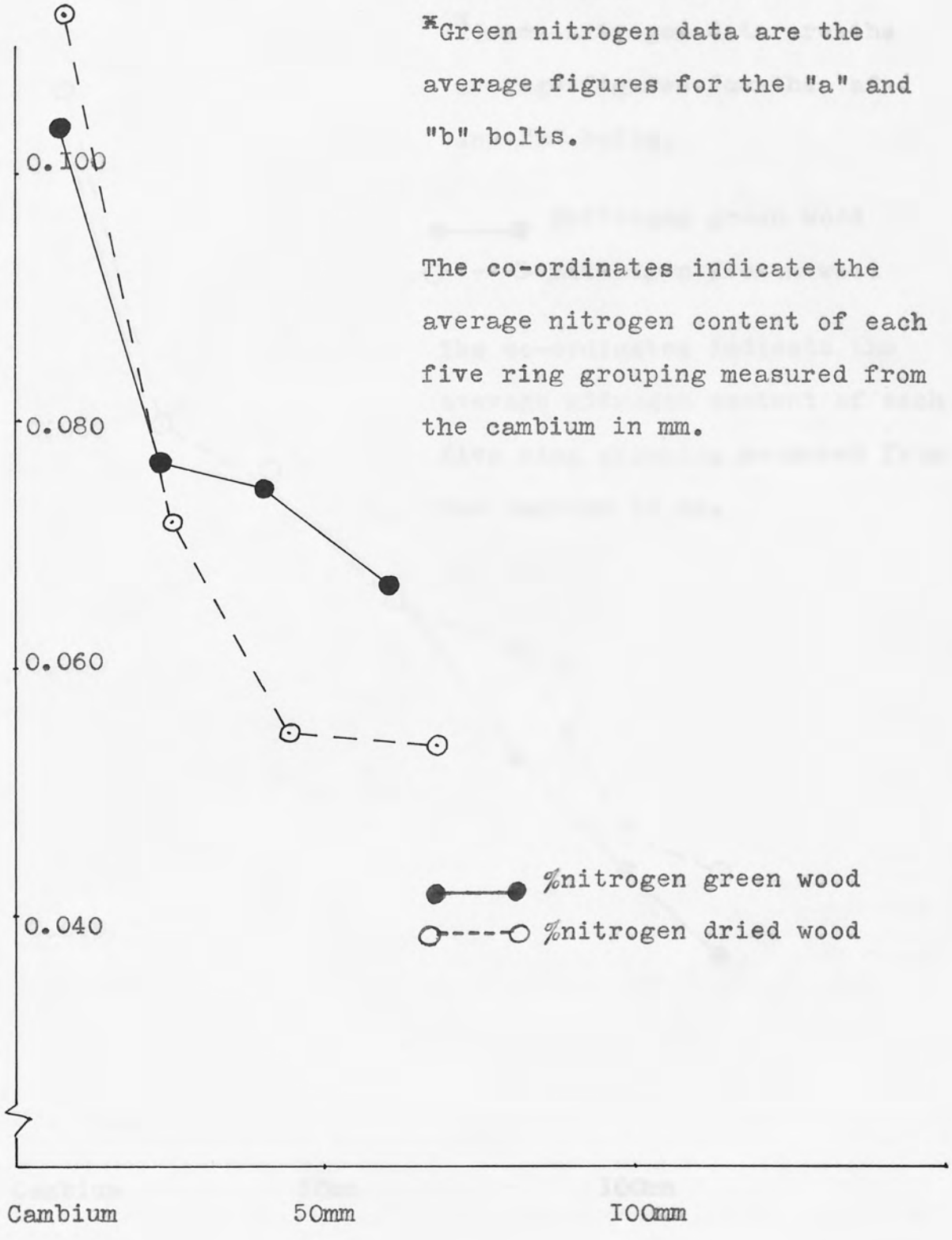


FIGURE 2.18 Nitrogen distribution in green* and "deep frozen" pine.

*Green nitrogen data are the average figures for the "a" and "b" bolts.

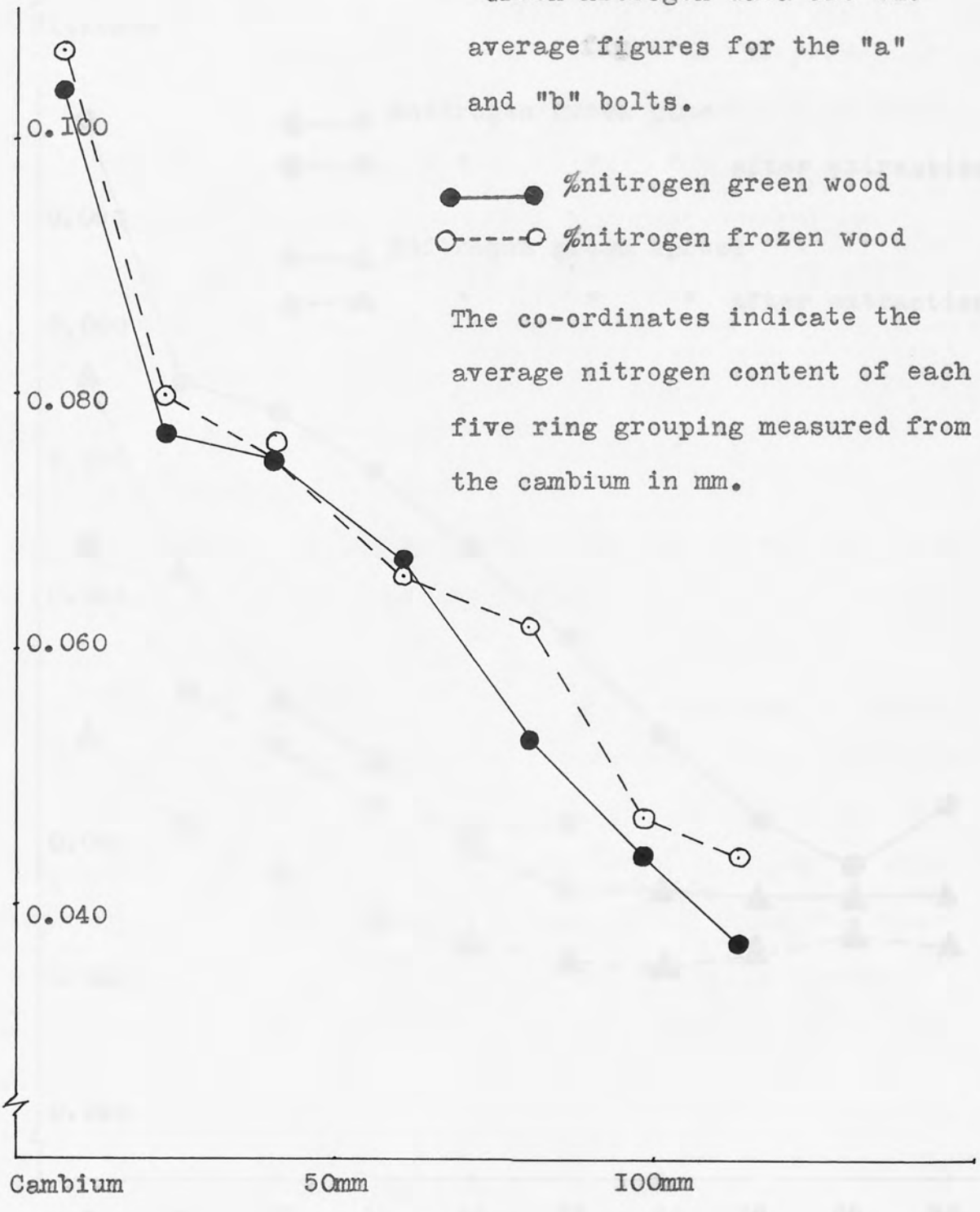
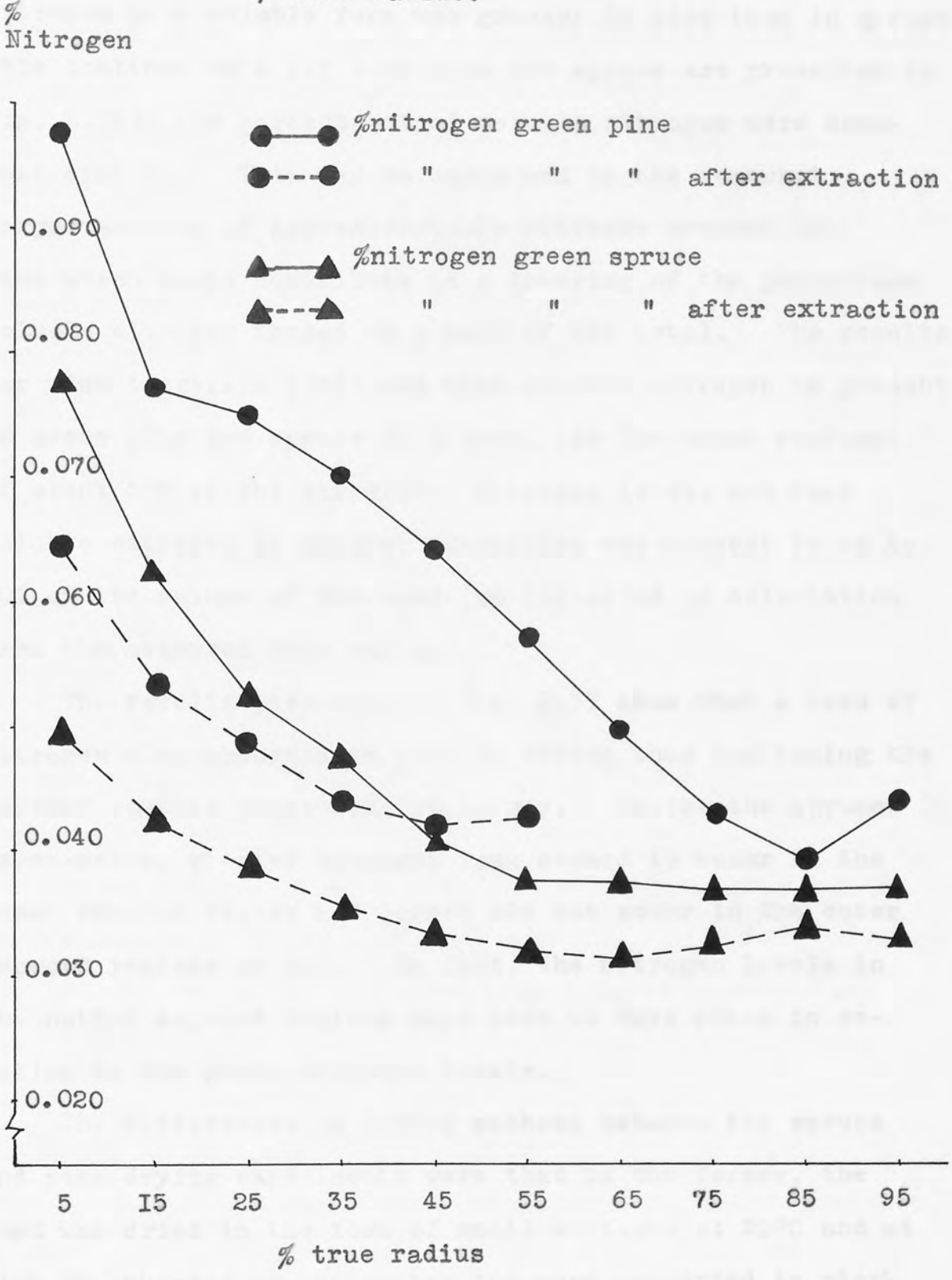


FIGURE 2.I9 Mean distribution of nitrogen in the six spruce trees and the pine tree related to % true radius.



30% of the nitrogen in green pine was in a soluble form in the outer 30% of the wood. Although the amount of the total nitrogen in a soluble form was greater in pine than in spruce (the combined data for both pine and spruce are presented in Fig. 2.19), the percentages of soluble nitrogen were somewhat similar. This may be explained by the somewhat larger amounts of non-extractable nitrogen present in pine which would contribute to a lowering of the percentage soluble nitrogen formed as a part of the total. The results for pine therefore confirmed that soluble nitrogen is present in green pine and spruce at a level (in the outer regions) of about 50% of the structural nitrogen level, and that soluble nitrogen in smaller quantities was present in up to 80% of the volume of the wood (as indicated by calculation from the measured true radius).

The results presented in Fig. 2.17 show that a loss of nitrogen also occurred in pine on drying thus confirming the earlier results presented for spruce. Unlike the spruce experiments, greater nitrogen loss seemed to occur in the inner sapwood region and losses did not occur in the outer sapwood regions at all. In fact, the nitrogen levels in the outher sapwood regions were seen to have risen in relation to the green nitrogen levels.

The differences in drying methods between the spruce and pine drying experiments were that in the former, the wood was dried in the form of small sections at 20°C and at high RH, whereas in the latter the wood was dried in plank form at 4°C and at a presumed/relatively low RH. It seemed from the pine experiment that not only had nitrogen been "lost" from

the inner sapwood of the pine but had been "gained" in the outer sapwood, which suggested that, not only was a loss of soluble nitrogen taking place, but that either nitrogen was being deposited on the outer layers of the wood from the atmosphere or that a radial migration of nitrogen was occurring during the drying of wood which was only being detected in the larger samples.

The results presented in Fig.2.18 showed that loss of nitrogen did not occur during storage of wood in the deep freezer, and that after a storage period of two months, the nitrogen content of pine and its distribution across the stem were substantially the same as those presented for green wood. It was therefore concluded that storage of green wood in a deep freeze unit did not apparently result in any significant redistribution or loss of nitrogen.

2.2.5. Nitrogen Distribution in wood before and after drying (Experiment 2. 5.).

It was evident from the results of Expts. 2.1.-2.4 that (a) nitrogen was present in a soluble form in the sapwood of both spruce and pine; (b) that this soluble nitrogen was easily extractable; and (c) that nitrogen could be "lost" from wood during the drying process, particularly when small specimens were dried under conditions providing reduced evaporation. It was also evident from the results presented in Fig. 2.17 that either nitrogen had been deposited in the outer rings of the dried plank from the atmosphere, or that some form of nitrogen redistribution within the wood had occurred.

It was noted that during drying of spruce planks for

preparation of test blocks for soil burial, and also during the drying experiments with pine, that the surfaces of the drying planks had slowly darkened during the drying process. The colour of the wood in the green condition had been white, whereas when fully dried had turned to a pale yellow colour. This colour change was particularly noticeable on the dried surfaces of the larger planks.

It was considered that this colour change indicated some change in the condition of the wood, which might also be related to the changes in nitrogen content observed during drying. It was therefore decided to investigate the changes in nitrogen content in wood during drying in the light of the hints given by surface discolouration.

A. Materials and Methods

Two experiments were undertaken to examine the phenomenon described above:

(i) A survey of the nitrogen contents of the stock of dried spruce and pine planks stored in the laboratory was undertaken. These planks, when converted, had been quarter-sawn and consequently included all the sapwood contained in the tree at the point from which the plank was removed. The sapwood of these planks (rings 1-25 measured from the cambium) was planed on the radial longitudinal surfaces from which drying had occurred, until the pale yellow discolouration evident on the surfaces (described earlier) was no longer visible. This usually occurred after 1-2mm depth of the wood surface had been removed. Nitrogen content was determined on the wood shavings

removed during the planing procedure after all the shavings had been homogenised and sampled as described in Expt. 2.1.

The planed planks were sawn radially into two halves and a sample was removed by planing from the freshly-sawn sapwood surface in the manner described above. The shavings produced were similarly homogenised and sampled for nitrogen determination. Five replicate nitrogen determinations were made on each sample of planings.

(ii) A comparison of the nitrogen contents of the inner material and outer surface of one pine plank in the green (deep frozen) condition was undertaken, in similar fashion to above, to determine the nitrogen status of wood when no significant evaporation had taken place from the surface.

B. Results.

The results are presented in Tables 2.2. and 2.3.

TABLE 2.2

Nitrogen Content (percentage by weight) of Centre and Surface of dried Pine and Spruce

Sample		0-2mm	Plank Centre
Air-dried spruce* (30mm thick)	Plank 1	0.118%	0.042%
	Plank 2	0.118%	0.049%
	Plank 3	0.112%	0.060%
Air-dried pine** (55mm thick)	Plank 1	0.135%	0.065%
Oven-dried pine*** (55mm thick)	Plank 1	0.187%	0.043%
	Plank 2	0.223%	0.041%
	Plank 3	0.220%	0.041%

* Dried at University of Aston
** "Air dried" at a low fluctuating temperature averaging 4°C
*** "Oven dried" at 50°C

TABLE 2.3

Nitrogen Content of Inner and Outer Surfaces of Pine

Green Pine

Nitrogen Content	Surface of Plank	Centre of Plank
	0.076%	0.072%

Discussion

The results presented in Tables 2.2 and 2.3. show that in the stored planks, the nitrogen contents of the evaporating surfaces of the planks were in all cases greatly higher than the nitrogen contents of the inside layers. It was further apparent that the thickness of the planks had some effect on the nitrogen levels of the outside layers. The spruce planks were 30mm thick and the pine planks were 55mm thick and these thicknesses are reflected in the degree of difference between the nitrogen levels of the outside layers of the pine and spruce respectively. However, this issue was complicated by the temperature at which drying occurred. Whereas all the spruce planks were slowly dried during storage in the laboratory, the pine planks were dried in two groupings. One group, as described earlier, was dried in a fume cupboard in a forced-air stream at 4°C approximately, whereas the second group was dried at 50°C in a drying oven. This influence of drying is shown in Table 2.2. The data presented in this table indicate that generally, the nitrogen levels of the inner layers of the wood dried at higher temperatures, were lower than those dried at

the lower temperatures, and that the nitrogen levels of the outer layers of pine dried at higher temperatures were higher than the nitrogen levels of outer layers of pine dried at lower temperatures. The difference between outside and inside nitrogen contents for spruce was about 100%; for cold-sir-dried pine was about 80%, and for the pine dried at 50°C between 400% and 600%, all sets of planks having, presumably, similar levels of soluble nitrogen in the first instance.

The results presented in Table 2.3 show that significant differences did not exist between the nitrogen contents of the inner unexposed sapwood surfaces and the outer exposed but undried sapwood surfaces of the pine plank maintained in a green or undried condition in the deep-freeze unit for two months. It was therefore concluded that the increased nitrogen content of the outer surfaces of the dried wood had occurred during drying and were as a result of the drying process.

2.2.6. Migration of soluble Nitrogen in Pine during Drying (Expt. 2.6)

It was evident from the results presented in Expt. 2.5 that changes had occurred in the nitrogen content of both pine and spruce during drying and that the increased nitrogen noted in the outer rings of pine in Expt. 2.4 would seem to have been deposited there during the drying process. The results presented in Expt. 2.4. also suggested that some loss of nitrogen had occurred during drying but Expt. 2.5 showed that in fact nitrogen was gained by the outer surfaces of wood during the

drying process and was not lost. Although the phenomenon of higher nitrogen content at the drying faces of wood might well have been caused by migration of soluble nitrogen to wood surfaces and its deposition there during drying on evaporation of wood moisture, the alternative hypothesis was proposed that such gains might be associated with absorption of ammonia from the atmosphere by the slightly acid plank surfaces (pH 4.7, Expts. 3.3., 3.4.).

A. Materials and Methods.

An experiment was carried out to test this hypothesis. A green plank matching that used for determination of inner and outer nitrogen levels in green pine (Expt. 2.5.) was selected for testing. The inside and outside nitrogen values for this green plank were known to be substantially the same (Table 2.3.). This plank was dried in an ammonia free atmosphere at 50°C. To achieve this state, the air supply to the oven used for drying was passed through 10% sulphuric acid, was then washed in distilled water and then passed through silica gel to dry. This air supply was the major air supply to the oven and was passed into the oven at its base. After the wood had dried, nitrogen content was determined on the radial surfaces of the sapwood, and on material removed from the sapwood at the centre of the plank, as described in Expt. 2.5. Five replicate nitrogen determinations were carried out on each surface tested.

B. Results and Discussion.

The results of this experiment are presented in Table 2.4. These results show that the nitrogen content of the outer surface of the pine plank had a greatly increased

nitrogen content, even after drying in an atmosphere which did not contain ammonia. It was therefore concluded that nitrogen from the atmosphere was not "deposited" on the plank surfaces but that the high surface N level was related only to nitrogen within the wood itself. The nitrogen

Table 2.4

Nitrogen Content of inner Material and outer Surfaces of Pine before and after drying in an Ammonia-free Atmosphere

	Plank Surfaces	Inner Material
Before drying	0.076%	0.072%
After drying	0.220%	0.041%

content of the wood removed from the centre of the dried plank was considerably lower than that removed from the centre of the matching green plank, consequently it was concluded that solbule nitrogen migrated from the inside of planks to the evaporating surfaces of wood during drying where it was deposited.

2.2.7. Migration of soluble Carbohydrates and other soluble materials in Pine during drying

To further check the hypthesis that soluble nitrogen migrated to the evaporating surface of wood during drying, it was decided to determine the presence of other soluble materials at this surface. (It was presumed that if

soluble nitrogen, a minor component of the soluble cell contents of wood, migrated to an evaporation surface, it was highly probable that other components, e.g. simple sugars, also soluble in cell contents, might equally migrate to evaporating surfaces during drying.)

A. Materials and Methods

Samples, matching those tested for nitrogen content on the outside and inside of planks dried in an ammonia-free atmosphere, were extracted to determine (a) alcohol-benzene solubles, which include waxes, fats and resins; (b) alcohol-water solubles which include tannins and some carbohydrates; and (c) cold-water solubles which include non-structural carbohydrates. All determinations were carried out according to the methods in Laidlaw and Smith (1965) and TAPPI Standard Nos.TI M-59 (1959), and four replicates were determined for each analysis.

B. Results

The results of this experiment are presented in Table 2.5. These results show that the soluble extractives in the outer pine surface were very different to the amounts of extractable material remaining in the inside. Alcohol benzene solubles on the outside were 71% greater than on the inside; alcohol-water solubles on the outside were 161% greater than on the inside, and cold-water solubles on the outside were 60% greater than on the inside.

Table 2.5

Extractable materials from the inner material and outer surface of pine

	Plank Surface	Inner Material
Alcohol Benzene Solubles	4.19%	2.45%
Alcohol Water Solubles	2.78%	1.04%
Cold Water Solubles	1.35%	0.84%
Total Solubles	8.32%	4.35%

C. Discussion

The results presented above confirmed that migration of soluble nitrogen in wood during drying was also accompanied by deposition of other soluble materials at evaporation surfaces. The extent of deposition of other soluble materials was not as great as that for soluble nitrogen (the surface nitrogen levels for pine were 5-6 times greater than the inner material). However, it was considered that this was in keeping with the work of Levy and Olofinboba (1967) who showed that loss of soluble carbohydrates occurred during drying of wood. Consequently, the extent of total soluble deposition after drying would not be expected to be as great as that for soluble nitrogen.

The results of Expts. 2.6 and 2.7 therefore confirmed that migration of soluble nutrients occurred during drying of wood, and it was concluded that the accumulated nitrogen at the drying surfaces of wood was the soluble nitrogen referred to in Expts. 1-4. This conclusion was confirmed when pine blocks containing evaporation surfaces were prepared for concurrent soil burial experiments. These experiments showed (Fig. 3.27) that the higher nitrogen levels of outside

layers could be reduced to the same level as centre samples by the extraction procedure used throughout this series of experiments, and that at no stage did nitrogen contents for outside surfaces fall below the structural nitrogen levels.

2.2.8. Confirmation of Observations of soluble Nitrogen migration (Expt. 28)

As the observation and quantification of significant soluble nitrogen migration during drying of wood had not been reported in the literature, it was considered desirable to determine if the phenomenon was localised to drying conditions at this laboratory and its environs or whether it was of more widespread nature. To examine this aspect, it was therefore decided to obtain material, dried at institutions outside this laboratory, for analysis.

A. Materials and Methods

Samples of home-grown beech (*Fagus sp.*), air dried at the Mid-Warwickshire College of Technology; dried imported Scandinavian pine (*Pinus sp.*, designated X, Y and Z in the results); and home-grown Sitka spruce (*Picea sitchensis*), kiln-dried at Princes Risborough Laboratory, Building Research Establishment, were obtained in plank form. The radial evaporation surfaces of the sapwood of these planks were planed to a depth of 1mm approximately, and a second sample was obtained by planing to a depth of one further mm. The planks were split, and the surfaces thereby exposed were planed to provide "inside" samples. Five replicate nitrogen determinations were undertaken on each planed sample, the preparation sampling etc. of which was in the manner described earlier.

B. Results.

The results of this experiment are presented in Table 2.6. These results show that the outer surface, and sub-surface samples of all planks tested, had higher nitrogen contents than samples removed from the plank centres.

Table 2.6

Nitrogen Contents of Surface, inner Surface, and centre Samples removed from a range of commercially dried Woods

Plank No.	0-1mm	1-2mm	Inside
Pine plank X	0.103%	0.055%	0.048%
Pine plank Y	0.092%	0.076%	0.048%
Pine plank Z	0.074%	0.070%	0.050%
Beech	0.173%	0.141%	0.131%
Sitka spruce	0.080%	0.057%	0.029%

C. Discussion.

The results presented above confirmed that the migration of soluble nitrogen and its accumulation at the drying or evaporation surface of wood was not confined to this laboratory, but occurred in material obtained from other varied sources. It was therefore concluded that migration of soluble nitrogen was probably of fairly common occurrence during wood drying, as it was not limited to the two softwood species but also occurred in the hardwood species and both the softwood species commercially dried before analysis.

2.3. Discussion.

The findings in 2.2. Experimental may be summarised as follows.

Soluble nitrogen was shown to be mainly located in the sapwood regions of wood in the green condition. Greatest amounts of soluble nitrogen were consistently found in the outer sapwood regions while only small amounts of soluble nitrogen were found in the heartwood regions (Expts. 2.2., 2.3., 2.4.).

Two drying experiments (included in Expts. 2.1. and 2.4.), one with small radial strips of green spruce, maintained at laboratory temperatures and high relative humidity, and the other with short planks of Scots pine air dried at low temperature, showed that during drying of wood, nitrogen could be either "lost" or "gained" during the drying process. A survey of nitrogen contents of both spruce and pine planks dried at this laboratory showed that the outer surfaces of all planks had nitrogen levels far in excess of the inside nitrogen contents (Expt. 2.5), and it was shown in Expt. 2.6. and 2.7. that these higher nitrogen contents were due to migration of soluble nitrogen and other soluble materials to the drying or evaporating surfaces of planks during the drying process. The results of Expt. 2.8. showed that the phenomenon of migration of soluble nitrogen during drying was not confined to this laboratory, but also occurred during drying of wood commercially and at other institutions.

The results for total nitrogen distribution in wood were in general agreement with the trends presented in previously published data (Becker, 1952; Merrill and Cowling, 1966); however, the data on soluble nitrogen distribution in green wood and its redistribution on drying provides information

on the nutritional status of wood, as a material susceptible to biodeterioration, which has not previously been reported.

The presence of soluble nitrogen in wood is well recognised, but it has generally been considered by other workers only in the context of the total nitrogen content of wood. This, of course, was an entirely reasonable working basis, as the total nitrogen content of wood is a very small proportion of the total wood substance (Cowling and Merrill, 1966), and soluble nitrogen content has been considered only as a fraction of a minor wood component. Consequently, much of the earlier work on the biological role of nitrogen in wood has consisted not of examining the nitrogen content of the wood itself but of adding nitrogen to wood to enhance decay rate (Findlay, 1934; Schmitz and Kaufert, 1936; 1938, and as recently as Kaune, 1970).

Crook and Holden (1948) showed that nitrogen was present in a soluble form in the leaves of woody tissues and it was further shown (Bletchly, 1957; 1966a; Bletchly and Farmer, 1959) that "traces" of soluble nitrogen were removed from the wood of Corsican pine after extraction with water at 30°C, 60°C and 100°C. Bollard (1953, 1957a, b & c) and Fukuda (1963) determined the composition of the free amino acids of a number of wood species and Cowling and Merrill (1966) showed that quantities of nitrogen were removable from wood with a range of solvents, and that nearly all the nitrogen could be removed with 6N Hydrochloric acid. Baker, Laidlaw and Smith (1970) determined the composition of the free amino acids in home-grown Scots pine.

Although the above work has been carried out on the identification of the individual amino acids in some woods, few quantitative data are available on their gross distribution

in wood. This lack of knowledge may generally be attributed to the understandable concentration of attention on the total nitrogen content of wood, and the inherent difficulties in obtaining even this information taking into account differences in wood species, different geographical regions of growth, age of the tree from which the wood is removed, and the location of specialised tissue within the wood from which samples are removed for analysis.

The variations to be expected in total nitrogen content between wood species may be found in the literature reviews (Merrill and Cowling 1966; Cowling and Merrill, 1966; Griffin, 1972), and data for variation in nitrogen contents within trees may be found in Wright and Will (1958), Bletchly and Farmer (1959), Fukuda (1963) and Bletchly, 1966(c).

Quantification of soluble nitrogen in wood may have been subject, however, to one further consideration which has resulted in its unstressed role. Most previous work on nitrogen in wood has been carried out in one of two ways, the first in which nitrogen was determined immediately after felling, and the second in which nitrogen has been determined on already dried material. The latter method may be typified by the work of Becker (1962) and to a lesser extent Laidlaw and Smith (1965) the aims of which respectively were to quantify total nitrogen distribution in wood and to determine the chemical composition of the nitrogenous compounds in wood. The former may be typified by the work of Cowling (1970), the aim of which was to clarify the role of nitrogen in the ultimate deterioration of wood.

The first of these methods suffered the defect that nitrogen in wood was apparently presumed to be stable and

the liability of soluble nitrogen to be redistributed during drying or storage was not recognised. An example of this is the work of Baker, Laidlaw and Smith (1970) on the nutrition of Anobium punctatum larvae. Test blocks used for this work were removed from the outer sapwood of Scots pine air dried immediately after felling. At the termination of a two-year incubation period, the blocks were analysed for nitrogen content and the results showed that 13% of the nitrogen in the wood was in a soluble form. It was also shown that up to 40% of the nitrogen ingested by the larvae was not accounted for by the nitrogen in the wood, and that soluble nitrogen was more easily digested, and digested in greater quantities than the cell-wall protein. However, as the blocks were not analysed until at least two years after they were made and the amounts of soluble N_2 to be expected in outer sapwood would be about 30%, it is possible that nitrogen migration or loss, during drying and incubation, may have occurred and might have explained and corroborated the phenomenon of loss in nitrogen observed in the initial experiments of this thesis as well as the 40% nitrogen ingested by the Anobium larvae.

The second type of work mentioned above was carried out on material analysed immediately after felling, or within a short time period after this. This has been typical of the work in the field of cambial chemistry and is best exemplified by the work of Cowling (1970). (Recent literature, however, refers to storage of fresh material in deep-freeze units as a substitute for immediate analyses, e.g. Clark and Mills, 1970; Grozdits and Ifju, 1973). The material used for these analyses was converted into individual growth rings or five ring groupings and rapidly dried within 48 hours after removal

of samples from the test material (the procedure used at this laboratory for nitrogen determinations on wood in the green condition), consequently, changes in the gross distribution of nitrogen in wood had not occurred because conversion of the material into small test units prevented movement of nitrogen within the wood itself.

A third factor, the methods by which wood is prepared prior to analysis, might also have influenced the lack of recognition of redistribution of soluble nitrogen during drying. As a matter of standard procedure for analysis of wood, it is common practice to take a representative sample, which would normally include both surface and inner material, and thoroughly mix the sample produced. Planks of wood with redistributed soluble nitrogen on the outer edges and a consequently decreased total nitrogen content in the inner portions if analysed after a preparation as described above, would yield a result which represents the mean of inner and surface material and does not distinguish between them.

This is exemplified by the data presented in Fig. 2.17 and Table 2.4 which represent matched planks. In Fig. 2.17, the nitrogen conten t of the first five rings from the cambium of green pine was 0.104% and for dried pine was 0.113%, suggesting negligible effect of drying. These samples, however, were taken to include the whole thickness of the plank including surface and inner material. This compares with data from the matching green and dried planks presented in Table 2.4, showing that when the wood was in the green condition, nitrogen content was substantially the same in the inner and surface wood but when the wood was dried, the nitrogen levels in the centre of the wood could be as low as 0.041% and on the plank surface could be as high as 0.220%.

2.3.1 Importance of soluble Nitrogen to Wood Biodeteriogens

The importance of soluble nitrogen has not been stressed in the literature. Merrill and Cowling (1966) suggested that protein and peptides in heartwood and amino acids and nucleic acids in sapwood were the available nitrogen sources for fungal growth, and Levi and Cowling (1969) suggested that the presence of free amino acids and cytoplasmic materials in parenchymal tissue might explain the colonisation of those tissues by staining and mould fungi.

Direct unstressed evidence and circumstantial evidence is available in the literature to show the importance of soluble nitrogen to wood-colonising fungi. It has frequently been suggested (Rudman, 1965) that toxic extractives in wood, particularly in heartwood, inhibit colonisation by fungi and insects. Sapwood, which generally does not contain these substances, is consequently more susceptible to decay than heartwood. Weight loss experiments with extracted heartwood, extracted sapwood and unextracted sapwood of Thuja plicata D. Don (Southam and Ehrlich, 1943) and the brown-rot fungus C. cerebella, showed that weight losses produced in the extracted sapwood were less than those produced in the unextracted sapwood. These differences were at first attributed to a possible removal of carbohydrates and nitrogenous materials during the extraction process. However, as the nitrogen content of the heartwood was lower than that of the sapwood, but the heartwood samples had shown greater weight losses than either extracted or unextracted sapwood samples, they considered that nitrogen could not have been responsible for the lower susceptiblity of the extracted sapwood samples. The studies of Peterson and Cowling (1964) were in agreement with those of

Southam and Ehrlich (1945). Peterson and Cowling (1964) showed that the effect of water extraction on both Sitka spruce and Southern pine sapwood was to significantly increase resistance to decay, unextracted sapwood samples showing weight loss values nearly twice as great as extracted samples. It was found that when nitrogen was added to matching extracted blocks, weight loss production increased. Peterson and Cowling (1964) concluded that "the evidence suggests that water extraction removed low molecular weight carbohydrates, or nitrogenous materials, or both, and these substances are necessary for the test fungi to become established in Sitka spruce wood." However, nitrogen analyses of the extracted wood were not carried out, nor was the role of soluble nitrogen stressed in the further work of Cowling et al. outlined in 2.1.

Gaumann (1930) (in Findlay 1931) showed that wood was more susceptible to decay when felled in winter than when felled in summer, and Hepting (1945) showed that reserve food materials were present in wood in greater quantities before foliation than after foliation. Bletchly (1966 a , 1969a), and Levi and Cowling (1968) showed that nitrogen was also present in greater quantities before foliation than after foliation. To prove Gaumann's (1930) observation on susceptibility of wood to decay in relation to season of felling, Levi and Cowling (1968) extracted cores from oak trees before and after foliation of the wood and inoculated them with Polystictus versicolor and Lenzites trabea. After the incubation period, it was found that the wood with higher nitrogen content showed a significantly higher weight loss than the wood with the lower nitrogen content. It was then concluded that wood felled before foliation might well be more susceptible

to decay than wood felled after foliation because of the higher total nitrogen content.

It has been shown that seasonal variations in soluble nitrogen occur in the cambial area (Clarke and Mills, 1970) in the sap of wood (Bollard, 1957a, b and c),&in reserve carbohydrates (Hepting, 1945). Levi and Cowling (1968) considered that seasonal nitrogen variation was associated with changes in reserve food materials. These latter authors, however, did not relate the increased decay rate observed with increases in soluble nitrogen, but rather with increases in total nitrogen of wood, and were able to demonstrate a relationship between total nitrogen and decay.

The evidence contained in some of the literature on nutrition of wood-colonising insects provides circumstantial evidence that soluble nitrogenous materials also play a considerable role in influencing insect colonisation of wood.

It has been shown (Bletchly, 1966b; Bletchly and Farmer, 1959; Bletchly and Taylor, 1964) that larval growth rate of wood-colonising insects is closely dependent on nitrogen in wood, and Baker and Bletchly (1966) considered that little development of Anobium punctatum larvae could take place in wood containing less than 0.03% total nitrogen (the approximate lower level of structural nitrogen found in spruce, Fig. 2.19). Bletchly (1966a), however, also showed a correlation between the growth of Anobium larvae and the seasonal fluctuation of nitrogen content of Salix branch material which, it seems likely, may be attributable only to soluble nitrogen variation. Consequently, it may be suggested that growth of Anobium larvae is related not

only to total nitrogen content in wood but more particularly to the soluble nitrogen component. Studies by White (1962) and Bletchly (I969b) showed that if wood is colonised by blue staining fungi, subsequent development of A. punctatum larvae is markedly decreased. As these fungi are believed to utilise the soluble nitrogen materials in wood, their colonisation may have resulted in a depletion or alteration of these substances which resulted in their not being available to A. punctatum larvae. This suggested mechanism would explain their inhibited growth in blue-stained wood.

This hypothesis that A. punctatum might be greatly dependent on soluble nitrogen in wood is, however, at variance with Becker (1942) (in Baker, Laidlaw and Smith, 1970), who suggested that A. punctatum could "survive and even gain in body weight on a diet of nearly pure cellulose". Knudsen Cymorek and Bakke (1969) provide evidence for the role of soluble nitrogen in colonisation of wood by another insect, this insect, Hylotrupes balulus. In studies with/the growth and development of which is also correlated with wood nitrogen (Becker, 1963), they showed that ponding of both pine and spruce immediately after felling produced a significant inhibitory effect on development of larvae. Nitrogen analyses on the sapwood of the pine showed the total nitrogen content of the sapwood had been decreased from 0.065% to 0.040% as a result of the ponding process. It has been shown (Expt. 2.2) that water extraction of spruce resulted in a depletion of the soluble nitrogen present and 0.040% nitrogen corresponds to the level of nitrogen remaining in the centre of pine planks after much of the soluble nitrogen had migrated to drying surfaces (Expts. 2.5 and 2.6). It is consequently apparent that soluble nitrogen might well play an important role in

the development in wood of H. bajulus (along with A. punctatum).

Recent work (Parry, 1974) on aphids in Sitka spruce needles support these conclusions, when it was shown that seasonal variation in certain amino acids correlated directly with aphid population numbers.

Bletchly (1966b) carried out a series of studies on the influence of kiln-drying schedules on the development of H. bajulus in Sctos pine sapwood, and found that high temperature in combination with high humidity markedly reduced the susceptibility of the wood to colonisation; drying at high temperature without high humidity produced less significant effects and drying with lower temperatures did not greatly inhibit colonisation in relation to the higher temperatures.

The data produced on drying of wood (Expts. 2.1, 2.4, 2.5, 2.6, 2.8) showed that soluble nitrogen could be lost or redistributed in wood during the drying process, and that the extent of these reactions seemed to be controlled by combinations of temperature, humidity and sample size.

It has been shown that a relationship exists between soluble nitrogen and wood colonisation by H. bajulus (Knudsen et al., 1959) and a possible explanation for Bletchly's (1966b) observation was that soluble nitrogen and other soluble materials were distributed to the drying surfaces of the wood during drying at high temperatures. Nitrogenous materials in an available form would therefore have been "less available" to the colonising larvae which live below the surface, thus they would have had to expend greater energy in finding nitrogenous materials in the wood which would be expressed in the form of

lower larval weights, and a consequent overall inhibition of infestation (Baker Laidlaw and Smith, 1970). Becker, Hof, and Walchii (1970) have found that higher wood-drying temperatures resulted in lower toxicity values for some insecticides; however, nutrient redistribution during drying does not seem to have been examined as a contributory factor; and Baker (1972) has shown that A. punctatum larvae tended to colonise the outer regions of test blocks leaving the inner regions uninhabited. In blocks treated with preservatives after inoculation, the larvae in the outer layers were killed and the survivors migrated to, and remained in the core. However, in blocks treated with the solvent without the preservative included, a similar initial effect was observed, but after the solvent had evaporated, survivors remigrated and colonised the surface layers.

Both these observations may be explained in terms of nitrogen redistribution during drying. In the case of the wood dried at higher temperatures (Becker, Hof and Walchii, 1970), the lower toxicity values are explicable by supposing that the larvae have to ingest greater quantities of wood (consequently greater quantities of insecticide) to obtain their nitrogen requirements, as greater soluble nitrogen redistribution occurs at higher drying temperatures. The observations of Baker (1972) may be explained by the presence of soluble and consequently easily-available nitrogenous and carbohydrate material in the outer 2-3mm of plank surfaces.

It is therefore suggested in the light of the circumstantial evidence presented above that soluble nitrogen has a greater role in the deterioration of wood than is suggested by the literature. There is significant evidence to show that this is the case for both fungi and insects. For fungi,

direct evidence is available from the work of Levi and Cowling (1968) who related increased weight loss in wood with soluble nitrogen increased by 0.03% associated with seasonal variation. The accumulated soluble nitrogen levels at the surfaces of planks dried at this laboratory were increased by considerably more than 0.030% and consequently, it might be expected that the extent of decay produced by fungi in these regions would be severe.

Cowling and Merrill (1966) and Levi and Cowling (1969) have respectively shown that the cellulase systems of fungi are regulated by the C : N ratios present in wood, and the C:N required by microfungi before cellulase production could occur would be less than 200 : 1. By calculation of C (Levi and Cowling, 1968) from the total cellulose present in wood (Butcher and Drysdale 1974), it is possible to show/ the probable C : N ratios present in the stems of the Sitka spruce and pine trees examined at this laboratory (Fig. 2.21). This figure also shows the probable C : N ratios of the soluble components only. Soluble carbohydrates were derived from the alcohol-water and cold-water solubles data in Figure 2.20, and Table 2.5.

The ratios of total nitrogen to the carbon in cellulose of both pine and spruce would be, according to Levi and Cowling's data, unsuitable for the production of soft-rot cavities by microfungi, whereas the ratio of soluble carbon to soluble nitrogen in wood are low enough (below 200 : 1) in the sapwood to support cellulolytic activity. However, King and Smith (1973) have shown that it is not only the ratio of C : N which is important in wood but also the absolute amounts of these materials present. Consequently,

it may be deduced that whereas the ratio of soluble C to soluble N in pine and spruce may be suitable for production of soft-rot cavities, the absolute amounts of nitrogen present may be insufficient to support these activities in wood in the green condition.

Lundström (1972), by adding nitrogen in the form of ammonium nitrate and asparagine to Betula sp. sapwood, raised the total nitrogen content of the sapwood from 0.07% and 0.09% to 0.27% and 0.20% respectively, and when ammonium tartrate, and casein hydrolysate were added, the levels were raised to 0.15%. In studies on the rate of cavity formation in Betula, he then found that it was difficult to follow cavity formation in nitrogen-impregnated wood after 100 hours' incubation due to the rapidity of formation of cavities. Data presented in this thesis show that soluble nitrogen accumulated at evaporating surfaces sufficiently to raise the total nitrogen from an average value of 0.051% to 0.116% in spruce, and from 0.048% to 0.191% in pine. It may therefore be suggested, in the light of Lundström's work that these raised levels of nitrogen may similarly raise the susceptibility of these wood surfaces to decay and also satisfy King and Smith's criteria for absolute amounts of nutrients present.

Microfungi colonising wood have generally been characterised by the broad nature of the phenomena they cause, e.g. "blue-staining fungi", "mould fungi", "soft-rot fungi", etc. It has been shown, however (Savory, 1954b; Krapivina, 1960; Merrill, 1965; Nilsson, 1973) that many microfungi characterised by one particular effect, such as decay of cotton textiles, staining of wood, or causing surface discolourations of wood,may also be included among the soft-rot fungi.

FIGURE 2.20 Soluble materials present in spruce as a % of wood dry weight.
(Discs from five trees were analysed in five ring groupings; the data presented are the averages for these discs.)

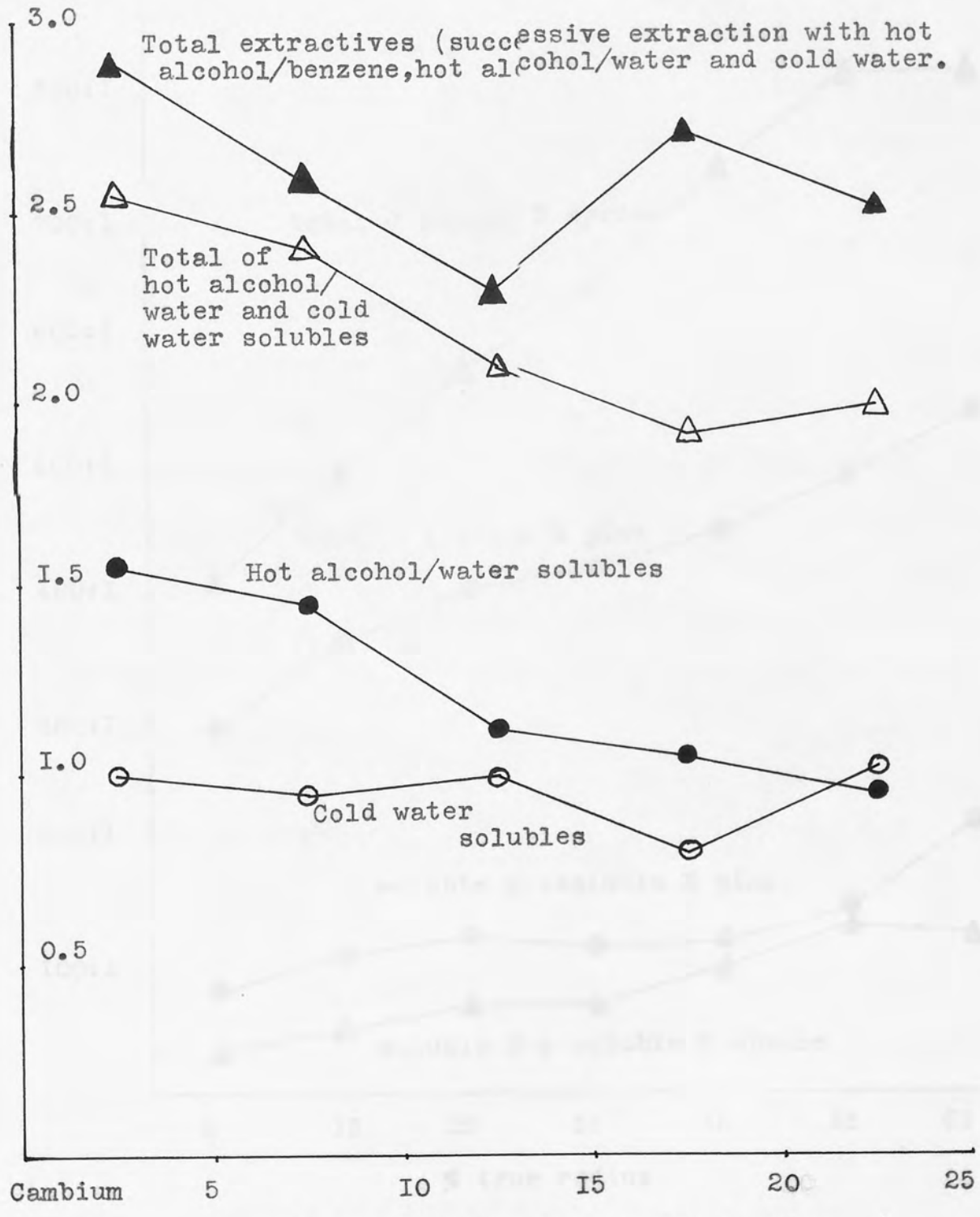
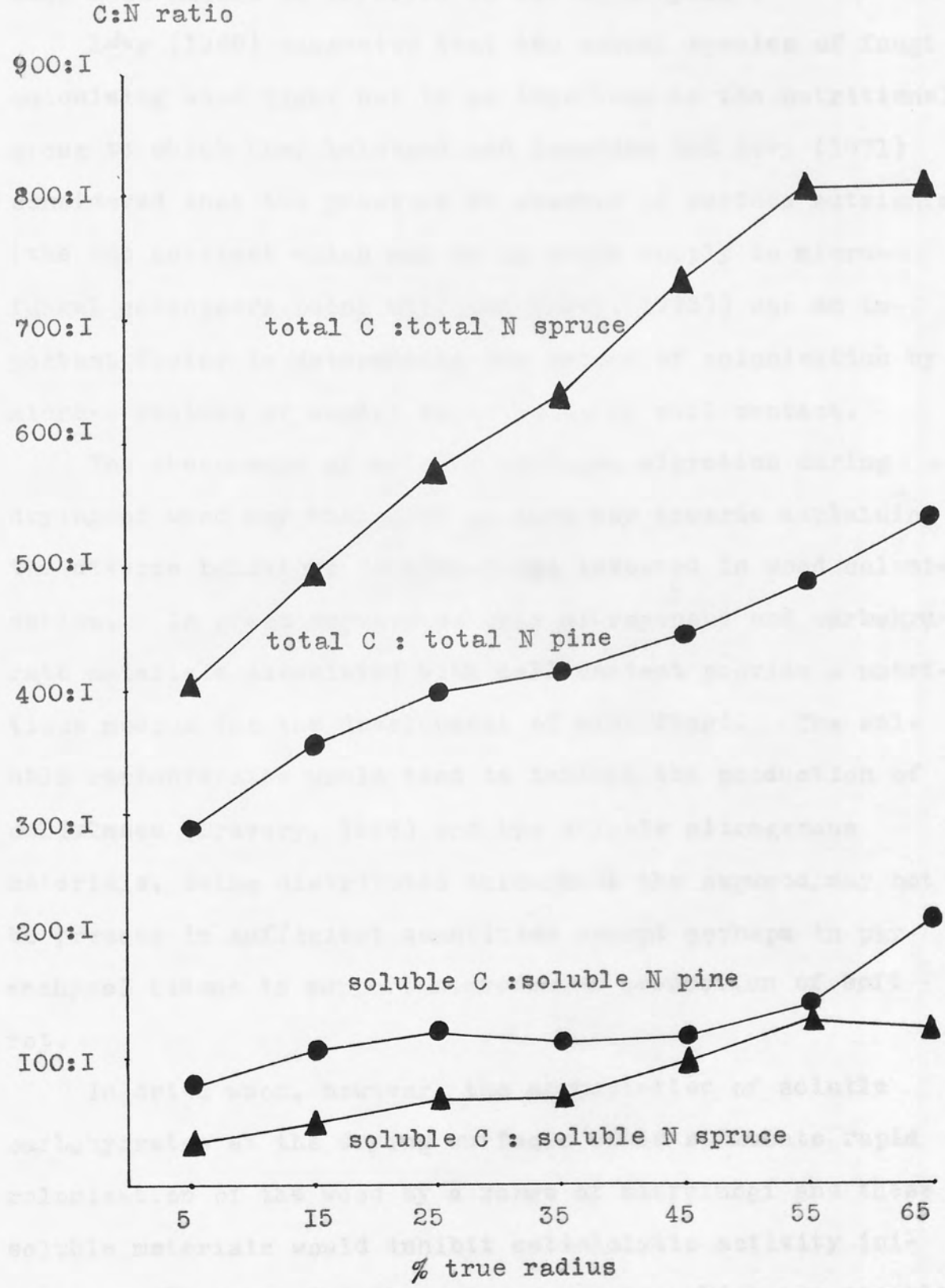


FIGURE 2.2I Carbon (C) to Nitrogen (N) ratios in pine and spruce sapwood in the green condition.



Gorshin and Krapivina (1969) have suggested that the use of descriptive terms such as "mould","staining" and "soft rot" should be discontinued and that all microfungi colonising wood should be referred to as "micromyces".

Levy (1969) suggested that the actual species of fungi colonising wood might not be as important as the nutritional group to which they belonged and Banerjee and Levy (1971) considered that the presence or absence of surface nutrients (the one nutrient which may be in short supply to microfungal colonisers being nitrogen (Levy, 1973)) was an important factor in determining the extent of colonisation by micro-organisms of wooden fence posts in soil contact.

The phenomenon of soluble nitrogen migration during drying of wood may therefore go some way towards explaining the diverse behaviour of microfungi involved in wood colonisation. In green sapwood soluble nitrogenous and carbohydrate materials associated with cell content provide a nutritious medium for the development of microfungi. The soluble carbohydrates would tend to inhibit the production of cellulases (Bravery, 1968) and the soluble nitrogenous materials, being distributed throughout the sapwood,may not be present in sufficient quantities except perhaps in parenchymal tissue to support microfungal production of soft rot.

In dried wood, however, the accumulation of soluble carbohydrates at the drying surfaces would stimulate rapid colonisation of the wood by a range of microfungi and these soluble materials would inhibit cellulolytic activity initially. However, for those fungal species which are capable of acting as "sugar fungi" in the presence of simple nutrients and also capable of acting as soft-rot fungi (Banerjee

and Levy, 1971), enough soluble nitrogen would be accumulated at the drying surfaces of wood to support soft-rot production when soluble carbohydrates were depleted. In terms of C : N ratios, the hypothesis of Cowling et al. on C : N requirements of microfungi for cellulase production would also be largely fulfilled since the C : N ratios at the surface of spruce would be approximately 259 : 1 and for pine would be 157 : 1.

It was therefore concluded that wood is not deficient in nitrogen at the evaporating surfaces, even though nitrogen is generally considered to be the limiting factor to decay. Soft rot, the form of wood decay produced by microfungi, generally occurs at plank surfaces, and Banerjee and Levy (1971) suggested that the nutrient status of the surface of wood might influence the sequence of colonisation which occurred there. These authors also noted that wood colonisation by maximal numbers of fungal species occurred in the outer 5mm of wood, the area to which migration of soluble nitrogen occurred in the experiments described in this chapter.

As nitrogen is considered to play a critical role in the decay of wood by microfungi, it was hypothesised that soluble nitrogen in easily-available (Griffin, 1972) water soluble forms, might provide a considerable stimulus to colonisation by micro-organisms at a soil-contact face. This influence of redistributed soluble nitrogen has hitherto not been examined, although the phenomena associated with redistribution might well have been described in terms of soft rot. It was therefore decided to examine directly the influence of redistributed soluble nitrogen on production of soft rot by micro-organisms in wood. The experiments to determine this influence are described in Chapter 3.

CHAPTER III

SOIL BURIAL STUDIES

3.1. INTRODUCTION

The purpose of this work was to determine the extent of nitrogen movement into wood from soil with which it is in contact, and to relate this to the amount of soft-rot produced in the wood as a result of soil burial. It was initially decided (Chapter 1, Section 3) to use green Sitka spruce as the test material and the results of initial experiments showed that the nitrogen content of green spruce was greater than that of dried spruce and was apparently unstable. As such differences existed, it was obvious that green spruce could not be used to measure nitrogen migration from soil, and similarly, that the differences themselves would also merit investigation. The investigations into the latter phenomenon are described in Chapter 2.

Concurrent with these latter investigations, the initial aim of the project was also pursued, viz. to monitor nitrogen migration into wood from soil. This took the form of a series of soil-burial experiments carried out between April 1973 and November 1974 using both spruce and pine. The first of these experiments, the spruce experiment, was carried out without knowledge of nutrient redistribution or nitrogen migration, and the results were initially considered only in the context of nitrogen movement into wood. However, when the phenomenon of nitrogen migration during drying of wood was discovered, the results became pertinent to the biological role of redistributed soluble nutrients. The experiments with pine were undertaken <u>after</u> the discovery of nutrient distribution and consequently were related both to nitrogen movement into wood and to the biological role of redistributed soluble nutrients.

The format of presentation of Chapter 2 has been followed in this chapter, the experiments being described in the order in which they were carried out in Section 3.2. (Experimental), and being collectively discussed in Section 3.3. (Discussion).

3.2. EXPERIMENTAL

3.2.1. Soluble Nitrogen in Soil (Experiment 3.1.).

The technique used to determine nitrogen in the previous chapter was not designed to quantitatively detect nitrates. This did not detract from the results as the nitrate content of wood is negligible, its structural nitrogen being generally in the form of proteins, and the soluble nitrogen in wood, either in storage tissue or in material being translocated from the roots being in the form of amino acids (Bollard, 1953; Merrill and Cowling, 1966). However, the purpose of the research was to determine the extent of nitrogen movement from soil into wood, and it was envisaged that movement of soluble nitrogenous materials including nitrates would take place as a result of soil contact. It was therefore considered that the technique used to determine nitrogen content on wood blocks should also be capable of determination of nitrogen addition caused by nitrates which has passed into the wood from soil.

The nitrate content of an unfertilised soil such as is used in soil burial experiments at Princes Risborough Laboratory could be expected to be small, as most of the available nitrogen in soil is rapidly utilised by micro-organisms (Garrett, 1963) and Nikolskin (1959) has suggested that most of the nitrogen in soil would be in the form of its component micro-organisms. The micro-kjeldahl technique for nitrogen determination used previously was capable of measuring small quantities of nitrates present in organic material but required modification before large amounts could be detected. This modified technique was considerably longer than the unmodified technique.

If the nitrate content of the soil to be used for wood

contact experiments was low, nitrogen determinations could be undertaken on the wood using the unmodified technique with considerable saving of time, whereas if the nitrate content of the soil was high, it would be necessary to analyse the wood after soil contact with the modified technique. It was therefore decided to investigate the nitrogen status of the soil with reference to the presence of nitrates and other soluble nitrogenous materials, and with particular reference to the analytical method used to determine these substances.

A. Materials and Methods.

Two samples of soil were analysed. These were the soil used for the spruce experiment (analysed after the burial period) and the soil used for the burial experiments with pine analysed before the burial period. Both samples were obtained from Princes Risborough Laboratory. The soil used for the spruce experiment was an unamended sandy loam removed from the same sample plot as that used for the thin strip experiments of Smith (1971). The soil used for the pine burial experiments was obtained from a new soilpit close to this site.

The soil samples to be analysed were thoroughly mixed in plastic sacks before sampling. A sample was then removed from each sack and was dried at 100°C overnight. These samples were then ground in a pestle and morter. Total nitrogen content both including and excluding nitrates was determined according to the micro-kjeldahl methods of Humphries (1956) on soil samples in an unextracted form, and when successively extracted with alcohol-benzene, alcohol water, and hot water (the same method used to extract soluble nitrogen from wood in Chapter 2 and by Laidlaw and Smith, 1965).

Three replicate determinations were carried out for each treatment on the post spruce burial material and five replicate determinations were carried out on the ante pine burial material.

B. Results

The results for this experiment are presented in Table 3.1. Analyses of variance showed that the differences

Table 3.1

Nitrogen Content of Soil

Total Nitrogen Content (% of Soil Dry Weight)				
Soil Type	Extracted Soil		Unextracted Soil	
	(a)	(b)	(a)	(b)
Spruce experiment	0.230	0.232	0.258	0.256
Pine experiment	0.275	0.268	0.304	0.307

(a) total nitrogen content including nitrate content

(b) total nitrogen content excluding nitrate content

between means for all treatments was only significant for the post spruce burial soil at $p = 0.2$ ($F = 2.36$) and these differences could be attributed to the extraction of the soil.

The differences between the nitrogen contents of both extracted soil and unextracted soil, when determined using either technique including nitrates or excluding nitrates, were not significant (t = 0.14 and t = 0.13 respectively).

Analysis of variance of the means of all treatments for the ante pine burial soil showed that the differences between means were significant at p = 0.001 (F = 14.4). However, as in the post spruce burial soil, this difference in means could be solely attributed to the effects of extraction. The differences between the means of both extracted soil and unextracted soil when nitrogen was determined using either a process including or excluding nitrates were not significant (t = 0.76 and t = 0.64 respectively).

C. Discussion

The results showed that approximately 10% of the total nitrogen content of the soil was in a soluble form, and that there was no measurable amount of nitrate in either of the soil samples tested. As the extraction process was severe, it was considered that the 10% soluble nitrogen in the soil would have included nitrogenous cytoplasmic constituents of the microbial population which would normally not be considered as a part of the "soluble nitrogen" present in soil. Similarly, the washing and filtration of the soil would have removed numbers of the bacterial and actinomycete population the biomass of which is also nitrogen rich. It was therefore concluded that the amount of soluble nitrogen present in soil in an available form to micro-organisms was probably less than the 10% suggested by the data in Table 3.1.

A previous estimate of the nitrogen content of P.R.L. test soil by another worker (I.R.G. Document, Bravery 1971) was 0.251%, a figure consistent with the figure produced for the post spruce burial soil. Comparative data are not available on the nitrogen content of the new soil plot to the author's knowledge. It was concluded that the differences in nitrogen content between the two soil samples could be attributed to site differences.

The fact that no measurable amount of nitrate was present in the soil was also consistent with the literature viz. in unamended soil most of the nitrogen present would have been utilised by the component micro-organisms. It was therefore concluded that the unmodified micro-kjeldahl technique would adequately determine nitrogen changes in wood as a result of soil contact as there were no measurable amounts of nitrates to move from soil into the wood.

3.2.2. The relationship of Soil Nitrogen to Soft Rot Production in Spruce (Experiment 3.2.).

The spruce burial experiment described in this section was an attempt to replicate the initial experiment described in the Introduction (1.3) which was undertaken as the first experiment in a series to determine the threshold level of nitrogen movement from soil into wood which would permit soft rot formation. (It was envisaged that, by using a standard time period, e.g. 12-14 weeks, for incubation of test blocks, and by varying the proporation of that time period during which the wood was in soil contact or was incubated at high humidity, it would be possible to determine this threshold level. Therefore, the first experiment included a three-day soil contact period and a twelve-week incubation period, the

next experiment was planned to have a two-week soil-contact period and a ten-week high-humidity incubation period, etc.) As the duration of the first experiment and the subsequent analyses was quite lengthy, and there were neither very great changes in the nitrogen distribution in the test blocks, nor any soft-rot production therein, as a result of the limited soil contact period, it was decided to change the order of the series of experiments. Instead of a soil contact experiment, it was decided that wood blocks would be buried in soil for the entire test period and would be examined afterwards for additional nitrogen content as the first experiment in the series. Once the amount of nitrogen migration from soil into wood had been quantified for the maximal soil exposure period, and the amount of soft rot produced as a result of soil burial was assessed, it would then be possible to plan a further series of experiments to determine the threshold nitrogen "pick-up" level at which soft-rot occurrence could be observed.

To overcome the difficulties experienced with the use of green material, it was decided that only air-dried wood would be used for this experiment.

It was decided also to examine the original hypothesis that cell contents in wood might influence the translocation of nitrogenous materials into wood. This could be carried out by using the dried sapwood and inner sapwood of spruce to provide material with, and largely without, cell contents respectively. It was proposed that initially four parameters would be examined in relation to wood buried in soil; these would be moisture content, total nitrogen content, presence of soft-rot cavities and respiration of wood blocks. Central

to this analysis scheme was that, as far as possible, all measurements could be made on each test block used so that variations in the physical or chemical composition between the wooden test blocks would not interfere unduly with the parameters being examined. It was hoped that by examining these parameters in relation to wood containing and not containing cell contents, changes in the wood condition after burial could qualify or amplify the phenomenon of the occurrence of soft rot in wood, and possible nitrogen movement from soil.

A. Materials and Methods

(i) Materials

The materials used for this experiment were the wood of Sitka spruce, and soil obtained from Princes Risborough Laboratory, Aylesbury, Bucks. The spruce used was obtained at the same time as the material used for Experiments 2.1-2.3.

The Sitka spruce wood was removed in the form of a bolt from a standing tree (2.2.1.A.) and was immediately converted (while still in the green condition) into 30mm thick quarter-sawn (i.e. radial) planks. Some of these planks were retained in a deep-freeze unit (at -18°C), while the remainder were slowly air dried in the forced-air current of a fume cupboard with an air supply from outside the laboratory. When the planks had been dried, they were converted into test blocks by tangentially sawing them into strips 10mm thick and then transversely cutting the strips into 90-mm lengths. Matching blocks, each of 90 x 30 x 10mm were prepared from each strip, each matching block containing the

same number of growth rings, and including the same growth ring groupings. Strips were not removed from the juvenile wood area of the planks which was arbitrarily designated as rings one to fifteen measured from the pith. Sapwood samples were taken from strips which were included within rings one to fifteen measured from the cambium, while inner sapwood samples were removed from strips which were included within rings 15-25 measured from the cambium (Figure 3.1).

After the blocks were prepared they were carefully numbered, and one block from each converted strip was reserved for control determinations. The tangential and transverse faces of each block were then coated in "Araldite" epoxy resin (the commonly-found commercial brand), leaving only the radial edges uncoated. When dry, the blocks were then cured overnight at 100°C. The blocks were then ready for burial.

The soil used for burial was supplied by Princes Risborough Laboratory, and is described in 3.2.1AA. This soil was thoroughly mixed and was then moistened to its water-holding capacity (Savory 1972) of about 32% moisture content.

(ii) Methods

(a) Burial and Incubation of test Blocks

Polythene boxes 85mm deep by 330 x 230mm were filled to a depth of 35mm with soil at water-holding capacity. Six to eight test blocks were placed with tangential faces in the horizontal plane on the soil surface. The blocks were then covered with a further 35mm of soil until the container was just filled. The soil was slightly compacted and the boxes of soil and test blocks were then weighed.

FIGURE 3.I Conversion of planks to test blocks

A. Quartersawn plank, 30mm thick, slowly air-dried in fume cupboard

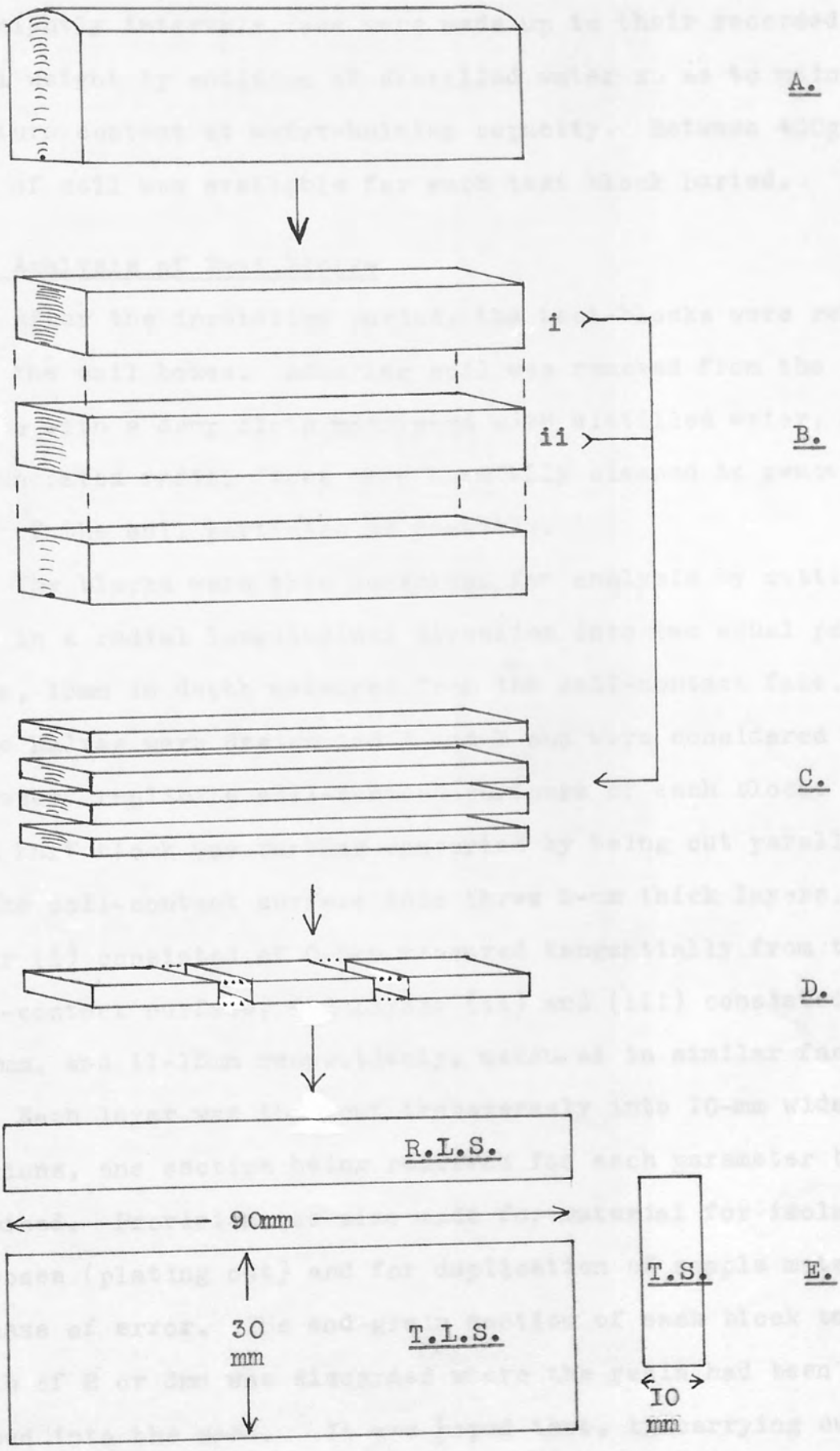
B. Air-dried plank converted into (i), a sapwood region which included rings one to fifteen measured from the cambium, and (ii), an inner sapwood region which contained rings sixteen to twenty five measured from the cambium. The juvenile /heartwood region of the plank was discarded. The conversion was carried out by sawing tangentially.

C. Conversion of sapwood and inner sapwood regions into 10mm thick strips (the radial measurement of the strips being 10mm)

D. Conversion of strips by transverse sawing into test blocks

E. Exact dimensions and orientation of test blocks

Note: Both opposing radial longitudinal faces (R.L.S.) uncoated to allow soil contact. Both opposing tangential longitudinal faces (T.L.S.) and transverse faces (T.S.) sealed with "Araldite" epoxy resin.


A.
i
ii
B.
C.
D.
R.L.S.
90mm
30
mm
T.L.S.
T.S.
E.
10
mm

The buried test blocks were incubated at 25°C for 14 weeks. During this time, the polythene boxes were weighed at fortnightly intervals, and were made up to their recorded original weight by addition of distilled water so as to maintain moisture content at water-holding capacity. Between 400g and 600g of soil was available for each test block buried.

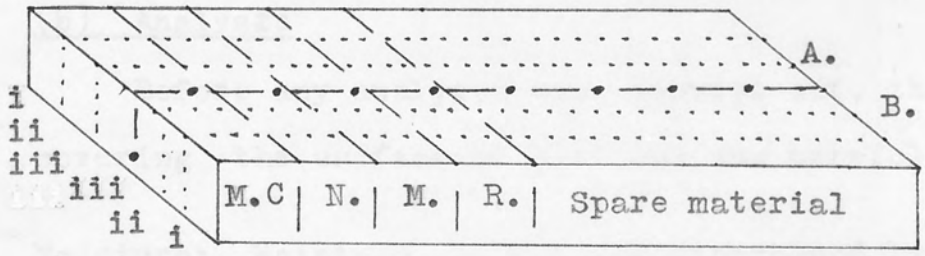
(b) Analysis of Test Blocks

After the incubation period, the test-blocks were removed from the soil boxes. Adhering soil was removed from the blocks with a damp cloth moistened with distilled water, and the uncoated radial faces were carefully cleaned to remove as many of the soil particles as possible.

The blocks were then converted for analysis by cutting each in a radial longitudinal direction into two equal portions, 15mm in depth measured from the soil-contact face. These halves were designated A and B and were considered to represent replicate soil-contact surfaces of each block. Each half block was further converted by being cut parallel to the soil-contact surface into three 5-mm thick layers. Layer (i) consisted of 0-5mm measured tangentially from the soil-contact surface, and Layers (ii) and (iii) consisted of 6-10mm, and 11-15mm respectively, measured in similar fashion.

Each layer was then cut transversely into 10-mm wide sections, one section being reserved for each parameter being examined. Provision was also made for material for isolation purposes (plating out) and for duplication of sample material in case of error. The end-grain section of each block to a depth of 2 or 3mm was discarded where the resin had been absorbed into the wood. It was hoped that, by carrying out each

FIGURE 3.2 Conversion of wood blocks after burial



—•— Conversion of block into two sides, A and B

..... " " sides " layers, i, ii, and iii.

— — " " layers " specimens for individual analyses

M.C. Moisture content

N. Nitrogen content

M. Microscopic examination

R. Respirometric evaluation of wood colonisation

analysis on the matching layers of each section, a measure of the movement of organisms and nitrogen, and the development of soft rot in depth in wood could be established. The conversion of test blocks to layers and sections after soil burial is illustrated in Fig. 3.2. Blocks individually sealed in polythene sheets, were stored in an unconverted form in a refigerator (4°C) until analysed.

(b) Analyses

Before any analyses were carried out, the epoxy resin covering the surface of sections was carefully removed.

Moisture: Moisture content was determined by loss of weight on drying in an oven and expressed as a percentage of the wood dry weight (Cartwright and Findlay, 1958).

Nitrogen: Nitrogen content was expressed as a percentage of the wood dry weight and was determined as outlined in 2.2.1.A.

Soft Rot: Soft-rot presence was determined by microscopic examination. Tangential longitudinal sections 25µ thick (i.e. sections having the soil exposed surface at one edge) were taken from each layer with a sledge microtome. These were stained with aqueous safranin and picro-analine blue (Gurr 1956) after the manner of Jane (1956). Best results were obtained if the sections covered with picro-analine blue were boiled for about 15 seconds. The sections were examined under polarised light after staining. The depth to which soft-rot cavities were found was measured from the radial soil-contact surface. Two sections were measured in each layer.

Respiration: Respiration in each layer was measured using a warburg respirometer. Each layer section (5mm x 10 mm x 10mm) was split into slivers of less than 3 x 3mm cross section to permit movement of gasses within tissue sections (Kenten 1956), and was placed in a 15-ml flask which contained 0.5 ml of 20% Potassium hydroxide in the centre wall to absorb CO_2 produced. A piece of folded filter paper to increase the absorption area was also included. The respirometer water bath maintained a flask temperature of 25°C, and each experiment was carried out over a four-hour time period (Behr, 1972), initiated by a one-hour equilibration period during which the flask was open to the atmosphere. Manometer readings were taken each hour. Respiration in each layer was calculated in microlitres (Halabisky and Ifju, 1968) as the average amount of oxygen used per hour per cc of wood. As the manometer reading for the first hour of incubation after the system had been closed to the atmosphere was frequently unusually large, the manometer readings for the second and third hours of incubation were used as a basis for this average.

(The large reading for the first hour of incubation has been explained by Behr op. cit. in terms of continued respiration of micro-organism mycelium not actively growing in the wood but growing on traces of inoculum material still adhering to wood surfaces. This contained respiration was seen by Behr to stop upon exhaustion of these extra nutrients. A further explanation is provided by the fact that when the monometric system is sealed from the atmosphere, apart from carbon dioxide produced by the organisms being absorbed in the potassium hydroxide in the centre wall, the carbon

dioxide contained in the air in the flask will also be absorbed, thus "inflating" the first reading.)

Teyegaga (1975) has shown, using wood specimens converted as described for the experiments related here, that during the second and third hours' incubation in the respirometer flask, oxygen consumption in soil-inoculated wood blocks is at the logarithmic phase, and is hence a reliable comparative indication of biological activity.

Respiration was calculated on a volume rather than a weight basis as it was considered that the "weight" figure would have been subject to too much variation due to variation in moisture content with consequent effects upon the calculated figure.

B. Results

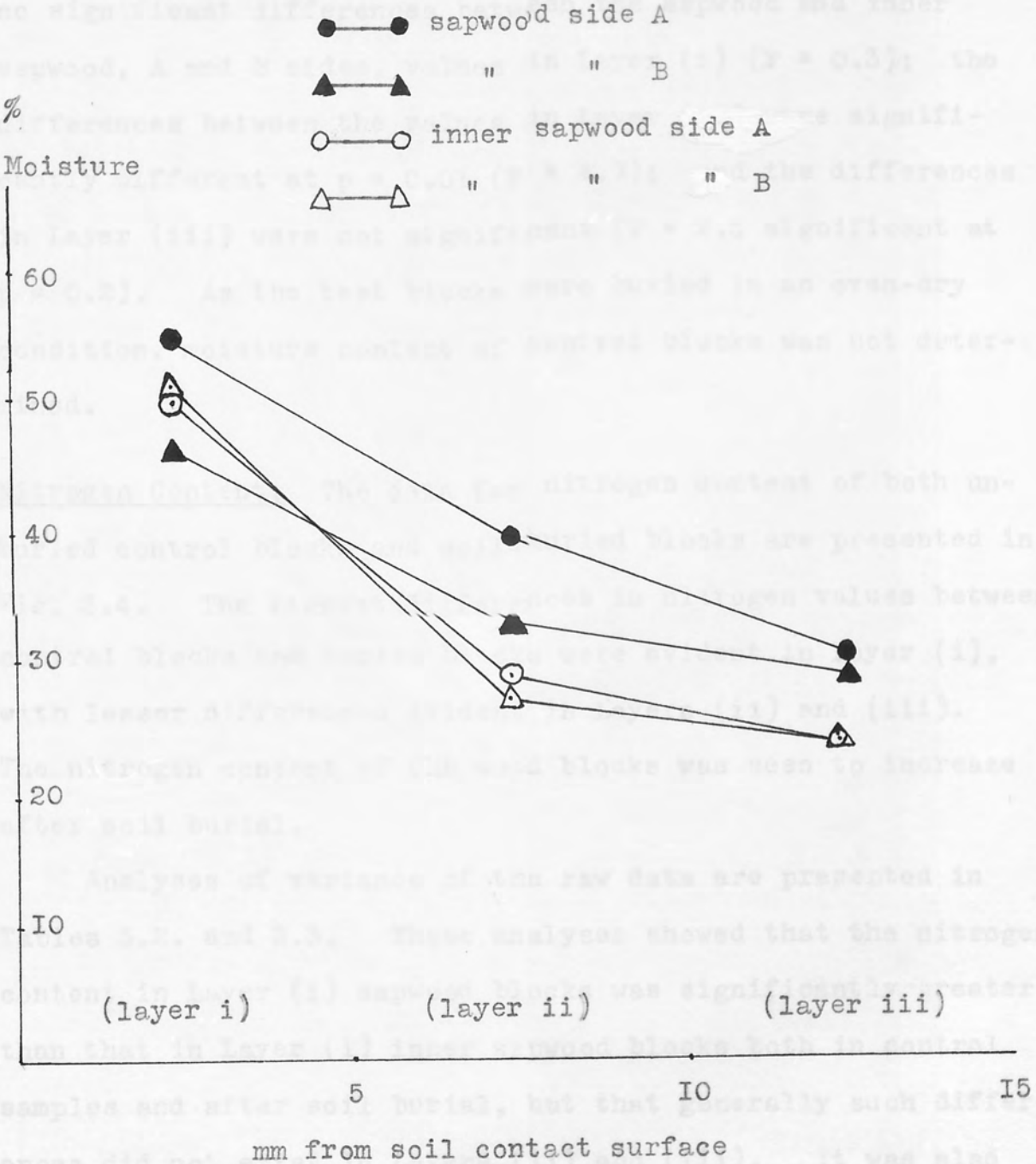
The results for these analyses are presented graphically in Figs. 3.3., 3.4, 3.5, and 3.6. Fourteen sapwood and 14 inner sapwood blocks were examined after soil burial. Eight unburied control blocks of each type were also analysed.

A statistical analysis giving the number of replicate determinations, mean, standard deviation and standard error of the raw data contributing to the figures is presented in Appendix I.

Moisture Content

The data for moisture content are presented in Fig. 3.3. Generally, the inner sapwood samples had somewhat lower values than the sapwood values, and the figure also shows that for the A and B sides of the test blocks the moisture values were very similar. Moisture in Layer (i) ranged from 55% to 46%;

FIGURE 3.3 Moisture in spruce blocks after burial



in Layer (ii) from 27% to 40% and in Layer (iii) from 24% to 31%.

Analysis of variance, however, showed that there were no significant differences between the sapwood and inner sapwood, A and B sides, values in Layer (i) ($F = 0.3$); the differences between the values in Layer (ii) were significantly different at $p = 0.01$ ($F = 4.7$); and the differences in Layer (iii) were not significant ($F = 2.5$ significant at $p = 0.2$). As the test blocks were buried in an oven-dry condition, moisture content of control blocks was not determined.

Nitrogen Content: The data for nitrogen content of both unburied control blocks and soil-buried blocks are presented in Fig. 3.4. The biggest differences in nitrogen values between control blocks and buried blocks were evident in Layer (i), with lesser differences evident in Layers (ii) and (iii). The nitrogen content of the wood blocks was seen to increase after soil burial.

Analyses of variance of the raw data are presented in Tables 3.2. and 3.3. These analyses showed that the nitrogen content in Layer (i) sapwood blocks was significantly greater than that in Layer (i) inner sapwood blocks both in control samples and after soil burial, but that generally such differences did not exist in Layers (ii) and (iii). It was also shown that the nitrogen contents of Layers (i) of both sapwood and inner sapwood blocks were significantly greater after burial than their respective control blocks, but that the nitrogen contents of layers (ii) and (iii) of both sapwood and inner sapwood had not increased as a result of burial.

FIGURE 3.4. Nitrogen content of spruce blocks both before and after burial

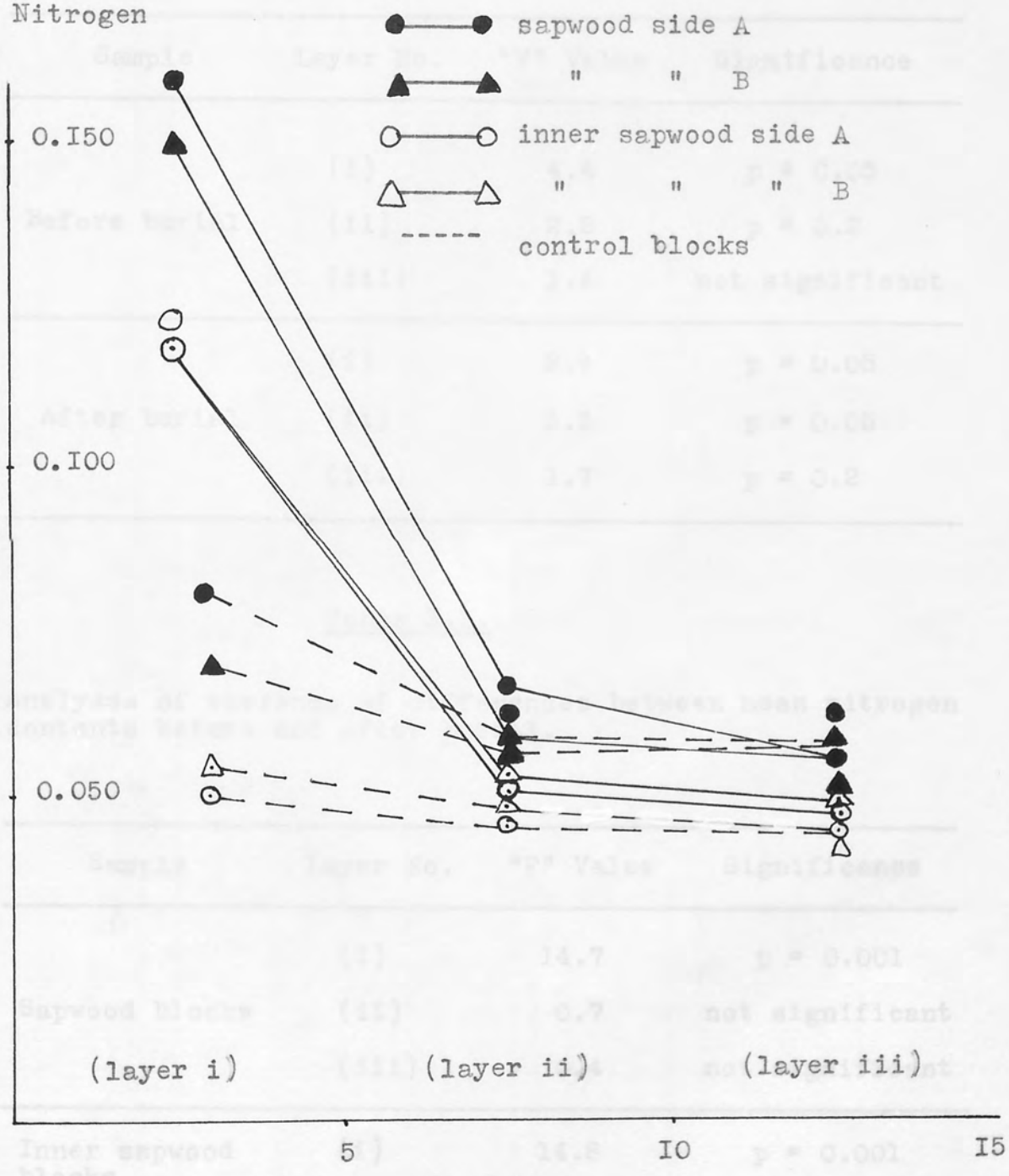


Table 3.2.

Analyses of variance of differences between mean nitrogen contents of sapwood and inner sapwood blocks before and after burial.

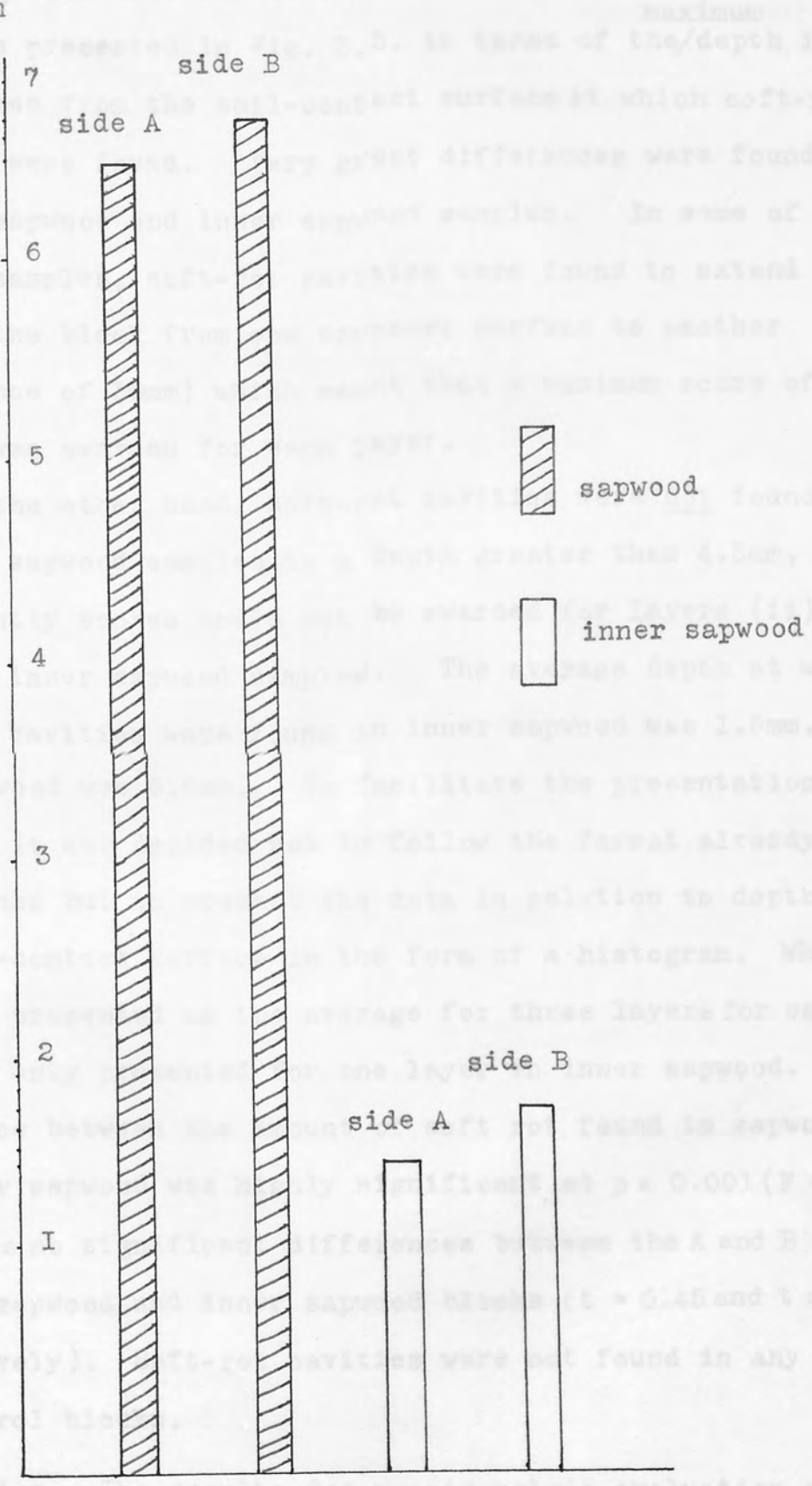
Sample	Layer No.	"F" Value	Significance
Before burial	(i)	4.4	p = 0.05
	(ii)	2.2	p = 0.2
	(iii)	1.4	not significant
After burial	(i)	2.9	p = 0.05
	(ii)	3.3	p = 0.05
	(iii)	1.7	p = 0.2

Table 3.3.

Analyses of variance of differences between mean nitrogen contents before and after burial.

Sample	Layer No.	"F" Value	Significance
Sapwood blocks	(i)	14.7	p = 0.001
	(ii)	0.7	not significant
	(iii)	0.4	not significant
Inner sapwood blocks	(i)	14.8	p = 0.001
	(ii)	0.3	not significant
	(iii)	0.3	not significant

FIGURE 3.5 Soft rot, measured in mm from soil contact surface, in spruce blocks after burial

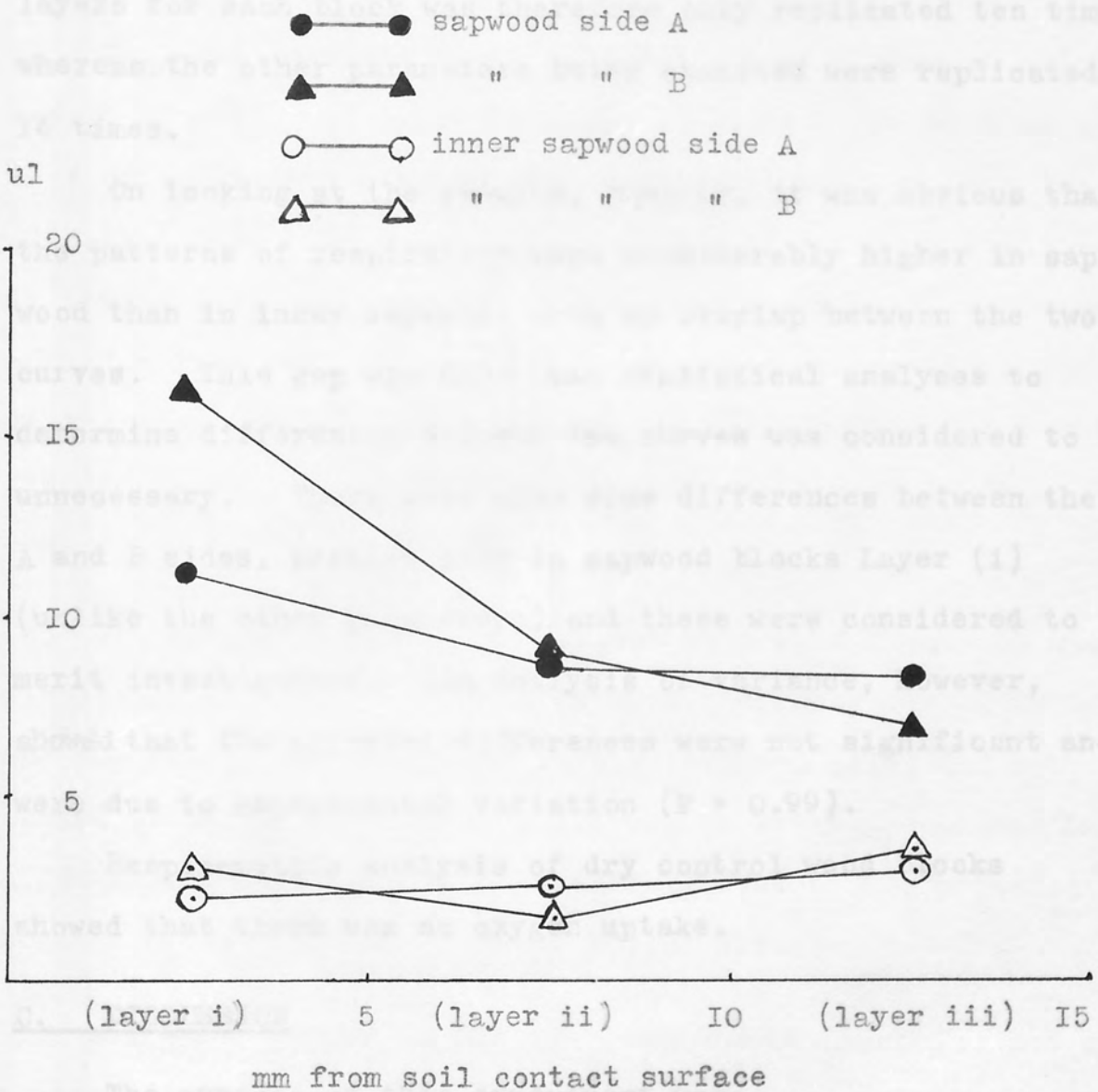


Soft Rot: The data for soft-rot presence in buried soil blocks is presented in Fig. 3.5. in terms of the maximum depth in millimetres from the soil-contact surface at which soft-rot cavities were found. Very great differences were found between sapwood and inner sapwood samples. In some of the sapwood samples, soft-rot cavities were found to extend through the block from one exposure surface to another (a distance of 30mm) which meant that a maximum score of five mm was awarded for each layer.

On the other hand, soft-rot cavities were not found in inner sapwood samples to a depth greater than 4.3mm, consequently scores could not be awarded for Layers (ii) and (iii) of inner sapwood samples. The average depth at which soft-rot cavities were found in inner sapwood was 1.6mm, and in sapwood was 6.6mm. To facilitate the presentation of results, it was decided not to follow the format already established but to present the data in relation to depth from the soil-contact surface in the form of a histogram. Whereas data are presented as the average for three layers for sapwood data are only presented for one layer in inner sapwood. The difference between the amount of soft rot found in sapwood and inner sapwood was highly significant at $p = 0.001$ ($F = 24$). There were no significant differences between the A and B sides of both sapwood and inner sapwood blocks ($t = 0.45$ and $t = 0.89$ respectively). Soft-rot cavities were not found in any of the control blocks.

Respiration: The results for respirometric evaluation of colonisation are presented in Fig. 3.6. These experiments were carried out at 25°C in a small room which at certain

FIGURE 3.6 Respiration in spruce blocks after burial (micro-litres of oxygen consumed per hour per cc of wood)



times was subject to direct sunlight, consequently the air temperature on one or two occasions rose above 25°C. Due to this factor, the respirometric data for those days was subject to some variation. These data have not been included in the presented results. Data for respiration for sapwood layers for each block was therefore only replicated ten times whereas the other parameters being examined were replicated 14 times.

On looking at the results, however, it was obvious that the patterns of respiration were considerably higher in sapwood than in inner sapwood, with no overlap between the two curves. This gap was such that statistical analyses to determine differences between the curves was considered to be unnecessary. There were also some differences between the A and B sides, particularly in sapwood blocks Layer (i) (unlike the other parameters) and these were considered to merit investigation. An analysis of variance, however, showed that the apparent differences were not significant and were due to experimental variation ($F = 0.99$).

Respirometric analysis of dry control wood blocks showed that there was no oxygen uptake.

C. DISCUSSION

The purpose of this experiment was:

(a) to determine if nitrogen moved from soil into wood;

(b) to determine if soft rot development was related to any nitrogen movement; and

(c) to determine whether the presence or absence of cell contents in wood would influence the extent of nitrogen movement.

The results presented showed the following:

(a) nitrogen content of the wood layer in contact with the soil had increased as a result of soil burial in both sapwood and inner sapwood blocks. The nitrogen contents of Layers (ii) and (iii), however, did not change.

(b) Soft-rot cavities were produced in wood blocks as a result of soil burial but this was not correlated with movement of additional nitrogen from soil into wood. This is illustrated in Fig. 3.7. The presence of soft-rot cavities in Layers (ii) and (iii) of sapwood samples without nitrogen addition from soil was consistent with this observation, and it therefore seemed that there was no relationship between nitrogen addition and soft-rot production in wood in soil contact.

(c) The amount of nitrogen movement into wood from soil was similar in Layers (i) in both sapwood and inner sapwood blocks, and the nitrogen contents of layers (ii) and (iii) did not change as a result of burial. It was therefore concluded that nitrogen movement into wood was not related to the presence or absence of cell contents in the buried material.

Although changes in nitrogen content of Layer (i) had been produced as a result of soil burial, moisture content changes had also occurred. As high moisture levels have generally been associated with soft-rot production and it was considered that the moisture levels, particularly in Layer (i), were adequate to support this activity, it was decided to determine if moisture content was related to the amounts of soft rot produced.

FIGURE 3.7 Correlation of soft rot, measured in mm from soil contact surface, with increase in nitrogen content as a result of soil burial

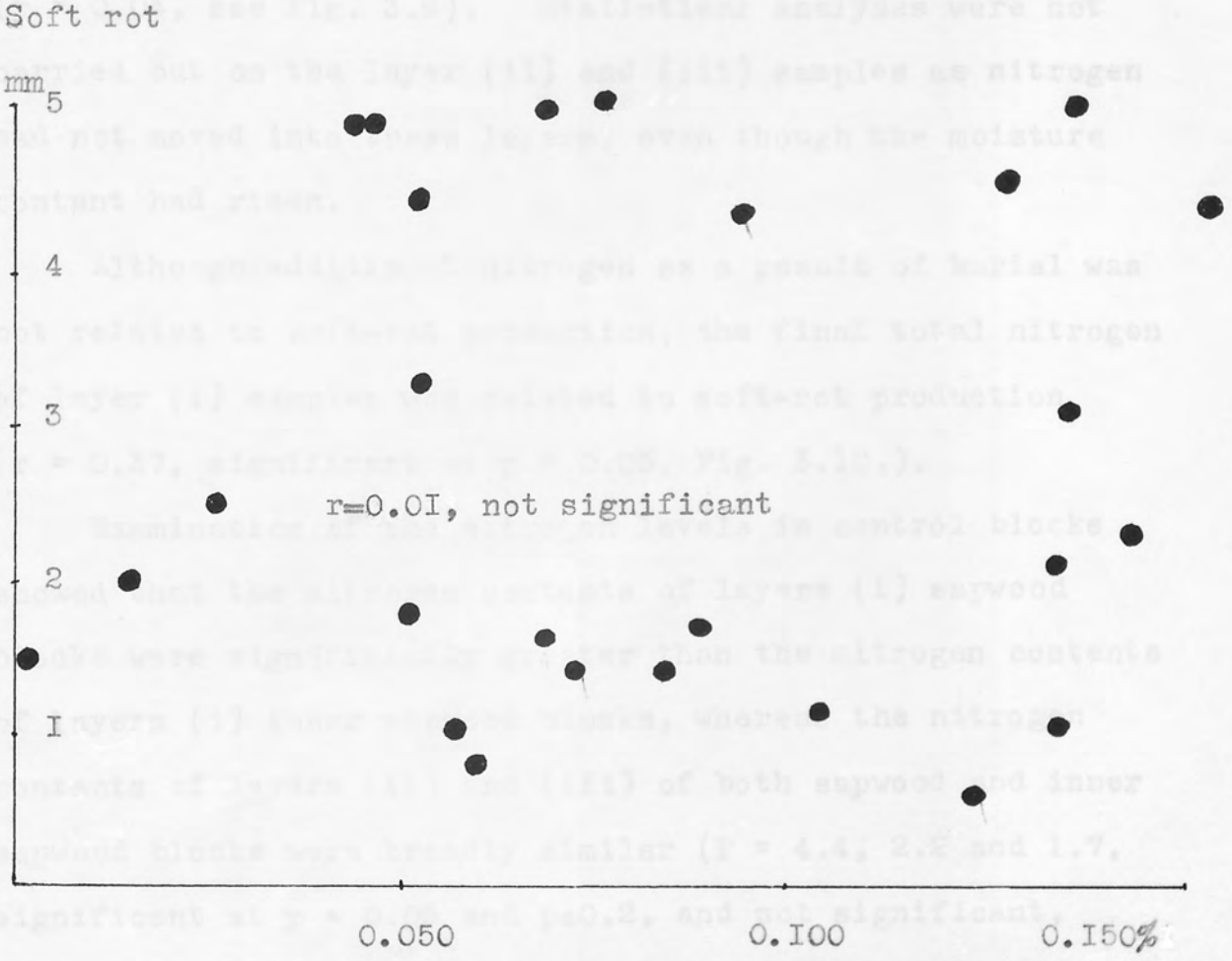


Figure 3.8 shows the lack of relationship between moisture content and soft-rot production ($r = 0.12$), and it therefore seemed that neither moisture nor additional nitrogen had an influence on the extent of soft rot produced. Nitrogen added to wood as a result of burial was likewise not related to the moisture content of the layer (i) samples ($r = 0.04$, see Fig. 3.9). Statistical analyses were not carried out on the layer (ii) and (iii) samples as nitrogen had not moved into these layers, even though the moisture content had risen.

Although additional nitrogen as a result of burial was not related to soft-rot production, the final total nitrogen of layer (i) samples was related to soft-rot production ($r = 0.37$, significant at $p = 0.05$, Fig. 3.10.).

Examination of the nitrogen levels in control blocks showed that the nitrogen contents of layers (i) sapwood blocks were significantly greater than the nitrogen contents of layers (i) inner sapwood blocks, whereas the nitrogen contents of layers (ii) and (iii) of both sapwood and inner sapwood blocks were broadly similar ($F = 4.4$, 2.2 and 1.7, significant at $p = 0.05$ and $p=0.2$, and not significant, respectively.

It was at this stage of the project that indications of nitrogen migration were becoming apparent in the concurrent investigation into the nitrogen status of green and dried wood. The hypothesis was adopted that redistribution of soluble nitrogen to wood surfaces during drying of spruce planks prior to conversion had probably occurred. The sapwood samples removed from rings 1-15 (measured from the cambium) came from those parts of the planks with the higher soluble nitrogen content in the green condition.

FIGURE 3.8 Correlation of soft rot with moisture in spruce blocks after burial

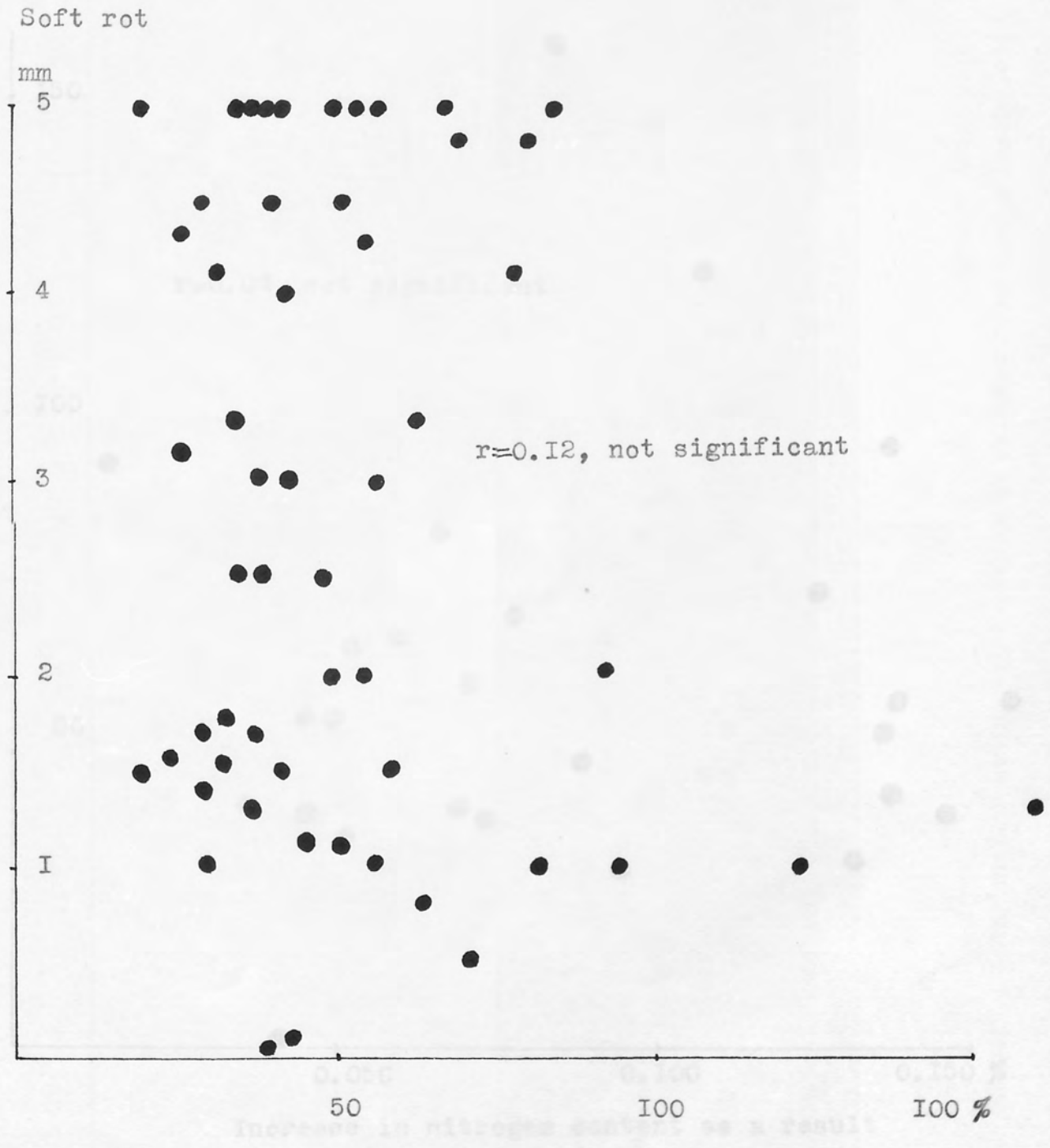


FIGURE 3.9 Correlation of moisture content after burial with increase in nitrogen content as a result of burial

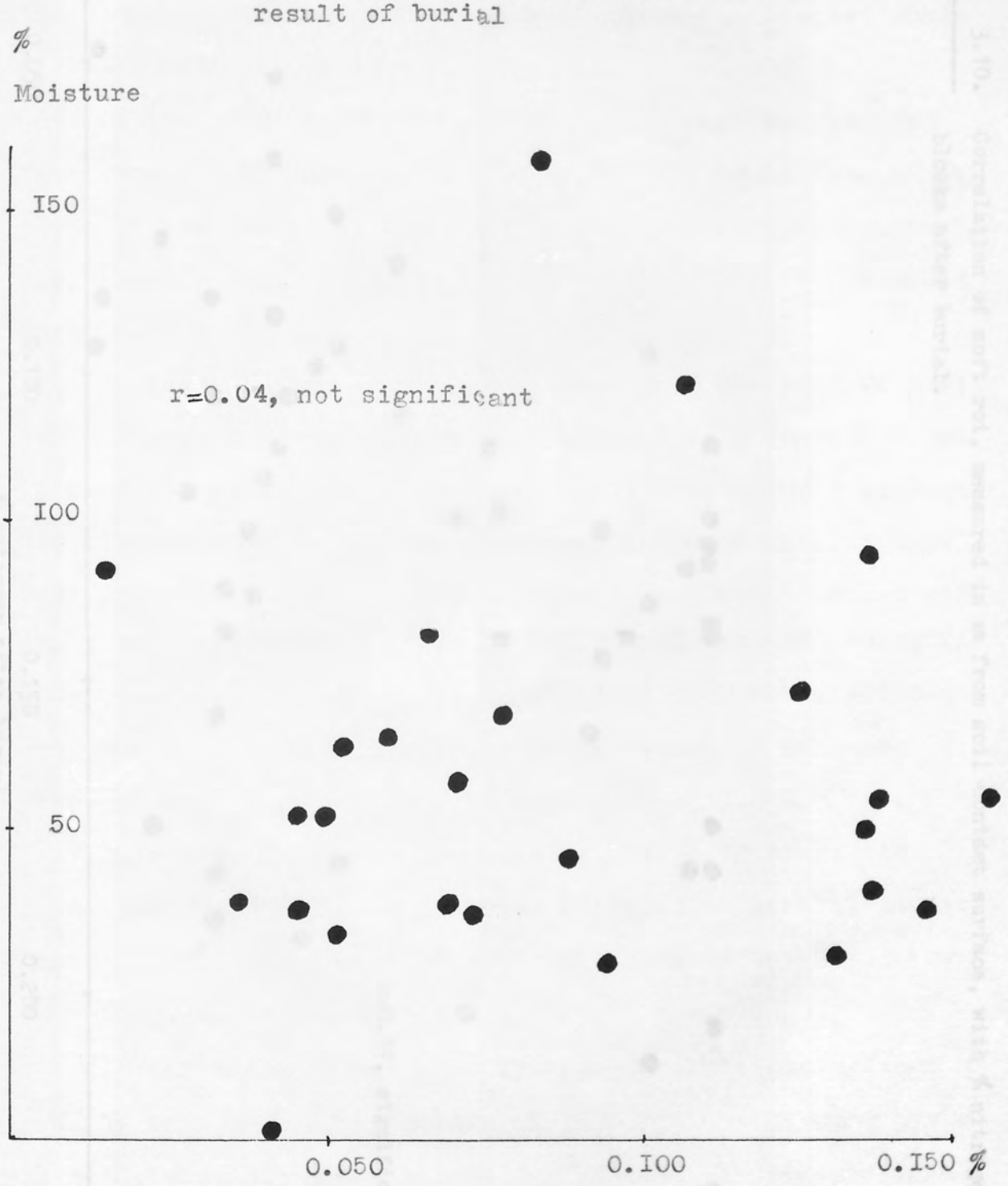
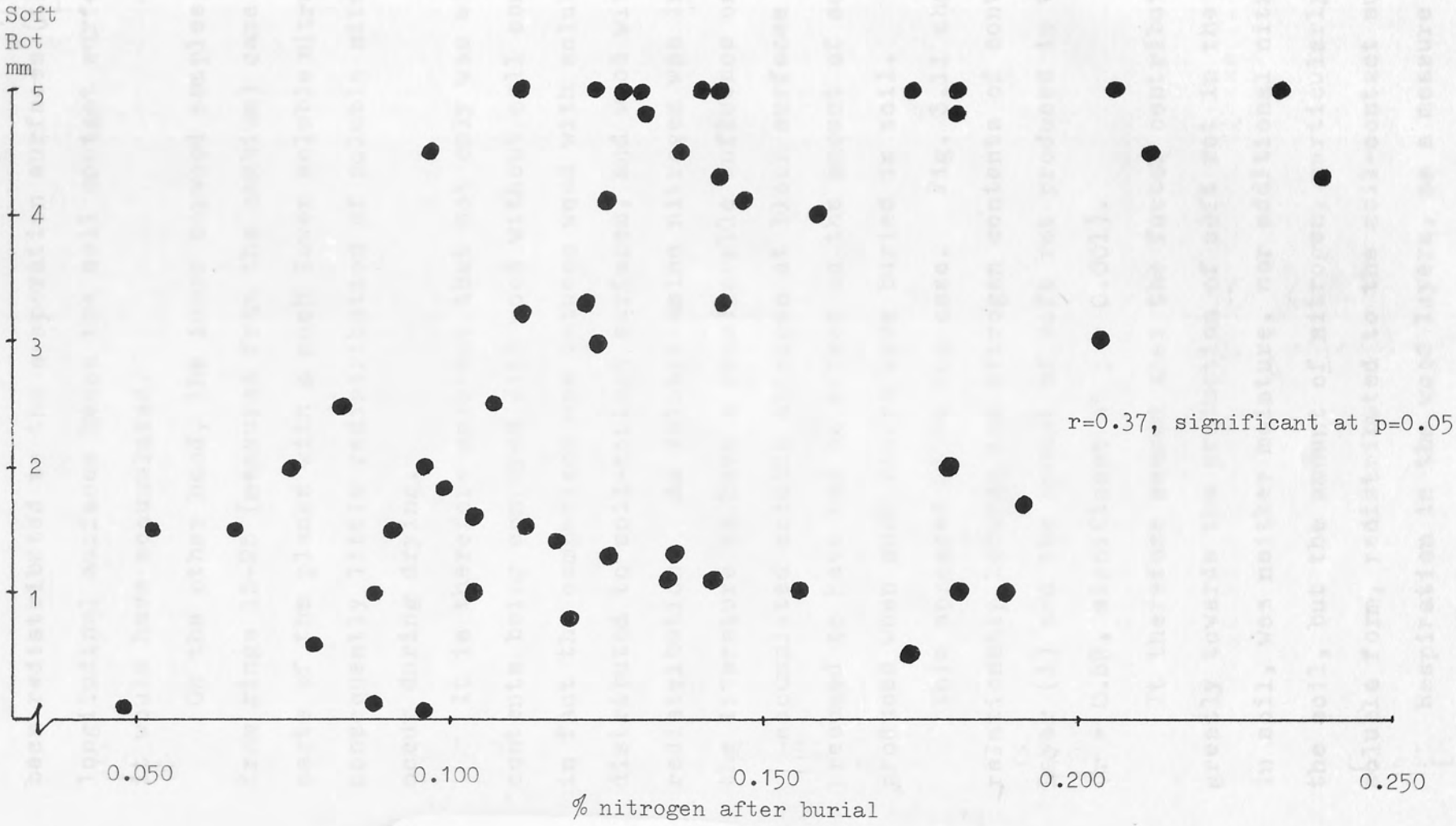


FIG. 3.10. Correlation of soft rot, measured in mm from soil contact surface, with % nitrogen of layers (i) of blocks after burial.



On drying of the planks, this soluble nitrogen would have been redistributed to the evaporation surfaces (the radial longitudinal surfaces hence the soil contact surfaces) where it would have accumulated.

On the other hand, the inner sapwood samples, removed from rings 15-25 (measured from the cambium) came from those parts of the planks with a much lower soluble nitrogen content, consequently little redistribution of soluble nitrogen could occur during drying.

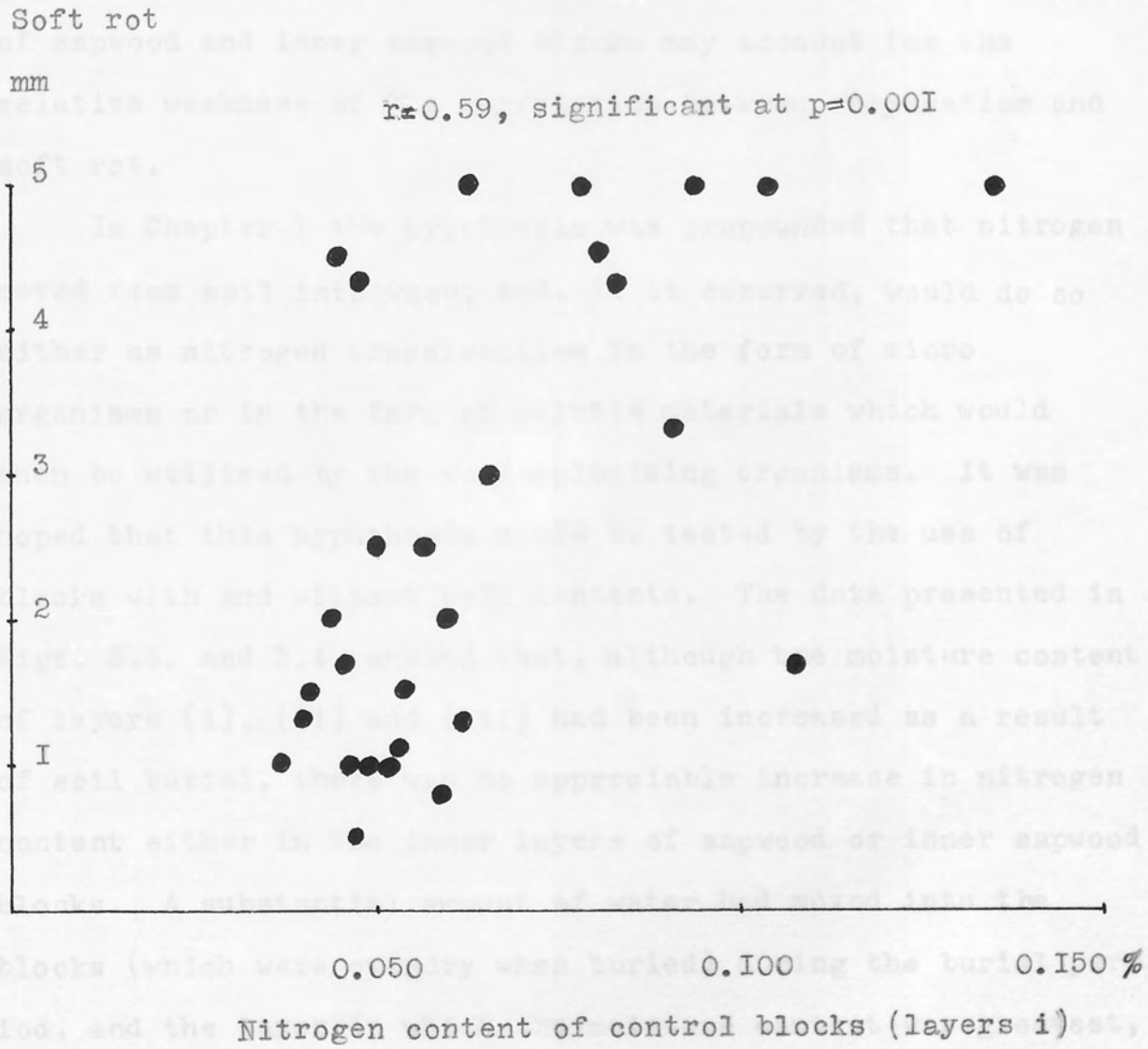
It is therefore apparent that not only was wood with cell contents being compared with wood without cell contents, but in fact the comparison was between wood with soluble nitrogen distributed to soil-contact surfaces, and wood without this redistribution. As soluble amino nitrogen was inferred from the literature to have a considerable influence on decay, the accumulated soluble nitrogen at block surfaces might be presumed to have had an effect on the amount of soft-rot produced when such blocks were buried in soil.

This appeared to be the case. Fig. 3.11 shows the relationship between the nitrogen contents of control blocks Layer (i) and the amount of soft rot produced in that layer ($r = 0.59$, significant at $p = 0.001$).

It therefore seemed that the factor contributing most greatly towards the production of soft rot in the wood buried in soil, was neither moisture, nor additional nitrogen from the soil, but the amount of nitrogen, particularly in a soluble form, redistributed to the soil-contact surfaces.

Respiration in the wood layers, as a measure of biological activity, also correlated, though not strongly, with the amount of soft rot produced ($r = 0.39$, significant at $p = 0.05$) and did not correlate significantly with moisture content

FIGURE 3.II Correlation of soft rot with nitrogen content of control blocks layers (i)

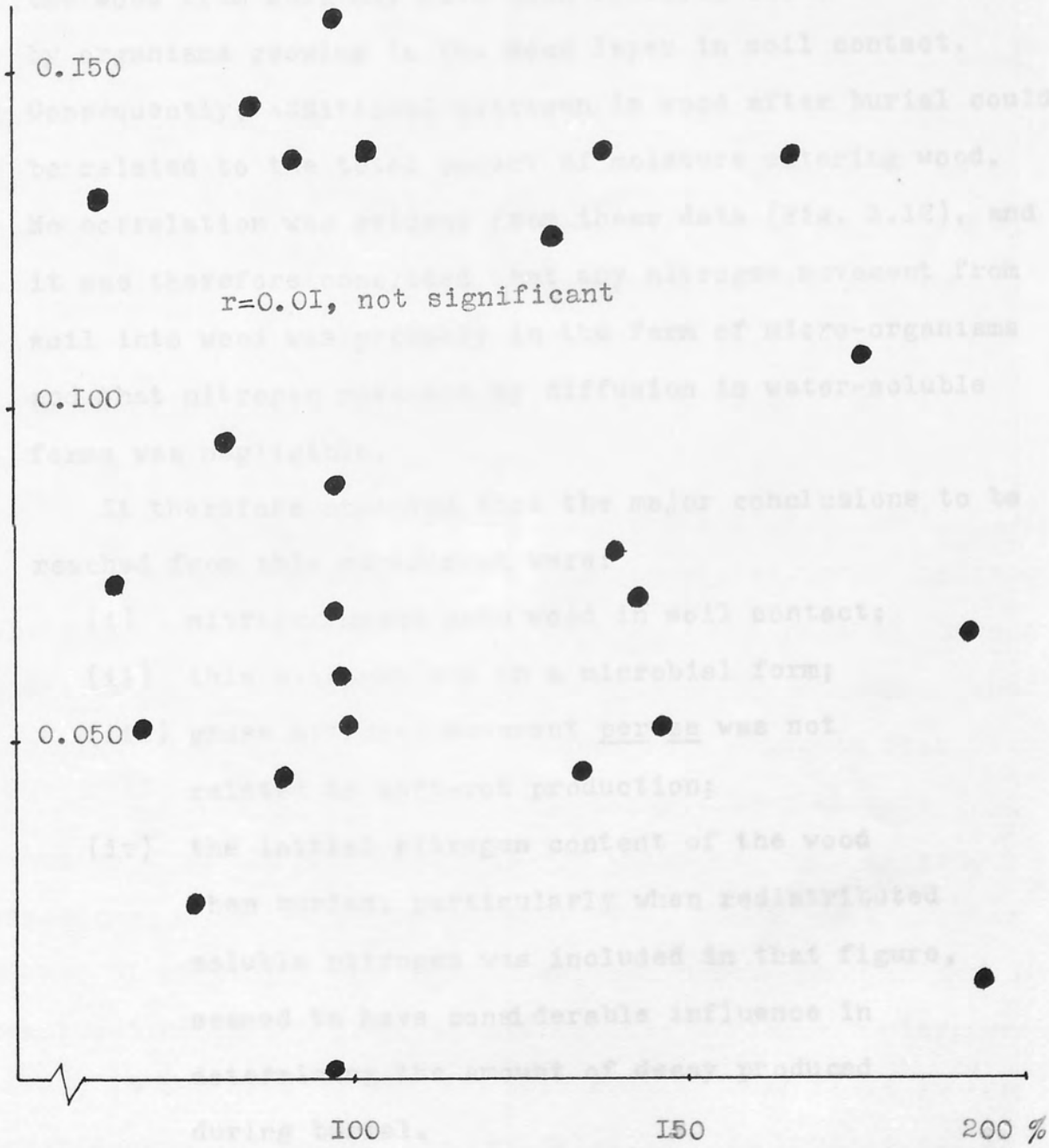


(r = 0.02), nitrogen content after burial (r = 0.14) or nitrogen content before burial (r = 0.11). It was considered that the higher respiration levels in sapwood, evident from Fig. 3.6, might be attributed to the amount of passive colonisation which occurred. During microscopic examination of wood blocks, it was noted that generally fungal penetration had occurred, whereas inner sapwood blocks did not display this to the same extent. This passive colonisation of sapwood and inner sapwood blocks may account for the relative weakness of the correlation between respiration and soft rot.

In Chapter 1 the hypothesis was propounded that nitrogen moved from soil into wood, and, if it occurred, would do so either as nitrogen translocation in the form of micro organisms or in the form of soluble materials which would then be utilised by the wood-colonising organisms. It was hoped that this hypothesis could be tested by the use of blocks with and without cell contents. The data presented in Figs. 3.3. and 3.4. showed that, although the moisture content of layers (i), (ii) and (iii) had been increased as a result of soil burial, there was no appreciable increase in nitrogen content either in the inner layers of sapwood or inner sapwood blocks. A substantial amount of water had moved into the blocks (which were ovendry when buried) during the burial period, and the layer in which the moisture content was greatest, layer (i), was the only layer which contained an increase in nitrogen after burial.

If soluble nitrogen from soil had entered the blocks in solution, it was presumed that its distribution should have corresponded with the distribution of soil water in the wood.

FIGURE 3.I2 Correlation of increase in nitrogen content after burial (layers i) with total moisture movement into wood



However, this was not the case. Similarly, Fig. 3.9 showed that the increase in nitrogen content in Layers (i) after burial was not related to the amount of moisture found there. It was considered, however, that soluble nitrogen entering the wood from soil may have been filtered out or retrieved by organisms growing in the wood layer in soil contact. Consequently, additional nitrogen in wood after burial could be related to the total amount of moisture entering wood. No correlation was evident from these data (Fig. 3.12), and it was therefore concluded that any nitrogen movement from soil into wood was probably in the form of micro-organisms and that nitrogen movement by diffusion in water-soluble forms was negligible.

It therefore appeared that the major conclusions to be reached from this experiment were:

(i) nitrogen moved into wood in soil contact;

(ii) this nitrogen was in a microbial form;

(iii) gross nitrogen movement *per se* was not related to soft-rot production;

(iv) the initial nitrogen content of the wood when buried, particularly when redistributed soluble nitrogen was included in that figure, seemed to have considerable influence in determining the amount of decay produced during burial.

Whereas conclusions (i) and (ii) were to some extent expected, conclusions (iii) and(iv) were totally contrary to those envisaged at the beginning of the experiment. It was therefore decided to investigate the matter more carefully, and this experiment is described in Expt. 3.3.

3.2.3. The Influence of redistributed soluble Nutrients on soft-rot production in Pine (Experiment 3.3.).

Experiment 3.2 was designed to measure the amount of nitrogen movement into wood in soil contact, to relate this movement to any decay produced, and to see if the presence or absence of cell contents influenced the extent of nitrogen movement. The results showed that nitrogen moved into wood buried in soil, that the extent of this movement was not related to soft-rot production, and was not associated with the presence or absence of cell contents. The results also showed, however, that soft-rot production was related to the initial nitrogen content of the wood, and that nitrogen movement into wood from soil was in a microbial form, and not in the form of soluble salts.

The design of the experiment, particularly of test blocks, may have contributed to or exaggerated these findings. While the results presented for soft rot were related to wood with and largely without redistributed soluble nitrogen, the wood blocks displaying the phenomenon were selected from different areas of the wood. The sapwood samples were selected from the outer sapwood, in which the rate of growth was slower than the inner sapwood; i.e. there was a greater number of rings per inch in the sapwood blocks than in the heartwood blocks. Along with this, the structural nitrogen levels in the sapwood samples were greater than those in the inner sapwood, consequently any relationships hypthesised on the basis of the spruce experiments in relation to nitrogen content would have to take both of these factors into account. This was illustrated to some extent by the statistical analyses carried out. Whereas in many cases the levels of significance for correlation were high (e.g. $p = 0.01 - 001$)

the levels of the correlation co-efficients were low (e.g. 0.4-0.5), indicating a certain vagueness in the relationships or a considerable spread of results.

The concurrent experiment on the nitrogen content of wood in the green and dried condition was still in progress at this stage, and investigations were being initiated on the wood of Scots pine. It was therefore decided to carry out a soil burial experiment with pine, broadly similar to that carried out with spruce but taking into account those factors which might have contributed to an exaggeration in findings.

A. Materials and Methods.

(i) Materials

The materials used for this experiment were the wood of the Scots pine tree used in Experiments 2.4.-2.6. and the soil from P.R.L. described in Experiment 3.1.

A bolt from the Scots pine tree was converted at the forest into 55m thick radially-cut planks immediately after felling. These planks were returned to the laboratory on the day of felling and conversion where they were stored in the deep-freeze unit (-18°C) until they were dried. To overcome any variation in the extent of nitrogen migration during drying of wood, and in order that the experiments could be replicated, the wood was dried at 50°C in an oven with a circulation fan and with an atmosphere scrubbed free of ammonia as described in Experiment 2.6.

The planks were converted into sapwood and inner sapwood strips as described in Experiment 3.2. However, in this instance the strips were 55m wide. One evaporation surface to a depth of 5mm was removed from each strip, and

this was designated the "A" side of each strip. The other side, which retained an evaporation surface, was designated the "B" side. These 50-mm wide strips were then converted into 90-mm long blocks by transverse sawing after the end-grain section of each strip had been removed to a depth of 20-30mm in order that transverse end-grain sections containing redistributed soluble nitrogen should not be included in test blocks removed from the ends of strips.

Six soil-buried blocks and four control blocks were prepared for both sapwood and inner sapwood, the control blocks being selected from immediately adjacent material to the soil burial blocks (Fig. 3.13). The blocks were coated, on all except the radial surfaces, with "Aradite" expoxy resin, cured, and buried as described in Experiment 3.2.

It was initially envisaged that wood blocks should be buried in sterile soil to monitor diffusion of soluble nitrogen from soil into wood. It was decided, however, that this procedure would be both unnecessary and confusing because:

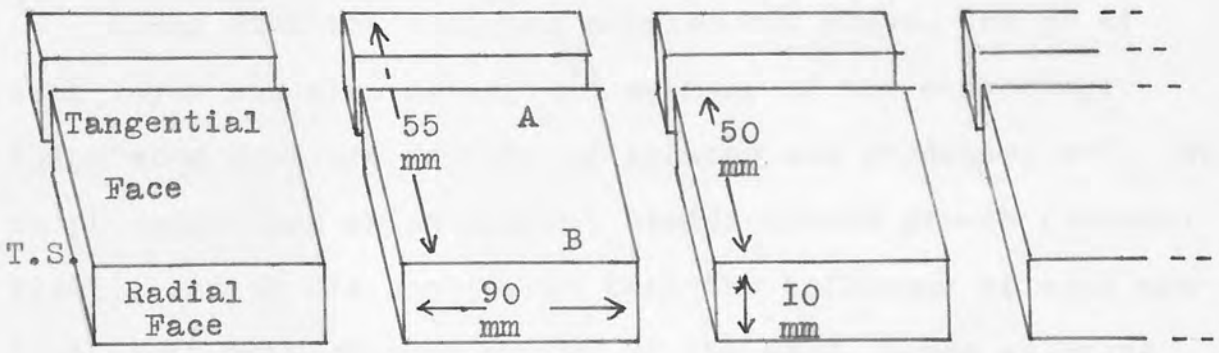
(a) Experiment 3.2 showed that soluble nitrogen did not move from soil into wood;

(b) Experiment 3.1 showed that there was not very much soluble nitrogen in the soil under study ($> 10\%$);

(a) Soil sterilisation procedures generally tend to increase the soluble nitrogen content of the soil (Parkinson, Grey and Williams, 1971), hence conclusions on migration of soluble nitrogen from sterile soil into wood could at best only inaccurately indicate the extent of soluble nitrogen migration into wood from soil with a still viable micro-flora.

The blocks, both sapwood and inner sapwood, were buried in soil for 13 weeks. Six sapwood and five inner sapwood test blocks were buried, while a matching control block to each buried block was retained for analysis.

Fig. 3.13. Conversion of Strips into Test Blocks*



Soil Burial Block | Control Block | Soil Burial Block | Control Block

A = soil exposure face not including redistributed soluble N_2

B = soil exposure face including redistributed soluble N_2

T.S. Transverse Face

* (not to scale)

(ii) Methods

After the soil-burial period the blocks were removed from the soil-burial boxes, and adhering soil was scrubbed free from the soil-exposure faces with an old toothbrush. The blocks were converted for analysis in similar fashion to Experiment 3.2. except that in this instance five 5-mm layers (designated layers (i), (ii), (iii),(iv)&(v) measured from the soil-contact surface) were available from the "A" and "B" side of each block. The blocks were individually wrapped in polythene and were stored in a fridge until analysed.

The parameters to be examined were the same as those for Experiment 3.2 viz. moisture content, nitrogen content,

presence of soft rot and respiration. (Respiration experiments were carried out at 28°C instead of 25°C as used in Experiment 3.2.). Each of these analyses were carried out on layers (i), (ii), (iii), (iv) and (v) of each side of each block, except for nitrogen content, which was not determined on layer (iv).

Along with the analyses carried out above, the pH of each layer was also determined as many of the microfungi colonising wood are capable of growing and producing soft rot in pH conditions which inhibit basidiomycete growth (Duncan, 1960b), and it was considered that the influence of soil contact might have changed the pH of the wood, hence altering the susceptibility of the wood to colonisation and decay.

The method used to determine pH was derived from those of Gray (1959a) and Butcher (1968). These methods essentially relied on the use of one-gram samples of wood being suspended respectively in 3mls and 20mls distilled water for 20-minute or one-hour time-periods before pH determination.

It was decided that, as each layer of each wood block used for the various analyses weighed approximately 150mg., it would be possible to determine pH in those layers by using each layer (milled to pass a 40-mesh sieve) suspended in 3ml of distilled water. The wood-water suspension was contained in specimen tubes 50mm long and 16mm internal diameter so that the electrode could be fully immersed in the suspension. A Pye 78 pH meter and an Activian standard electrode were used for these determinations.

B. Results

The results for these analyses are graphically presented in Figs. 3.14; 3.15; 3.16; 3.17 and 3.18. A statistical analysis giving the number of replicate determinations, mean, standard deviation and standard error of the raw data contributing to these figures is presented in Appendix II.

Moisture Content: The results for moisture determinations are presented in Fig. 3.14. This figure shows that the moisture levels were considerably higher in layers (i) than in the other four layers and that the inner sapwood blocks had lower moisture contents than the sapwood blocks. Nevertheless, a substanial amount of water had penetrated to the full depth of the blocks which were initially oven dry.

An analysis of variance showed that the differences between the means for layers (i) were significant at $p = 0.05$ ($F = 1.9$). These differences in moisture contents could be attributed to the difference between sapwood and inner sapwood blocks since there was a lack of significant differences between the moisture in A and B sides of inner sapwood blocks for both layers (i) and (ii) ($t = 0.16$ and $t = 0.4$ respectively).

Due to the similarities between the curves for layers (iii), (iv) and (v), and because of the results of statistical analyses presented above, further statistical analyses were considered to be unnecessary. Moisture content was not determined on control blocks.

Nitrogen Content: The data for nitrogen content of both buried test blocks, and unburied control blocks are presented in Fig. 3.15. The biggest differences in nitrogen values between control blocks and buried blocks were evident in layers (i) with apparently only slight differences in layers (ii) to

FIGURE 3.14 Moisture in pine blocks after burial

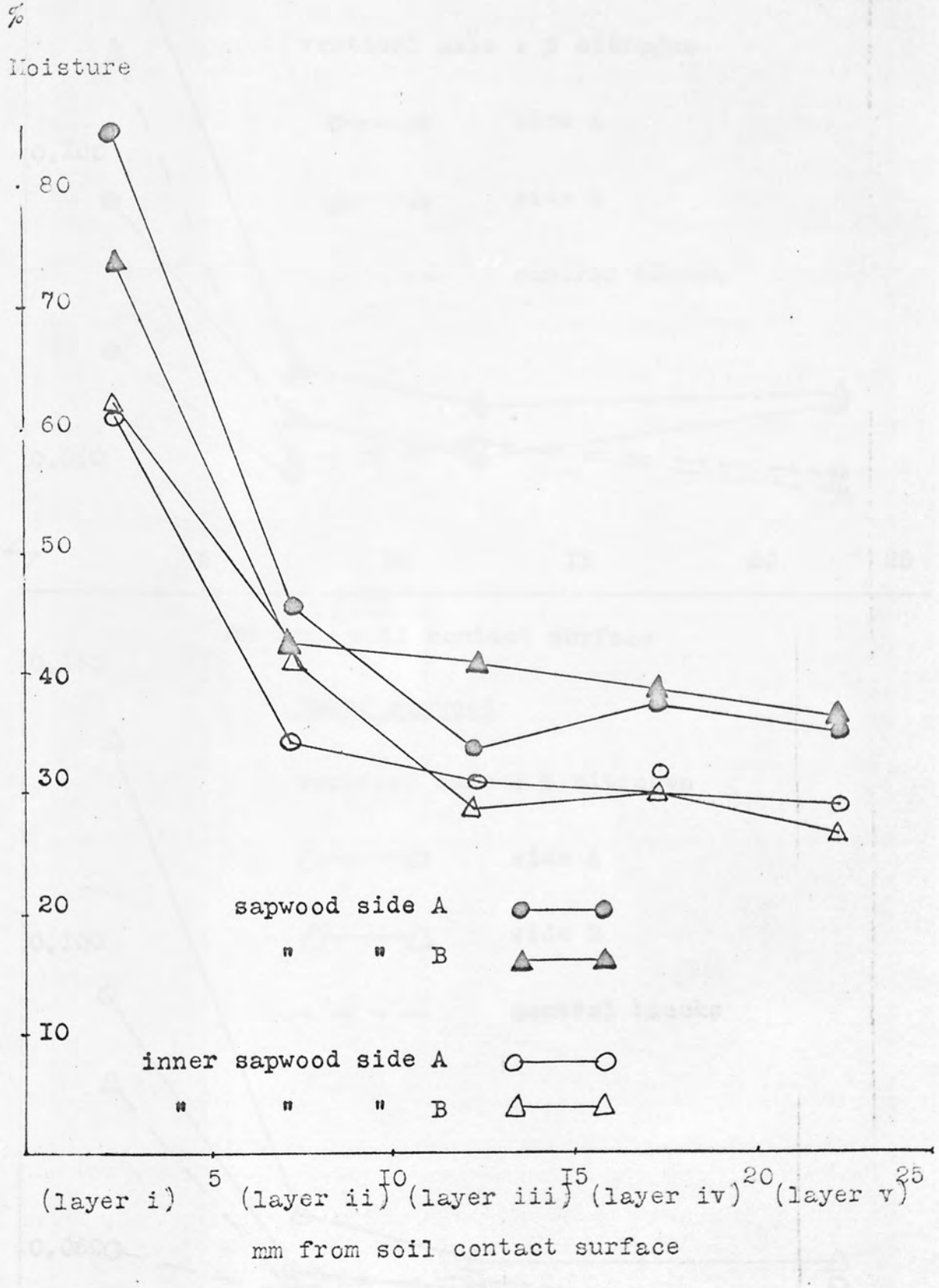
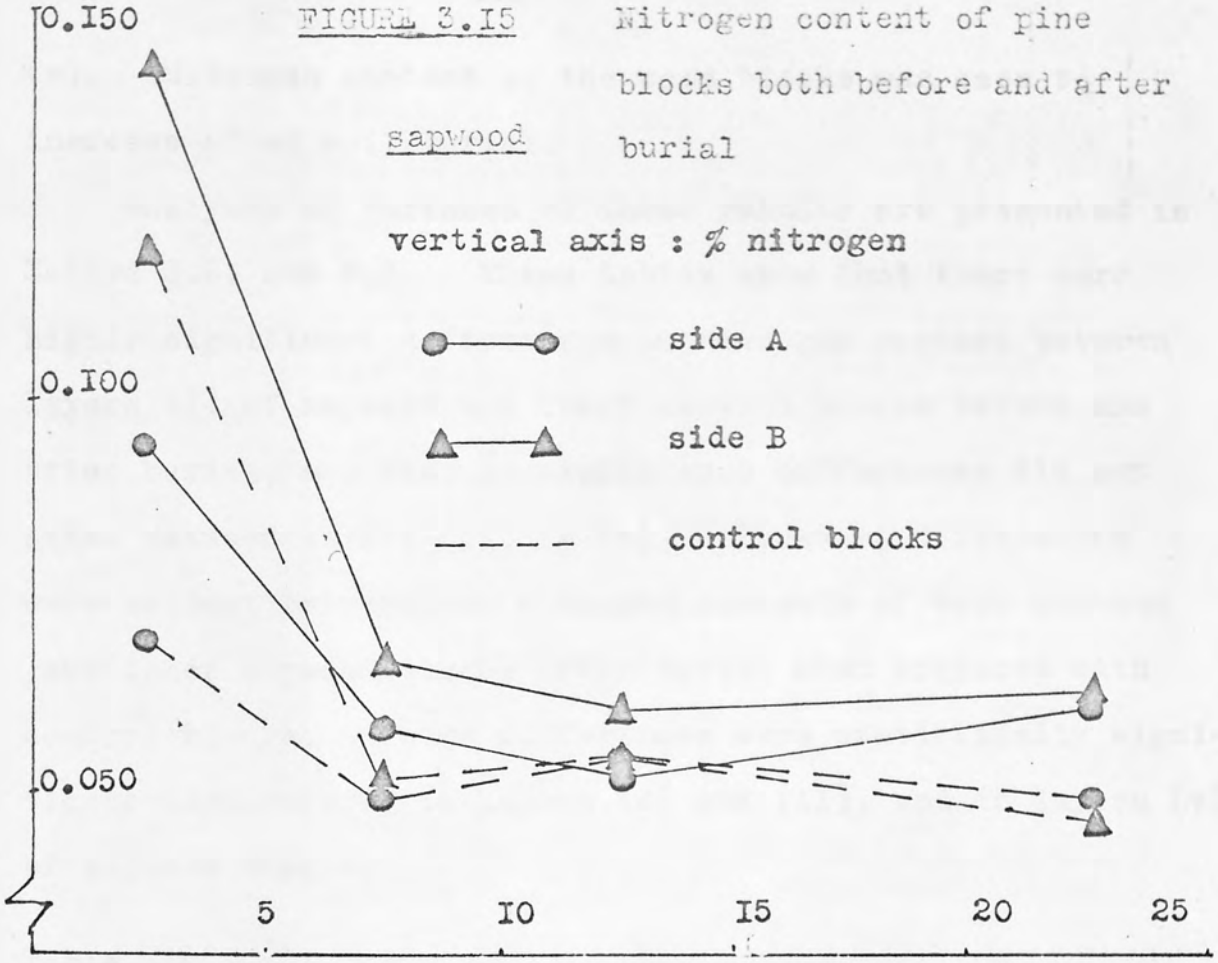
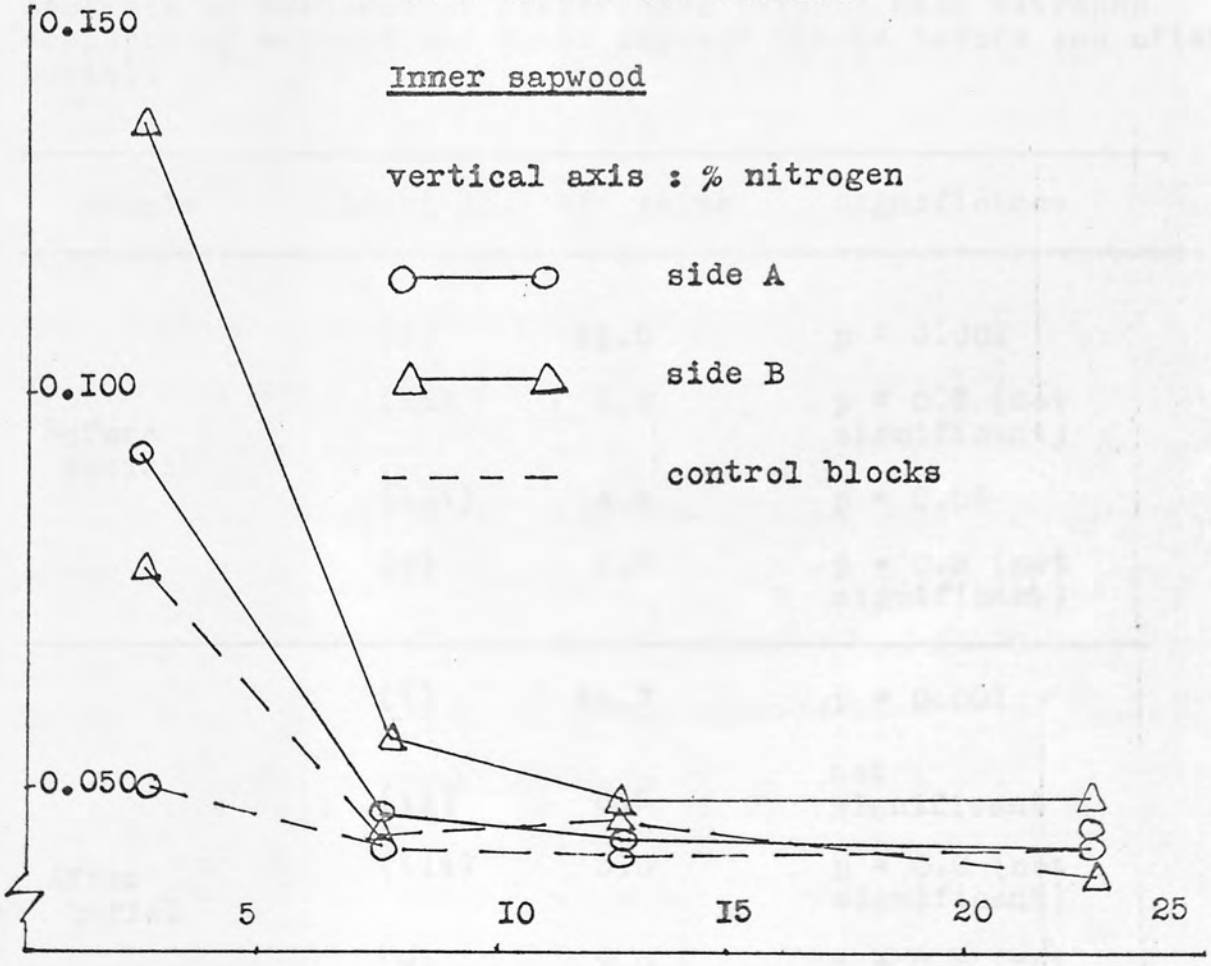


FIGURE 3.15 Nitrogen content of pine blocks both before and after burial





(v). Nitrogen content of the wood blocks was seen to increase after soil burial.

Analyses of variance of these results are presented in Tables 3.2. and 3.3. These tables show that there were highly significant differences in nitrogen content between layers (i) of sapwood and inner sapwood blocks before and after burial, and that generally such differences did not exist between layers (ii) to (v). However, differences were evident between the nitrogen contents of both sapwood and inner sapwood blocks after burial when compared with control blocks. These differences were statistically significant particularly in layers (i) and (ii), and in layers (v) of sapwood blocks.

Table 3.4

Analysis of variance of differences between mean nitrogen contents of sapwood and inner sapwood blocks before and after burial.

Sample	Layer No.	"F" value	Significance
Before burial	(i)	31.0	p = 0.001
	(ii)	2.1	p = 0.2 (not significant)
	(iii)	4.8	p = 0.05
	(v)	2.2	p = 0.2 (not significant)
After burial	(i)	24.7	p = 0.001
	(ii)	0.9	not significant
	(iii)	3.0	p = 0.2 (not significant)
	(v)	2.0	p = 0.2 (not significant)

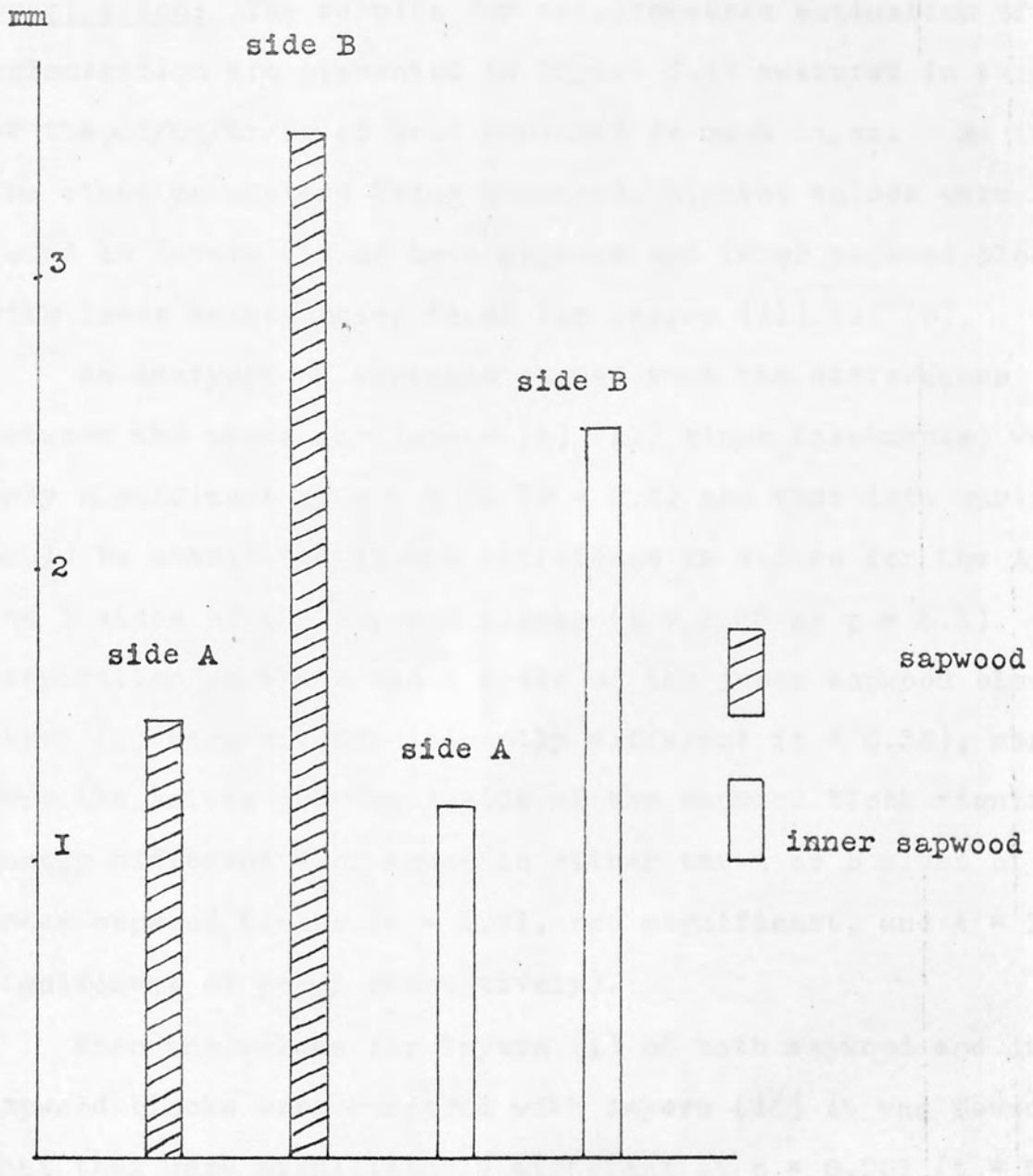
Table 3.5

Analysis of variance of differences between mean nitrogen contents of blocks before and after burial.

Sample	Layer No.	"F" value	Significance
Sapwood blocks	(i)	20.5	p = 0.001
	(ii)	4.9	p = 0.01
	(iii)	1.2	not significant
	(v)	4.3	p = 0.05
Inner Sapwood blocks	(i)	17.3	p = 0.001
	(ii)	2.5	p = 0.2 not significant
	(iii)	1.0	not significant
	(v)	2.9	p = 0.2 (not significant)

Soft Rot: The data for soft-rot presence in soil-buried blocks is presented in Fig. 3.16 in terms of the depth in mm from the soil-contact surface at which soft-rot cavities were found. Soft-rot cavities were found only to a depth of 5.4mm i.e. confined almost completely to layer (i) (the layer in contact with soil), and were not noted in control blocks. Analysis of variance of the mean depths to which soft-rot cavities were found showed that the results were significantly different at p = 0.01 (F = 5.3). It was found, however, that there was not a significant difference between the values for the "A" sides of sapwood and inner sapwood blocks (t = 0.99), nor were the differences in the amounts of soft rot

FIGURE 3.16 Soft rot, measured in mm from soil contact surface, in pine blocks after burial



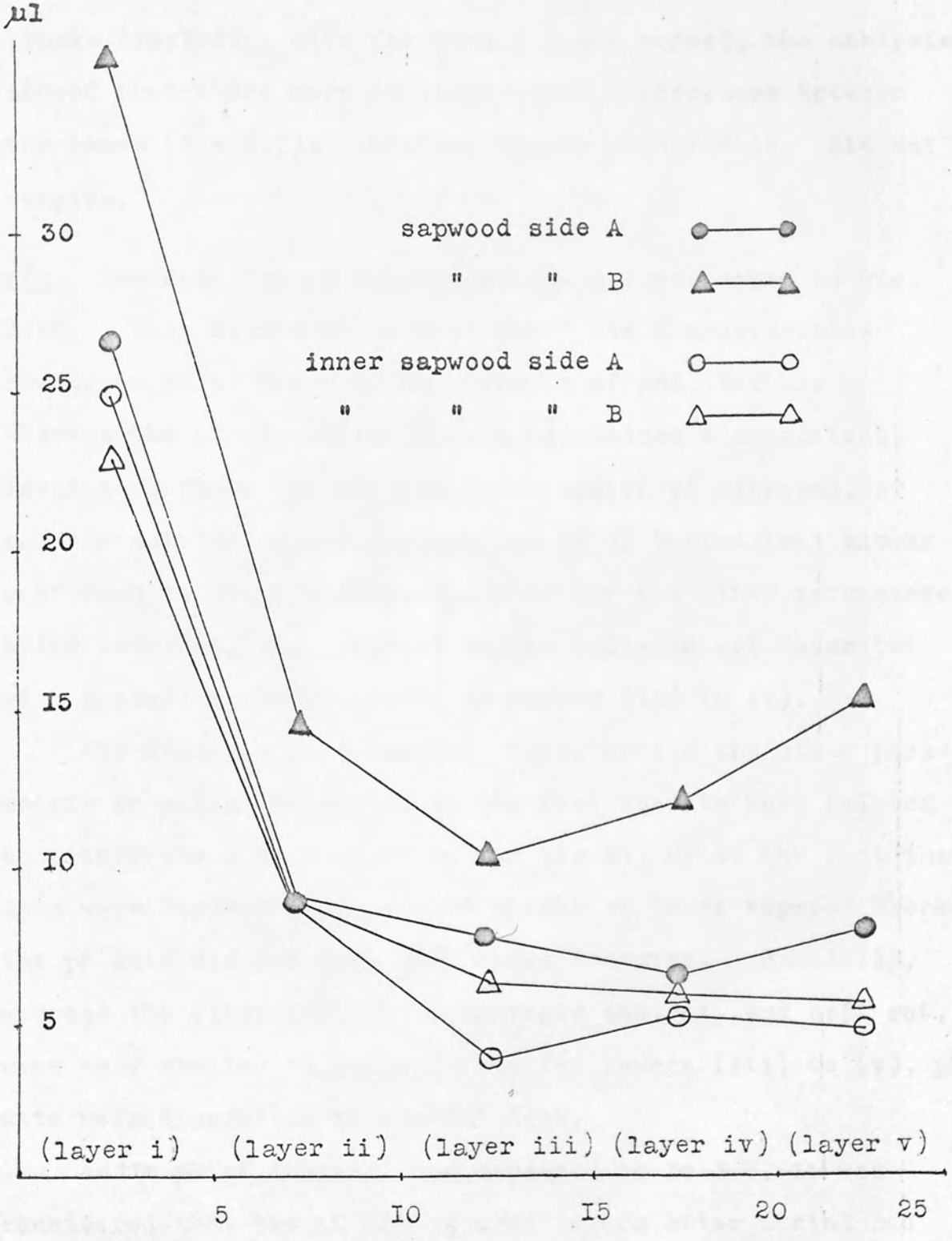
present in the "B" sides significant (t = 1.02). The mean soft rot depths for "A" sides, however, were significantly different to those of "B" sides at p = 0.01 (t = 3.7).

Respiration: The results for respirometric estimation of colonisation are presented in Figure 3.17 measured in terms of the $\mu l/O_2/hr/cc$ of wood consumed in each layer. As in the other parameters being examined, highest values were found in layers (i) of both sapwood and inner sapwood blocks, with lower values being found for layers (ii) to (v).

An analysis of variance showed that the differences between the means for layers (i) (all block treatments) were only significant at p = 0.2 (F = 2.8) and that this variance could be attributed to the difference in values for the A and B sides of the sapwood blocks (t = 1.88 at p = 0.1). Respiration in the A and B sides of the inner sapwood blocks layer (i) were not significantly different (t = 0.36), nor were the values for the A side of the sapwood block significantly different from those in either the A or B sides of the inner sapwood blocks (t = 1.71, not significant, and t = 1.97, significant at p=0.1 respectively).

When the values for layers (i) of both sapwood and inner sapwood blocks were compared with layers (ii) it was found that they were significantly different at p = 0.001 (t = 509) but analyses of variance of the means of layers (ii) to (v) for sapwood blocks showed that they were drawn from the same population (F = 0.9). Similarly, the values for layers (ii) to (v) of inner sapwood blocks were also drawn from the same population (F = 0.09). However, when the sapwood blocks layer (ii) to (v) were compared with layers (ii) to (v) for inner sapwood, they were found to be significantly greater

FIGURE 3.17 Respiration in pine blocks after burial (micro-litres of oxygen consumed per hour per cc of wood)



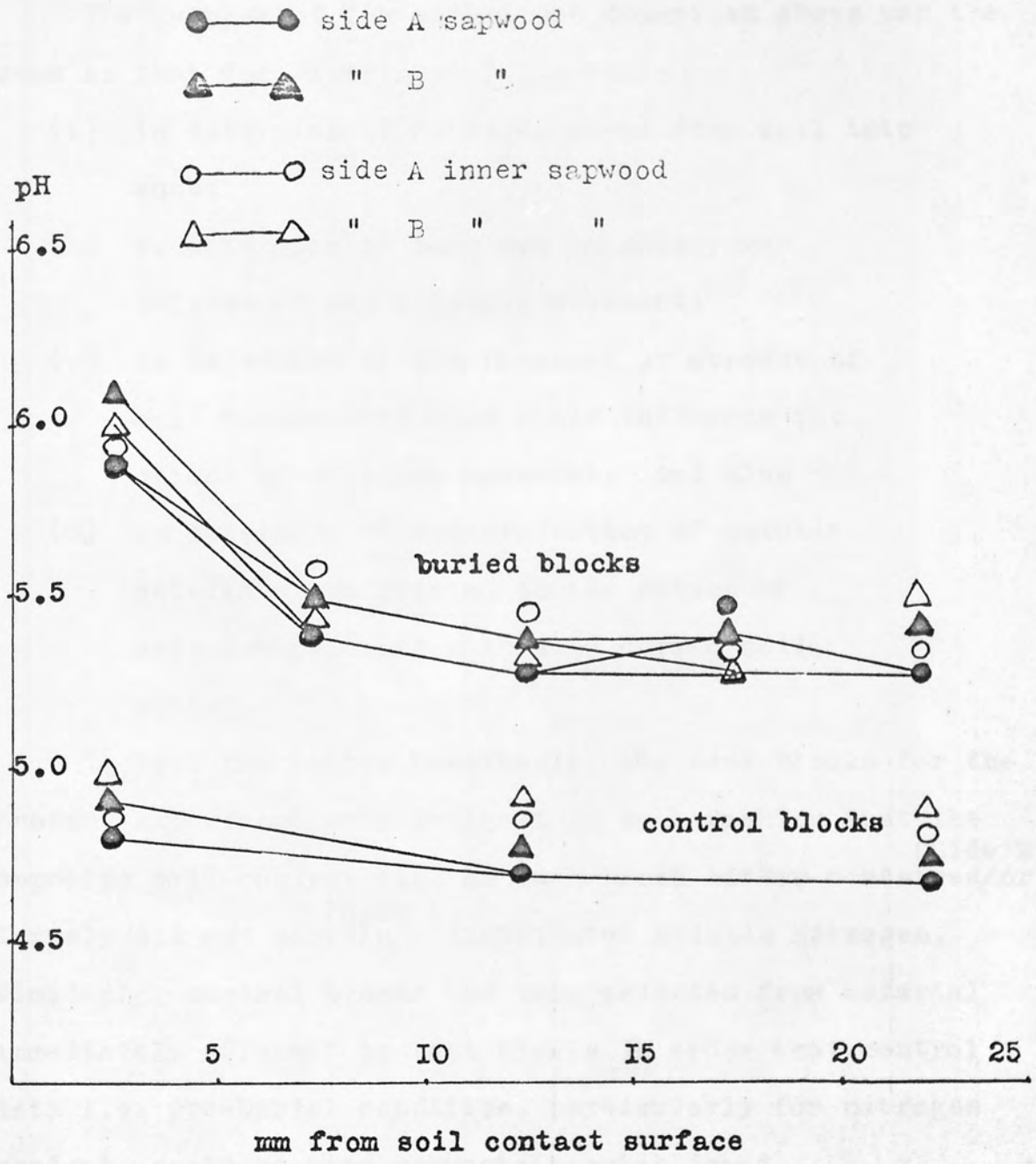
at p = 0.05 (F = 2.6). Significantly, when the values for sapwood blocks layer (ii) to (v) exclusive of the "B" side were compared with the values for the inner sapwood blocks (including data for both A and B sides), the analysis showed that there were no significant differences between the means (F = 1.1). Control blocks when tested did not respire.

pH: The data for pH determinations are presented in Fig. 3.18. This figure shows that there was a considerable change in pH of the wood as a result of soil burial. Whereas the pH of control blocks maintained a consistent level throughout the layers, irrespective of nitrogen, or soluble nutrient distribution, the pH of buried test blocks conformed to the trends established for the other parameters being examined, viz. highest in the soil-contact layer but with a similar, lower, level in layers (ii) to (v).

The figure shows, however, that, unlike the other parameters in which variations in the test results were related to either the A or B sides of the blocks, or to the fact that data were derived from sapwood blocks or inner sapwood blocks, the pH data did not vary with these features. Similarly, whereas the other data, viz. nitrogen content, and soft rot, were very similar to control data for layers (iii) to (v), pH data were dissimilar to control data.

As the pH of the soil was assessed to be 7.3, it was considered that the pH of the wood layers after burial had been influenced by the soil pH figure, and that the change in wood pH had been brought about by moisture movement from soil into wood.

FIGURE 3.18 pH of pine blocks after burial



As the data for pH was so similar within each layer, statistical analyses were not carried out.

C. Discussion

The purpose of the experiment described above was the same as that for Experiment 3.2., viz.:

(a) to determine if nitrogen moved from soil into wood;

(b) to determine if soft rot intensity was related to any nitrogen movement;

(c) to determine if the presence or absence of cell contents in wood would influence the extent of nitrogen movement; and also

(d) to determine if redistribution of soluble nutrients was related to the extent of soft-rot production in wood during soil burial.

To test the latter hypothesis, the test blocks for the present experiment were designed in such fashion that the opposing soil-contact face of each block either contained/or (Side B) largely did not contain/redistributed soluble nitrogen. (Side A) Similarly, control blocks had been selected from material immediately adjacent to test blocks in order that control data i.e. pre-burial condition, particularly for nitrogen content, could be more accurately established.

The results presented showed:

(a) the nitrogen content of the wood layer in contact with the soil (layer (i)) had increased as a result of soil contact for both sapwood and inner sapwood blocks; the nitrogen contents of layers (ii) had similarly increased (but

to a lesser extent); layers (iii) had not changed, and the nitrogen content of layer (v) of sapwood, but not of inner sapwood, had increased slightly.

(b) Soft-rot cavities were also produced as a result of soil burial but were largely confined to layers (i) of both sapwood and inner sapwood blocks. Soft rot, however, was not associated with the movement of additional nitrogen from soil ($r = 0.20$, Fig. 2.19), nor was it associated with movement of moisture from soil ($r = -0.09$, Fig. 3.20), nor was additional nitrogen movement from soil into wood associated with moisture ($r = -0.35$, Fig. 3.21).

(c) The presence or absence of cell contents, in contrast to the results of Experiment 3.2, influenced the amount of nitrogen movement from soil. Wood with higher nitrogen contents at the beginning of the burial period (the sapwood blocks) showed lesser increases in nitrogen contents as a result of burial than the wood with the lower control nitrogen contents (the inner sapwood blocks). However, this division tended to be blurred as the B sides (with some redistributed soluble nutrients) of the inner sapwood blocks had the same control nitrogen levels as the A sides (these largely without redistributed

FIGURE 3.I9 Correlation of soft rot with increase in nitrogen content as a result of soil burial (layer i)

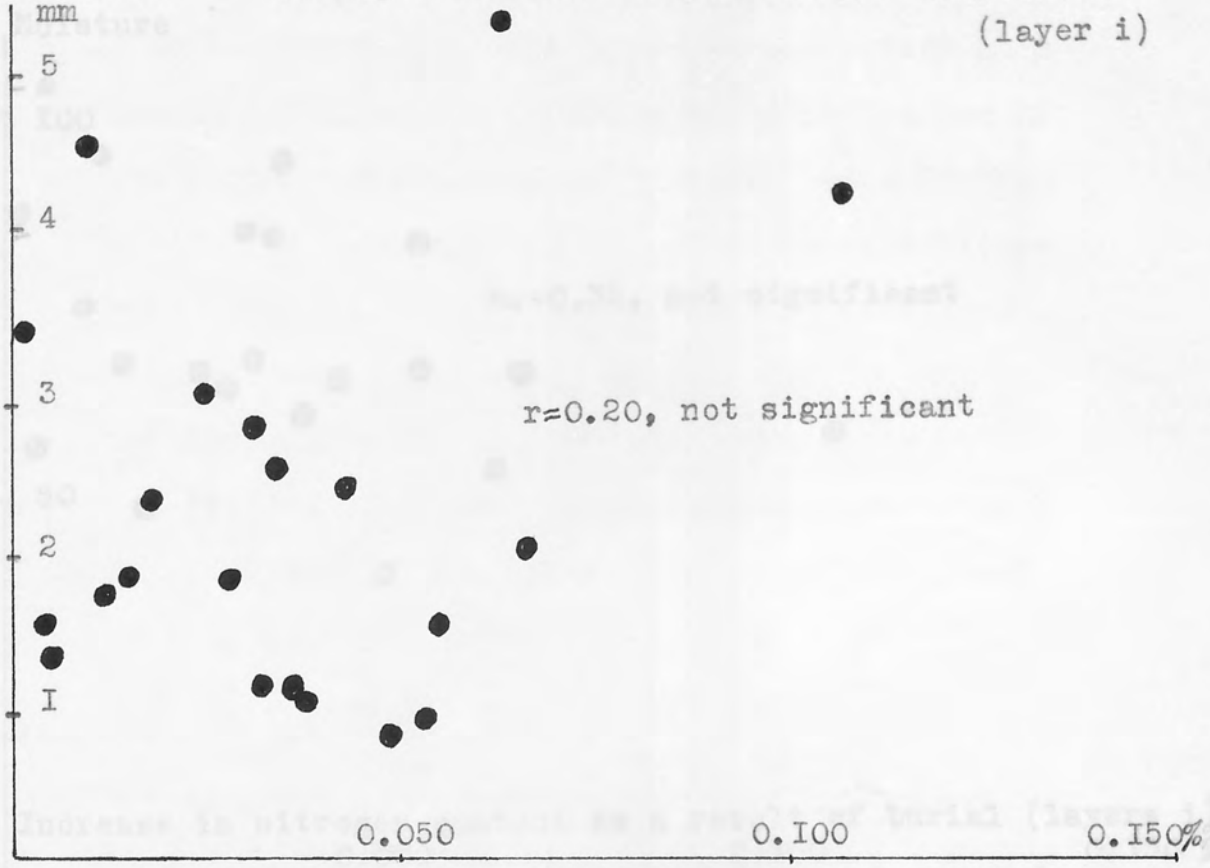


FIGURE 3.20 Correlation of soft rot with moisture in pine blocks after burial (layer i)

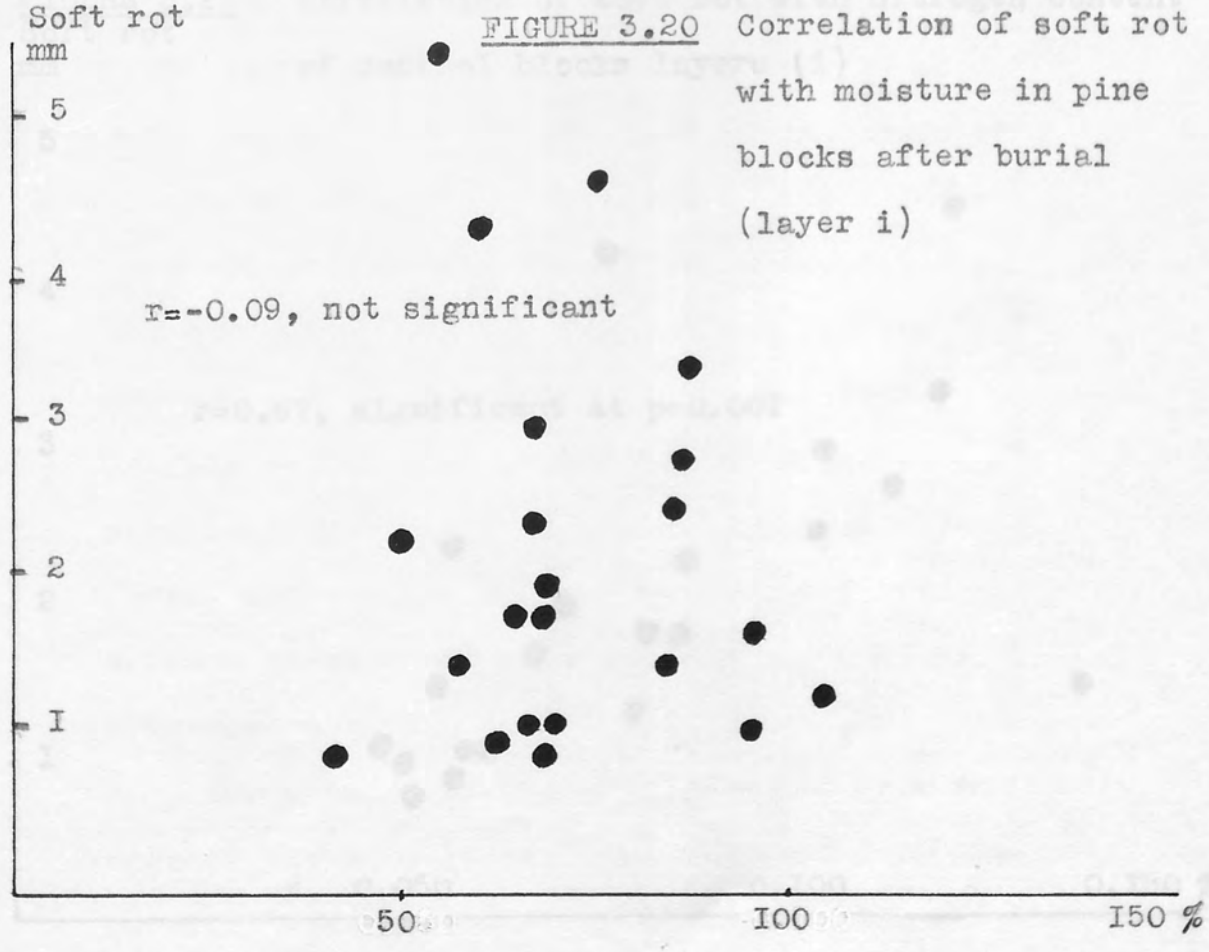


FIGURE 3.2I Correlation of moisture content with increase in nitrogen content as a result of burial

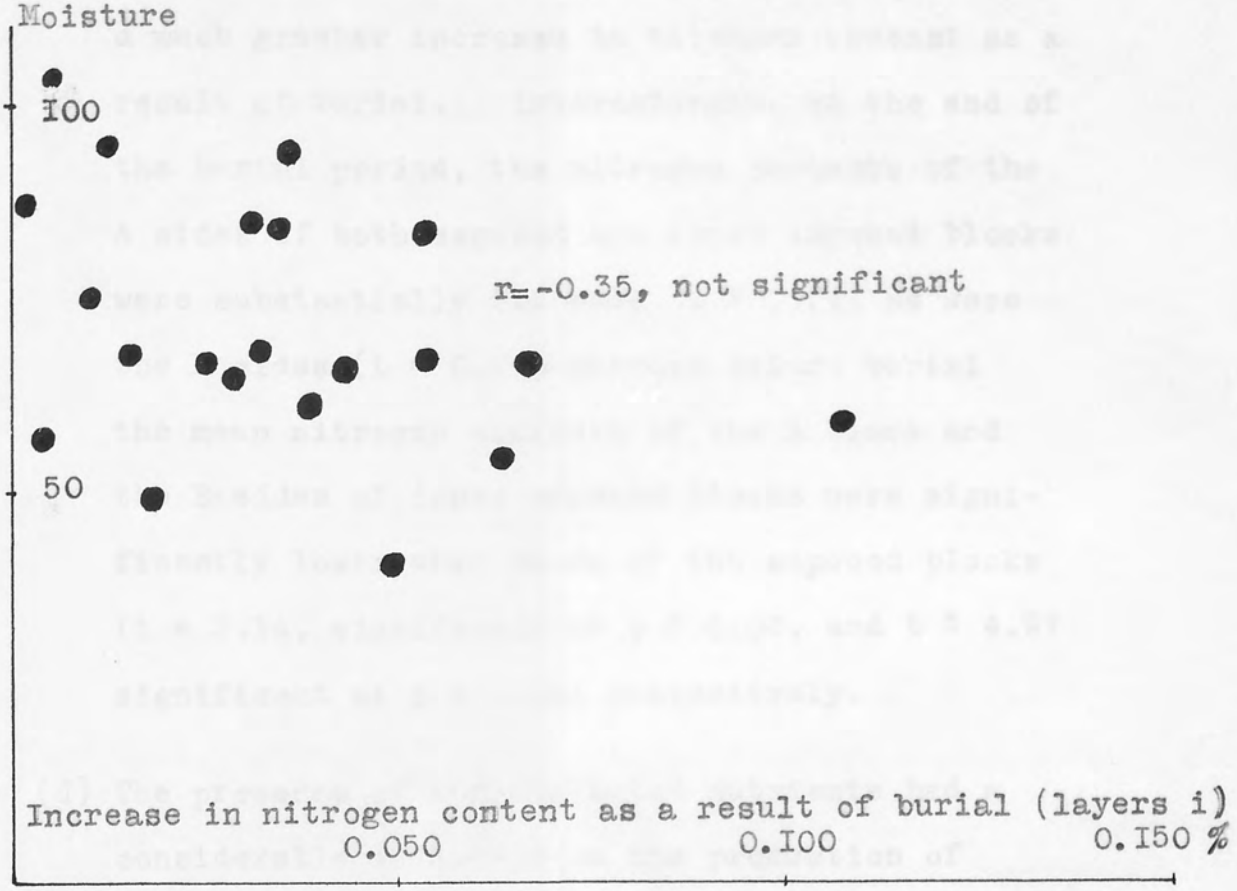
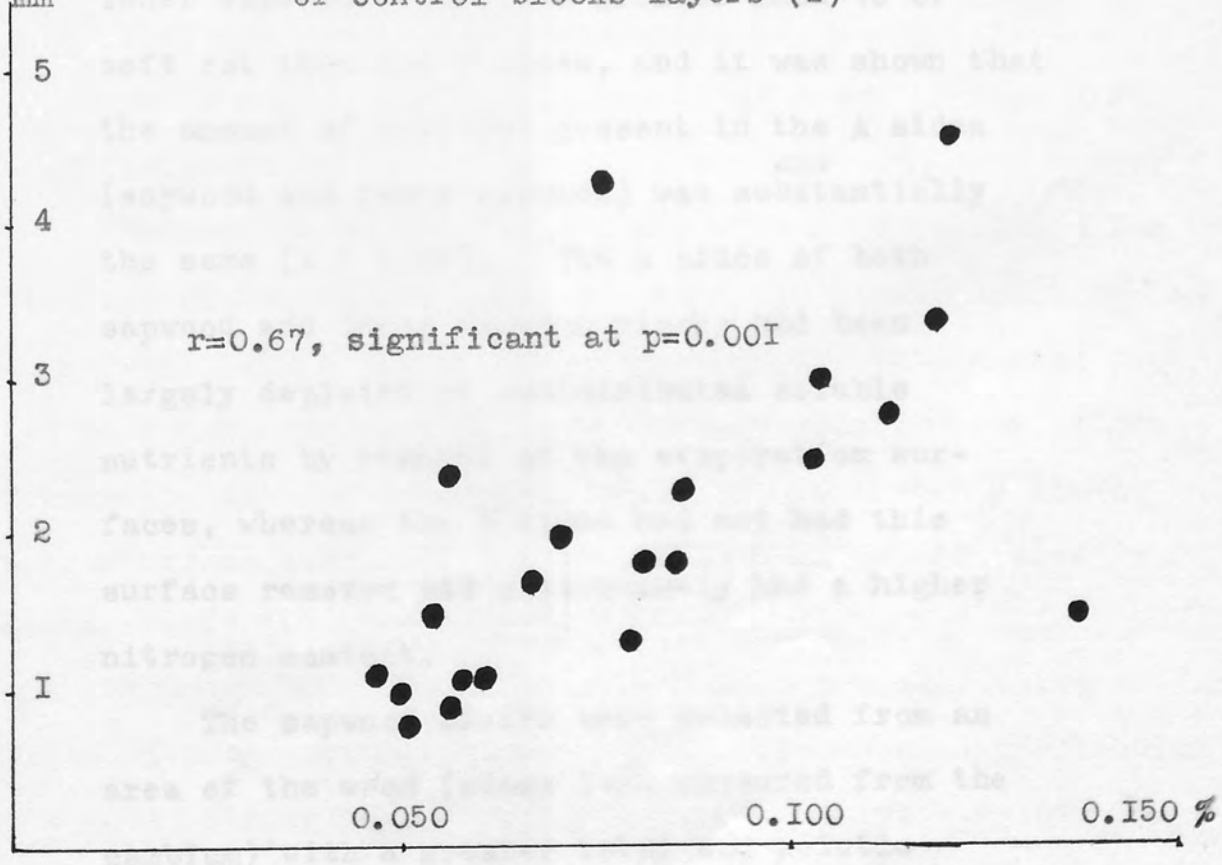


FIGURE 3.22 Correlation of soft rot with nitrogen content of control blocks layers (i)



soluble nutrients) of the sapwood blocks, yet had a much greater increase in nitrogen content as a result of burial. Interestingly, at the end of the burial period, the nitrogen contents of the A sides of both sapwood and inner sapwood blocks were substantially the same ($t = 0.14$) as were the B sides ($t = 0.27$) whereas before burial the mean nitrogen contents of the A sides and the B sides of inner sapwood blocks were significantly lower than those of the sapwood blocks ($t = 3.14$, significant at $p = 0.02$, and $t = 4.97$ significant at $p = 0.001$ respectively.

(d) The presence of redistributed nutrients had a considerable influence on the production of soft rot. The B sides of both the sapwood and inner sapwood blocks had greater amounts of soft rot than the A sides, and it was shown that the amount of soft-rot present in the A sides (sapwood and inner sapwood) was substantially the same ($t = 0.99$). The A sides of both sapwood and inner sapwood blocks had been largely depleted of redistributed soluble nutrients by removal of the evaporation surfaces, whereas the B sides had not had this surface removed and consequently had a higher nitrogen content.

The sapwood blocks were selected from an area of the wood (rings 1-15 measured from the cambium) with a greater total and soluble nitrogen content than the inner sapwood blocks

(which were selected from rings 16-25 measured from the cambium) consequently, the nitrogen available for redistribution in the wood before drying was greater in the sapwood blocks than in the inner sapwood blocks. This is reflected in the control nitrogen contents of the B sides of blocks, that of layer (i) sapwood blocks being greater than layer (i) of inner sapwood blocks.

It was noted however, that although the mean depth of soft rot apparent in the B sides of sapwood blocks was greater than that of the B sides of inner sapwood blocks (reflecting the respective layers (i) control nitrogen status), these mean soft-rot depths were not statistically different. The relationship between the initial nitrogen contents of blocks and the amount of soft rot produced after soil burial is shown in Fig. 3.22 (significant at $p = 0.001$, $r = 0.67$).

The results of this experiment therefore confirmed the findings of Experiment 3.2, viz. that the factor contributing most to the extent of soft-rot production in wood buried in P.R.L. soil was not moisture, or additional nitrogen from soil, but the amount of nitrogen (and probably other nutrients) in a soluble form redistributed to the faces in contact with the soil.

Respiration in wood layers was also somewhat correlated with the control nitrogen content ($r = 0.49$ significant at $p = 0.05$, Fig. 2.23), but did not correlate with moisture content ($r = 0.16$) excess nitrogen after burial ($r = -0.34$) or total nitrogen content after burial ($r = 0.16$).

FIGURE 3.23 Correlation of respiration in pine blocks after burial with nitrogen content of control blocks (layers i)

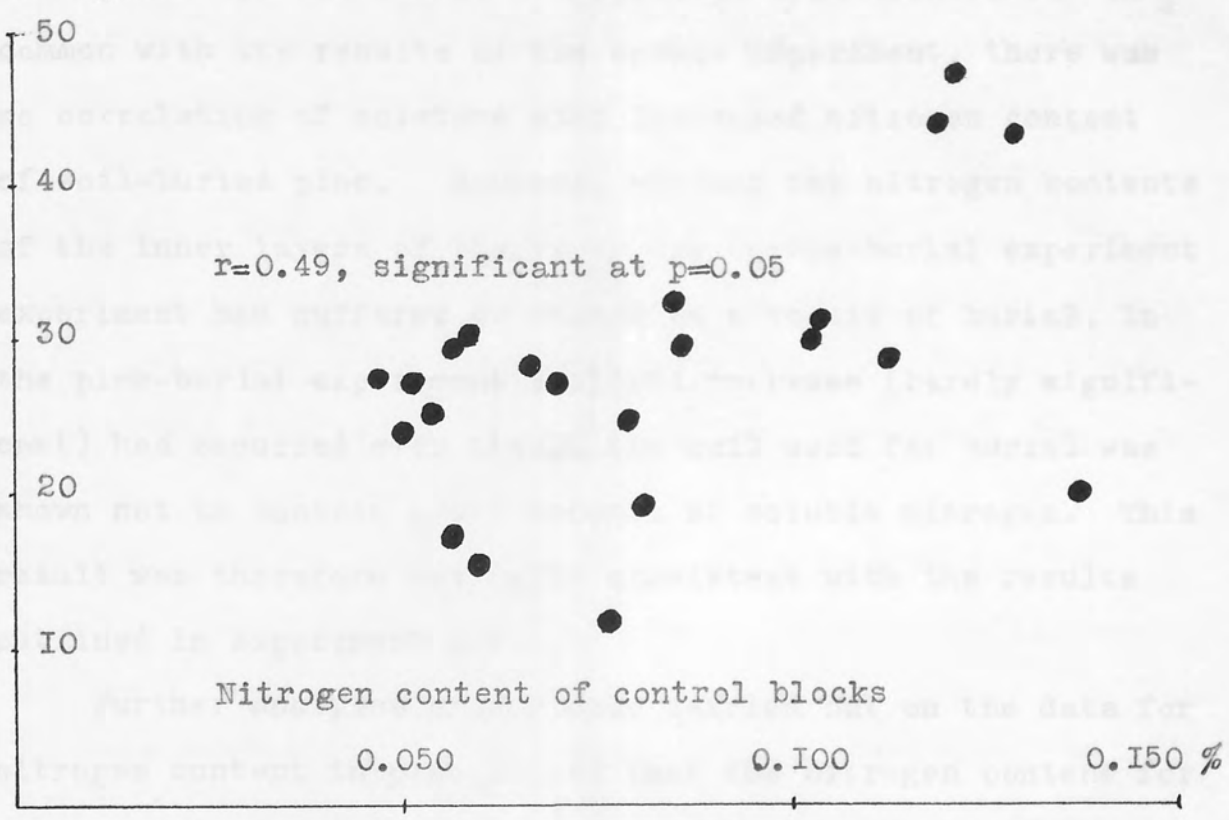
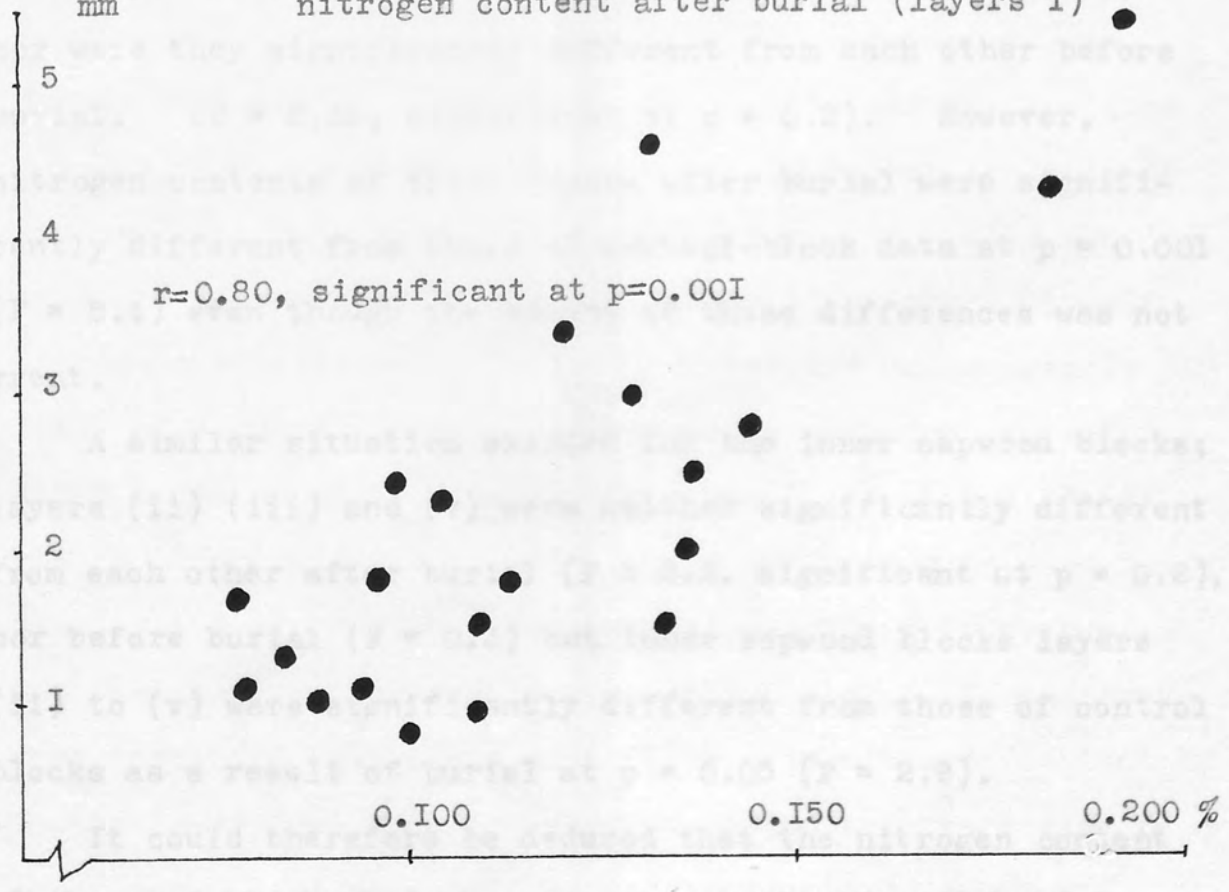


FIGURE 3.24 Correlation of soft rot in pine blocks with nitrogen content after burial (layers i)



It was shown in Experiment 3.2. that nitrogen did not move from soil into wood in the form of soluble materials but rather was the result of microbial translocation. In common with the results of the spruce experiment, there was no correlation of moisture with increased nitrogen content of soil-buried pine. However, whereas the nitrogen contents of the inner layers of blocks in the spruce-burial experiment experiment had suffered no change as a result of burial, in the pine-burial experiment a slight increase (barely significant) had occurred even though the soil used for burial was shown not to contain great amounts of soluble nitrogen. This result was therefore not fully consistent with the results outlined in Experiment 3.2.

Further analyses of variance carried out on the data for nitrogen content in pine showed that the nitrogen content for layers (ii), (iii) and (v) of sapwood blocks were not significantly different from each other after burial ($F = 1.2$), nor were they significantly different from each other before burial. ($F = 2.35$, significant at $p = 0.2$). However, nitrogen contents of these layers after burial were significantly different from those of control-block data at $p = 0.001$ ($F = 5.4$) even though the extent of these differences was not great.

A similar situation existed for the inner sapwood blocks; layers (ii) (iii) and (v) were neither significantly different from each other after burial ($F = 2.2$. significant at $p = 0.2$), nor before burial ($F = 0.3$) but inner sapwood blocks layers (ii) to (v) were significantly different from those of control blocks as a result of burial at $p = 0.05$ ($F = 2.9$).

It could therefore be deduced that the nitrogen content of the wood blocks had changed as a result of soil burial,

which indicated that some form of nitrogen movement had occurred. Slight fungal colonisation without decay had taken place in the inner layers of blocks and this might have contributed to nitrogen movement.

Consideration of the design of the test blocks, however, provided a further explanation for this phenomenon. The sapwood and inner sapwood blocks were removed from wood with high and low soluble nitrogen contents respectively. As the planks from which these blocks were removed dried, the soluble nitrogen migrated to the evaporation surfaces. However, not all soluble nitrogen would be expected to migrate during drying, and some could be expected to remain in the interior of the wood. It was presumed that the amounts of soluble nitrogen remaining in the sapwood blocks would have been higher than that remaining in the inner sapwood blocks and this higher amount of nitrogen might have been capable of redistribution on wetting of wood blocks during soil burial. However, one evaporation surface (the A side) was removed from all test blocks and an analysis of variance was undertaken to compare layers (ii) (iii) and (v) of the A sides of inner sapwood blocks (i.e. those largely without redistributed soluble nutrients and an inherently low level of soluble nitrogen) after burial with the control A and B sides of those blocks. The result of this analysis showed that there was no significant difference between these data ($F = 0.8$), and that movement of soluble nitrogenous material had not occurred during soil burial. It was therefore concluded that the slight changes in nitrogen content in the inner layers of the sapwood blocks and the B side of inner sapwood blocks could be attributed to movement of

redistributed soluble nitrogen back into the interior of those blocks as a result of moisture movement during burial, and not to movement of soluble nitrogen from soil. It was therefore concluded that, as with the spruce experiment, nitrogen movement into pine buried in soil was probably in a microbial form.

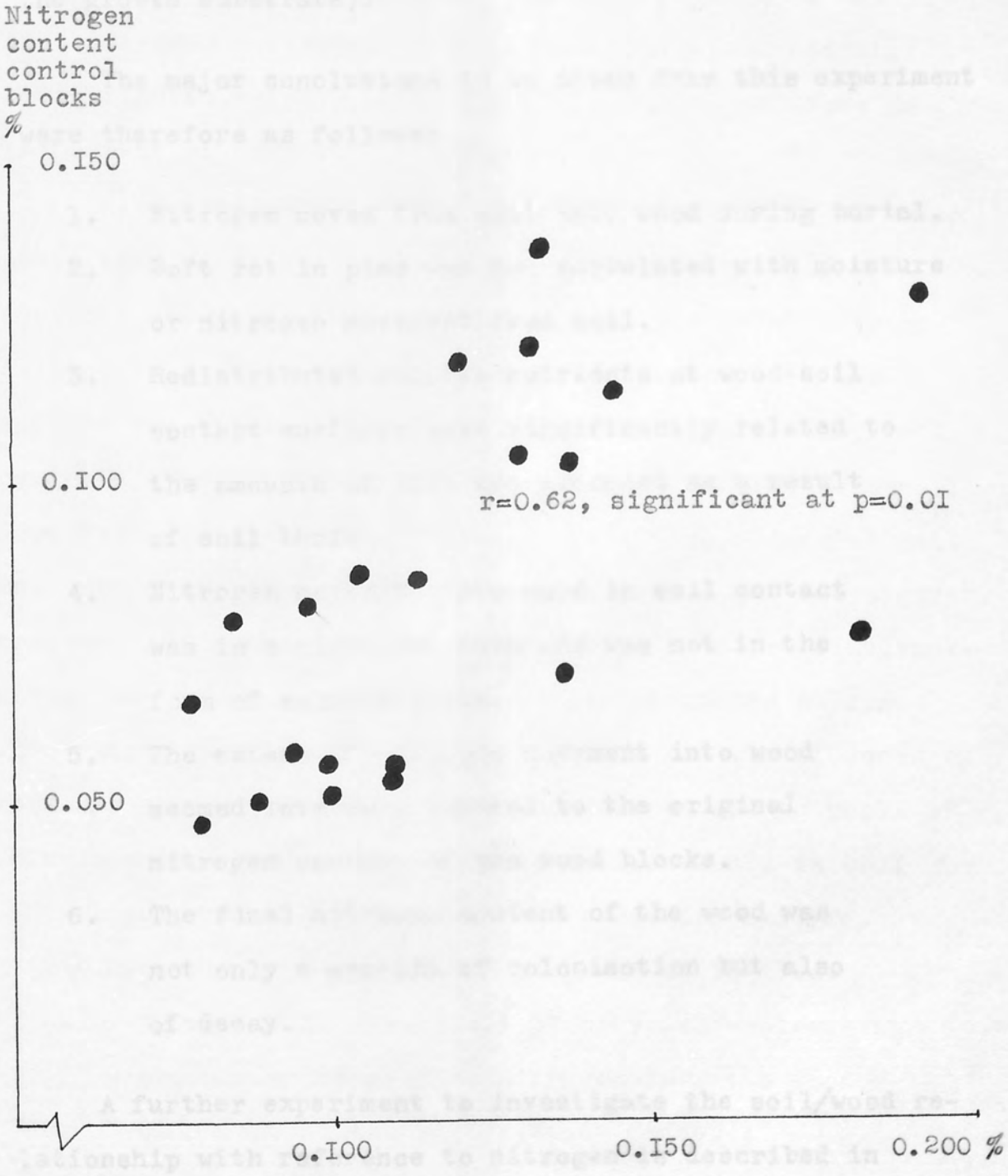
It was clear from the results that the presence of cell contents in wood did not stimulate translocation of nitrogen from soil, but rather that a lack of cell contents promoted nitrogen movement from soil into wood. In contrast with this, however, the wood with the least amount of nitrogen translocated to it during burial exhibited the greater amount of decay, whereas the wood with a greater amount of translocated nitrogen suffered lesser degrees of decay, although this distinction was not clear as stated earlier. It therefore seemed that two phenomena were being examined simultaneously, one in which nitrogen was being translocated to nitrogen-deficient wood before soft rot was produced, and another in which organisms were utilising pools of nitrogen and carbohydrate redistributed to soil-contact surfaces and rapidly producing soft-rot cavities.

It was also apparent that the nitrogen contents of the A sides of inner sapwood and sapwood blocks were similar after soil burial as were the B sides, whereas they had been significantly different before burial. Similarly, the soft-rot levels in the A sidesof inner sapwood and sapwood blocks were similar to each other as were those in the B sides. A pattern was not apparent in the datafor nitrogen movement into wood, other than at certain total nitrogen contents after burial, specific levels of soft rot were

present. As soluble nitrogen did not move from soil into wood, it was considered that the final nitrogen content (after burial) could only consist of (i) microbial biomass and (ii) the wood nitrogen content.

It is known that, as wood decays, its nitrogen content increases due to loss by respiration of carbohydrate materials (Hungate 1941; Fisher 1941); consequently, nitrogen analyses of decaying wood would be expected to show an increased nitrogen content (measured as a percentage on a weight basis) relative to sound wood, and wood with greater amounts of decay would have higher nitrogen contents than wood with lesser amounts of decay. This relationship is evident from the data presented in Fig. 3.24 ($r = 0.80$, significant at $p = 0.001$). It therefore appeared that, not only was the nitrogen content of wood blocks after burial a measure of translocation of nitrogen, but it was also an arbitrary measure of decay within the context of the experiment described. As the final nitrogen content was also a measure of decay, it would be expected to correlate with the nutrient status of the wood which promoted that decay, viz. the initial nitrogen content of the wood. This relationship is demonstrated in Fig. 3.25. (While the final total nitrogen of the wood includes the original nitrogen, if no decay had occurred, no <u>change</u> in nitrogen content would have taken place and the correlation co-efficient would have been 1. As changes in nitrogen content occurred as a result of burial and the final nitrogen content includes the original nitrogen, it is presumed that this latter had been transformed into fungal tissue, wood-decay enzymes, etc. Consequently, the correlation is not simply between wood

FIGURE 3.25 Correlation of nitrogen content of control blocks with nitrogen content of pine blocks after burial



nitrogen and wood nitrogen but rather between the end result of a biological process involving wood, measured as nitrogen content, and the initial nitrogen content of the growth substrate).

The major conclusions to be drawn from this experiment were therefore as follows:

1. Nitrogen moved from soil into wood during burial.
2. Soft rot in pine was not correlated with moisture or nitrogen movement from soil.
3. Redistributed soluble nutrients at wood-soil contact surfaces were significantly related to the amounts of soft rot produced as a result of soil burial.
4. Nitrogen movement into wood in soil contact was in a microbial form and was not in the form of soluble salts.
5. The extent of nitrogen movement into wood seemed inversely related to the original nitrogen content of the wood blocks.
6. The final nitrogen content of the wood was not only a measure of colonisation but also of decay.

A further experiment to investigate the soil/wood relationship with reference to nitrogen is described in Experiment 3.4.

3.2.4. Nitrogen translocation from Soil into Pine and the Influence of precise location of redistributed soluble Nutrients on soft-rot Production (Expt. 3.4)

The results of Expt. 3.3 confirmed those of Expt. 3.2, viz. that nitrogen moved from soil into wood, that this nitrogen movement was not directly related to decay, that nitrogen movement was in a microbial form, and that redistributed soluble nutrients had a major influence on the extent of soft-rot production.

In contrast with the results of Experiment 3.2, in which there were no relationships between the presence of cell contents and nitrogen movement from soil, a relationship existed in Experiment 3.3. This relationship did not support the hypothesis (propounded in Chapter 1) that cell contents stimulated the translocation of nitrogen from soil into wood, but rather the reverse, i.e. that a lack of cell contents stimulated nitrogen translocation. It was however, considered that two phenomena were being examined simultaneously: one in which organisms were translocating nitrogen into nitrogen-deficient wood before soft rot was produced by them and another, in which organisms were utilising pools of soluble nitrogen and carbohydrates redistributed to soil contact surfaces and were there producing soft rot.

A hypothesis was therefore proposed as follows:

(a) In wood with a low level of nitrogen, colonising organisms translocate nitrogen into the wood as a part of the colonisation process;

(b) In wood with a high level of nitrogen (comprised of both structural nitrogen and redistributed soluble nitrogen) colonising organisms grow and multiply at the expense of the nitrogen present in the wood and do

not translocate significant amounts of nitrogen into the wood (Hypothesis 1).

It has been shown in the literature that the amount of nitrogen in wood considerably influences the amount of decay which is produced therein, and Experiments 3.2. and 3.3. showed that redistributed soluble nitrogen, as a part of the total nitrogen content, considerably enhanced the susceptibility of both pine and spruce to soft rot.

For example, the mean nitrogen content in layers (i) side B of pine control blocks was 61% greater than that in layers (i) side A due to soluble nutrient redistribution and its deliberate removal from the latter. The mean depth of soft rot in the B sides was correspondingly 138% greater than that in the A sides after burial. The mean nitrogen content of layers (i) in spruce sapwood blocks was 52% greater than that of layers (i) of the inner sapwood blocks. The mean depth to which soft-rot cavities were found in sapwood blocks was correspondingly 276% greater than that in the inner sapwood blocks.

A previous report (Merrill and Cowling 1965b) showed that threefold increases in nitrogen content attributed to ring-to-ring variation resulted in twofold increases in decay rates. A further report (Levi and Cowling, 1968) showed that mean increases in nitrogen content of 20%, attributed to seasonal variation in total nitrogen (this variation, as stated earlier, is in reality due to seasonal fluctuation of soluble nitrogen) resulted in mean weight losses in oak sapwood of 53%, e.g. an increase in nitrogen content to increase in decay rate ratio of 1 : 2.5. It was therefore apparent that greater amounts of decay were related to increases in

total nitrogen content in wood produced by soluble nitrogen fluctuation, and lesser amounts of decay were associated with increases in the total nitrogen content produced by ring-to-ring variation.

The increase in nitrogen content to increase in decay rate ratio for oak, 1 : 2.5 ,(Levi and Cowling op. cit.) was similar to that evident for Expt. 3.3., 1 : 2.3, but both ratios were less than that for the spruce-burial experiment, 1 : 5.3 . Recent information (Baker, Miller, Morgan and Savory, 1973) has shown that the sapwood of pine contains a water-soluble fungistatic material and it was considered this material would probably have accumulated (along with carbohydrates and resinous materials) at the evaporation surfaces. This may have inhibited colonisation of pine blocks in relation to that of spruce. However, this did not explain the difference in ratios between the oak and the spruce. The oak had been tested shortly after felling whereas the spruce had been tested after it had slowly dried. One possible explanation for the difference, therefore, is that the distribution and location of soluble nitrogen within wood after drying rather than the gross nitrogen content would determine the decay pattern (Hypothesis 2).

These hypotheses were examined in the following experiment.

A. Materials and Methods.

(i) Materials

The material used for this experiment was the wood of the Scots pine tree used for Experiment 3.3. The selected quartersawn plank (55mm thick) was converted while still deep frozen into 10mm thick strips removed from the sapwood

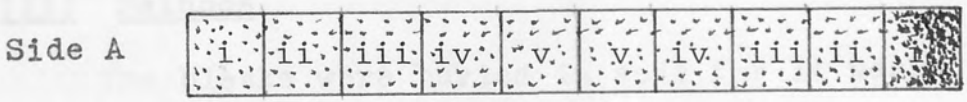
and inner sapwood. The strips were then dried at 50°C in an ammonia-free atmosphere. A 5-mm strip was removed from one radial edge of each strip to produce an "A" exposure face, the intact edge of the strip being designated "B". The dried strips were converted by transverse cuts into blocks 50 x 10 x 90mm as described in Experiment 3.3., the two ends of the strips being rejected.

The current experiment, however, differed from 3.3., for, in the latter experiment, the wood had been dried in plank form, consequently soluble nutrients had been redistributed to the transverse and radial faces of the plank only. As the plank was converted after drying, and transverse edges were never included in test material, the only parts of the blocks used to which redistribution of soluble nutrients had occurred were the radial soil-exposure faces.

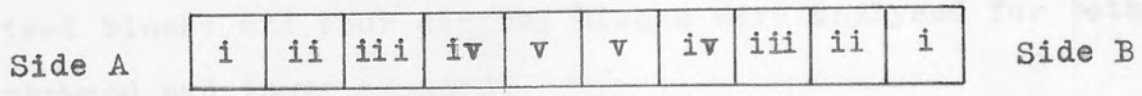
In the current experiment, however, the planks were converted into strips while still green; consequently during drying, soluble nutrients were redistributed to the radial and tangential faces both of which formed faces of the finished blocks. Redistributed soluble nutrients were therefore concentrated at the radial and tangential faces. The probable redistribution of soluble nutrients in blocks used in Experiment 3.3. and the current experiment is illustrated in Fig. 3.26.

The inner sapwood blocks were extracted in a 1 L sohxlet apparatus for 12 hours, successively with hot alcohol/benzene and hot alcohol/water solutions (Laidlaw and Smith, 1965). The blocks were then washed in water for 48 hours at room temperature in a shaking incubator and then dried at 50°C. This procedure was used to extract cell contents from the wood to provide "cell-content free" material in order to test

FIGURE 3.26. Diagramatic representation of transverse cross-sections of blocks used for Experiments 3.3. and 3.4.



Expt. 3.3. Sapwood and inner sapwood blocks



Expt. 3.4. Extracted inner sapwood blocks

Expt. 3.4. Sapwood blocks

Roman numerals indicate layers removed for analysis at successive 5mm depths from the soil contact surface.

Stippling indicates probable areas of blocks in which redistributed soluble nutrients were concentrared.

Hypothesis 1. The unextracted sapwood blocks were used to test Hypothesis 2.

All blocks, both sapwood and inner sapwood, were coated with epoxy resin except on the radial, soil-exposure faces, and were "cured" overnight at 100°C.

(ii) Methods

The blocks were buried in soil for 14 weeks as described for Experiment 3.3., and after the burial period were analysed by layers in similar fashion for moisture, nitrogen content, presence of soft rot, respiration, and pH. Six test blocks and four control blocks were analysed for both sapwood and inner sapwood.

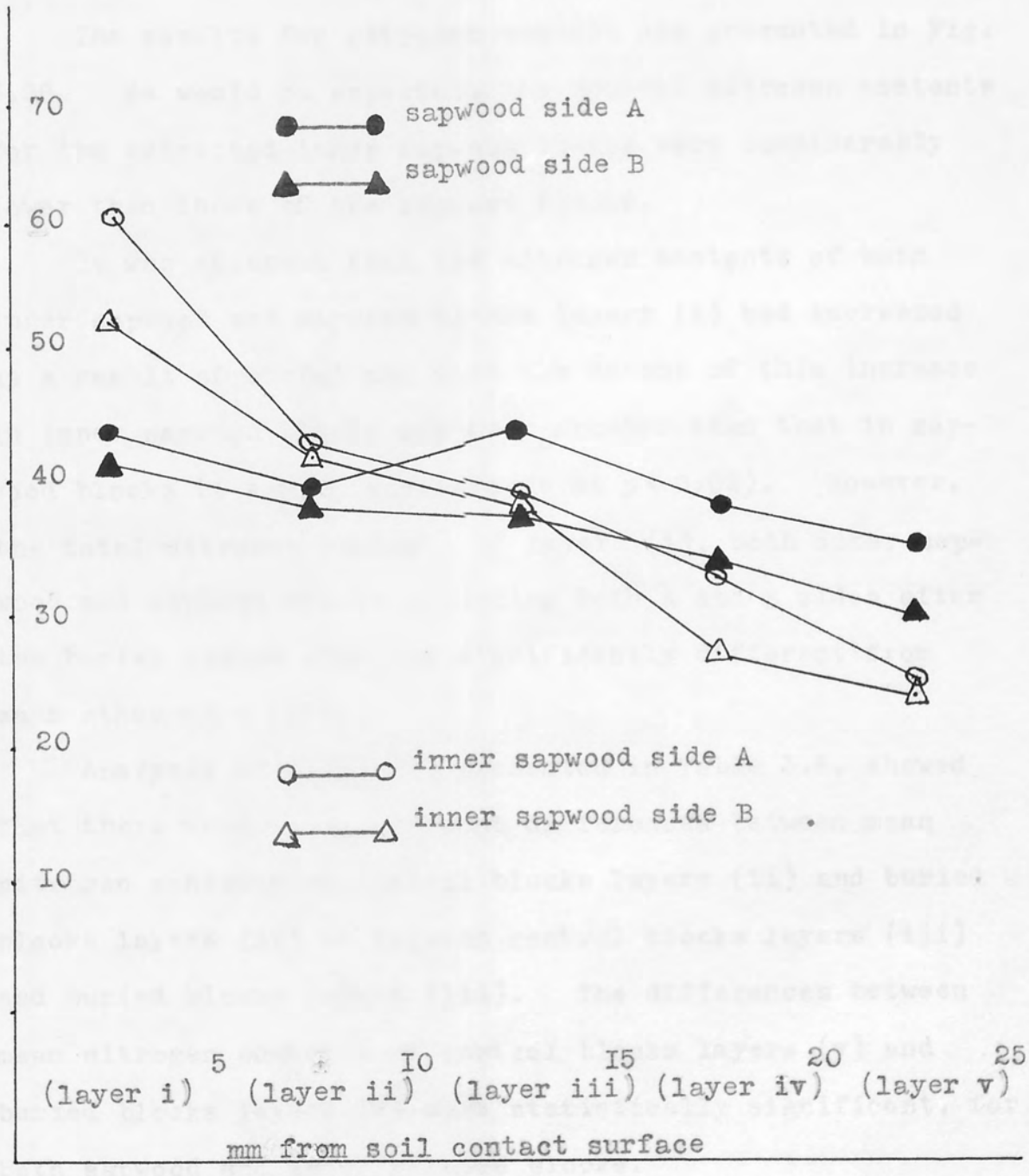
B. Results.

The results for these analyses are presented graphically in Figs. 3.27-3.31. Statistical analyses including number of samples, mean, standard deviation and standard error of the raw data contributing to these figures are presented in Appendix III.

Moisture Content

The results for moisture determinations are presented in Fig. 3.27. The results conformed to the pattern established in the earlier soil-burial experiments although the inner layers appeared to be somewhat higher than the earlier results. Analyses of variance showed that there was not a significant difference between the layers for the sapwood blocks ($F = 0.7$) but that the differences between the layers for the extracted inner sapwood blocks were significant at $p = 0.001$ ($F = 17.7$). There was not a significant differ-

FIGURE 3.27 Moisture in pine blocks after burial



ence, however, between layers (ii) and (iii) of these latter blocks (t = 1.35). Moisture determinations were not undertaken on control blocks.

Nitrogen Content

The results for nitrogen content are presented in Fig. 3.28. As would be expected, the control nitrogen contents for the extracted inner sapwood blocks were considerably lower than those of the sapwood blocks.

It was apparent that the nitrogen contents of both inner sapwood and sapwood blocks layers (i) had increased as a result of burial and that the extent of this increase in inner sapwood blocks was much greater than that in sapwood blocks (t = 2.78 significant at p = 0.02). However, the total nitrogen contents of layers (i), both inner sapwood and sapwood blocks including both A and B sides after the burial period were not significantly different from each other (F = 0.3).

Analyses of variance, presented in Table 3.6, showed that there were no significant differences between mean nitrogen contents of control blocks layers (ii) and buried blocks layers (ii) or between control blocks layers (iii) and buried blocks layers (iii). The differences between mean nitrogen contents of control blocks layers (v) and buried blocks layers (v) were statistically significant, for both sapwood and inner sapwood blocks.

It was apparent that the differences between mean nitrogen contents of layers (v) sapwood control and buried blocks were due to increases in the values for the buried blocks. The A and B sides of buried blocks layers (v) were not statistically different from each other (t = 0.21) nor were the values for the A and B sides (t = 0.42) of control blocks.

FIGURE 3.28 Nitrogen content of pine blocks both before and after burial

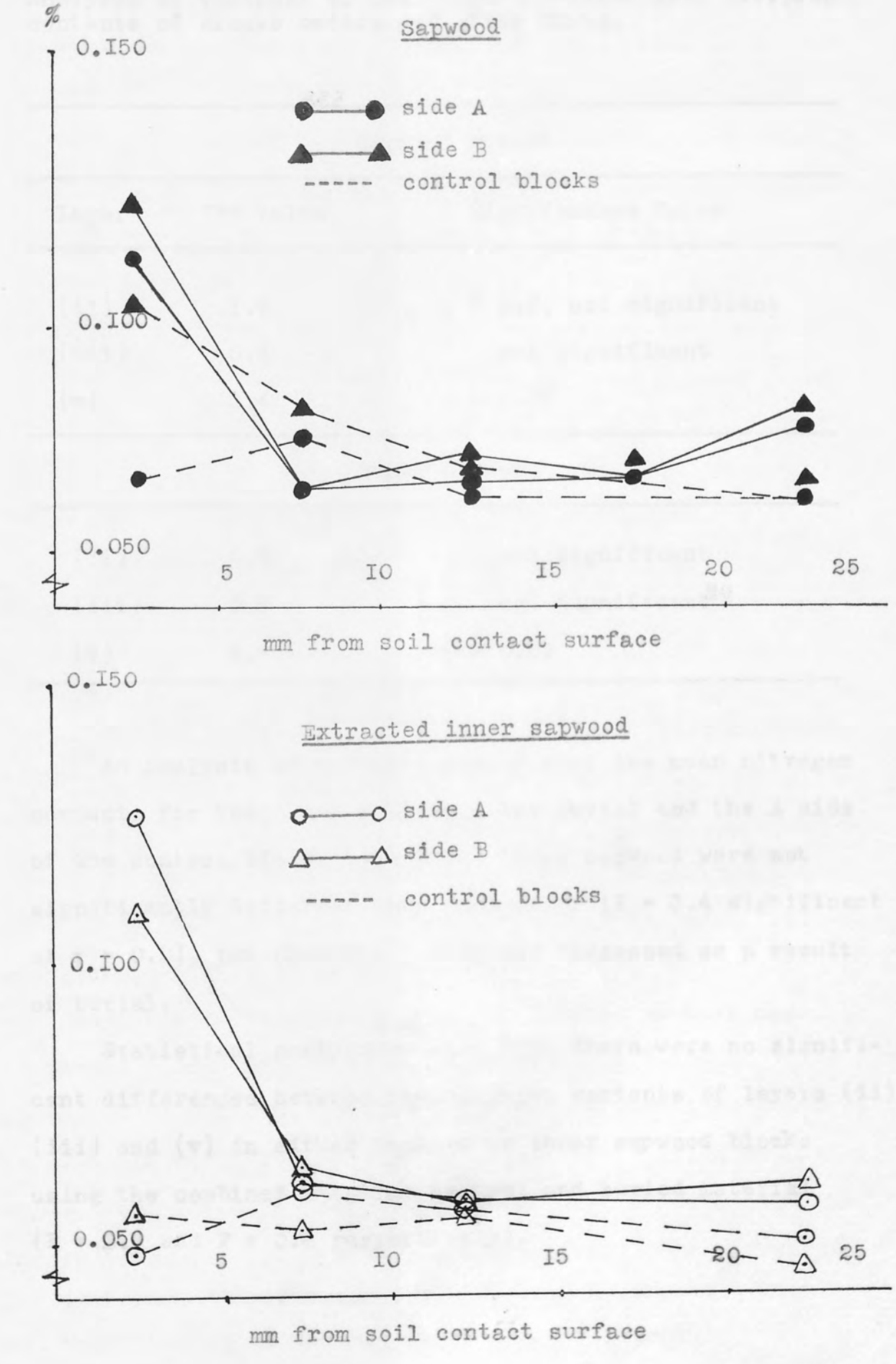


Table 3.6

Analyses of variance of differences between mean nitrogen contents of blocks before and after burial.

Layer	"F" Value	Significance Value
Sapwood Blocks		
(ii)	1.9	$p \doteq 0.2$, not significant
(iii)	0.4	not significant
(v)	3.4	$p = 0.05$
Inner Sapwood		
(ii)	0.5	not significant
(iii)	0.2	not significant
(v)	4.8	$p = 0.05$

An analysis of variance showed that the mean nitrogen contents for the A and B sides after burial and the A side of the control blocks layers (v) inner sapwood were not significantly different from each other ($F = 3.4$ significant at $p = 0.2$), but that the B side had increased as a result of burial.

Statistical analyses also showed that there were no significant differences between the nitrogen contents of layers (ii) (iii) and (v) in either sapwood or inner sapwood blocks using the combined data for control and buried material ($F = 0.8$ and $F = 0.6$ respectively).

Presence of Soft Rot

The data for presence of soft-rot cavities are presented in Fig. 3.29. These results showed that soft rot was produced in the layer in contact with soil and that greater amounts of soft rot were produced in the extracted inner sapwood blocks than in the sapwood blocks. It was clear from the results that there were no significant differences between the values for the A and B sides of the sapwood blocks ($t = 0.23$) and that the amount of soft rot in the A sides of the inner sapwood blocks was greater than that in the B sides ($t = 2.44$ significant at $p = 0.05$). Analyses of variance, however, showed that there was not a significant difference between the A and B sides of the sapwood blocks and the B sides of the extracted inner sapwood blocks.

Soft rot was not detected in the outer layers of control blocks.

Respiration

The data for respirometric evaluation of wood colonisation are presented in Fig. 3.30. These results confirmed the biological activity present in layer(i) and also conformed to the patterns already established in Experiment 3.3 viz., highest in layer (i), with a sharp decrease in layer (ii) but with levels rising in the inner layers.

Analyses of variance of the differences between mean respiration levels in both inner sapwood and sapwood blocks are presented in Table 3.7. These analyses showed that there were no significant differences between mean respiration levels of sapwood and inner sapwood blocks or between A and B sides in layers (i), (iii), (iv) and (v). The significant difference between means evident in layer (ii) was attributable to the higher value of the B side of the

FIGURE 3.29 Soft rot, measured in mm from soil contact surface, in pine blocks after burial

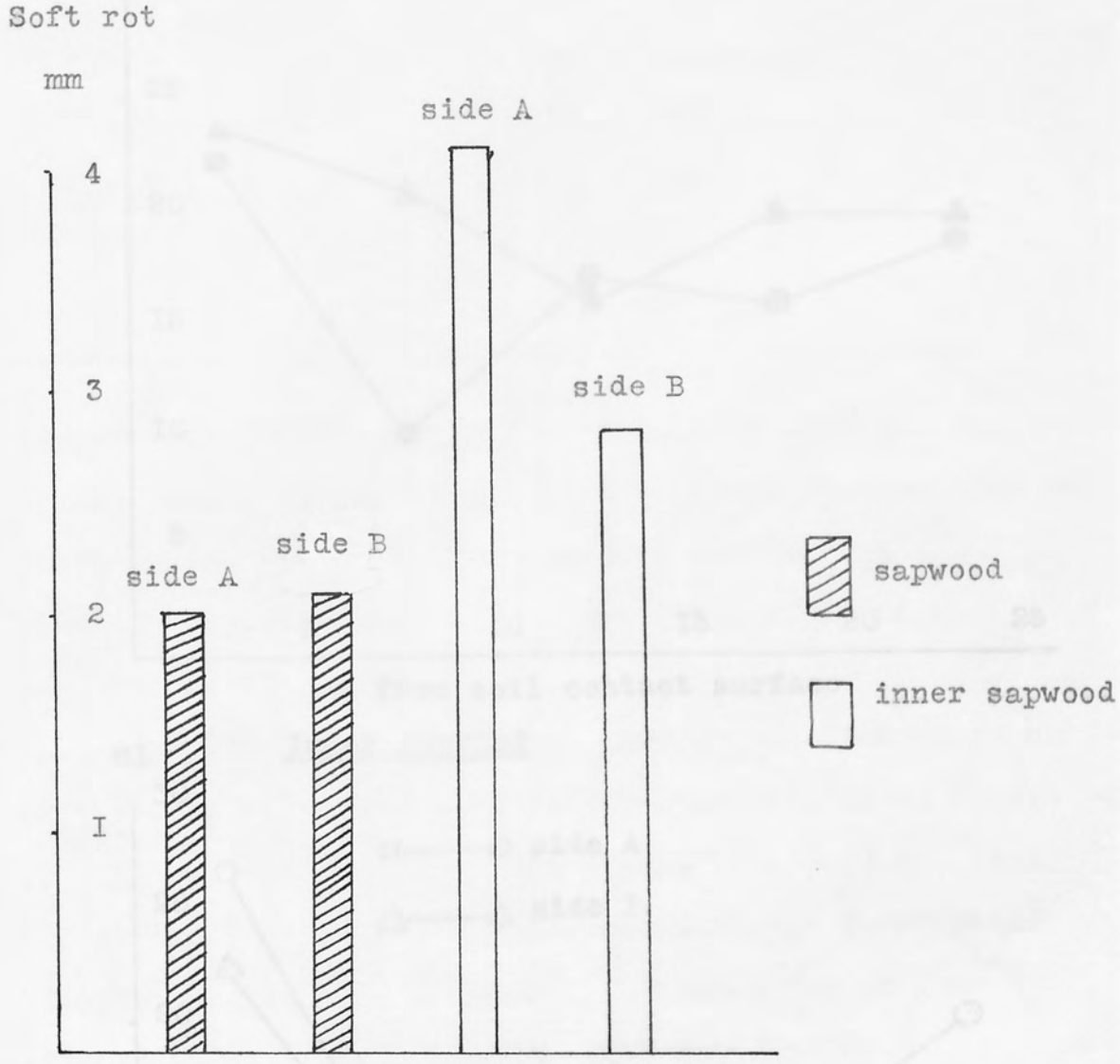
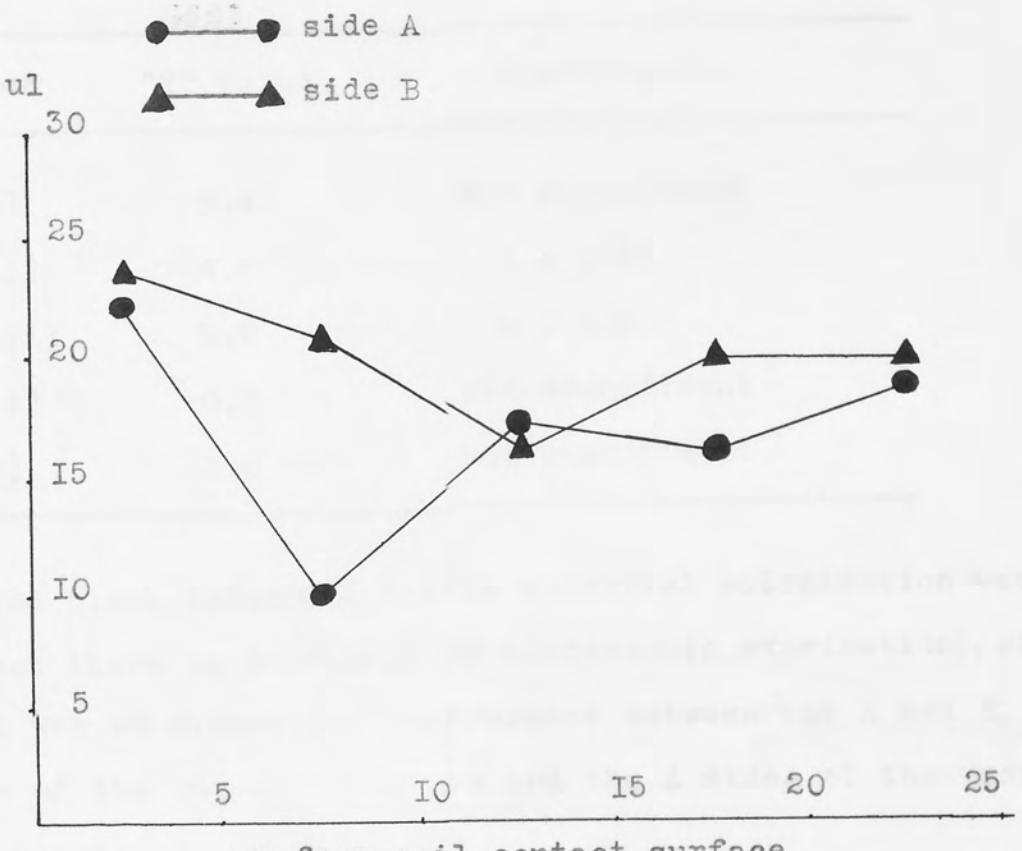


FIGURE 3.30 Respiration in pine blocks after burial (micro-litres of oxygen consumed per hour per cc of wood)



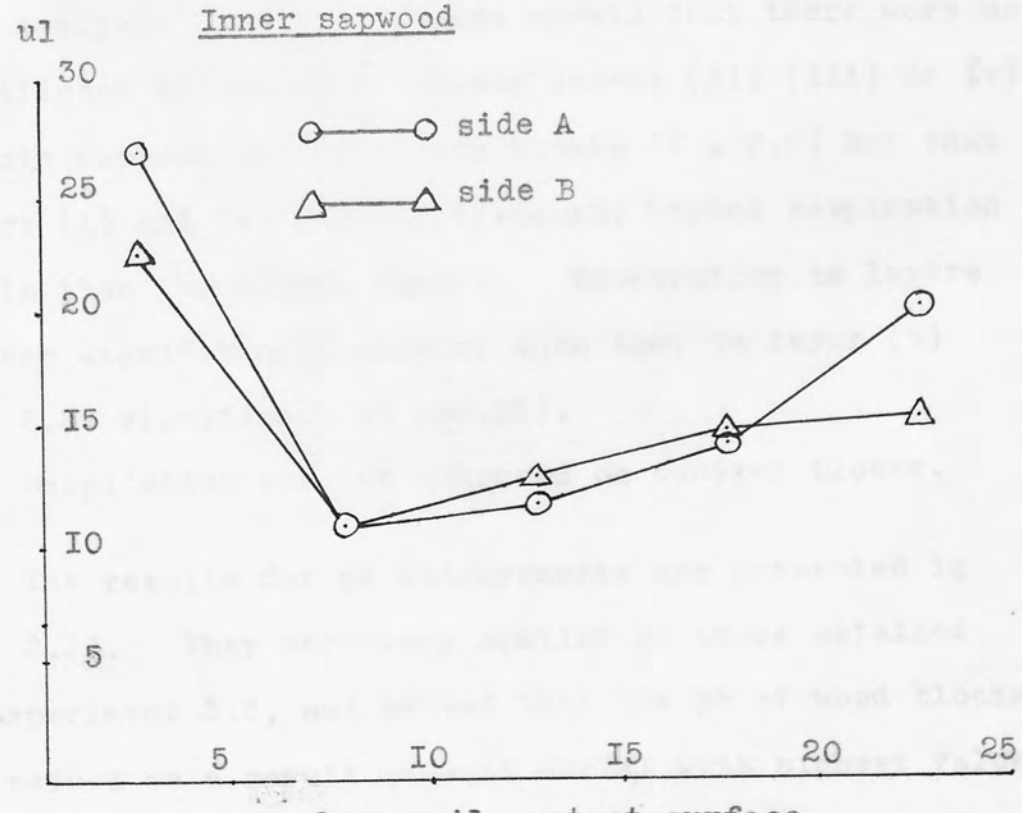


Table 37

Analyses of variance of differences between mean respiration levels in layers of blocks.

Layer	"F" value	Significance
(i)	0.4	not significant
(ii)	4.9	p = 0.01
(iii)	1.9	p = 0.2
(iv)	0.7	not significant
(v)	1.0	not significant

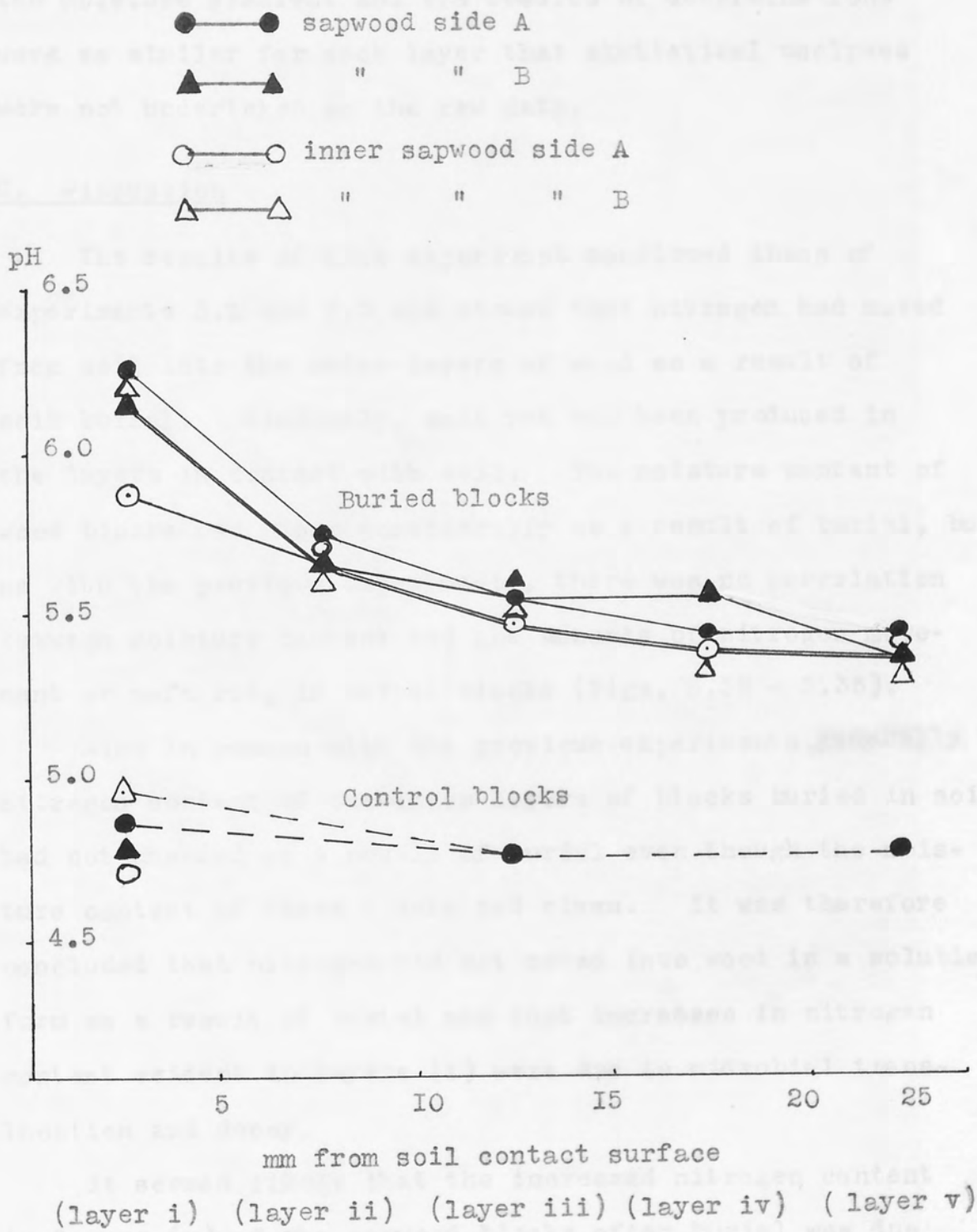
sapwood block (although little microbial colonisation was present there as indicated by microscopic examination), as there was no significant difference between the A and B sides of the extracted blocks and the A sides of the sapwood blocks ($F = 0.4$).

Analyses of variance also showed that there were no significant differences between layers (ii) (iii) or (iv) of both sapwood and extracted blocks ($F = 0.8$) but that layers (i) and (v) had significantly higher respiration levels than the middle layers. Respiration in layers (i) was significantly greater than that in layer (v) ($t = 2.39$ significant at $p=0.05$).

Respiration was not measured on control blocks.

pH: The results for pH measurements are presented in Fig. 2.3I. They were very similar to those obtained in Experiment 3.3, and showed that the pH of wood blocks was raised as a result of soil burial with highest values

FIGURE 3.31 pH of pine blocks after burial



recorded for the layer in contact with the soil (the pH of the latter was 7.3). The pH gradient followed the moisture gradient and the results of determinations were so similar for each layer that statistical analyses were not undertaken on the raw data.

C. Discussion

The results of this experiment confirmed those of Experiments 3.2 and 3.3 and showed that nitrogen had moved from soil into the outer layers of wood as a result of soil burial. Similarly, soft rot had been produced in the layers in contact with soil. The moisture content of wood blocks had risen considerably as a result of burial, but as with the previous experiments, there was no correlation between moisture content and the amounts of nitrogen movement or soft rot, in buried blocks (Figs. 3.32 - 3.35).

Also in common with the previous experiments, generally the nitrogen content of the inner layers of blocks buried in soil had not changed as a result of burial even though the moisture content of those blocks had risen. It was therefore concluded that nitrogen had not moved into wood in a soluble form as a result of burial and that increases in nitrogen content evident in layers (i) were due to microbial translocation and decay.

It seemed likely that the increased nitrogen content in layers (v) of the sapwood blocks after burial was due to redistribution of nutrients concentrated at the surface back into the inner layers with movement of moisture when blocks were buried. However, it could also have been due to a microbial presence, as fungal hyphae were observed

FIGURE 3.32 Correlation of moisture content with increase in nitrogen content as a result of burial

(layer i)

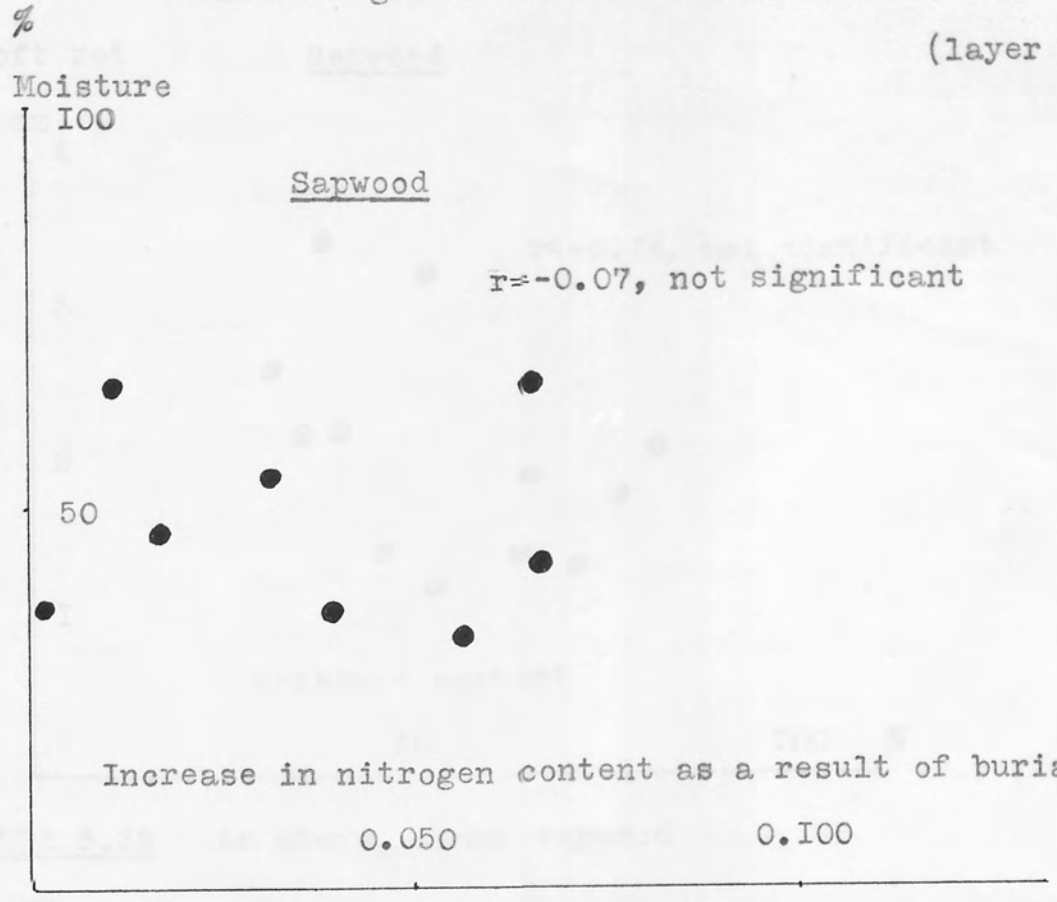


FIGURE 3.33 As above, inner sapwood

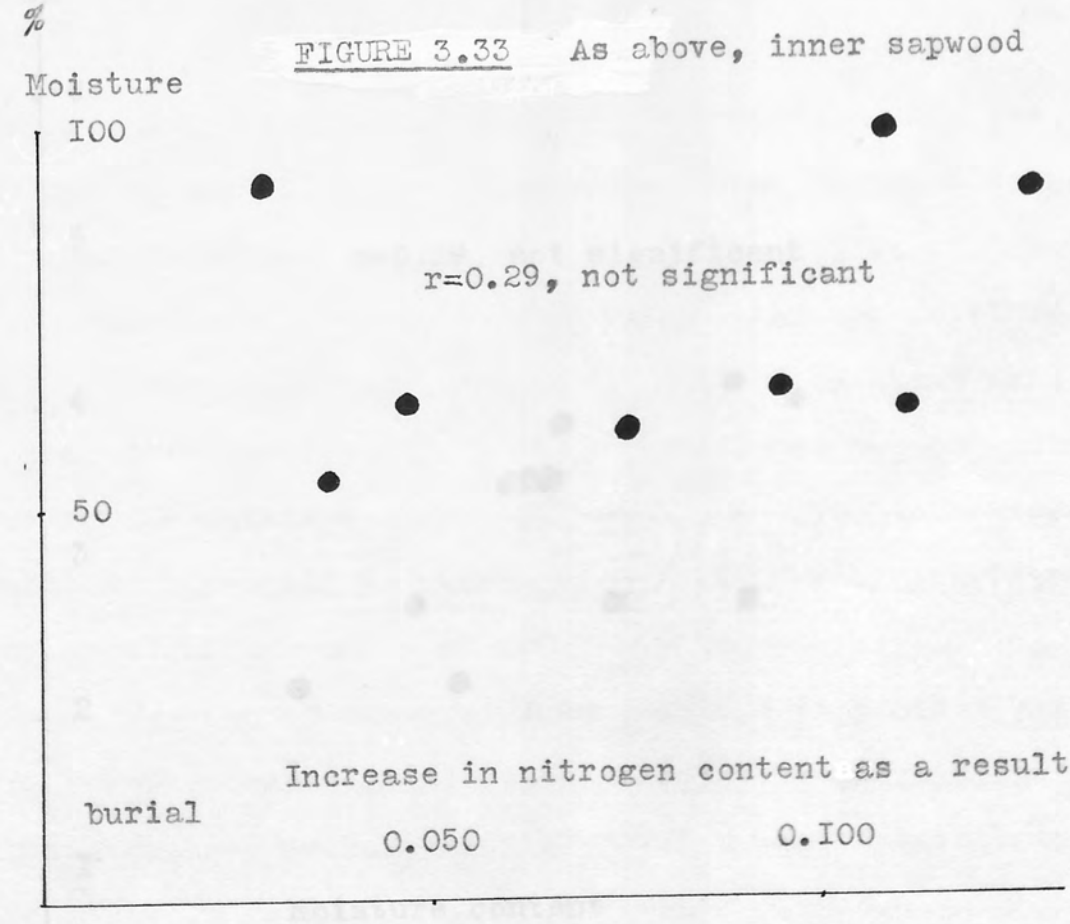


FIGURE 3.34 Correlation of soft rot with moisture content

(layer i)

Soft rot Sapwood

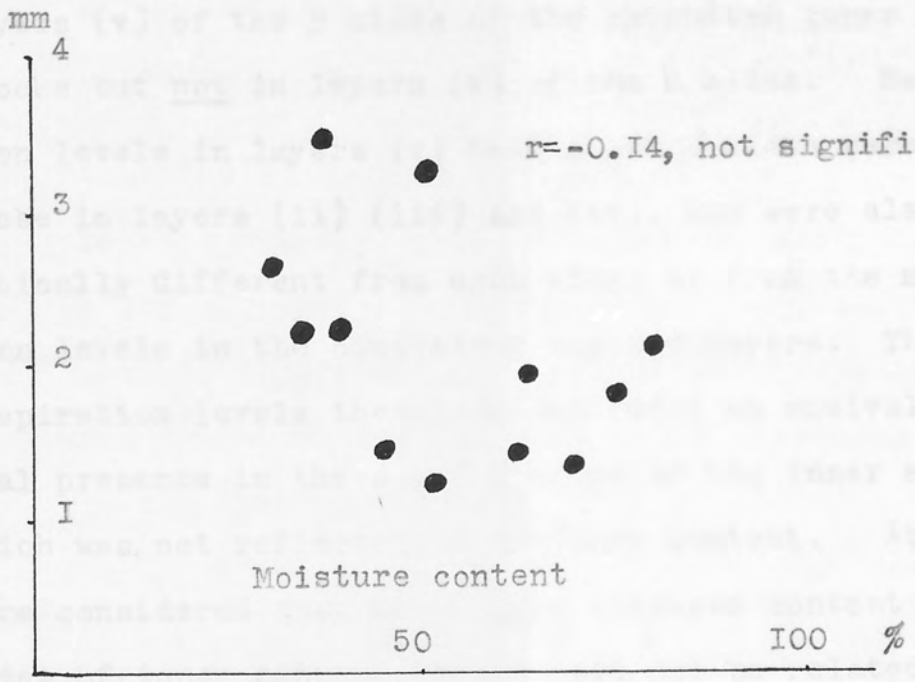
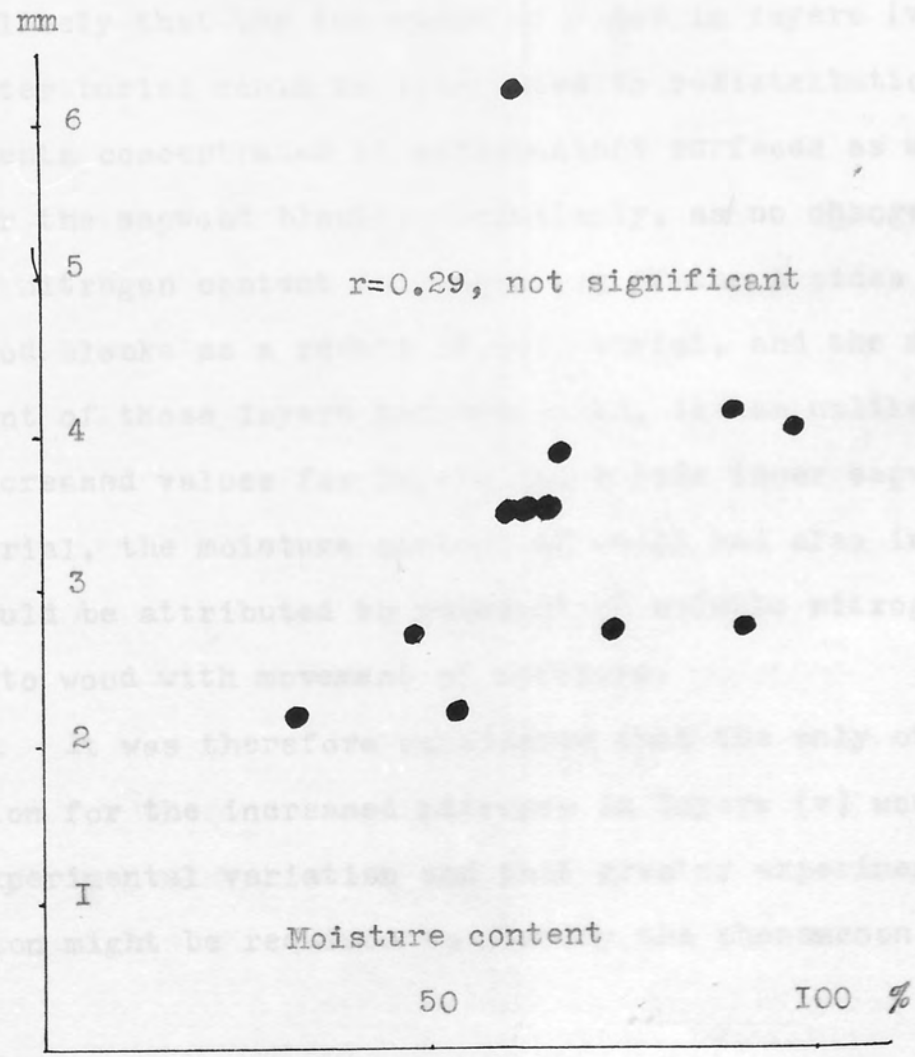


FIGURE 3.35 As above, inner sapwood



in layers (v) and respiration levels in those layers were higher than those in layers (ii), (iii) and (iv).

An increased nitrogen content was also observed in layers (v) of the B sides of the extracted inner sapwood blocks but not in layers (v) of the A sides. Mean respiration levels in layers (v) both A and B sides were greater than those in layers (ii) (iii) and (iv), and were also not statistically different from each other or from the mean respiration levels in the equivalent sapwood layers. These higher respiration levels therefore indicated an equivalent microbial presence in the A and B sides of the inner sapwood blocks which was not reflected in nitrogen content. It was therefore considered that the higher nitrogen content in the B sides of inner sapwood blocks need not be related to microbial presence. Because the blocks were extracted, it was unlikely that the increased nitrogen in layers (v) B side after burial could be attributed to redistribution of nutrients concentrated at soil-contact surfaces as was the case for the sapwood blocks. Similarly, as no change had occurred in nitrogen content in layers (v) of the A sides of inner sapwood blocks as a result of soil burial, and the moisture content of those layers had increased, it was unlikely that the increased values for layers (v) B side inner sapwood after burial, the moisture content of which had also increased, could be attributed to movement of soluble nitrogen from soil into wood with movement of moisture.

It was therefore considered that the only other explanation for the increased nitrogen in layers (v) would lie in experimental variation and that greater experimental replication might be required to clarify the phenomenon.

The purpose of this experiment was to examine two hypotheses:

1) that the nitrogen status of wood at the time of burial could determine the amount of nitrogen movement into wood; and

2) that the importance of redistributed soluble nutrients in enhancing the susceptibility of wood to soft-rot fungi was related more to the locations to which nutrients were redistributed than to simple increases in the total nitrogen content of the wood.

Hyrthesis 1: It is clear from the results that this hypothesis was correct. The inner sapwood blocks had been extracted to remove soluble nutrients while the sapwood blocks had contained soluble nutrients redistributed to the tangential and radial faces. The nitrogen contents of layers (i) of sapwood blocks were consequently considerably higher than those of the extracted blocks at the beginning of the experiment, but were substantially the same at the end of the burial period.

As the amount of soft rot produced in the sapwood blocks was similar to that produced in the B sides of the extracted blocks (the difference between the amounts of soft rot found in the A and B sides of extracted blocks were only significant at $p = 0.05$), it was concluded that the larger increased in nitrogen content of the extracted blocks was attributable to microbial translocation (i.e. as the amounts of soft rot found were substantially the same, arithmetic increases in nitrogen content due to removal by respiration of carbohydrates would also be the same for both sapwood and inner sapwood

blocks). Consequently, the greater increases in inner sapwood blocks after burial could only be attributable to microbial translocation.

It was therefore apparent that:

(a) in wood with a low level of nitrogen, colonising organisms translocated nitrogen into wood as a part of the colonisation process;

(b) in wood with a high level of nitrogen (comprised of both structural and redistributed soluble nitrogen) colonising organisms grew and multiplied at the expense of the nitrogen present in the wood, and did not translocate significant amounts of nitrogen from the soil into wood, i.e. the differences between mean nitrogen contents of sapwood buried and control blocks B side layer (i) was not significant ($t = 1.96$ significant at $p = 0.1$).

Hypothesis 2

The probable distribution of soluble nutrients in test blocks used for Experiments 3.3. and 3.4. is illustrated in Fig. 3.26. This figure shows the accumulation of soluble nutrients at the radial soil-contact evaporation surface for the blocks used in Experiment 3.3. and the probable nitrogen redistribution pattern to both the tangential faces and the radial soil-contact faces in blocks used in Experiment 3.4.

In sapwood blocks, the nitrogen contents of layers (i) sides B were considerably greater than those of the A sides at the beginning of the latter experiment but at the end of this experiment the nitrogen contents were the same for both A and B sides as were the soft-rot patterns. This differed from the evidence of the earlier soil-burial experiments

which showed that the extent of decay was greater in those sides of the blocks with accumulated soluble nutrients. It is suggested that the distribution of nutrients within the layers explains this phenomenon. In previous experiments the soluble nutrients were redistributed tangentially and concentrated at the radial face during drying of wood. The redistributed nutrients were thus perpendicular to the line of advancing microbial colonisation i.e. the cross section of wood in soil contact was nutrient rich. In the current experiment, however, soluble nutrients were redistributed both radially and tengentially thus producing nutrient concentrations largely in parallel with the plane of fungal colonisation (at the tangential faces) but leaving the greater proportion of the radial cross section of the wood in contact with the soil largely depleted of soluble nutrients. Consequently, even though the gross nitrogen content of layers (i) side B of sapwood blocks were greater than the A sides, the amount of nitrogen immediately available to colonising organisms at least in the short term was less than would have been expected from the total nitrogen content. Furthermore, judging from the soft-rot patterns in the A and B sides (not significantly different from each other) nitrogen available in the B sides of blocks only amounted to that available in the A sides (which had had their radial evaporation surfaces removed). Whereas control nitrogen contents were correlated ($p = 0.001$) with soft rot in Experiments 3.2 and 3.3, such a correlation did not exist for the sapwood blocks examined in Experiment 3.4. (Fig. 3.36).

The decay pattern in sapwood blocks was thus seen to support Hypothesis 2 namely that the pattern of redistribution

FIGURE 3.36 Correlation of soft rot with nitrogen content of control blocks layers (i)

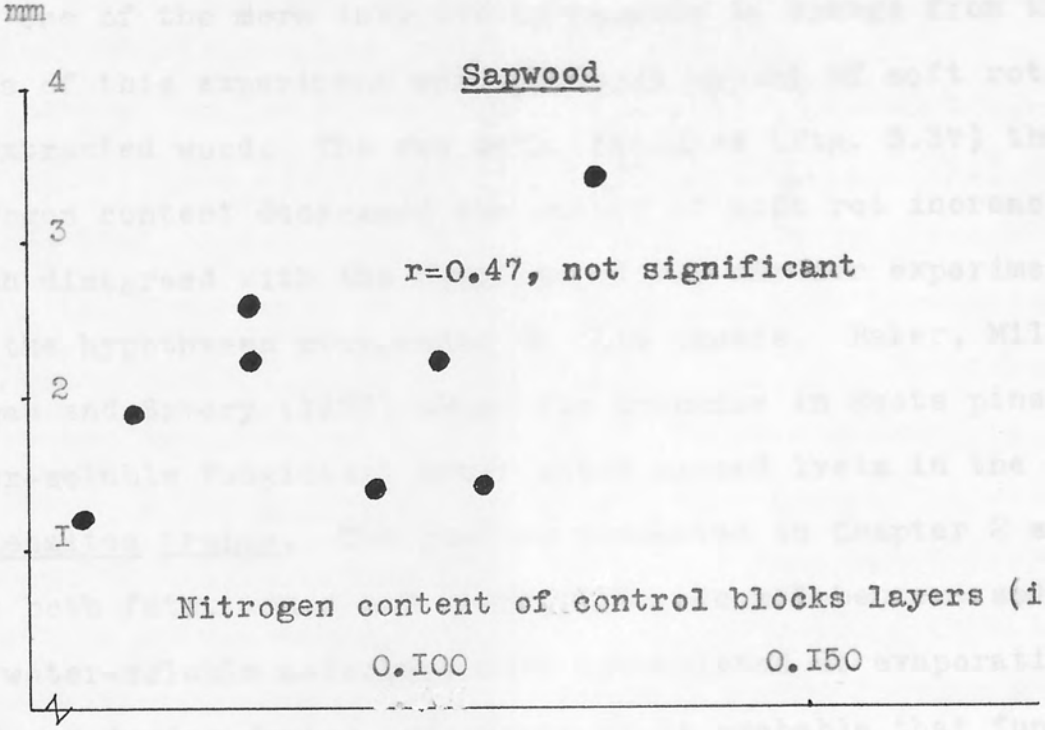
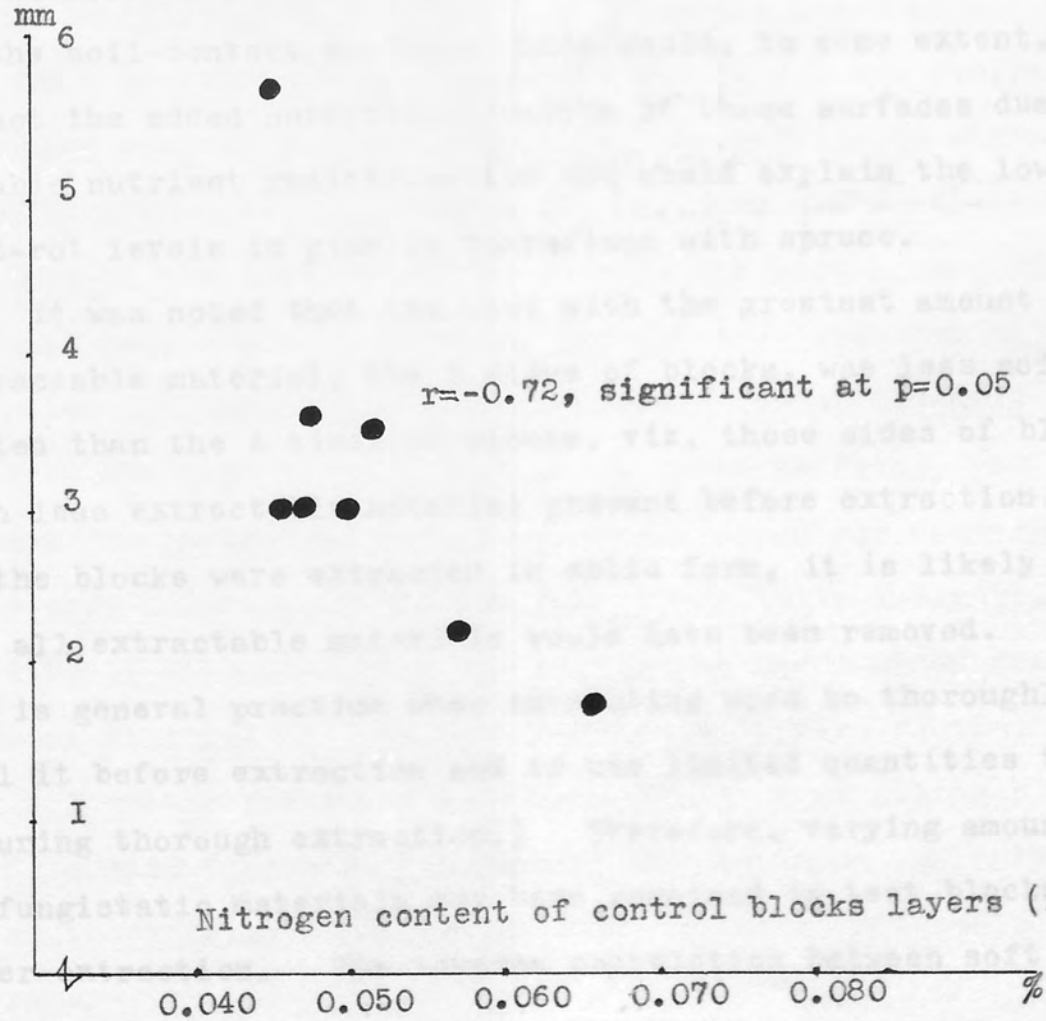


FIGURE 3.37 As above, inner sapwood



of soluble nutrients during drying of wood, and the subsequent location of soluble nutrients, is more important than the gross nitrogen content of the wood in determining decay.

One of the more interesting aspects to emerge from the results of this experiment was the large amount of soft rot found in extracted wood. The raw data indicated (Fig. 3.37) that, as nitrogen content decreased the amount of soft rot increased, which disagreed with the findings of the earlier experiments and the hypotheses propounded in this thesis. Baker, Miller, Morgan and Savory (1973) noted the presence in Scots pine of a water-soluble fungicidal agent which caused lysis in the spores of Lenzites trabea. The results presented in Chapter 2 showed that both fats, waxes and resins (the alcohol-benzene solubles) and water-soluble materials were accumulated at evaporation surfaces during drying, and hence it is probable that fungistatic soluble materials present in pine were also concentrated at the soil-contact surfaces. This would, to some extent, counteract the added nutritional status of those surfaces due to soluble nutrient redistribution and would explain the lower soft-rot levels in pine in comparison with spruce.

It was noted that the wood with the greatest amount of extractable material, the B sides of blocks, was less soft rotted than the A sides of blocks, viz. those sides of blocks with less extractable material present before extraction. As the blocks were extracted in solid form, it is likely that not all extractable materials would have been removed. (It is general practice when extracting wood to thoroughly mill it before extraction and to use limited quantities thus ensuring thorough extraction.) Therefore, varying amounts of fungistatic materials may have remained in test blocks after extraction. The inverse correlation between soft rot

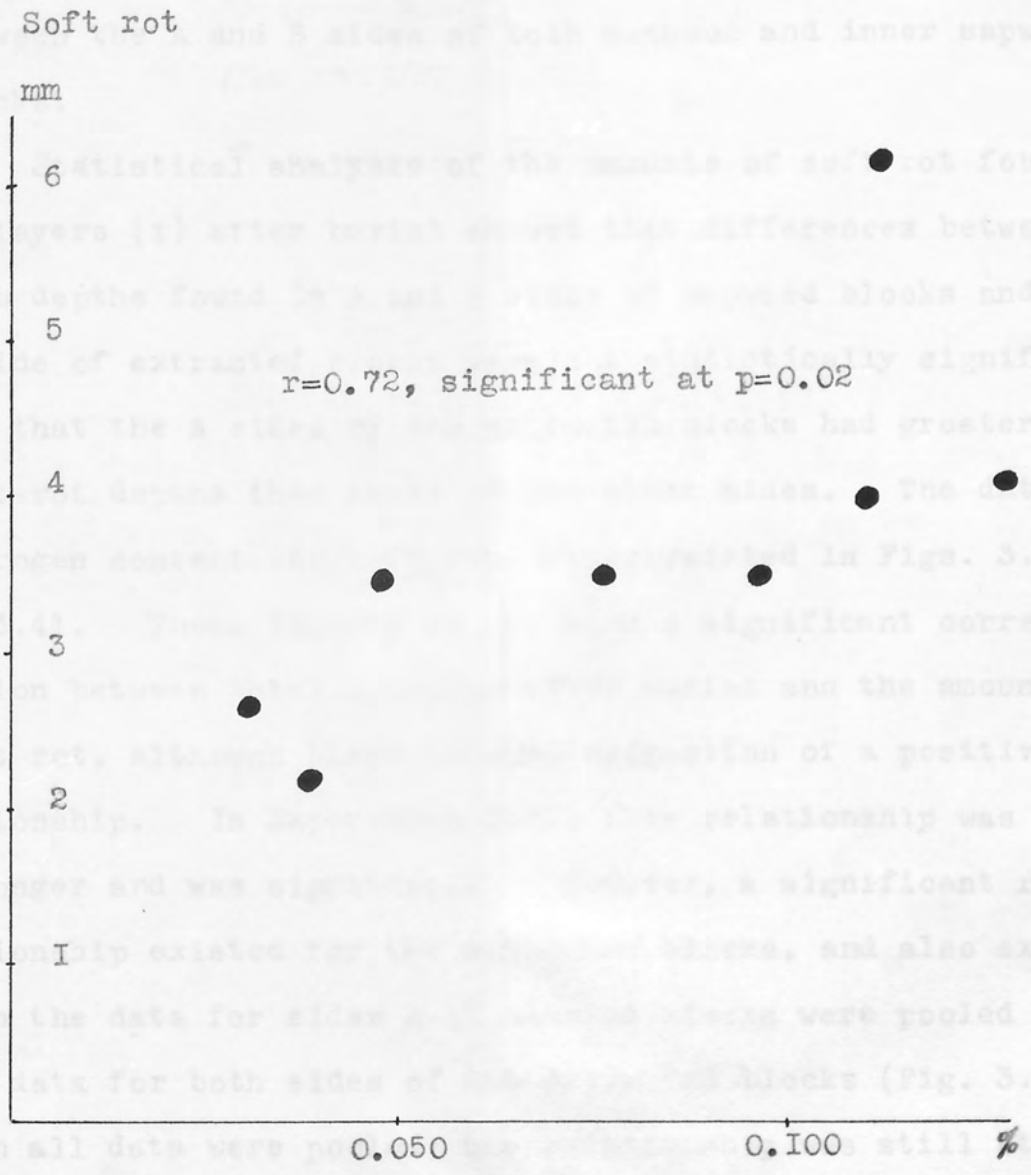
and control nitrogen content may therefore reflect the thoroughness of the extraction procedure, wood with the higher nitrogen contents containing greater amounts of inhibiting substances.

The effect of extraction on soft-rot production disagreed with the finding of Peterson and Cowling (1963) who showed that when Yellow pine was extracted with a range of solvents, there were no effects on weight loss production by Polyporus versicolor and Polyporus anceps. However, these workers also showed that alcohol-benzene extraction of Sitka spruce increased the weight loss production by P. versicolor and Teyegega (1975) has shown that respiration levels in extracted pine are considerably greater than those in unextracted pine. It has also been shown (Rudman and Da Costa, 1959) that extracted sapwood of Tectona grandis is more susceptible to decay than unextracted sapwood.

The inverse correlation between soft rot and control nitrogen content might, however, not be related to either fungistatic materials or nitrogen content. The effect of extraction may also have been to make the wood more porous and may have minutely affected the physical and chemical structure. Extraction may therefore have sufficiently altered the wood to make it more amenable to decay, and hence to make comparison of data between extracted and unextracted wood particularly unreliable. However, it is interesting to note that, although there was an inverse correlation between control nitrogen content and decay in extracted blocks, there was a direct correlation between the amount of nitrogen translocated from soil into wood, and the amount of soft rot found there (Fig. 3.38.) which confirmed a relationship of nitrogen with decay, even for extracted material.

FIGURE 3.38 Correlation of soft rot with increase in nitrogen content as a result of soil burial (layer i)

Inner sapwood



The results for Experiment 3.3. showed that the nitrogen content of wood blocks after burial correlated with the amount of soft rot produced. A statistical analysis of nitrogen contents after burial of layers (i) of blocks used in Experiment 3.4. showed that there were no significant differences between the sapwood and inner sapwood blocks or between the A and B sides of both sapwood and inner sapwood blocks.

Statistical analyses of the amounts of soft rot found in layers (i) after burial showed that differences between mean depths found in A and B sides of sapwood blocks and the A side of extracted blocks were not statistically significant but that the A sides of the extracted blocks had greater soft-rot depths than those of the other sides. The data for nitrogen content and soft rot are correlated in Figs. 3.39. to 3.41. These figures do not show a significant correlation between total nitrogen after burial and the amount of soft rot, although there is some suggestion of a positive relationship. In Experiment 3.3., this relationship was stronger and was significant. However, a significant relationship existed for the extracted blocks, and also existed when the data for sides A of sapwood blocks were pooled with the data for both sides of the extracted blocks (Fig. 3.41.). When all data were pooled, the relationship was still statistically significant although the correlation co-efficient was rather weak ($r = 0.47$, significant at $p = 0.05$).

It was therefore apparent that some relationship existed between the total nitrogen of wood after burial and the amounts of soft rot present thus corroborating the evidence of Experiment 3.3. However, it was considered that the locations to which nutrients were redistributed considerably

FIGURE 3.39 Correlation of soft rot with nitrogen content after burial (layers i)

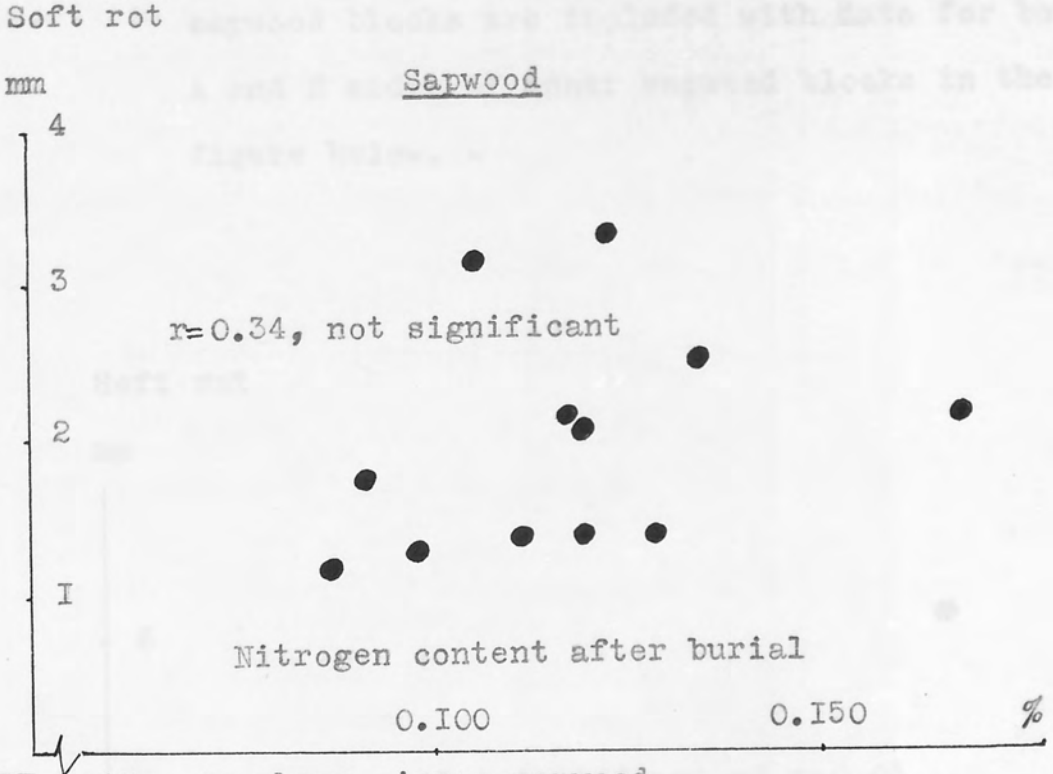


FIGURE 3.40 As above, inner sapwood

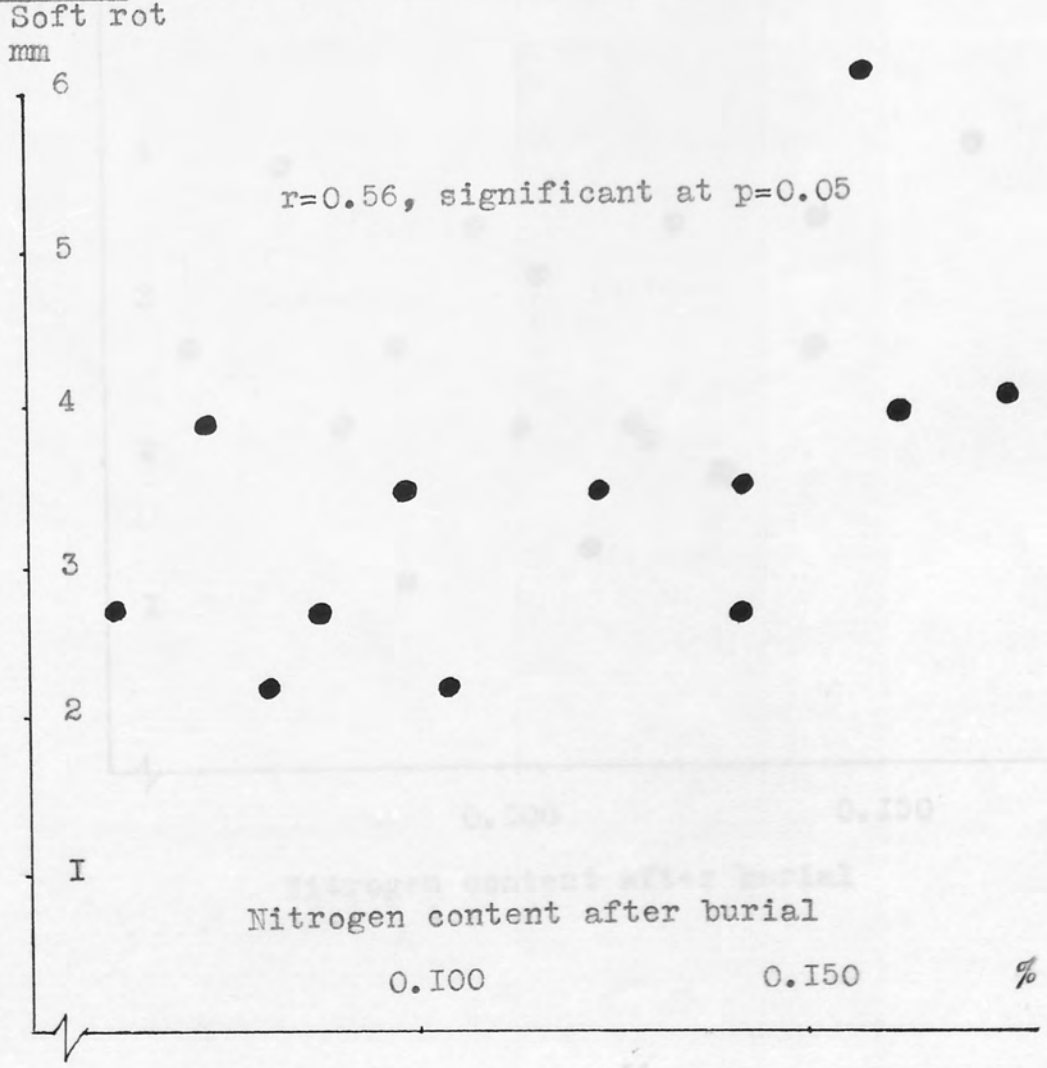
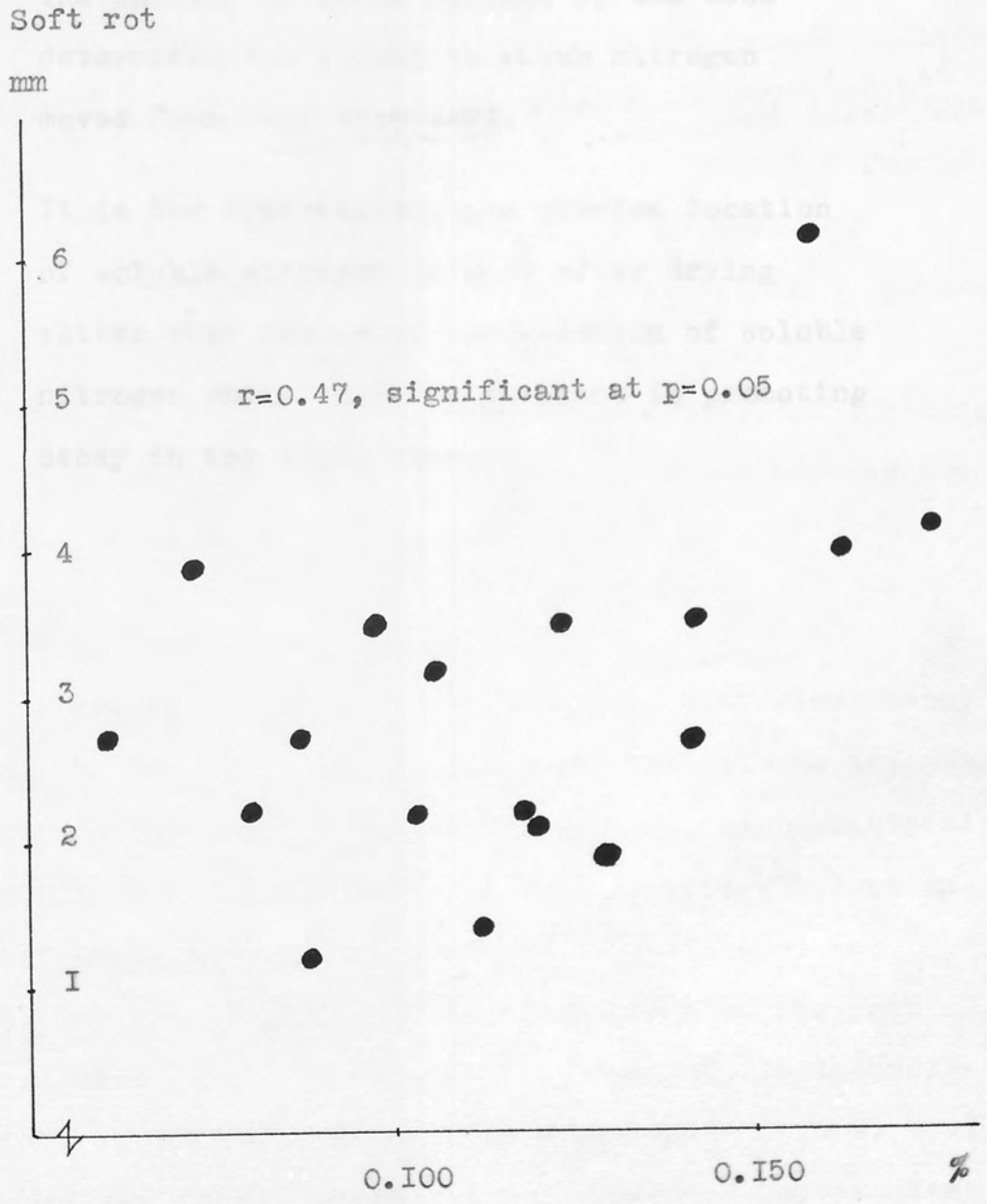


FIGURE 3.4I Correlation of soft rot with nitrogen content after burial (layers i). Data for A sides of sapwood blocks are included with data for both A and B sides of inner sapwood blocks in the figure below.



influenced the pattern of decay in the sapwood blocks resulting in low correlation co-efficients. This aspect has been discussed under the heading Hypothesis 2.

It therefore appeared that the results of Experiment 3.4. confirmed the results of Experiments 3.2. and 3.3. and that the following additional conclusions could be drawn:

1. The initial nitrogen content of the wood determined the extent to which nitrogen moved from soil into wood.

2. It is the distribution and precise location of soluble nitrogen in wood after drying rather than the gross accumulation of soluble nitrogen which is more important in promoting decay in the short term.

3.3. DISCUSSION

The purpose of the soil-burial experiments was to determine the relationship of nitrogen movement from soil into wood with soft-rot production, and, as a consequence, to the discovery of nitrogen migration described in Chapter 2, to determine the biological effects of redistributed soluble nutrients to soil-contact surfaces. The results of these experiments have been individually discussed, and it was decided that the general relationship of the soil-burial experiments, and the results presented in Chapter 2 should be collectively discussed in Chapter 4 "Discussion".

Several aspects of the soil-burial experiments, particularly experimental design, and the techniques used to measure colonisation and decay and the results for specific parameters, merit separate discussion. These aspects are discussed below.

Test Block Design.

When examining parameters relating to biological decay of wood, it is common to use different test blocks for each parameter being examined. For example, it is impractical to test for both weight loss and bending strength loss on one test block because the physical dimensions of the blocks, and the incubation conditions required for each test are different. When possible, however, it is desirable to test more than one parameter on an individual test block because physical and chemical variation between test blocks is then not a complicating factor when correlating the parameters after testing, e.g. moisture content may be easily correlated with decay when both moisture content and

weight loss are both determined on the same test piece.

Ecological studies of the decay process have particularly demanding requirements for test-specimen design because the complex of interacting biological, physical and nutritional factors require so many different analyses to clarify the decay phenomenon. Some recent ecological studies have not confined themselves purely to the isolation, morphological or decay-rate studies which been common in the past, and have tried to use more than one feature to qualify the phenomenon of decay. Good, Basham and Kadzielawa (1968) used measurements of respiration, pH and moisture to qualify the range and stages of decay of Acer saccharum by Fomes ignarius. By careful use of matching adjacent 2-in. discs, they were able to match and relate each of the parameters being examined.

Butcher (1968) used a similar technique to examine colonisation of wooden fence posts buried in soil. He removed three adjacent $\frac{1}{4}$-in. thick transverse sections from wooden fence posts and analysed each alternate disc for moisture, presence of organisms, and pH, at three levels in the posts: 10in. below ground level, ground level, and 4in. above ground level to build up a profile of microbial activity.

A similar principle was adopted for the experiments described in this Chapter except that the sample dimensions were adapted for burial in the laboratory, and conversion after burial was on a micro or on a semi-micro scale. Five parameters (four for spruce) were examined in each test block viz. moisture content, total nitrogen content, presence of soft rot, respiration, and pH. The design of the test blocks was such that changes in these parameters could

be examined at increasing depths from the soil-contact surfaces without physical variation in the sample material unduly influencing the results obtained.

The use of matching control blocks to determine the state of the wood before burial was a useful guide to detecting the spectrum of changes which occurred in the wood during soil contact. The results of these experiments showed that if test material was carefully selected and designed, even the small samples used for individual analyses did not detract from the reproduceability of results, or the facility with which raw data could be correlated. Difficulty in interpreting results from wood analyses is frequently attributed to the inherent variability of wood. Due to the design of test blocks this was not a complicating factor when relating the parameters examined.

Moisture Content.

Wood will not decay unless it is moist and basidiomycetes can decay wood at moisture contents in excess of 18% (Cartwright and Findlay, 1958). Soft rot is frequently produced by microfungi in moisture conditions unconducive to basidiomycete colonisation, e.g. wet conditions such as cooling tower timbers, undrained soil etc. (Savory 1954b, Duncan, 1960b), however it may also be produced over a range of moisture conditions (Savory and Bravery 1971) which may also be suitable for basidiomycete decay.

It is common to maintain soil at its water-holding capacity (W.H.C.) in soil-burial tests. This, in turn, maintains an adequate moisture level in wood for decay to occur. The lower moisture limit for decay production by soft-rot fungi is 30-35% and its upper limit is about 60-80% for pine

sapwood (Becker and Kaune, 1966). This lower limit agrees with that produced for Chaetomium globosum and a Paecilomyces sp. when growing on Fagus sylvatica by Liese and Ammer (1964). These latter workers also showed an increasing decay capacity with increased moisture content. Carey and Savory (1973) showed that soils from 10 institutions when wetted to their moisture-holding capacities, produced final moisture contents in Scots pine blocks after burial ranging from 7% to 106%, with the moistures for eight of these soils ranging from 34% to 97% moisture. The decay range measured as percentage weight loss corresponding to the extremes of the moisture range was 2.9% and 19.8%, while that for the remaining eight soil samples was 0.7% to 27.6%.

The soils described in 3.2., in which wood blocks were buried, was maintained at its W.H.C. of approximately 32% for the duration of the burial period. This moisture content agrees with a previously-determined value for P.R.L. soil (Savory and Bravery, 1970). The wood blocks, buried in an oven-dry condition, all increased in moisture content as a result of burial. The distribution of moisture in the buried blocks, shown in the individual experiments, was in all cases greatly in excess of the minimum required for soft-rot production (30%) in layers (i) and even in the inner layers was generally in excess of this minimal requirement.

Previous reports of a correlation between moisture content and soft-rot were not borne out in any of the soil burial experiments, and it was considered that, whereas this relationship might exist for individual micro-organisms, it might not necessarily exist for microbial populations. It

was considered, however, that a further explanation might exist for this lack of correlation. Corbett (1965) showed that penetration of a number of fungal species through both birch and Scots pine was most rapid when the transverse face was exposed to colonisation, was less rapid when the tangential face was exposed and was slowest when the radial face was exposed. It has also been shown (Okigbo, Greaves, and Levy, 1966) that the anatomical orientation of wood to organisms colonising from soil may influence the extent of decay produced by them. The test blocks used in 3.2. had only radial faces exposed, consequently, although moisture was seen to move from soil into wood, the easiest pathways for fungal colonisation (transverse and tangential faces) were sealed with epoxy resin thus blocking the easiest access points for fungi. This may have contributed to the lack of correlation observed between moisture and soft rot.

Nitrogen Content.

It was clear from the results that soluble nitrogen had not moved from soil into wood and that microbial translocation of nitrogen and loss of carbohydrates due to decay were the factors which contributed to increases in wood nitrogen content as a result of burial. A possible explanation for lack of soluble nitrogen movement from soil into wood, other than that only small quantities are present there exists. Petty (1974) has suggested that moisture movement in wood occurs in a vapour phase. If this is the case, it is unlikely that transfer of solutes in water within wood and consequently soluble nitrogen movement from soil could

occur. It was also noted that the nitrogen contents of layers (v) of wood blocks increased as a result of burial and this was attributed earlier to redistribution of already redistributed nutrients back into the wood when wetted. If Petty's theory is correct, this explanation is probably false.

The evidence of migration of soluble nitrogen and other soluble materials to evaporation surfaces of wood during drying conflicts with Petty's theory. It was therefore considered that water movement from the full cells in green wood during drying must occur, at least in part, in the liquid phase, consequently soluble materials viz. amino acids and carbohydrates are trasported to evaporation surfaces. It is presumed, however, that moisture movement from wood in the final stages of drying from the green state may occur in the vapour phase, as water in wood-cell capillaries (which may be as small as 10A°, Bosshard,1969) is more probably in the form of a saturated vapour (dependent on concentration, temperature and pressure). All soluble materials would therefore not be transported to evaporation surfaces of wood during drying and some would be left behind due to movement of the remaining liquid in the vapour phase. This theory would plausibly explain the presence of soluble wood nitrogen in the inner layers of control blocks.

Similarly, as wood in soil-contact is moistening up to fibre-saturation point, it may be presumed that much of the moisture movement within wood will be in a vapour phase and soluble nitrogen in soil could not move within the wood. Where wood-moisture conditions were in excess of fibre saturation point, "free" water, i.e. that present in cell

lumina and not in cell-wall capillaries, would be present in wood and it is theoretically feasible that, under these conditions, soluble nitrogen from soil is transferred within wood in water in the liquid phase.

Soft Rot.

The method used to assess decay was a measurement of the depth to which soft-rot cavities were formed from the soil contact surface. This was a subjective method. However, as the nitrogen content after burial is also a measure of decay, and it correlated with depth of soft rot, it was considered that microscopic examination was adequate for the purposes of this initial investigation.

Assessment of colonisation was made more difficult because soft-rot development was in two stages, a band of intense rot in which the cells had nearly totally lost birefringence (as described by Courtois, 1963) located at and parallel with the soil-contact face, and a second band of less intense rot immediately adjacent to it. Measurement of soft rot was made to the inner limit of this second band.

The soft-rot cavities in the first band conformed generally to those described by Corbett (1965) as type 1 (cavities within the S_2 layer, formed after T branching), while those in the second band consisted of a range of decay forms including both type 1 and Corbett's type 2 (cavities formed by erosion from the lumen) and a range of indeterminate or apparently indeterminate forms similar to those described by Courtois (1963) and also to those produced in Betula sp. and described by Lundström (1972). The width of these bands varied with the amount of redistributed nutrients present.

The cavities in spruce were located in both the early and latewood of the growth rings. In pine, however, soft rot cavities were largely confined to the earlywood zone, and to the zone of transition between early and latewood. Nilsson (1973) has made a similar observation in which certain fungal species only produced cavities in earlywood, while others produced cavities exclusively in the latewood of softwoods. In further studies with pine and spruce wood, Nilsson (1974a) showed that only soft-rot cavities of type 1 were produced by Humicola alopallonella but that these were produced in both early and latewood of the two woods tested. With reference to the experiments carried out in this thesis, the different soft-rot patterns may therefore reflect either one or more of the following:

(i) a relative difference in susceptibility of pine and spruce to soft rot;

(ii) different microbial populations in the soils used;

(iii) different dominant species in those populations;

(iv) the influence of different nutrient statuses at the soil-contact surfaces of the woods.

It has been suggested that softwoods are more resistant to soft rot than are hardwoods, and that generally softwoods less typically demonstrate type 2 cavities than hardwoods (Levy, 1967; Greaves and Levy, 1968; Liese, 1970; Nilsson, 1973, 1974b). In the experiments described in this thesis, both types were found in both woods (both of which are soft woods). This is consistent with the data of Nilsson (1974c), who found cavities of both types in birch wood, pine sulphate

pulp, spruce holocellulose, and fibres of cotton, jute, sisal and kapok.

It was not within the scope of this project to investigate the type and form of soft-rot cavity formation in wood in soil contact. However, in relation to the results and the literature briefly discussed above, it is apparent that the formation of type 2 cavities and the localisation of cavity formation to the earlier parts of the annual rings in pine would merit more detailed investigation.

Respiration

The respiration levels in wood blocks followed the general trends of the other parameters examined after burial, i.e. highest in layers in contact with the soil with lower levels in the inner layers. In contrast with the other parameters, there seemed to be considerable variation both within and between the respiration levels in the various layers of blocks. While the activity of soft-rot organisms may have contributed to the high respiration rate in the soil contact layer, soft-rot production did not extend below this layer in the pine experiments or in the inner sapwood in spruce. Passive colonisation extended beyond layer (i) in all experiments but this aspect was not examined in detail. This passive colonisation without decay would, however, explain the lower respiration levels in the inner layers. It is difficult to explain the increase of respiration with depth from the soil-contact surface in the pine experiments. One explanation may be that, with movement of moisture from soil into the wood, nutrients redistributed to soil-contact surfaces move back into the wood with the advancing moisture front. Microfungal utilisation of these nutrients in the

inner layers may have contributed to the higher respiration levels observed. Another explanation is that bacteria might have been moved in with the advancing moisture front on burial and the presence of these in the inner layers might also have contributed to enhanced respiration level. Microscopic examination of blocks did not reveal a significant bacterial presence although these organisms might have been lost from sections during cutting, dehydration, and mounting. This is in keeping with the observation of Good, Basham and Kadzielawa (1968), who found that carbon dioxide output could be high in regions apparently without visible decay.

The results considered as a whole are consistent with the literature, which suggests that oxygen uptake and carbon dioxide production are useful methods to measure microbial colonisation and decay of wood. Klingstrom (1965) found that carbon dioxide production could be used to project weight-loss figures, but that carbon dioxide evolution was subject to large variation with reference to temperature, moisture content, and mixed microbial populations. It has been suggested (Smith, 1969) that, whereas respiration measured by the Warburg respirometer is a well-tried and rapid method, it may be limited in application by precluding measurement of the influence of atmospheric composition on microfungal activity (because respiration occurs in a closed system). Hintikka and Laine (1970) have agreed with Smith op. cit. and have suggested that respiration measurements using the Warburg apparatus may give "rather variable" results due to concentrations of carbon dioxide within wood blocks. Good and Darrah (1967) observed that stained wood had about double the oxygen consumption of unstained wood

and that there was a direct linear relationship between respiration and moisture content.

It was therefore considered that, while the respiration measurements corroborated the other findings in the soil burial experiments, the differences in mean readings for layers observed could have been related either to variation in the extent of colonisation, or to inherent limitations in the use of the technique as a sensitive measure of fungal colonisation, or combinations of both.

pH

It has been shown that the activity of wood-decay enzymes may differ with changes in pH, e.g. a cellulase of Aspergillus niger may produce a weight loss in cellulase of 33.2% when tested at pH 4.5, and 14.6% when tested at pH 6.5 (Walseth, 1952). The optimal pH for basidiomycete growth in wood tends to be in the acid regions, and recent information (Savory, 1975) has shown that basidiomycetes produce considerable decreases in the pH of wood. It has been suggested (Duncan, 1960b; Butcher, 1968; Sharp and Eggins, 1970b) that many microfungi colonising wood could grow under alkaline conditions and be actively cellulolytic. It has also been shown that pH may influence the behaviour of preservatives; the activity of pentachlorophenol for instance decreased by 100 times as pH was changed from 5 to 8 (Wessels and Adema, 1968). Previous studies (Grey, 1959a, 1959b; Farmer, 1959; Sandermann and Rothkamm, 1959) have concentrated on producing data for pH of wood in an undecayed condition, and few studies other than those of Butcher (1968) and Good, Basham and Kadzielawa (1968) have examined changes in pH of decaying wood and wood in soil contact.

The results for pine control blocks were broadly in agreement with published data for undecayed wood (Grey, 1959, op. cit.). The pH of buried blocks, however, had increased as a result of burial and tended towards the soil pH (neutrality); pH levels decreased with distance from the soil-contact surface. Butcher (1968) showed that the pH of stakes buried in soil decreased as a result of burial. However, this decrease was not related to soil pH, nor was the pH of the soil given. The data for pH change in wood buried in soil therefore disagreed with Butcher's data, although the apparent differences might be resolved if the pH of the soil Butcher used was lower than the pH of the wood. Butcher, however, also showed that basidiomycete colonisation had occurred in the stakes examined by him, and this might have contributed to the depressed pH values.

It therefore appeared that the result of soil burial with PRL soil was to increase the pH of Scots pine to soil pH levels. This would have resulted in making the layers in soil contact less amenable to basidiomycete colonisation and highly suitable for microfungal and bacterial growth (many of the microfungi isolated by Butcher grew optimally between pH 5 and pH 7). Similarly, as the pH levels in the inner layers had suffered less change than the outer layers, these would have been less amenable to micro-fungal colonisation and were suitable for basidiomycete colonisation. It has been observed (Banerjee and Levy, 1971) that maximal numbers of micro-fungal species colonise the outer 5mm of wooden fence posts (corresponding with those parts of test blocks with raised pH levels), and that basidiomycetes are not found within distances of approximately 25mm from soil-contact surfaces (corresponding with those

parts of test blocks with lower pH levels). Apart from nutritional factors, and dependent on soil pH levels, the phenomenon of organism succession and decay in wood might also be related to wood-soil pH interrelationships. This phenomenon, as with the other parameters examined, would also merit further more detailed investigation.

CHAPTER IV

GENERAL DISCUSSION

4. GENERAL DISCUSSION

The work described in this thesis was undertaken initially on the premise, derived from the literature, that the gross distribution of nitrogen in wood was fully understood. This premise was incorrect, and it was shown conclusively in Chapter 2 that the distribution of nitrogen in green wood differed from that in the dry material. Soluble nitrogen was shown to be present in green pine and spruce at levels of approximately 50% of the nitrogen of extracted outer sapwood or one third of the total nitrogen content. Most previous nitrogen determinations had been undertaken on dried material, and it was not apparent from the literature that soluble nitrogen was present in such concentrations in wood.

This work has shown that the distribution of nitrogen in dried wood differs from that in green wood insofar as soluble nitrogen, along with other soluble materials, including carbohydrates, migrate to evaporation surfaces of wood during drying where they accumulate. This phenomenon was observed not only in wood dried at this laboratory, but was also observed in material dried at other institutions. The phenomenon occurred in the hard and softwood species examined, namely, beech, pine and spruce.

The hypotheses propounded in Chapter 1 that nitrogen moves into wood from soil within which it is buried, and that this movement was related to soft-rot production, were examined in Chapter 3. The results confirmed that nitrogen moved into wood from soil, and also showed that the movement was primarily in a microbial form (Experiments

3.2. and 3.3.). Movement of soluble nitrogen from soil into wood was not detected. There were no correlations between increases in nitrogen content as a result of burial and the amounts of soft rot found in the wood. It was shown, however, that, if wood was depleted of soluble materials by extraction, there was a direct correlation between the increase in nitrogen content as a result of burial and the amount of soft rot found (Experiment 3.4.).

A major conclusion drawn from the results of Chapter 3 was the relationship between redistributed soluble nutrients concentrated at soil-contact surfaces and the amounts of soft rot produced there. Wood with large amounts of redistributed nitrogen were much more soft rotted than wood surfaces without this redistribution. Considering all the soil-contact faces which were not chemically extracted and in which redistributed soluble nitrogen was not accumulated (viz. the A and B sides of inner sapwood blocks used in Expt. 3.2. and the A sides only of both sapwood and inner sapwood blocks used in Expt. 3.3.), it was found that, although the mean soft-rot depths found in these faces after burial were different, the differences were small and were not statistically significant ($F = 0.6$). The original nitrogen content of these faces differed appreciably, ranging from 0.05% to 0.08%. This suggests that there is no effect of nitrogen content at the time of burial on the amount of soft rot production. However, in these faces there was little soluble nitrogen, and the nitrogen content was largely comprised of structural nitrogen.

It is therefore clear that it is only variation in

the soluble nitrogen component which influences soft-rot production and thus, variations in total nitrogen content may not be a guide to probable soft-rot attack unless the proportions of variations due to variations in the soluble nitrogen component are known. Many hardwood species have higher nitrogen contents than commercial softwoods (Merrill and Cowling, 1966), and are more susceptible than softwoods to soft-rot attack. Hardwoods were not subject to detailed investigation in this thesis, and it is therefore considered that the nitrogen status of commercial hardwoods with reference to the distribution of soluble nitrogen before and after drying would merit further more detailed investigation.

In contrast with the hypothesis propounded in the Introduction (Chapter 1), the presence of wood-cell contents did not promote nitrogen translocation from soil, rather there was an inverse relationship between increases in nitrogen content during burial and the initial nutrient status of the wood. Wood with low levels of nitrogen when buried had greater increases in nitrogen content (and was less decayed) than wood with high nitrogen levels. This unexpected observation was explicable by the nutrient status of the wood surfaces exposed to soil contact as follows.

It was shown from the literature that cellulolytic microfungi require more nitrogen than is found in wood to produce soft-rot cavities, and it was considered that wood containing largely only structural nitrogen viz. the sides of blocks from which evaporation surfaces had been removed, would be nutritionally deficient to soft-rot fungi, i.e. would require extra before they could be decayed. It is

suggested that such wood is passively colonised, thus increasing its nitrogen content before limited amounts of soft rot, perhaps by secondary-colonising organisms, are produced. The results of the soil-burial experiment are consistent with this suggestion.

The sides of pine blocks containing redistributed soluble nutrients were seen to provide sufficient soluble nitrogen to support rapid soft rot production without the requirement of nitrogen translocation from soil. These sides did not increase greatly in nitrogen content, consequently it may be deduced that the colonising organisms largely derive their nitrogen nutrition from within the wood.

The accumulated sugars may have contributed to this situation. Garrett (1963) has shown that simple sugars stimulate rapid colonisation of cellulosic substrates. The alteration of the biological status of soil produced by contact with sugar-rich surface wood may have stimulated germination of dormant spores of "sugar fungi" which would have colonised the wood. Colonisation by such species would account for the small amount of nitrogen translocated into the surfaces of wood blocks containing redistributed soluble nutrients. The complex of sugars and nitrogen would have initially promoted rapid colonisation (Darbyshire, Wade and Marshall, 1969) but cellulolytic enzyme production would have been inhibited by the presence of soluble carbohydrates (Bravery, 1968; Nilsson, 1974d). On depletion of cell contents, however, it is quite probable that soft-rot cavities were produced by these organisms, as secondary or passive colonisation from soil by further organism groupings, did not occur as indicated by total nitrogen content after burial.

It would therefore seem that a number of microbial groupings may have interacted with wood dependent on whether it contained redistributed soluble nutrients or not. This is in agreement with the suggestion of Levy (1969) and Banerjee and Levy (1971) that a nutritional basis may exist for the successions of organisms colonising wood. It is considered that the wood blocks used in the soil-burial experiments may act as a model for the nutritional state of wood posts in soil contact to which many succession studies relate. Wooden fence posts are normally dried before insertion into soil and consist largely of sapwood. Consequently, soluble nutrients contained by them will have been redistributed to the evaporation surfaces. It is suggested that this redistribution will have created a nutrient gradient with greatest accumulation at the soil-contact surface, and with lesser amounts available with creasing distance from that surface.

The biological consequence of the nutrient gradient is to produce a matching organism gradient from the outside of the wood to the interior. The wood, because of the presence of soluble nutrients, is non-selective to micro-organisms in the soil-contact area, but with decreasing soluble nutrient status becomes more selective and ultimately, with only traces of soluble nutrients present, is inhibitory to organisms other than basidiomycetes.

It was hoped, when initially planning the work described in this thesis, that isolation of micro-organisms colonising wood would be undertaken in conjunction with the measurement of nitrogen movement into wood. However, it was decided that, as the time available was limited, it was

more important to clarify the phenomenon of nitrogen redistribution and its biological effects in relation to soft-rot production. It was obvious, however, that biological effects were not confined to soft-rot production, but also influenced the successions of organisms colonising wood. The phenomenon of redistribution of soluble nutrients, therefore, provides much further scope for ecological and isolation studies.

Few studies have been undertaken to follow nitrogen migration from soil into wood. However, it has been suggested (Levy, 1968) that soluble salts move into wood exposed to soil contact. No evidence was found in the work described in this thesis to support this suggestion although only nitrogen was measured; analyses for other soluble salts were not undertaken. Sharp and Millbank (1973) and Sharp (1974) have shown that a nitrogen-fixation activity occurs in wood in soil contact. Their studies showed that 0.029 n moles of N_2 could be fixed per hour, measured over a 24-hour period, in Scots pine veneers of a total weight of 2.4 g. If this data is transposed to μg of N_2 per gram of wood over a 14-week burial period, the total amount of nitrogen fixed is less than 1 μg/g.

Further studies (Levy, Millbank, Dwyer, and Baines, 1974) showed that after a 67-day burial period in soil, approximately 7000 n moles of ethylene were produced per g of wood per hour of incubation using the acetylene reduction method of assessing nitrogenase activity. Transposing these data to μg of nitrogen fixed per gram of wood, it would seem that potentially 65 μg of nitrogen per gram of wood could be fixed per hour in wood in soil contact.

These data are larger than those of Aho, Seidler, Evans and Raju (1974) who showed a nitrogen-fixation activity on the part of the majority of 1200 bacterial strains isolated from living white fir trees. These latter workers also showed that, in agar culture and using a micro-kjeldahl technique for analysis, up to 4 μ moles of N_2 were fixed in a 90-hour test period. This reading corresponds with a fixation rate of approximately 1 μg nitrogen per hour.

Levy et al. (1974) suggested that nitrogen fixation could be assumed to be taking place throughout the blocks of wood examined by them. However, the data obtained in this thesis did not suggest that significant changes in nitrogen content were taking place in the interior layers of the wood blocks, except perhaps in layers (v) of pine blocks. It may therefore be suggested that either (a) nitrogen fixation was generally not occurring in the inner layers of blocks buried at this laboratory, or (b) that the nitrogen fixing activity shown by Levy et. al., op. cit., and the other workers referred to, was not actually taking place in wood, but was a measure of the potential activity which could take place on the part of the bacterial component of the succession of organisms colonising wood.

This latter suggestion may well be the case as it has been shown that the nitrogen-fixation activity is essentially an anaerobic or micro aerophilic process (Yates, 1971; Postgate, 1971) whether the organisms are aerobic or anaerobic, and that the nitrogenase enzyme itself is oxygen labile (Evans and Russell, 1971). Consequently, although many organisms may be potentially nitrogen fixing

as determined by the acetylene reduction method, in practice unless the wood provides an anaerobic environment or micro-anaerobic environments, nitrogen fixation in wood is probably slight and may not supply the total nitrogen needs for cellulolytic organisms to decay wood.

It would therefore seem that the soluble nitrogen in wood may be the only nitrogen source easily available to the early colonisers of wood. Henningsson (1968) carried out growth studies on the component amino acids of soluble nitrogen in wood and found that these supported good growth of fungi. Further studies (Henningsson, Henningsson and Nilsson, 1973) showed that the nitrogen nutrition of fungi could greatly influence the type of wood-decay enzyme production. Henningsson et.al., op. cit. showed that a cellulolytic wild strain of Peniophora when grown on a diet of Asparagine as nitrogen source, was nearly entirely lignolytic, 50% of the lignin and only 2% of the cellulose in the test material being utilised. The influence of nitrogen on the cellulolytic activity of soft-rot fungi is well documented, but the range of forms and shapes of cavities have been attributed largely to the physical influence of lignin (Nilsson, 1974b). The influence of concentrations of soluble nitrogen at soil-contact surfaces on lignin decay has not yet been examined, and it may be that such concentrations not only influence the amount of soft rot produced but also influence the type of cavity formed. This matter, too, is considered to require further investigation.

Redistribution of soluble nutrients during drying of wood may also influence the behaviour of wood treated with

copper-chrome-arsenic solutions. During treatments of this type, the preservative in a water-soluble form is impregnated into the wood. The wood is then stored while still full of water to allow the preservative to "fix" and is then allowed to dry slowly. It is possible that this latter procedure will result in a depletion of the interior of the wood of soluble nutrients and a concentration of these nutrients at the evaporation surface when dried. This concentration of both carbohydrates and nitrogen may considerably influence the subsequent behaviour of the treated material when in service. Apart from the CCA possibly "complexing" with the soluble carbohydrates at the soil-contact face, concentrations of nutrients may enable colonising organisms to sacrificially colonise the wood (Levi, 1968) thus depleting it of CCA and enabling the outer layers to be decayed.

A major conclusion to be drawn from results of the experiments was, in contrast with the hypothesis in Chapter 1, that there is not a relationship between nitrogen movement from soil into wood and the amount of decay produced. This is not because there is no such relationship (there is, in extracted wood), but because the wood used contained nitrogen redistributed to soil-contact surfaces, which was not known when deriving this hypothesis. Wood in service will also contain redistributed soluble nutrients unless precautions are taken to avoid this occurring. Consequently, much of the indeterminate behaviour of micro-fungi colonising wood in respect of their decay activity may be related, not to differences between organisms, or the nutritional status of the wood as a whole, but rather to the nutritional status of the wood at the point of contact.

The work described in this thesis has established the existence of a nutritional status of wood which has not previously been recognised, and has related this status to decay incidence in two commercial softwoods. These findings provide a basis for the improved understanding of, and further investigation into, the fields of ecological succession, preservative performance and enzyme production of micro-organisms involved in the decay process.

APPENDICES

APPENDIX I. Spruce burial experiment (Expt. 3.2.)

Statistical analyses

Moisture content % after burial - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	14	55	18	4
Aii	14	40	13	3
Aiii	14	31	10	3
Bi	14	46	17	4
Bii	14	33	7	2
Biii	14	29	8	2

Moisture content % after burial - Inner sapwood

Ai	14	50	26	7
Aii	14	29	11	3
Aiii	14	24	10	3
Bi	14	51	37	10
Bii	14	27	10	3
Biii	14	24	8	2

Nitrogen content % after burial - Sapwood

Ai	14	0.152	0.043	0.012
Aii	14	0.067	0.024	0.007
Aiii	14	0.054	0.012	0.003
Bi	14	0.150	0.046	0.012
Bii	14	0.059	0.015	0.004
Biii	14	0.056	0.016	0.004

Appendix I - continued

Nitrogen content % after burial - Inner sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	14	0.118	0.034	0.009
Aii	14	0.050	0.009	0.002
Aiii	14	0.050	0.011	0.003
Bi	14	0.118	0.040	0.010
Bii	14	0.049	0.020	0.006
Biii	14	0.048	0.013	0.003

Control nitrogen content % - Sapwood

Ai	8	0.082	0.028	0.010
Aii	8	0.059	0.018	0.006
Aiii	8	0.057	0.012	0.004
Bi	8	0.070	0.013	0.005
Bii	8	0.057	0.014	0.005
Biii	8	0.056	0.018	0.006

Control nitrogen content % - Inner Sapwood

Ai	8	0.050	0.08	0.003
Aii	8	0.045	0.07	0.002
Aiii	8	0.047	0.07	0.002
Bi	8	0.055	0.022	0.008
Bii	8	0.050	0.010	0.004
Biii	8	0.048	0.010	0.004

Appendix I - contd.

Soft rot in mm - Spruce sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	13	4.1	1.3	0.4
Aii	14	1.2	2.1	0.6
Aiii	14	1.1	2.1	0.6
Bi	13	4.1	1.1	0.3
Bii	13	1.5	2.3	0.6
Biii	14	1.1	2.1	0.6

Soft rot in mm - Spruce inner sapwood

Ai	14	1.4	1.0	0.3
Aii	14	-	-	-
Aiii	14	-	-	-
Bi	14	1.7	1.0	0.3
Bii	14	-	-	-
Biii	14	-	-	-

Respiration in sapwood (μl O_2/hr/cc of wood)

Ai	10	11.2	11.1	3.5
Aii	10	8.5	10.4	3.3
Aiii	10	8.3	9.2	2.9
Bi	10	16.4	13.2	4.2
Bii	10	9.0	11.8	3.7
Biii	10	7.0	7.3	2.3

Appendix I - contd.

Respiration in inner sapwood (ul O_2/hr/cc of wood)

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	14	2.4	4.0	1.1
Aii	14	2.5	3.7	1.0
Aiii	14	3.2	3.7	1.0
Bi	14	3.3	4.8	1.3
Bii	14	2.3	2.7	0.8
Biii	14	3.9	4.2	1.1

APPENDIX II. Pine burial experiment (Expt. 3.3.)

Statistical analyses

Moisture content % after burial - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	85	16	6
Aii	6	45	7	3
Aiii	6	33	9	4
Aiv	6	36	6	3
Av	6	35	10	4
Bi	6	74	15	6
Bii	6	41	13	5
Biii	6	41	8	3
Biv	6	39	14	6
Bv	6	37	5	2

Moisture content % after burial - Inner sapwood

Ai	5	61	12	5
Aii	5	33	8	4
Aiii	5	29	6	2
Aiv	5	30	10	4
Av	5	29	12	5
Bi	5	62	8	3
Bii	5	37	17	7
Biii	5	29	12	5
Biv	5	30	11	5
Bv	5	27	11	5

Appendix II. - continued

Nitrogen content % after burial - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	0.096	0.013	0.006
Aii	6	0.057	0.006	0.002
Aiii	6	0.053	0.010	0.004
Av	6	0.062	0.005	0.002
Bi	6	0.142	0.025	0.010
Bii	6	0.066	0.013	0.005
Biii	6	0.061	0.014	0.004
Bv	6	0.063	0.013	0.005

Nitrogen content % after burial - Inner sapwood

Ai	5	0.095	0.012	0.005
Aii	5	0.047	0.011	0.005
Aiii	5	0.045	0.008	0.004
Av	5	0.042	0.011	0.005
Bi	4	0.138	0.033	0.016
Bii	4	0.058	0.009	0.004
Biii	4	0.050	0.004	0.002
Bv	4	0.050	0.005	0.003

Appendix II. - contd.

Control nitrogen content % - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	0.071	0.013	0.005
Aii	6	0.049	0.008	0.003
Aiii	6	0.055	0.008	0.003
Av	6	0.050	0.009	0.004
Bi	6	0.120	0.011	0.005
Bii	5	0.049	0.007	0.003
Biii	6	0.055	0.003	0.001
Bv	6	0.049	0.006	0.003

Control nitrogen content % - Inner sapwood

Ai	5	0.052	0.004	0.002
Aii	4	0.044	0.010	0.005
Aiii	5	0.043	0.008	0.004
Av	5	0.044	0.009	0.004
Bi	5	0.079	0.017	0.007
Bii	4	0.045	0.004	0.002
Biii	5	0.047	0.003	0.001
Bv	5	0.040	0.003	0.001

Appendix II. - contd.

Soft rot in mm - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	1.5	0.2	0.1
Aii	6	-	-	-
Aiii	6	-	-	-
Aiv	6	-	-	-
Av	6	-	-	-
Bi	6	3.4	1.4	0.5
Bii	6	-	-	-
Biii	6	-	-	-
Biv	6	-	-	-
Bv	6	-	-	-

Soft rot in mm - Inner sapwood

Ai	5	1.2	0.7	0.3
Aii	5	-	-	-
Aiii	5	-	-	-
Aiv	5	-	-	-
Av	5	-	-	-
Bi	5	2.5	1.2	0.5
Bii	5	-	-	-
Biii	5	-	-	-
Biv	5	-	-	-
Bv	5	-	-	-

Appendix II. - contd.

Respiration in sapwood (μl O_2/hr/cc of wood)

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	26.5	4.7	1.9
Aii	5	8.7	3.4	1.5
Aiii	6	7.6	5.4	2.2
Aiv	6	6.4	4.3	1.7
Av	4	8.0	1.3	0.7
Bi	6	35.3	10.8	4.4
Bii	6	14.3	5.7	2.3
Biii	6	9.2	7.7	3.2
Biv	6	12.0	5.8	2.4
Bv	6	15.5	8.1	3.3

Respiration in inner sapwood (μl O_2/hr/cc of wood)

Ai	5	25.0	4.9	2.2
Aii	5	8.7	5.4	2.4
Aiii	5	3.8	5.4	2.4
Aiv	5	5.1	6.5	2.9
Av	5	4.7	4.4	2.0
Bi	5	22.9	9.0	4.0
Bii	5	8.4	8.1	3.6
Biii	5	6.2	5.7	2.5
Biv	5	5.8	6.3	2.8
Bv	5	5.7	5.6	2.5

APPENDIX III. Pine burial experiment (Expt. 3.4.)

Statistical analyses

Moisture content % after burial - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	55	16	7
Aii	6	51	15	7
Aiii	6	55	20	8
Aiv	6	49	16	7
Av	6	46	16	6
Bi	6	53	19	8
Bii	6	49	15	6
Biii	6	49	18	7
Biv	6	45	17	7
Bv	6	42	14	6

Moisture content % after burial - Inner sapwood

Ai	6	71	19	8
Aii	6	54	6	3
Aiii	6	50	10	4
Aiv	6	44	9	4
Av	6	36	3	1
Bi	6	64	20	8
Bii	6	53	5	2
Biii	6	49	5	2
Biv	6	38	5	2
Bv	6	35	4	2

Appendix III. - continued

Nitrogen content % after burial - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	0.112	0.014	6
Ai	6	0.071	0.014	6
Aii	6	0.074	0.014	6
Aiii	6	0.074	0.014	6
Aiv	6	0.074	0.014	6
Av	5	0.083	0.006	3
Bi	6	0.123	0.028	11
Bii	6	0.069	0.006	2
Biii	6	0.079	0.010	4
Biv	6	0.073	0.013	5
Bv	5	0.086	0.019	8

Nitrogen content % after burial - Inner Sapwood

Ai	6	0.125	0.049	0.019
Aii	6	0.058	0.008	0.003
Aiii	6	0.058	0.008	0.003
Av	5	0.054	0.007	0.003
Bi	6	0.109	0.027	0.011
Bii	6	0.061	0.017	0.007
Biii	6	0.057	0.009	0.004
Bv	6	0.059	0.005	0.002

Appendix III. - contd.

Control nitrogen content % - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	4	0.074	0.017	8
Aii	4	0.081	0.020	10
Aiii	4	0.069	0.014	7
Av	3	0.069	0.009	5
Bi	4	0.104	0.018	9
Bii	4	0.086	0.007	3
Biii	4	0.074	0.006	3
Bv	4	0.069	0.006	3

Control nitrogen content % - Inner sapwood

Ai	3	0.048	0.004	0.002
Aii	4	0.063	0.007	0.003
Aiii	4	0.055	0.007	0.004
Av	4	0.050	0.005	0.002
Bi	4	0.055	0.009	0.004
Bii	4	0.052	0.003	0.001
Biii	4	0.054	0.012	0.006
Bv	4	0.045	0.008	0.004

Appendix III. - contd.

Soft rot in mm - Sapwood

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	2.0	0.7	0.3
Aii	6	-	-	-
Aiii	6	-	-	-
Aiv	6	-	-	-
Av	6	-	-	-
Bi	6	2.1	0.8	0.3
Bii	6	-	-	-
Biii	6	-	-	-
Biv	6	-	-	-
Bv	6	-	-	-

Soft rot in mm - Inner sapwood

Ai	6	4.0	1.1	0.5
Aii	6	-	-	-
Aiii	6	-	-	-
Aiv	6	-	-	-
Av	6	-	-	-
Bi	6	2.8	0.6	0.2
Bii	6	-	-	-
Biii	6	-	-	-
Biv	6	-	-	-
Bv	6	-	-	-

Appendix III. - contd.

Respiration in sapwood (μl O_2/hr/cc of wood)

Layer No.	No. of Samples	Mean	Standard Deviation	Standard Error
Ai	6	22.5	10.5	4.1
Aii	6	12.5	3.5	1.4
Aiii	6	16.7	3.5	1.4
Aiv	6	16.3	2.9	1.2
Av	6	19.0	5.7	2.3
Bi	6	24.0	11.0	4.5
Bii	6	20.9	9.5	3.9
Biii	5	16.3	6.1	2.7
Biv	6	19.9	10.9	4.5
Bv	6	21.2	4.8	2.0

Respiration in inner sapwood (μl O_2/hr/cc of wood)

Ai	6	27.2	7.8	3.2
Aii	6	10.9	2.6	1.1
Aiii	5	11.7	1.7	0.7
Aiv	6	14.5	6.9	2.8
Av	6	20.4	6.0	2.4
Bi	6	22.7	4.3	1.8
Bii	6	11.1	2.8	1.1
Biii	5	13.0	4.0	1.8
Biv	6	15.1	4.8	2.0
Bv	6	15.8	7.3	3.0

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