CELL WALL DEGRADATION IN COPPER CHROME ARSENIC TREATED WOOD

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by

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ABSTRACT

A technique has been developed which allows the cell wall degradation of treated wood, by fungi and bacteria to be used as a means of rapidly determining the effectiveness of the preservative treatment. The first part of the work described in this thesis deals with the development of the technique and the second part examines the effectiveness of a range of preservatives containing various amounts of copper, chromium, arsenic and boron, against soft rot fungi and S_2 degrading bacteria using the technique.

Thin sections of variously treated wood were buried in a number of different soils, removed after varying time periods and examined in the light microscope for degree of fungal or bacterial attack.

Conditions suitable for culturing the bacteria in the laboratory were established after a series of preliminary tests. A saturated garden loam was ideal for producing bacterial attack in buried wood sections. Soil moisture content affected the type of attack found, fungi predominated in drier soils.

The decay potential of the different soils varied and ultimately affected the performance of the preservatives. A copper chrome arsenic formulation performed well against fungal attack but poorly against the bacteria. The bacteria were able to discriminate between different preservatives, those with a high arsenic content failed. Results obtained in weeks using the thin section technique, agree with those found in the field after years of exposure.

The hardwood, birch, was found to be very susceptible to fungal attack but less susceptible to the bacteria, whilst the softwood

Scots pine, was attacked predominantly by bacteria.

A brief attempt was made to characterise the bacteria found, using the scanning electron microscope.

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SECTION I: INTRODUCTION

1. Section I Introduction

1.1 Background

1.1.1 Biodegradation of Timber

In the natural environment, wood enters the soil ecosystem and as such is subject to the activities of decomposer organisms inhabiting the soil. These organisms, which include fungi, bacteria, actinomycetes and insects , degrade the timber into simple compounds for reabsorption into the carbon cycle. Preservation aims at interrupting this cycle in order to extend the service life of timber. To know how and where to apply preservatives requires a detailed knowledge of the mechanisms by which the microorganisms degrade the wood cell wall.

1.1.1.1 Fungi

In terrestrial temperate situations, fungi are the main cause of timber decay (Dickinson and Levy, 1982). A number of investigations have been carried out to determine the range and nature of their activities. Basidiomycetes are significant amongst the fungi for their wood decaying abilities. According to their mode of attack these fungi are grouped as brown rots or white rots. Brown rots attack the wood cellulose, leaving the lignin whilst white rots attack both lignin and cellulose. The hyphae of white rot fungi make bore holes in the wood cell walls surrounding the hyphae. With brown rot, a more generalised decay of the wood cell wall takes place which is not restricted to the immediate vicinity of the hyphae.

Until Findlay and Savory (1950) first suggested the economic importance of soft rot fungi, basidiomycetes were regarded as the

only fungi capable of causing severe economic degradation of timber in service. The micromorphology of these soft rot fungi had been illustrated by Schacht (1863) and later by Bailey and Vestal (1937). Savory (1954a,b, 1955) published a series of papers which established the term 'Soft Rot' and demonstrated that these fungi were responsible for degradation of the timber fill in water cooling towers,which had earlier been thought to be due to chemical breakdown. Soft rot caused by various ascomycetes and fungi imperfecti has been recognised as being of considerable significance in situations which are largely unsuitable for basidiomycete attack (Savory, 1954a,b, 1955). The soft rot problem in preservative treated wood is world-wide e.g. in Queensland, Australia, it is estimated that up to 400,000 copper-chrome-arsenic (CCA) treated eucalyptus transmission poles are affected to varying degrees by soft rot (Greaves, 1977). In Sweden up to four million 20-35 year old zinc salt-impregnated transmission poles of Pinus sylvestris are attacked by soft rot (Friis-Hansen, 1975). There are few reports of Basidiomycetes in preservative treated wood (Henningsson and Nilsson, 1976).

The soft rot fungi are of greatest importance in preservative treated timber, a situation in which the principal wood-destroying fungi, the basidiomycetes, are excluded. Many microfungi are capable of causing soft rot. Seehann, Liese and Kess (1975) have listed 305 true soft rot fungi, representing 172 genera, capable of producing cavities or erosion in woody tissue.

Soft rot fungal attack differs from that of basidiomycetes as the fungi grow in the S_2 layer of the secondary cell wall, forming chains of cavities with pointed ends, which are arranged in a helix parallel to the orientation of the cellulose microfibrils (Savory, 1954a). Soft rot cavity formation has been extensively studied and

discussed (Corbett, 1963, 1965; Corbett and Levy, 1963; Courtois, 1963a,b; Liese, 1964, 1970; Levi and Preston, 1965; Findlay, 1970; Crossley, 1979; Hale and Eaton, 1981, 1985a,b). Courtois (1963a,b) compared several patterns of soft rot attack and Corbett (1963, 1965) termed the cavity formation Type I attack in order to distinguish it from a second type of attack involving progressive erosion of the wood cell wall, termed Type II. Cavity formation (Type I) is perhaps the most diagnostic feature of soft rot decay. It is this feature of attack of the treated wood cell wall, that has been utilised in the technique developed in the work described in this thesis, to study the progress of decay and hence the performance of the preservative treatment.

In hardwoods and softwoods the hyphae invade the tissues preferentially through the vessels or rays, from where they grow into the lumina of adjacent fibres or tracheids (Courtois, 1963a,b; Liese, 1964; Corbett, 1965; Greaves and Levy, 1965; Levy and Stevens, 1966). Corbett (1963, 1965) was the first to observe that a short side branch of a hypha lying in the cell lumen, penetrated the S_3 layer perpendicular to the wall and on reaching the S_2 layer (or having passed into the S_2 layer of the adjacent cell wall), appeared to turn at right angles to lie parallel to the cellulose microfibrils and at the angle, branched at 180° to form a 'T - branch'. A cavity was formed by wall lysis round the hyphal branches forming the cross of the T and the hyphae then increased in girth. Fine hyphal strands, termed 'proboscis' hyphae (Corbett, 1963, 1965) form the initial extension growth from the distal ends of the hyphae in the cavity (Crossley and Levy, 1977; Hale and Eaton, 1985b).

Leightley (1977), Leightley and Eaton (1977) and Hale and Eaton (1981, 1985a) have studied development of proboscis hyphae

and cavity formation by time-lapse photography. The proboscis hypha develops from the tip of a cavity hypha and continues penetration through the wall in the same helix as the cavity hypha, in a direction parallel to the cellulose microfibrils. After a period of growth, further extension of this proboscis hypha ceases while it increases in girth and a new cavity forms around it. After some time, extension growth begins again by development of another proboscis hypha and the process is continually repeated. Helices of cavities eventually coalesce and the whole of the S_2 layer can be removed, leaving only the heavily lignified middle lamella and S_3 layer, the latter often collapsing.

1.1.1.2 Bacteria

In the past, research efforts have been directed towards the fungal attack of wood. However, recently it has become evident that bacteria may play an important role in the colonisation and decay of wood in service. The bacterial attack of wood occurs when wood is very wet, under saturated conditions e.g. wood stored in log ponds or rivers (Stutz and Stout, 1957; Suolahti and Wallen, 1958; Ellwood and Ecklund, 1959; Knuth and McCoy, 1962; Greaves, 1969; Highley and Lutz, 1970; Karnop, 1972), buried in soil (Hawker et al, 1960; Morton et al, 1969; Dwyer and Levy, 1976; Nilsson, 1984 ; Nilsson and Daniel, 1983a), in marine situations (Eaton and Dickinson, 1976; Leightley and Eaton, 1977, 1980; Kohlmeyer, 1980, where the attack was attributed to actinomycetes), in mines (Levy, 1964; Levy and Greaves, 1966), or when wood is used in water cooling towers (Greaves, 1969, 1971; Liese and Greaves, 1975).

The first report of bacterial decay in CCA treated timber was published by Greaves (1968). Other reports have followed (Greaves and Foster, 1970; Irvine, 1974). Eaton and Dickinson (1976) and

Leightley and Eaton (1977, 1980) described a peculiar form of degradation of CCA treated timber exposed in the marine environment. They suggested that the attack was caused by actinomycetes, but later Holt (1981), suggested that bacteria may have produced the degradation patterns found. Leightley (1981, 1982) reported bacterial decay in preservative treated eucalypt power transmission poles in Australia. Nilsson and Daniel (1983a), Daniel and Nilsson (1985, 1986), Nilsson and Holt (1983) and Mouzouras et al (1986) reported bacterial attack of treated wood. The recent findings of extensive bacterial degrade in CCA treated pine posts installed in horticultural soils in New Zealand (Drysdale and Hedley, 1984; Nilsson, 1984), further emphasise the role of bacteria as wood-degrading organisms in treated timber installed in certain environments.

As yet, bacteria have never been considered in tests of preservative performance in ground contact situations. The bacteria associated with the attack of pine posts in New Zealand represent a specialised group of wood-degrading bacteria. These bacteria penetrate into the S_2 layer of the secondary cell wall where they form tunnels or cavities and later complete destruction of the cell wall substance. This property of the bacteria, to attack the S_2 layer, makes them amenable to the technique developed in this work, to determine the performance of preservatives against soft rot fungi attacking the S_2 layer.

Several different forms of bacterial attack of treated and untreated wood have now been reported. Earlier studies using the light microscope described erosion troughs through the secondary wall, sometimes as far as the middle lamella (Harmsen and Nissen, 1965a,b; Courtois, 1966; Boutelje and Bravery, 1968; Greaves, 1969; Kohlmeyer, 1969), pittings and depressions in the cell wall (Courtois, 1966;

Liese and Karnop, 1968; Greaves and Foster, 1970; Greaves, 1973; Liese and Greaves, 1975). Later studies using the scanning electron microscope have described the ultrastructure of the various forms of degrade such as erosion, tunnelling and cavitation (Sieburth, 1975; Cundell and Mitchell, 1977; Holt et al, 1979; Holt, 1981, 1983; Nilsson and Holt, 1983; Nilsson and Daniel, 1983a; Daniel and Nilsson, 1985, 1986; Mouzouras et al, 1986).

It is still unclear whether the bacteria responsible for the various degradation patterns observed by many workers are the same or different species, or stages in the succession of bacterial wood-degrading species. Daniel and Nilsson (1986) suggest that there is a succession involved but that not all species are active in timber decay.

In the work reported here the bacterial patterns found after exposing thin sections of wood to bacterial attack were not identified. The patterns of attack have yet to be defined adequately by research workers. The bacteria attacking the S_2 layer were selected as being suitable for use with the thin section technique, designed to determine the effectiveness of preservative treatment of this wall layer.

1.2 Prevention of Biodegradation

1.2.1 General

In the past naturally durable timber species were used but the present demand for timber is so great that perishable timbers must be utilised. Such timber aquires an induced durability by the application of toxicants in the form of wood preservatives.

The most efficient method to avoid fungal decay is to keep wood dry and this can be achieved by structural design such as the incorporation of gutters to dispose of rainfall and damp-proof membranes to isolate structural wood from dampness in the soil or supporting structure. However, there are some situations where deterioration is unavoidable unless naturally durable or adequately preserved wood is used. One of the most important severe hazards for wood is in soil contact e.g. with transmission poles and fence posts. In this situation timber is frequently maintained at a suitable moisture content for decay by microorganisms. Soil supports a wide range of microorganisms capable of colonising and decomposing timber and may provide a source of additional nutrients taken up into the wood by "wick" action (Baines and Levy, 1979) and by microbial transfer (King, Mowe, Smith and Bruce, 1981).

1.2.2 Chemical Treatment

A large number of preservatives and methods of application are now available (Wilkinson, 1979; Cartwright and Findlay, 1958). The present study was concerned with inorganic, water-borne preservative formulations applied by vacuum-pressure impregnation and based on copper and chromium salts.

1.2.2.1 Water-Borne Multisalt Preservatives

Water-borne preservatives consist of solutions of salts selected because they possess an active ion e.g. copper is fungicidal and arsenic is fungicidal as well as being insecticidal.

A defect of all early water-borne preservatives was their liability to leaching. This severely limited their effectiveness and

their usage. In the mid 1920's the development of highly fixed formulations began with the introduction of the copper-chrome preservative "Celure" by Gunn (1926). Later Falck and Kamesan (1931, 1933) followed with a fixed chrome-arsenic mixture under the name "Falkamesan" and then soon afterwards, Kamesan (1933) introduced the first copper-chrome-arsenic mixture "Ascu". In all these three formulations fixation was achieved by the addition of an alkali metal dichromate, $K_2Cr_2O_7$.

Three further copper-chrome-arsenic formulations were introduced under the brand names of "Tanalith C" (1955), "Celure A" (1959-1960) and "Boliden K33". The latter consisted of a number of metal-chrome-arsenic formulations proposed by Häger (1969).

More than 60,000 tonnes of CCA preservatives are consumed worldwide annually (Lewis and Brooks, 1983). At first CCAs were used only in the UK as an alternative to creosote but later they spread to other areas such as Australasia where they are mostly used to treat hardwoods. The notable exception is New Zealand where they are used to preserve Pinus radiata, a softwood.

The CCA formulations currently in use were designed to give high fixation and incorporate increased amounts of copper. Copper has been shown to be the active ingredient in controlling soft rot organisms and many basidiomycete fungi (Findlay and Savory, 1950, 1954; Savory, 1954a; Duncan, 1960; Theden, 1961; Da Costa and Kerruish, 1963; Hulme and Butcher, 1977c; Henningsson and Nilsson, 1976), whereas the arsenic is effective against a wide range of insects and provides additional protection against a number of copper tolerant fungi (Tamblyn and Levy, 1981; Tillott and Coggins, 1981).

The main advantages of CCA as a wood preservative are its resistance to leaching, its wide spectrum of activity against wood-destroying organisms, its relatively low cost and the clean, non-oily finish to the timber after drying, enabling the treated wood to be glued or painted (Tillott and Coggins, 1981). CCA is suitable for use in ground contact or severe leaching situations such as the internal timbers of a water cooling tower.

Copper-chrome-boron (CCB) is another preservative based on the copper-chrome composition. It is used in many countries as a replacement for CCA, often because arsenic is unavailable or its use has been banned or is undesirable (Cockroft and Levy, 1973; Cavalcante, Geraldo and Freitas, 1982; Dickinson, 1983).

CCB has been found to be more effective than CCA against soft rot in birch in the laboratory, although both Scots pine and birch treated with CCB failed to copper tolerant basidiomycetes (Gray and Dickinson, 1982). In the field Tamblyn and Levy (1981) found that CCB treated Pinus radiata and Eucalyptus regnans stakes failed to copper tolerant brown rots. Tillott and Coggins (1981) found that CCB performed better than CCA in beech in the field. These results however, do not agree with the International Research Group on Wood Preservation collaborative soil burial trial (Carey and Savory, 1975), nor those obtained by Kerner-Gang (1975) in beech, where CCA was usually more effective than CCB. Boron is regarded as being unfixed (Wilkinson, 1979; Becker and Buchmann, 1966; Tillott and Coggins, 1981) and subject to leaching in wet conditions.

Gray (1983) found that the performance of CCB against soft rot fungi could be improved by following the treatment of CCB with a second treatment of arsenic (CCB + A).

1.2.2.2 The Effect of CCA Formulation on Performance

Henry and Jeroski (1967), Fahlstrom, Gunning and Carlson (1967) and Häger (1969) have all investigated the effect of varying the composition of the CCA mixture on its degree of fixation. Smith and Williams (1973 a,b), Hartford et al (1982) and Hedley (1984 a,b) have reported disparities in performance between the various CCA formulations when used in ground contact situations.

Smith and Williams (1973 a,b) concluded that maximum arsenic fixation took place when the chromium to arsenic ratio was 1 : 0.8 (in terms of active elements). A chromium to copper ratio of 1 : 0.42 defined maximum copper fixation.

Dahlgren (1975) made observations of the pH changes which govern the course of CCA fixation in wood. He found that formulations which conformed to those defined by the American Wood Preservers' Association (AWPA) as Type C (having a low arsenic and high chromium content), were more resistant to leaching than those of Type B (having a high arsenic and low chromium content). Plackett (1984) measured the leaching resistance of various CCA formulations and his results agreed with those of Dahlgren (1975).

The formulations giving maximum effectiveness have been studied by Hartford et al (1982) and Hedley (1984 a,b). Formulations approximating to Type C e.g. Celcure A salt and Tanalith C have been found to be superior to those of Type B e.g. K33 oxide,in conferring long-term resistance to decay. Type C and Type B CCA formulations were examined in the work reported here.

1.3 Soft Rot Attack of CCA Treated Wood

The association of the premature failure of CCA treated hardwoods in service to soft rot attack has been reported by Greaves (1972, 1977), Tamblyn (1973, 1975), Aston and Watson (1976), Henningsson (1976) and C.R.Levy (1978). This has been confirmed by *inter alia* Dickinson *et al* (1976), Fougerousse (1976) and Clubbe (1980a,b).

Leightley (1981) has examined the occurrence and severity of soft rot in CCA treated eucalypt transmission poles in Queensland, Australia. Of the seven million transmission poles in Australia, 5 - 7% are currently affected to varying degrees by the failure of preservative treatments to give adequate protection against soft rot in the sapwood (Greaves, 1977). Most of the poles have been treated with CCA preservatives.

Lewis and Brooks (1983) have reviewed the poor performance of CCA treated hardwoods and suggest that the problem in Australia arose as a result of the direct transference of retentions more pertinent in the treatment of softwoods under European conditions, to hardwood species which have since been found to be particularly susceptible to soft rot attack. A considerable amount of effort has been placed in determining the reasons for the variable performance of certain CCA treated hardwoods. Two main hypotheses have been developed to explain the difference in performance found between CCA treated hardwoods and softwoods.

The hypothesis of substrate susceptibility was developed by Hulme and Butcher (1977 a,b,c) when investigating the relationship between timber substrates and the CCA preservatives. They found that an increased preservative retention could compensate for the increased susceptibility of a species towards soft rot fungi. Butcher (1979) proposed that decay potential and preservative requirement could be related to the proportion

of fibres within a wood species.

The second hypothesis suggests that the cause of premature failure of CCA treated hardwoods is due to the poor macrodistribution of the preservatives in the wood tissues (Dickinson, 1974; Greaves, 1974; Dickinson et al, 1976; Levy and Greaves, 1978; Greaves and Levy, 1978) and also to the poor microdistribution of the CCA preservatives within the wood cell wall (Dickinson, 1974; Greaves, 1974; Dickinson et al, 1976; Drysdale, 1979; Drysdale et al, 1980). Drysdale et al (1980) noted that disproportionation of the copper, chromium and arsenic within the wood cell wall may be of significance in preservative performance.

Butcher and Nilsson (1982) and Nilsson (1982b) have suggested the importance of lignin in determining the susceptibility of a timber species towards soft rot fungi in association with the role of lignin in offering fixation sites for copper. Conradie and Pizzi (1985) have suggested that the chrome content on lignin correlates to soft rot resistance.

These aspects of the differences found between hardwood and softwood performance have been reviewed by Drysdale (1979) and Ofori (1980).

Recently there have been reports of serious failure of CCA treated softwoods used in horticultural situations in New Zealand (Butcher, 1984; Drysdale and Hedley, 1984; Nilsson, 1984). Soft rot fungi, together with bacteria have been associated with the premature decay.

1.4 Bacterial Attack of CCA Treated Wood

Bacterial attack of CCA treated wood has been reported in a variety of environments, in water cooling towers (Greaves, 1968; Irvine, 1974), in the marine environment (Eaton and Dickinson, 1976; Leightley and Eaton,

1977, 1980; Kohlmeyer, 1980, authors who attributed the attack to actinomycetes) and buried in soil (Banerjee, 1969; Butcher, 1971; Greaves, 1972; Levy, 1975; Clubbe, 1978, 1980a; Nilsson and Daniel, 1983a; Nilsson and Holt, 1983). CCA and Pentachlorophenol treated eucalypt transmission poles have shown patterns of bacterial decay in Australia (Leightley, 1981, 1982). As mentioned earlier, CCA treated radiata pine posts in New Zealand have also been attacked by bacteria (Butcher, 1984; Drysdale and Hedley, 1984; Nilsson, 1984).

Bacteria are able to attack wood which has been treated to retentions greater than those needed to prevent fungal decay (Nilsson and Daniel, 1983a). The bacteria may immobilise heavy metals intra- and extra-cellularly (Mouzouras et al, 1986; Blunn and Jones, 1986; Daniel and Nilsson, 1986).

There is a need to evaluate formulations in terms of their performance against soft rot fungi and bacteria. The bacteria are important in specialised situations.

1.5 Methods Available for Testing the Performance of Preservatives

A number of methods have been used for evaluating the efficacy of wood preservatives against soft rot fungi, these include fungal monoculture exposure (BS 838 Part 2, 1961; Schulz and Riewendt, 1962; Duncan, 1965; Bravery, 1968a,b), soil burial (Theden, 1961; Aston, 1978; Savory and Carey, 1978), vermiculite burial (Gersonde and Kerner-Gang, 1976). These methods do not allow an immediate examination of the interaction of the fungi with the treated cell wall layers, information such as this would enable the mechanism of preservative action to be studied and weaknesses to be overcome.

Bravery (1975) summarised various test methods for evaluating preservative efficacy. He concluded that there is a trend towards reducing the time for tests and improving their simplicity and reproducibility, together with a reduction in their cost. A laboratory test is required to rapidly screen preservatives for their performance against soft rot fungi.

Bacteria have never been considered in tests of preservative performance in ground contact, however, the recent findings of extensive bacterial and soft rot degrade in poles and posts in Australia and New Zealand, suggest that there is a need to include these decay organisms when evaluating preservatives. Therefore, a test method is required which can measure both fungal and bacterial attack, whilst at the same time give information about the mode of attack at the cell wall level.

1.6 Aims and Objectives

The aim of the work described in this thesis was to develop a technique whereby the cell wall degradation of treated wood could be examined. Once developed, the technique would be used to rapidly screen preservatives for their performance against soft rot fungi and bacteria. The conditions required for producing bacterial attack in the laboratory would be determined.

The technique developed has been used as part of a major project at Imperial College designed to examine the optimisation of the preservative action of copper within the wood cell wall. The microdistribution of the wood preservative elements within the layers of the wood cell wall should relate to that part of the wall structure attacked by decay microorganisms. The effectiveness of the treatment of the S_2 layer of the secondary wall could be assessed using the

technique developed.

Results obtained using this technique would be compared with those obtained by other workers examining the attack of treated wood in the laboratory and in the field.

The sapwood of a hardwood, birch and a softwood, Scots pine would be treated with a range of experimental formulations and commercially available preservatives. Their effectiveness against soft rot and bacterial attack would be assessed using the assay technique developed.

SECTION II: DEVELOPMENT OF ASSAY TECHNIQUES

2. Section II Development of Assay Techniques.

2.1 Introduction

In order to assess the effectiveness, as wood preservatives, of formulations containing copper, chromium, arsenic and boron against soft rot attack, a number of laboratory methods exist. These, although adequate in providing a basis for comparing the performance of preservatives give little information as to the relationships of the decay fungi to the variously treated wood, particularly at the cell wall level.

In the course of the work described in this thesis a new system was evolved whereby a thin section cell wall assay was applied to the soft rot attack of wood. The main objective of developing the assay was to produce a rapid technique whereby cell wall degradation could be examined. The assay acts as a screening technique, indicating the extent of the effectiveness of treatment of the S_2 cell wall layer. The effectiveness of various salt formulations against soft rot attack can be determined.

This assay has been developed and used as part of a project for research into the optimisation of the preservative action of copper within the wood cell wall.

A number of methods have been used for soft rot testing, using wood blocks. These include fungal monoculture exposure (BS 838 Part 2, 1961; Schulz and Riewendt, 1962; Duncan, 1965; Bravery, 1968 a,b), soil burial (Theden, 1961; Aston, 1978; Savory and Carey, 1978) and burial in inoculated vermiculite (Gersonde and

Kerner-Gang,1975,1976). Soil burial techniques are actively used in laboratories around the world, but problems arise with the inherent variability of soil taken from different locations. Pure culture soft rot tests will be better defined as to the organisms present, than unsterile soil tests, but they,together with vermiculite burial tests, do not simulate a field situation, where an interaction between the components of the natural microflora and the wood under test occurs. In addition, these soft rot tests do not allow an immediate examination of the interaction of the fungi with the cell wall layers. The thin section technique provides a simple method of direct examination of the wood sample, to enable a cell wall study of the initiation, development and extent of a soft rot attack to be undertaken. This test is more critical than existing tests and allows an assessment to be made of the performance of preservatives, by determining the extent of soft rot attack at the cell wall level. The burial of thin sections in unsterile soil still presents problems of inherent variability of different soils, when comparing results of attack of wood in soils from different locations, however the use of soil does attempt to simulate field conditions.

Bravery (1975) summarised various test methods for evaluating preservative efficacy and concluded that there is a trend towards reducing the time for tests and improving their simplicity and reproducibility, together with a reduction in their cost. The thin section assay developed in this work provides a method of rapidly screening the parameters of soft rot decay as well as the effectiveness of existing and alternative preservatives. The assay indicates the success of the preservative treatment of the S_2 cell wall layer by the absence of soft rot cavities when the cells have direct access to any soil microorganisms. The test is simple and

reproducible and can be carried out at minimum cost.

Once developed as an effective soft rot assay it became clear that it could be used to determine the activity of other wood-degrading microorganisms and it was decided to extend the work to include a means of assessing the attack of the wood cell wall by bacteria. Bacteria have never been considered in tests of preservative performance in ground contact. Recent reports of serious decay of softwoods in horticultural situations in New Zealand (Butcher et al, 1983; Drysdale and Hedley, 1984; Nilsson, 1984), caused by soft rot fungi together with bacteria, suggest that there is a need to include the bacterial hazard in any preservative screening test. The bacteria so far reported in New Zealand are able to attack the S_2 layer of the wood cell wall and produce tunnels or cavities within the wall. They attack at high preservative loadings, therefore it was decided to investigate whether these bacteria could be used as a means of discriminating between preservative formulations.

This group of bacteria attack the S_2 layer of the wood cell walls (Nilsson and Holt, 1983), as do soft rot fungi. The thin section assay allows an evaluation of preservative performance at the cell wall level against both the bacterial and fungal hazards. Existing and alternative preservative formulations have been tested in this work using the thin section assay.

2.2 Assay Technique

2.2.1 Introduction

Techniques for assaying preservative efficacy usually involve the colonisation, infection and decay by fungi of sample wood blocks. The standard test methods all utilise weight loss as the criterion of decay. Other methods rely on significant losses in one or other of the strength properties which can be measured by a range of techniques. However with all these methods, information about what is happening at the cell wall level with regard to the decay fungi is not determined.

In the thin section cell wall assay described here, the fungi and bacteria are allowed immediate access to the wood cell wall. This new approach allows the effects of the microdistribution of the preservative within the cell wall to be studied. With wood blocks, attack of the cell wall by soft rot fungi would occur from the lumen of the fibre (in a hardwood) or tracheid (in a softwood). In the known mode of soft rot attack, the fungal hyphae penetrate the wall of the fibre or tracheid from the lumen, via a side branch of the hyphae, which passes through the wall and the middle lamella and into the S_2 layer of the wall of an adjacent fibre or tracheid. There it forms a characteristic T-shape. The two branches of the T develop in the S_2 layer parallel to the orientation of the cellulose microfibrils in this layer (Corbett and Levy, 1963). In this work, the transverse sections examined showed no signs of T branch formation, suggesting that the fungus must have penetrated straight into the S_2 layer from above or below, then proceeded to attack this layer, without encountering the lumen. The use of thin sections thus

allows an assessment of the effectiveness of the preservative within the S_2 layer to be made, without any interference from the effects of preservative within the lumen. Both soft rot fungi and cell wall degrading bacteria attack the S_2 layer of the wood cell wall. Preservative efficacy in this layer can therefore be examined with regard to the two types of attack. As will be shown later, this gives rise to a rapid attack of the cell wall in the absence of a toxicant so that preservatives can be screened for their efficacy in three or four weeks rather than months.

2.2.2 Method

Treated 7 mm^3, orientated, birch and scots pine sapwood blocks and those untreated (as controls) were prepared, treated (where necessary) and leached as described in section 3.2, dried, then impregnated with water in a vacuum dessicator. A vacuum of 760 mm Hg was drawn for 30 minutes. This saturation of the wood blocks was necessary to facilitate the sectioning of the blocks.

The outer 0.5 mm of the face of each block to be sectioned was removed, using a Reichert sledge microtome, to ensure that the sections to be used were representative of the wood block. 30 μm thick, transverse sections were cut from each block.

Monofilament nylon bolting cloth with a 53 μm aperture (Henry Simon Limited, Stockport, Cheshire) was used to make mesh packets to hold the sections whilst they were buried in the soil. These consisted of two squares, 2.5 cm^2, of the nylon mesh heat sealed together on two edges to form a packet, the sealing being carried out by using a Bunsen burner flame. After the packets were sealed

the wood sections were placed via the open edges of the packets, into the packets, prior to soil burial. The mesh packets facilitated ease of removal of the sections from the soil, allowed the fungus access to the sections, but kept them clean and clear of soil particles for subsequent microscopic examination .

Six sections were placed in each mesh packet (figure 1). The packets were moistened with distilled water by soaking in water for 1 minute, before being buried in soil.

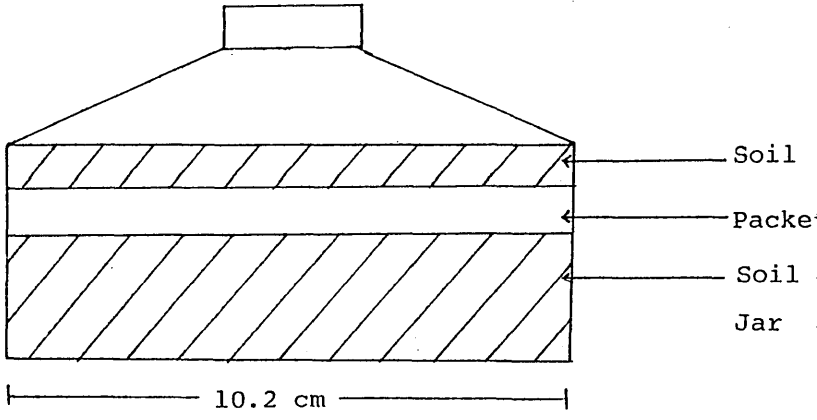
The amount of water required to wet the soil to the desired water holding capacity (w.h.c.) was calculated (section 2.4.2) and and added to the 200 g of soil in glass jars (supplied by French Flint and Ormco Limited, jars 500 ml nominal capacity). Eight moistened mesh packets, each containing six sections were laid down on a flat soil surface then covered with a 1 cm layer of soil at the same w.h.c. (figure 1).

The jars were weighed at intervals throughout the incubation period to ensure that the soil retained its original moisture content. The jars were incubated in a room at a controlled temperature of 28°C and relative humidity 80% in a fungal cellar .

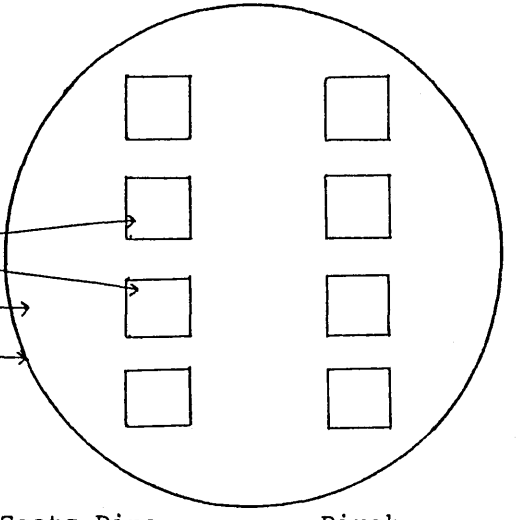
The wood sections were removed after varying time periods and stored in the fixative Formalin Acetic Alcohol (FAA) prior to staining for examination in the light microscope (section 2.4.4.3).

Figure 1 Thin Sections of Wood buried in Soil.

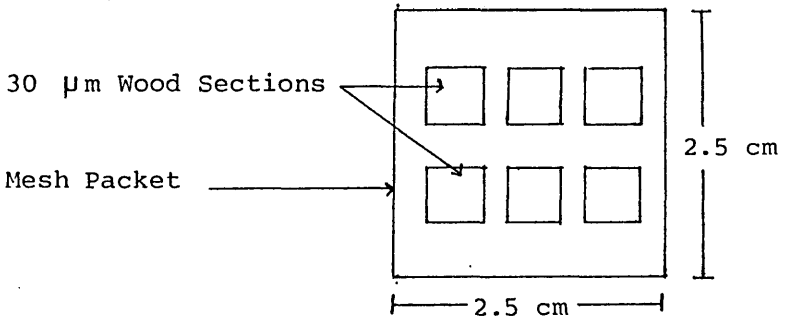
Incubation Jar



Cross Section of Jar with Mesh Packets containing Wood Sections on Soil surface.



One Mesh Packet containing six Wood Sections.



2.3 Soil Burial

2.3.1 Introduction

Unsterile soil was selected as the burial medium for the thin sections of wood. The aim was to provide conditions approximating to those found in the natural field situation.

Mixed culture soft rot tests are commonly carried out by the burial of wood blocks in vermiculite or unsterile soil (Gersonde and Kerner-Gang, 1976; Bravery, 1968 a,b; Savory and Bravery 1970, 1971; Savory and Carey, 1980; Nordic Wood Preservation Council 1.4.1.2/70).

The use of vermiculite has the disadvantage that it must be inoculated with a mixed culture (Savory and Carey, 1980) or a soil extract (Kaune, 1970). However, with unsterile soil, the natural soil flora can be used to effect decay (Theden, 1961; Becker and Kaune, 1966; Bravery, 1975). With soil there is no need to add an inoculum and also a wide range of soil types contain sufficient nutrients for decay (Theden, 1961; Becker and Kaune, 1966; Leutritz, 1946). It was for these reasons that soil was chosen as the burial medium. The use of natural mixtures of microorganisms in the soil enabled the biological exposure of thin sections to be close to conditions met in practice e.g. poles in ground contact.

2.3.2 Moisture Relationships

Vinden et al (1982), Baines (1984), Woodward (1985) and Mwangi (1986) have all shown the important relationships that exist between the moisture content of wood in soil contact, and the moisture

content of that soil, as well as the important difference that exists between soil moisture content and the soil water holding capacity (w.h.c.). The soil moisture content needs to be expressed as a percentage of the w.h.c. of the soil. Moisture content is measured as a percentage of the dry weight of the soil.

In this work, the moisture content and the w.h.c. of the soil were determined so that the moisture content of the wood substrate was the same in each test.

Vinden et al (1982) suggested that soil moisture content should be maintained within the range 80-100% water holding capacity for fungal attack of wood buried in soil. In soft rot studies here, soil at a moisture content of 95% of its w.h.c. was used. However these conditions were not found to be optimal for bacterial attack. A soil moisture content of 150% of soil w.h.c. was found to be the most effective for producing attack of wood by bacteria. Clearly there must be a relationship between the climax microflora and the moisture content of the wood. The moisture content of wood depends on the w.h.c. and the moisture content of the soil with which it is in contact. In view of this relationship it was decided to convert the soft rot assay into a bacterial assay in later experiments.

2.3.3 Temperature

The optimum temperature for the growth of most soft rot fungi lies between 28°C and 32°C (Duncan 1960, 1965; Kerner-Gang, 1966). Gersonde and Kerner-Gang (1976) found that a majority of the soft rot fungi they investigated decayed beech most heavily at 32°C in vermiculite, whereas the optima for pine sapwood were 24°C and 28°C. In the present

study, wood sections were incubated in unsterile soil at 28°C, this temperature was found to be suitable for both soft rot and bacterial attack. Nilsson and Holt (1983) used temperatures of 30°C and above when burying stakes in soil and found bacterial decay patterns in the stakes.

2.3.4 pH

Soil pH was not considered. Sharp and Eggins (1970) found that decay in unsterile soil was not markedly different over a pH range 3.7 - 8.6. They showed that some fungi were favoured by a low pH and others by a higher pH. This could explain the apparent tolerance of unsterile soil to changes in pH.

2.3.5 Aeration

Aeration requirements of soft rot fungi have been examined by Duncan (1961), Follstad(1967) and Griffin (1966). Duncan (1961) found that soft rot fungi were more tolerant of low oxygen tensions than the basidiomycetes tested. Savory (1955) stated that the small amount of oxygen present in well aerated water is sufficient to permit the growth of microfungi in buried wood.

2.3.6 Nutrients

Unsterile soil with no added nutrients was used in this work. Butcher (1975) has shown that the presence of alternative carbon sources in sterilised soil may be a contributing factor to the lower rate of decay when compared with decay in unsterile soil.

2.3.7 Leaching

The aim here was to provide conditions in the soil jar approximating, as closely as possible, to those found in the natural field situation. Treated wood blocks were leached with distilled water, (EN 84, BS 5761 Part 2) prior to the cutting of the thin sections of the wood which would be exposed to soft rot and bacterial attack. Leaching is characteristic of the ground contact environment to which the treated wood is exposed in service. Therefore, leaching the blocks prior to exposure of the sections will simulate (in a short period of time) the leaching that would be likely to occur through rain and movement of soil water over a long time period, in external field exposure of wooden posts and poles.

2.4 Soft Rot Fungi

2.4.1 Soil Types

Any soil in which plants will grow is likely to contain sufficient water and nutrients to sustain a wide variety of microorganisms. A wide range of soil types, therefore will contain the required microorganisms and sufficient salts to promote active decay (Theden, 1961; Becker and Kaune, 1966). A number of factors influence the soft rot degradation of wood buried in soil. These include soil water holding capacity, soil and wood moisture content, pH, organic matter and mineral content.

Three soil types were chosen for the following reasons:-

(1) Topsoil was dug from a site of known biological activity (Smith, 1980) in the grounds of the Princes Risborough Laboratory of the Building Research Establishment, Princes Risborough, Bucks. It was chosen as a source from which a great deal of information has been accumulated over the years and this forms the basic standard type, a form of control. This is referred to as Princes Risborough Soil in this thesis.

(2) Soil was dug from an area at Rentokil Laboratories, Felcourt, East Grinstead, West Sussex, which has been used for field trials of wood preservatives. In the last ten years it has formed one of the thirty-four sites of an international collaborative field trial of copper chrome arsenic (CCA) treated wood, particularly hardwoods, set up by the International Research Group on Wood Preservation (IRG). It has shown the most vigorous biological activity of all the sites so this soil was taken to ensure a vigorous test system of high microbial activity. This is referred to as Rentokil Soil in this thesis.

(3) A commercially available horticultural loam, John Innes No.1. This has been found by other workers (e.g. Mwangi,1986) to provide a readily available artificial soil of well defined and reproducible characteristics. It possesses a stable water holding capacity, high enough to ensure that wood buried in it would reach a high enough moisture content to encourage active decay, but low enough to ensure vigorous fungal development.This soil is referred to as John Innes No.1 in this thesis.

2.4.2 Moisture Relationships

Each soil was treated to the same procedure before the experiment was set up. The soils were partly dried by spreading out on plastic sheeting in a greenhouse. Occasionally the soil was turned until it was dry enough to sieve. A 2 mm mesh size sieve was used.

The water holding capacity (w.h.c.) of the soils was determined by the method of Carey and Grant (1975). The w.h.c. is defined as the amount of water retained by a soil sample after being subjected to a series of conditioning procedures.

The procedure for determining the w.h.c. was as follows:-

200 g of soil was placed in a 125 mm diameter Buchner Funnel over a coarse (Whatman No.4) filter paper. The sample was flooded with water and tamped down with the end of a glass rod. A vacuum was drawn for 10 minutes, then the wet soil was transferred to a foil container of known weight. The percentage moisture content of the soil was determined by oven drying, and taken as the w.h.c.

The procedure was repeated in the absence of soil to obtain wet and oven

dry weights of the filter paper.

Three water holding capacity determinations were made for each soil sample. The amount of water needed to raise the moisture content of the soil in each jar to 95% of its w.h.c. was calculated (Table 1).

Every effort was made to maintain a constant w.h.c. for the soil in each jar during the course of the experiment. To do this the jars containing the soil and thin sections were weighed at the start of each experiment and at frequent intervals throughout. Water was added in the form of a fine spray whenever necessary so that a w.h.c. of 95% could be maintained.

w.h.c. Calculation

weight (wt.) of soil as used	a
wt. of wet soil + filter paper + foil container	b
wt. of wet filter paper	c
wt. of container	d
wt. of oven dry soil + oven dry filter paper + container	e
wt. of oven dry filter paper	f
wt. of soil in each jar	g

(1) Moisture content of soil at w.h.c. (j)

$$= \frac{\text{wt. of wet soil - wt. of oven dry soil}}{\text{wt. of oven dry soil}} \times 100\%$$

wt.of wet soil = b - (c + d) h

wt. of oven dry soil = e - (d + f) i

(2) Moisture content of soil as used (k)

$$= \frac{\text{wt. of soil as used} - \text{wt. of oven dry soil}}{\text{wt.of oven dry soil}} \times 100\%$$

$$= \frac{a - i}{i} \times 100\%$$

(3) Volume of water needed to raise moisture content of soil in each jar to 95% of its w.h.c. (ml)

$$= \frac{a \times (j \times 95\%) - k}{100 + k}$$

$$= \frac{a \times (j \times {}^{95}/_{100}) - k}{100 + k}$$

Table 1

Water Holding Capacity Determinations for Soils

Soil		% Moisture Content of soil at w.h.c.	% Moisture Content of soil as used prior to wetting	Vol. of water added to bring soil to 95% of its w.h.c. (ml)
Princes	1	36.22	7.92	49.10
Risborough	2	37.70	8.64	50.03
Soil	3	35.42	8.61	46.11
Mean		36.45	8.39	48.41
Rentokil	1	32.56	6.32	46.31
Soil	2	36.95	6.38	54.00
	3	34.72	6.36	50.06
Mean		34.74	6.35	50.12
John Innes	1	50.93	10.34	68.96
No.1	2	52.32	10.71	70.44
	3	45.41	10.02	60.21
Mean		49.55	10.36	66.54

2.4.3 Soil Analysis

The three soils were analysed at Rothamstead Experimental Station, Harpenden, Herts. Organic carbon, total nitrogen, phosphorus, cation exchange capacity and pH of each soil were determined (Table 2). This analysis was carried out to attempt to explain any differences occurring in soft rot potential between the soil types.

Table 2

Composition of Soils Used for Soft Rot Tests

Soil	Organic Carbon (%)	Total Nitrogen (%)	Phosphorus (%)	Cation Exchange Capacity ($mEq\ 100g^{-1}$)	pH
Princes Risborough	4.9	0.43	4.33	28	7.51
Rentokil	2.3	0.16	1.12	16	5.65
John Innes No.1	4.7	0.34	7.62	22	6.68

2.4.4 Methods of Soft Rot Assessment

The assessment of decayed wood in the field or in a laboratory test, involves the measurement of a parameter which is altered in the course of decay. At present, in Britain, the standard test methods all utilise weight loss as the criterion of decay. Weight loss is simple to measure but there are a number of disadvantages associated with such methods. These are :-

(1) Weight loss is ideal for assessing basidiomycete decay which results in extensive degrade and weight loss, but may not be the best measure of attack by soft rot, which tends to progressively colonise from the surface and initially produces relatively little weight loss, but extensive decay.

(2) Wood blocks are exposed in weight loss tests for twelve or more weeks and destructively sampled at the end of the exposure period. Ideally a test method should provide indications of performance after only a few weeks exposure.

(3) Conventional weight loss tests only use decay as a measure of preservative performance. However, a preservative has other properties which must be tested such as penetrability, macro and microdistribution, fixation, leachability and disproportionation.

(4) Different wood densities affect weight loss results.

Significant losses in the strength properties of wood can develop more rapidly than losses in weight (Hartley, 1958). Strength has been commonly measured by static bending (Smith, 1980; Gray, 1983) and impact bending tests. These have disadvantages :-

(1) Careful sample preparation is required.

(2) Large numbers of replicates are required since these strength criteria are particularly sensitive to variation in experimental technique and the inherent variability of wood, especially wood undergoing decay.

(3) Sophisticated equipment is needed to measure loss of strength.

The thin section assay attempts to overcome the disadvantages incurred with other methods of assessment, decay is estimated on a visual basis in the light microscope. The advantages associated with the assessment of soft rot decay using the thin section assay are :-

(1) The assay gives results of performance of preservatives after only a few weeks. Attack is rapid.

(2) The development of decay can be followed in terms of the relationship of the decay fungi to the wood cell wall.

(3) The assay can be used to study preservative penetrability, macro and microdistribution, on the basis of attack occurring in an untreated zone. These properties can be assessed for sections from one wood block, thus problems of variations between samples are reduced.

(4) Sophisticated equipment is not required to assess soft rot decay microscopically.

Visual assessment is a common method of assessing decay in the field (Anon, 1972; ASTM D 1758-74) and in the laboratory (Duncan, 1965).

The stages of soft rot decay can be classified on the basis of the percentage loss of cross-sectional area of the cell wall, a number given to each class and average values determined. A soft rot degree system was first used by Hoffmeyer (1976). A modified version of this has been used in the present work.

The sections of wood are examined under the light microscope and the severity of the decay classified. This method of classification has been used in the examination of soft rot in salt-treated poles

in Scandinavia (Hoffmeyer, 1976) and has been shown to correlate well with weight loss and strength loss. It was decided that a modified classification was required for the present work using thin sections of wood. The classification used included a measure of loss of wall substance not just number of cavities in a cross-section (as in the classification by Hoffmeyer, 1976). The classification is described in section 2.4.4.2.

An attempt was made to establish the toxic values of the formulations and preservatives. The toxic value is defined as the preservative concentration below which the wood is no longer adequately protected and the concentration above which a product ensures protection.

2.4.4.1 Visual Assessment of Decay

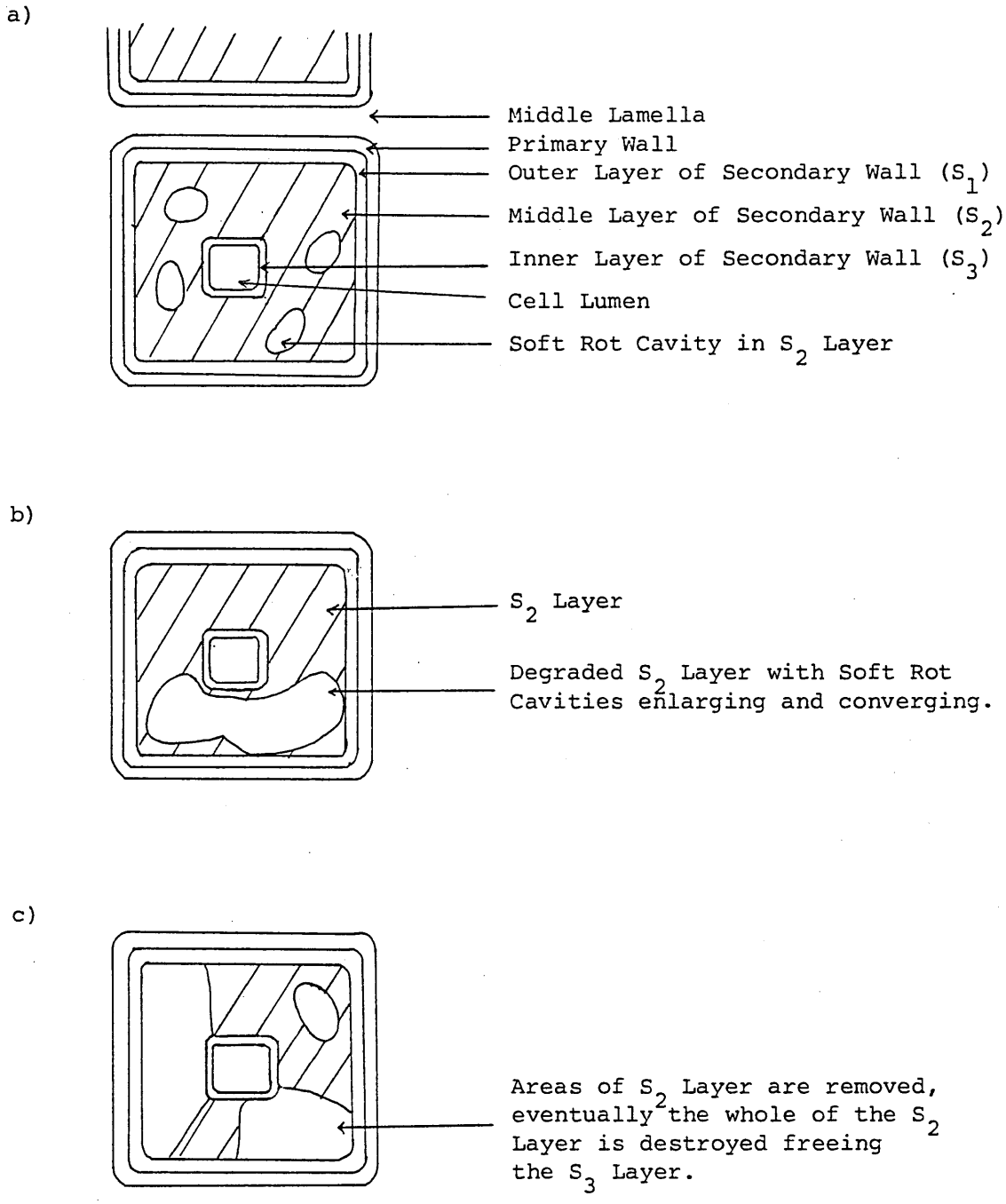
Corbett (1965) and Nilsson (1973, 1974) have shown that wood-attacking soft rot fungi produce two morphologically distinct types of degradation in wood. Type I attack (Corbett, 1965) is characterised by the formation of cavities within the wood cell walls. Type II attack is an erosion of the cell walls, starting from the lumen.

2.4.4.2 Soft Rot Degree Ratings in Birch and Scots Pine

Type I soft rot cavities begin as holes in the secondary wall (Figure 2a) and with increasing severity of attack the cavities join together (Figure 2b) and eventually the whole of the S_2 layer is removed (Figure 2c), completely freeing the S_3 layer.

Type II attack was seldom seen in the sections examined in this work.

Figure 2 The Development of Soft Rot Cavities within the Wood Cell Wall when viewed in Cross Section.



It is clear from Figure 2 that the decay of the wood cell wall by soft rot fungi, as seen in transverse section produces a progressive increase in the size of the cavities formed in the wall. The consequent loss of cross-sectional area of the wall can be estimated relatively easily as a percentage of the total area of the secondary wall, as seen in transverse section in the light microscope. This forms the basis of what is to be called the SOFT ROT DEGREE or SRD. This is defined in Table 3.

Photomicrographs were taken to illustrate the different soft rot degree classes (Plates 1 - 10), using a Zeiss Photomicroscope.

Table 3

Definition of Soft Rot Degree

SRD Class	Appearance of Cross Section	Area of Disintegrated Secondary Wall (%)
0	No cavities	0
1	A few scattered cavities	1 - 10
2	Many cavities in most cells, complete loss of wall in places.	10 - 40
3	Cavities abundant, often confluent, and complete loss of wall in places.	40 - 90
4	Total or almost total disintegration of secondary wall.	90 - 100

PLATES 1 - 5

Soft rot attack in birch, illustrating the five Soft Rot Degree (SRD) classes of decay. All specimens are transverse sections.

PLATE 1 SRD = 0.

S_2 layer intact, no soft rot cavities.

PLATE 2 SRD = 1.

A few scattered soft rot cavities (C) in S_2 layer.

PLATE 3 SRD = 2.

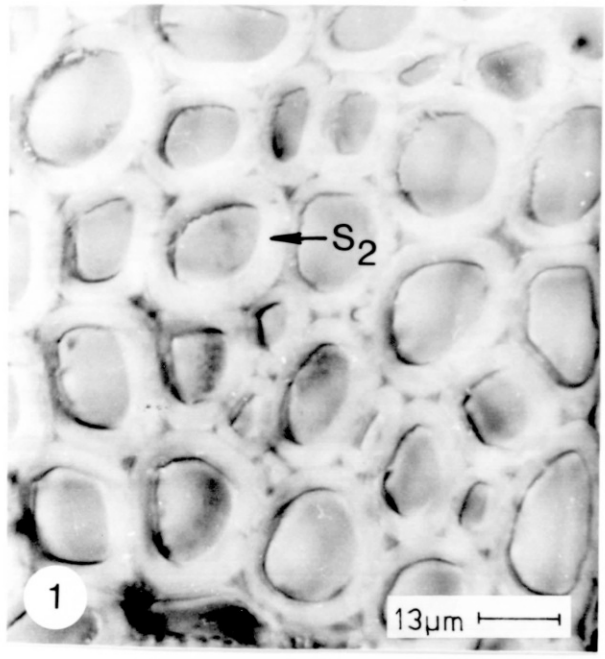
Many cavities (C) in most cells. The S_2 layer of the wall is removed in places (WR).

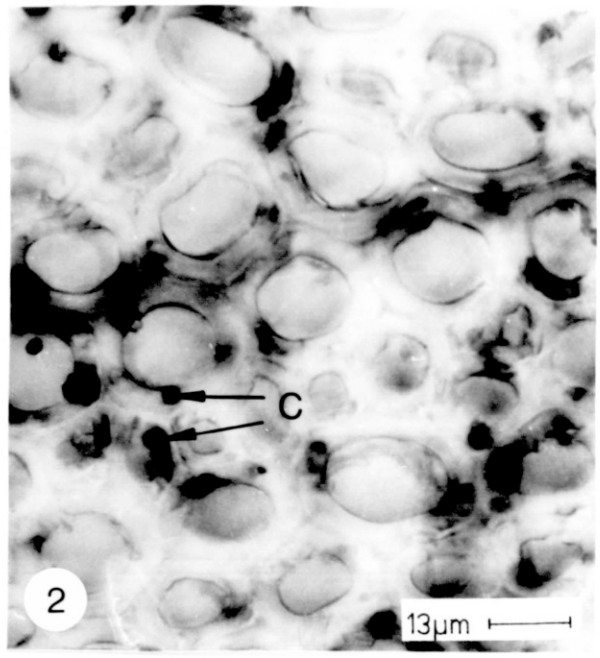
PLATE 4 SRD = 3.

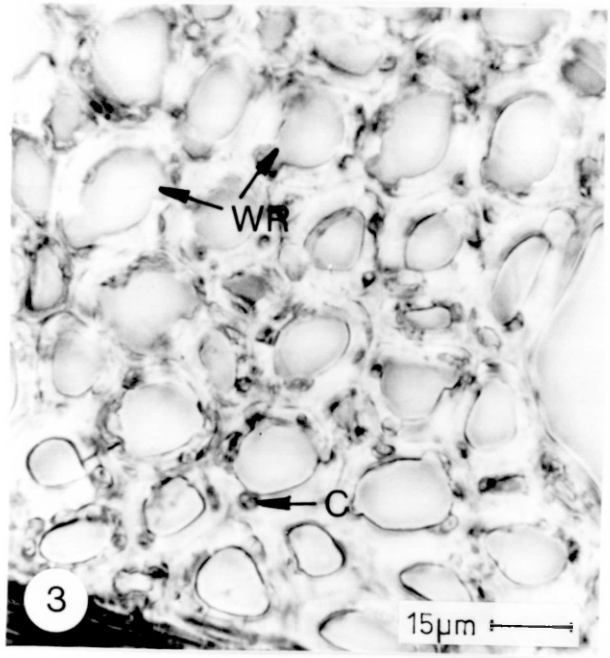
Cavities abundant, complete removal of the S_2 layer of the wall in many cells (WR).

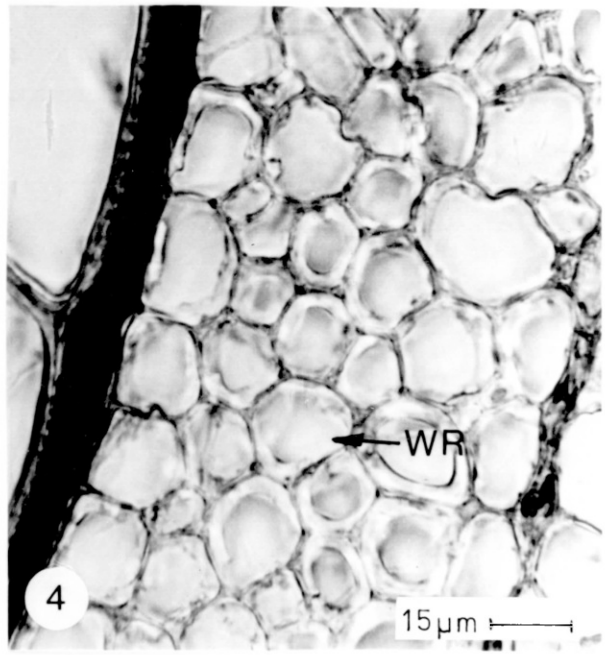
PLATE 5 SRD = 4.

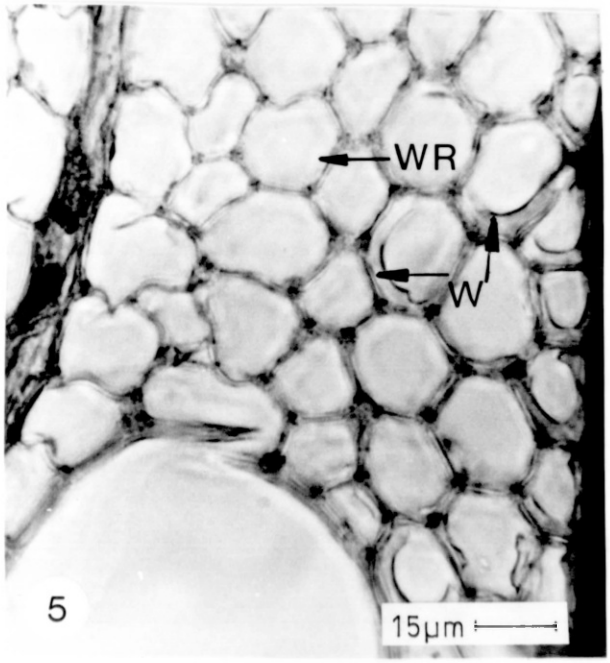
S_2 layer of wall (W) remaining in only one or two cells. The wall is removed in most cells (WR).


S_2
1
13µm


C
2
13µm


WR
C
3
15µm


WR
4
15µm


WR
W
5
15µm

PLATES 6 - 10

Soft rot attack in Scots pine, illustrating the five Soft Rot Degree (SRD) classes of decay. All specimens are transverse sections of latewood of scots pine.

PLATE 6. SRD = 0.

S_2 layer intact, no soft rot cavities.

PLATE 7. SRD = 1.

A few scattered soft rot cavities (C) in the S_2 layer.

PLATE 8. SRD = 2.

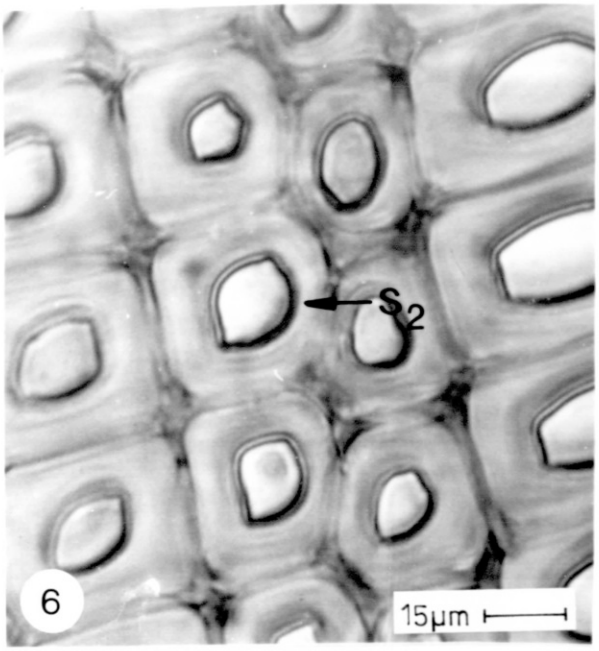
Many cavities (C).

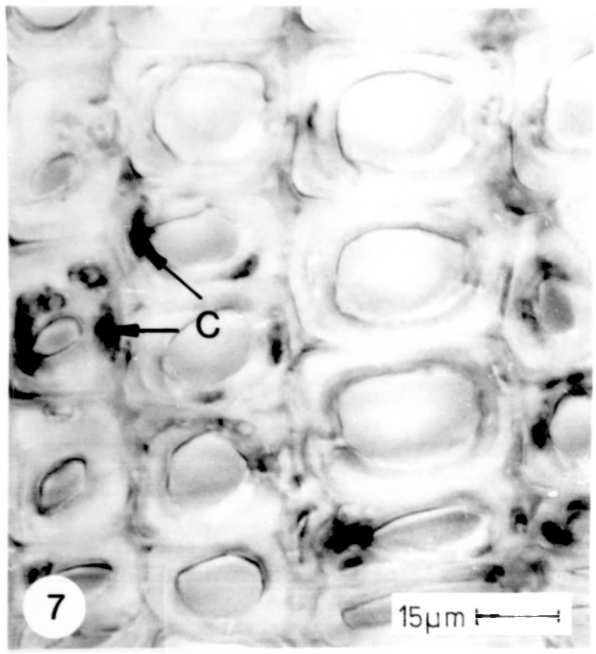
PLATE 9. SRD = 3.

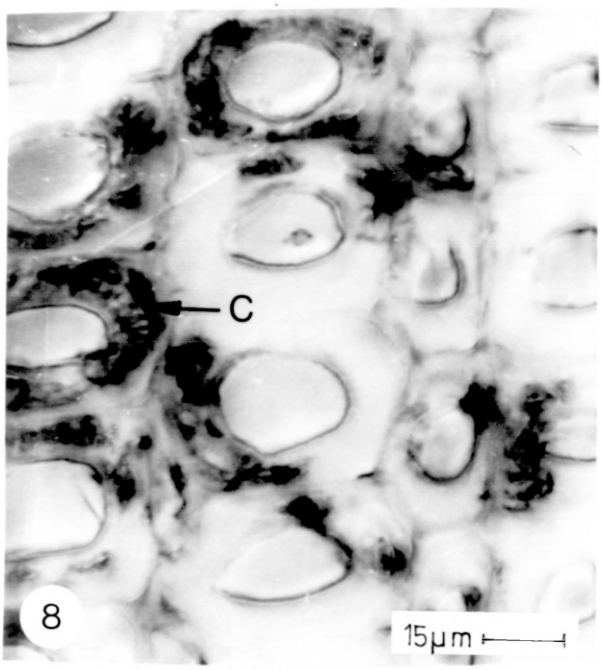
Many cavities in most cells (C).

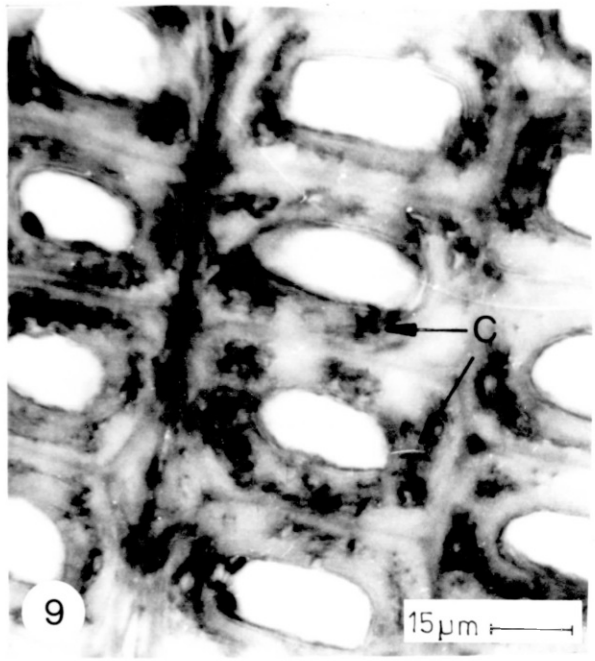
PLATE 10. SRD = 4.

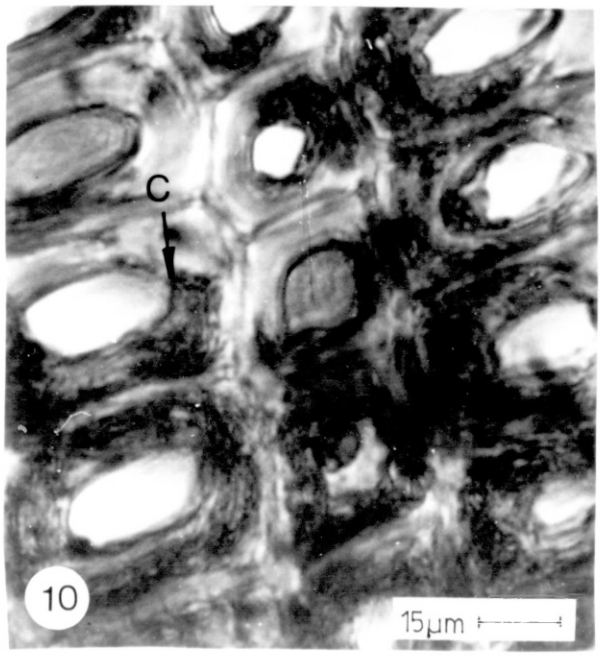
Cavities (C) confluent and abundant in all cells.


S_2
6
15µm


C
7
15µm


C
8
15µm


C
9
15µm


C
10
15µm

2.4.4.3 Method of Visual Assessment of Decay

After removal from unsterile soil, the sections were stored in formalin acetic alcohol (FAA) in McCartney bottles (capacity 28 ml), prior to examination. The fixative, FAA, consisted of:-

90 ml 70% ethanol

4 ml glacial acetic acid

6 ml formalin (37 - 41% formaldehyde)

Corbett (1963) and Greaves (1966) found FAA to be an effective fixative which did not damage the wood tissue or fungal hyphae.

2.4.4.3.1 Staining

Wilcox (1964) and Greaves (1966) compared several staining techniques for differentiating hyphae in wood sections, and concluded that the method of Cartwright (1929) using safranin and picro-aniline blue stains was the most effective.

A number of stains were tested in this work to determine which gave the greatest clarification of soft rot cavities within the secondary wall layer.

The following stains were used:-

(1) Safranin/Picro-aniline blue (Using the method of Cartwright, 1929).

The sections were stained in 1% aqueous safranin for about one minute. Excess stain was then washed out with distilled water before staining in picro-aniline blue. Picro-aniline blue was made up by mixing four parts of saturated picric acid with one part of 1% aqueous aniline blue. A drop of stain was then placed on the section which was warmed in

a flame just to the point of simmering and held there for a few seconds before being thoroughly washed with distilled water. The sections were then differentiated through 70% and absolute alcohol before mounting in molten glycerol jelly.

Using this mixture of stains, lignified tissue is stained red and fungal hyphae are stained blue. In these studies picro-aniline blue was found to have certain disadvantages, such as:-

(a) Precipitation.

(b) Blueing of the whole section, especially when severely decayed.

(c) Short lasting stain, which made it difficult to photograph stained sections after long periods of time.

(d) The soft rot cavities were not easily distinguished.

(2) Safranin/Fast green (Gram and Jørgensen, 1953)

This was found to be a good staining mixture and had the following advantages:-

(a) Soft rot cavities could be clearly seen within the S_2 layer of the cell wall and loss of wall easily observed.

(b) The mixture was easy to apply.

(c) The stain remained effective for long periods of time and was not prone to fading.

Sorkhoh, (1976) also achieved good results with safranin/fast green.

A mixture of safranin and fast green was prepared as follows:- 1.5 g Safranin O and 0.5 g Fast Green were dissolved in 200 ml of ethanol and two drops of concentrated hydrochloric acid were added. The final solution was dark violet. This stained lignified tissue red and fungal hyphae green.

The method of staining the sections consisted of:-

(i) Stain in safranin/fast green for 5 minutes.

(ii) Dehydrate in absolute alcohol.

(iii) Wash with distilled water.

(iv) Mount in molten glycerol jelly.

(v) Place a coverslip over the section.

(vi) Heat the slide until the glycerol jelly melts.

(vii) Apply pressure, by sandwiching the slide between two pieces of wood, all held together with a clothes peg, until the jelly cools.

The sections were examined under a Vickers light microscope for soft rot attack.

2.4.4.3.2 Assessment

1. Low Power (x 100). Five random fields of view were examined to assess the overall extent of decay, to cover the entire section. SRDs were determined for each field of view. (Appendix A).

2. High Power (x 450). Ten random fields of view were examined (Appendix A) and SRDs recorded. Four sections per treatment were used and an overall mean with standard deviation for forty fields of view determined for each treatment, and each time period. Two sections were removed from each of two replicate soil jars to take into account variations between jars. 95% confidence limits were attached to the means. These determined the range in which the mean fell 95% of the time. These means with confidence limits were plotted against incubation time.

2.5 Bacteria

2.5.1 Introduction

The bacteria examined in this work represent a very specialised group of wood-degrading bacteria which penetrate into wood, into the middle layer of the secondary cell wall, the S_2, where they form tunnels or cavities and later complete destruction of the cell wall substance. The S_2 layer is eventually removed. This group of cell wall degrading bacteria has not been characterised in this work due to time limitations. There is some confusion at present amongst research workers as to the exact nature of the bacteria involved. The type of attack found here may be due to the tunnelling bacteria described by Nilsson (1982a), Nilsson and Daniel (1983a), Daniel and Nilsson (1985), Mouzouras et al (1986), or the cavitation bacteria described by Nilsson (1982a), Holt (1983), Nilsson and Singh (1984) or a combination of both. The different types of cell wall attack may arise as a result of the same bacteria growing under varied conditions or different bacteria acting individually or as a mixed association or in succession. The two types were grouped together here and termed S_2 degrading bacteria. The important fact for the purpose of this study was the ability of this group of bacteria to degrade the S_2 layer of the wood cell wall, thus making them amenable to the thin section assay.

Attack by S_2 degrading bacteria has been reported in wet terrestrial and aquatic habitats (Holt, 1981; Nilsson, 1982a; Nilsson and Holt, 1983; Nilsson and Daniel, 1983a; Daniel and Nilsson, 1985; Mouzouras et al, 1986). Earlier reports from the marine environment attributed this type of attack to actinomycetes (Eaton and Dickinson,

1976; Leightley and Eaton, 1977; Kohlmeyer, 1980), Nilsson and Holt (1983) demonstrated that the causative organisms were single-celled bacteria of the tunnelling or cavitation (S_2 degrading) type.

The bacteria are able to attack timbers treated to high retentions with preservatives and also durable timbers considered to be very resistant to fungal decay (Nilsson, 1982a). Tunnelling and cavitation bacteria have been associated with the premature failure of treated softwoods in horticultural situations in New Zealand (Butcher et al, 1983, Drysdale and Hedley, 1984; Nilsson, 1984).

Tunnelling and cavitation bacteria had never previously been considered in tests of preservative performance in ground contact. However, like soft rot, once the initial observations had been made and an observer knew what to look for, the bacteria have been recognised more and more frequently. They have yet, however, to be considered in tests of preservative efficacy. Since, like the soft rot fungi, they also attack the S_2 layer of the lignified cell wall, it seemed logical that the thin section assay designed to monitor and assess the soft rot degree, SRD, of attack of the S_2 layer could, with little modification , be used to monitor and assess the decay of the S_2 layer caused by S_2degrading bacteria. It was therefore decided to produce a bacteria degree or BD, which could be used to assess their reactions to wood preservatives. Using the thin section assay, these bacteria may become a useful tool and might well act as an early indication of those preservative formulations likely to give better long term performance.

Firstly a series of preliminary tests were carried out to determine the conditions required for producing attack by S_2 degrading bacteria in untreated and treated wood buried in unsterile soil in the laboratory.

Previously this type of attack has only been reported in material collected from the field (Butcher et al, 1983; Drysdale and Hedley, 1984; Nilsson, 1984) and in certain fungal cellar soils (Nilsson, 1982a; Nilsson and Daniel, 1983a).

Once the conditions required for producing this type of bacterial attack in the laboratory had been determined, it was possible to use the thin section assay system to obtain indications of the long term performance of existing and alternative preservatives, against S_2 degrading bacteria. Results could then be correlated with those from soft rot studies of preservative performance.

2.5.2 Soil Types

The number and types of bacteria present in soil are governed to a large extent by soil type and cultivation practices (Alexander, 1977). In New Zealand, different soils were found to represent different decay hazards (Drysdale and Hedley, 1984), for treated horticultural radiata pine posts which had been planted for twelve years. Also for treated radiata pine posts exposed in a soil bed (Drysdale, 1984).

A number of soils were therefore examined in this work to find a suitable type for use with the thin section technique using S_2 degrading bacteria.

In preliminary tests a range of soil water holding capacities was used with each of the soils, to determine the optimum moisture conditions for bacterial attack.

2.5.3 Preliminary Tests

2.5.3.1 Preliminary Test I : Chemical Pretreatment

To determine rapidly those soils likely to be suitable for culturing S_2 degrading bacteria, wood sections were pretreated with concentrated hydrofluoric acid prior to burial in soil (T.Nilsson, pers. comm.).

Hydrofluoric acid removes the carbohydrates from wood (Clark, 1962), thus leaving a readily accessible lignin portion for bacterial attack. Therefore, a rapid determination of the most suitable soils for bacterial attack can be made.

S_2 degrading bacteria are known to attack the carbohydrate portion of wood but it has been disputed that they can degrade lignin. It has been reported that a pretreatment of the wood, a delignification in laboratory tests is required before the bacteria can attack the wood (Greaves, 1965, 1970, 1973; Holt, 1976, 1981; Furtado, 1978; Schmidt, 1978, 1980; Holt and Jones, 1978; Holt, Jones and Furtado, 1979; Schmidt and Liese, 1982). However, these reports do not clarify observations that bacteria can deteriorate timber in service. In this situation, attack may be facilitated by very specific bacteria, by prolonged incubations or by environmental conditions. It has now been proved invalid that a mild delignification of the wood is required for attack by S_2 degrading bacteria (Nilsson, 1982a; Nilsson and Daniel, 1983a). Recently the capacity of some wood degrading bacteria for lignin biodegradation has been demonstrated by biochemical studies using ^{14}C-labelled and synthetic lignins (Daniel and Nilsson, 1985, 1986). Daniel and Nilsson (1986) and Mouzouras *et al* (1986) have shown that all layers of the wood cell wall are degraded by the

S_2 degrading bacteria. In the work reported in this thesis, patterns attributed to these bacteria have been found in wood, in which the carbohydrates have been removed (by hydrofluoric acid treatment), leaving only the lignin portion open to attack. This further emphasises the ability of this group of bacteria to degrade lignin.

2.5.3.1.1 Method

Thin sections, 30 μm, were cut from birch and Scots pine sapwood 7 mm^3 blocks using a Reichert sledge microtome. Transverse, tangential longitudinal and radial longitudinal sections were used in this test. The sections were placed in 70% hydrofluoric acid in a plastic beaker for four days at 4 ^{o}C. The acid was then drained off, and the sections washed in distilled water for a further five days. Wood blocks, 7 mm^3, were also treated with hydrofluoric acid and included in the soil burial tests. Untreated, control and treated blocks, together with the thin sections cut from them, were buried in the different soils. The sections of wood were placed in nylon mesh packets prior to burial in soil (Section 2.2 as for soft rot thin section studies), and dipped in distilled water.

Eight different soils were used in this test:-

(1) Garden loam. Collected from the garden of Mr J.G.Savory, Princes Risborough, Bucks. This soil is referred to as garden loam in this thesis.

(2) Rentokil Soil. (See section 2.4.1) From the same source as that used for soft rot studies.

(3) A horticultural loam, John Innes No.2.

(4) A garden soil dug from Silwood Park (Imperial College Field Station), Ascot, Berks.

(5) A potting soil from Silwood Park. A mixture of loam, peat and sand.

(6) Princes Risborough Soil from the same source as that used for soft rot studies (See section 2.4.1).

(7) A 50:50 mixture, by weight, of Silwood garden soil and John Innes No.2.

(8) A 50:50 mixture of Princes Risborough Soil and John Innes No.2.

The soils were partially dried by spreading out on plastic sheeting in a greenhouse. Occasionally, the soil was turned until it was dry enough to sieve. A 2 mm mesh size sieve was used.

The water holding capacity (w.h.c.) of the soils was determined using the method of Carey and Grant (1975) (section 2.4.2). Soils at three w.h.c.s were used, 80%, 100% and 120%, to determine the range of moisture contents over which bacterial attack could occur. The amount of water required to produce these w.h.c.s was added to the soil in ointment jars, as in section 2.4.2.

The thin sections of wood in the mesh packets, together with the wood blocks were buried in the eight different soils at each of the three w.h.c.s. The jars containing the soils were placed in a fungal cellar, temperature 28 ^{o}C, relative humidity 80%.

Sections and wood blocks were removed after 2, 4, 8, 12, 16, and 30 weeks, from two sets of replicate soil jars. Thin sections were cut from the outer faces of the blocks and these, together with the thin sections in the mesh packets, were stored in the fixative, formalin acetic alcohol, prior to staining and examination under the light microscope.

Nilsson and Daniel (1983a) recommended the use of chlorazol

sky blue in lactophenol or cotton blue in lactophenol for staining the decay patterns caused by the S_2 degrading bacteria in the cell walls.

Thin sections were stained in cotton blue in lactophenol for five minutes, washed with distilled water, then mounted in lactophenol.

The sections were examined under the light microscope for bacterial attack, any soft rot attack was also recorded.

2.5.3.1.2 Results and Discussion

Attack of the wood cell walls by S_2 degrading bacteria was absent in all sections examined from 2 to 16 weeks burial in the eight soils at the three w.h.c.s. Soft rot cavities were, however, found in the Scots pine and birch sections.

After 30 weeks burial, one or two untreated and hydrofluoric acid treated, Scots pine sections, were found to show attack by the bacteria. Birch sections were, again , only attacked by soft rot fungi. The bacterial attack was confined to sections buried in three of the eight soils, the garden loam, Rentokil soil and John Innes No.2.

The decay patterns were very similar to those found by other workers who attributed this type of attack to actinomycetes (Eaton and Dickinson, 1976; Leightley and Eaton, 1977; Holt et al, 1979; Holt, 1981). The patterns were also similar to those observed by Nilsson and Daniel (1983a) and Daniel and Nilsson (1985), who attributed the decay to tunnelling bacteria. Nilsson (1984), Drysdale and Hedley (1984) found these patterns in CCA treated horticultural radiata pine posts in New Zealand.

At this stage, a degree of attack was not recorded, only presence or absence.

From the lack of results obtained in the first 16 weeks of soil burial of the sections and wood blocks it was decided that the conditions were not suitable for bacterial attack. After reviewing the literature for bacterial attack of experimental stakes buried in fungal cellar soils (Nilsson, 1982a; Nilsson and Daniel, 1983a; Nilsson and Holt, 1983), it became clear that attack should have been more rapid. The use of thin sections provides a readily accessible substrate for attack by microorganisms. If the bacterial attack had been prevented by the use of the hydrofluoric acid pretreatment, then attack of untreated sections would have been expected.

The presence of soft rot cavities was not thought to be a factor responsible for preventing the colonisation of the S_2 degrading bacteria. The two forms of attack have been reported occurring together in a number of instances (Nilsson and Daniel, 1983a; Drysdale and Hedley, 1984).

It was concluded that both the predrying regime, employed for all soils used, was too severe and the w.h.c. of the soils was not high enough. Bacterial attack of wood occurs when wood is very wet, under saturated conditions e.g. wood stored in log ponds or rivers (Stutz and Stout, 1957; Suolahti and Wallen, 1958; Ellwood and Ecklund, 1959; Knuth and McCoy, 1962; Greaves, 1969; Highley and Lutz, 1970; Karnop, 1972), buried in soil (Hawker et al, 1960; Morton et al, 1969; Dwyer and Levy, 1976 ; Nilsson,1982a; Nilsson and Daniel, 1983a), in marine situations (Eaton and Dickinson, 1976 ; Leightley and Eaton, 1977; Kohlmeyer, 1980; Holt, 1981), in mines (Levy, 1964; Levy and Greaves, 1966),or when wood is used in water cooling towers (Greaves, 1969,

1971; Liese and Greaves, 1975).

In New Zealand, the incidence of bacterial decay in horticultural posts was found to be greater in soils with a high water holding capacity (Drysdale and Hedley, 1984).

After reviewing the literature on the occurrence of bacterial attack and in view of the New Zealand experience, it was decided that in future bacterial tests, soil should be used wet, without any form of predrying. The predrying of soil is a standard laboratory procedure when studying fungal decay of wood buried in soil. However, for bacterial tests, predrying of soil may restrict bacterial growth.

Soil at a higher water holding capacity would be used in future tests to promote saturated conditions for the bacteria.

2.5.3.1.3 Conclusions

Results obtained here show that the S_2 degrading bacteria are able to degrade lignin. Bacterial attack was found in wood which had been pretreated to remove the carbohydrates, leaving a lignin rich substrate.

Predrying of the soils for bacterial tests possibly inhibits bacterial decay. The lack of earlier reports of bacterial attack of wood buried in soil in the laboratory may have been due to the use of the standard predrying regime employed for soils in laboratory tests.

Saturated conditions may be required for bacterial attack. These may help to encourage bacterial attack and reduce fungal attack. A higher soil moisture content would be used in future tests.

The three soils which had been found to produce bacterial attack in this preliminary test were used in the next test which was devised to determine how effective saturation of the soils would be, to produce bacterial attack of wood in the laboratory.

2.5.3.2 Preliminary Test II : Wet Soil Test

A soil w.h.c. of 150% was chosen as likely to be most suitable for providing saturated conditions. Three soils were used, those producing bacterial attack in the previous test, section 2.5.3.1. These were the garden loam, Rentokil soil and John Innes No.2.

The hydrofluoric acid pretreatment of the wood sections was used as in the previous test (Section 2.5.3.1) to determine which of the soils used would be suitable for further bacterial tests. Untreated thin sections acted as controls. In addition, sections cut from blocks which had been treated with a CCA preservative, K33(oxide), were used to determine the capability of the bacteria for attacking this type of preserved wood. Untreated, hydrofluoric acid pretreated and K33 treated 7 mm^3 blocks were included in the test.

2.5.3.2.1 Method

Thin sections, 30 μm, were cut from birch and Scots pine 7 mm^3 blocks. Transverse, tangential longitudinal and radial longitudinal sections were used. Some of the sections were put aside for pretreatment with concentrated hydrofluoric acid (Section 2.5.3.1.1). Others remained untreated. Thin sections were also cut from blocks which had been treated with K33 oxide (Section 3.2.3).

The composition of K33 Oxide is as follows:-

CuO	14.8%
CrO_3	26.6%
As_2O_5	34.0%
H_2O	24.6%

A 2.45% solution was used.

The thin sections were placed in mesh packets, dipped in distilled water and buried in the three soils (Section 2.2.2). Untreated and treated whole wood blocks were also buried in the soils.

The three soils were not predried. They were collected wet and their w.h.c.s calculated, together with the amount of water required to bring the soils to 150% of their w.h.c. (Table 4), using the method in Section 2.4.2. This water was added to the soils in glass jars. The excess water on the surface of the soil produced saturated conditions within the soil.

Table 4

Water Holding Capacity Determinations For Soils

Soil		% Moisture Content of soil at w.h.c.	% Moisture Content of soil as used prior to wetting	Vol. of water added to bring soil to 150% w.h.c (ml)
Garden Loam	1	46.66	41.53	40.22
	2	55.35	44.04	54.13
	3	50.82	42.59	47.18
Mean		50.94	42.72	47.18
Rentokil Soil	1	24.13	26.12	15.98
	2	31.49	28.65	28.89
	3	25.17	26.89	17.13
Mean		26.93	27.22	20.67
John Innes No.2	1	35.20	16.80	61.64
	2	37.45	19.05	62.37
	3	34.87	15.90	62.82
Mean		35.84	17.25	62.28

The thin sections in the mesh packets, together with the wood blocks, were buried in the three soils at a moisture content of 150% w.h.c. and removed after 2, 4, 6, 12, 16, 20 and 30 weeks from two sets of replicate soil jars, for examination under the light microscope. Sections were also cut from the outer surfaces of the buried wood blocks. The sections were stained in cotton blue in lactophenol (Section 2.5.3.1.1) and mounted in lactophenol. The presence or absence of bacterial and soft rot attack was recorded.

2.5.3.2.2 Results and Discussion

Birch sections were only attacked by soft rot fungi. The S_2 layer of the Scots pine sections was attacked by both bacteria and soft rot fungi (Table 5).

The increased moisture content of the soil in this preliminary test clearly increased the occurrence of attack by S_2 degrading bacteria.

If the results for the occurrence of bacterial attack (Table 5) are compared with the figures obtained for the determination of the w.h.c. of the soils (Table 4), it can be seen that the Rentokil soil, producing the least amount of attack, had the lowest moisture content of the three soils used. Rentokil soil had a moisture content of only 26.93% compared with 35.84% for John Innes No.2 and 50.94 for the garden loam. The moisture content of the Rentokil soil was probably too low to wet the wood to a sufficient moisture content to permit active degradation of the wood cell wall by bacteria, even at 150% w.h.c., where the moisture content was 40.40%. This was marginal, certainly in the case of wood blocks. The blocks may have lowered the moisture content of the soil too far for the moisture content

of the wood to be adequate. The moisture content of the Rentokil soil was, however, suitable for soft rot attack which was recorded most frequently in this soil.

Bacterial attack of wood sections buried in John Innes No.2 occurred more frequently than in Rentokil soil. However, the best results were achieved with the garden loam which had a very high moisture content, 50.94%, almost twice that of the Rentokil soil.

The moisture content of the soil clearly affected attack by the S_2 degrading bacteria. Treatment with K33 did not prevent bacterial attack or soft rot fungal attack. The hydrofluoric acid pretreatment enhanced attack only in the Rentokil soil. In the other soils attack also occurred in untreated sections. Nilsson (1982a) found that tunnelling bacteria were very common in preservative treated experimental stakes exposed in unsterile soil in a fungal cellar. He reported that treated Scots pine sections were attacked by tunnelling bacteria, regardless of the preservative type or retention. In the field, tunnelling or cavitation bacteria, together with soft rot fungi have been responsible for the premature decay of CCA treated horticultural posts in New Zealand (Nilsson, 1984; Drysdale and Hedley, 1984). Very high concentrations of preservative form no barrier against tunnelling bacteria (Nilsson and Daniel, 1983a). The bacteria have been shown to immobilise heavy metals intra- and extra-cellularly (Mouzouras et al, 1986).

The soils used were analysed at Rothamstead Experimental Station, Harpenden, Herts, (Table 6), to assess the effects of differences between the soils on their ability to produce bacterial attack in buried wood sections.

Table 5

Occurrence of S_2 Degrading Bacteria and Soft Rot Fungi in Scots Pine Sections Buried in Soils at a Moisture Content of 150% of W.H.C

Soil	Treatment	Incubation Time (Weeks)						
		2	4	6	12	16	20	30
Garden Loam	Untreated	-	B	-	-	SR, B	B	B
	Hydrofluoric Acid (HF)	-	B	-	-	B	B	B
	K33	-	B	B	-	-	-	B
Rentokil Soil	Untreated	-	-	-	SR	SR	SR	SR
	HF	-	-	-	-	-	B	B
	K33	-	-	-	-	SR	SR	SR
John Innes No. 2	Untreated	-	-	-	-	-	-	-
	HF	-	-	B	-	-	B	B
	K33	-	-	-	B	B	B	B

B = Bacteria

SR = Soft Rot

- = No attack

Table 6

Composition Of Soils Used

Soil	Organic Carbon (%)	Total Nitrogen (%)	Phosphorus (%)	Cation Exchange Capacity ($mEq\ 100g^{-1}$)	pH
Garden Loam	4.9	0.41	11.31	35	7.61
Rentokil Soil	2.3	0.16	1.12	16	5.65
John Innes No.2	4.6	0.32	6.46	25	6.65

The decay of wood buried in soil is influenced by water content, organic carbon content, mineral salts, nitrogen and phosphorus content and pH of the soil (Alexander, 1977). The soil analysis results confirmed that Rentokil soil was lower in the above factors. This, combined with the low moisture content at w.h.c., recorded in Table 4, suggests that the Rentokil soil would be expected to yield poor results in terms of producing attack by S_2 degrading bacteria in the buried wood sections.

Nitrogen and phosphorus are important inorganic nutrients required by plants and microorganisms. The community size in mineral soils is directly related to organic matter content, humus-rich soils having the largest bacterial numbers (Alexander, 1977).

In New Zealand, those soils producing the most active decay of CCA treated radiata pine posts by soft rot fungi, together with tunnelling and cavitation bacteria, had been heavily fertilised and had the highest phosphate content of all the soils examined (Drysdale,

1984). In the work reported here, results show that the garden loam had a very high nitrogen and phosphorus content, compared with the other soils. This soil proved to be the most effective used, producing bacterial decay patterns after only four weeks burial of wood sections. This garden loam had a very similar composition to that of Princes Risborough soil used in the previous preliminary test (Section 2.5.3.1). The two soils differed only in their phosphorus content, that of the garden loam being 11.31% whilst that of Princes Risborough soil was only 4.33%. The Princes Risborough soil was not effective in the previous test and did not produce attack by the S_2 degrading bacteria. The phosphorus content of the Princes Risborough soil was lower than that of John Innes No.2 which was 6.46%. John Innes No.2 produced bacterial decay patterns both in this test and the previous test. Clearly there may be a correlation between phosphorus content of the soil and attack by S_2 degrading bacteria in that soil.

Da Costa (1971, 1972) reported that the addition of inorganic phosphate to the testing medium, or to test blocks, reduced the effectiveness of a CCA preservative. The soils in New Zealand may have had high enough phosphate contents to reduce the effectiveness of the CCA in the horticultural posts, thus allowing fungal and bacterial attack. Boutelje and Göransson (1972) found that the degree of degradation by bacteria was correlated with the amount of organic nitrogen and phosphate in samples taken from foundation pilings below the ground water table.

Soil pH is important, bacteria and actinomycetes are uncommon in acid habitats but fungi thrive. The Rentokil soil had a low pH which, in conjunction with low moisture content, probably contributed to the increased occurrence of soft rot attack in this soil.

Birch sections were not found to be susceptible to bacterial attack, these had been attacked by soft rot fungi. This confirms the findings of Nilsson (1982a) who recorded that tunnelling bacteria could not be found in hardwood samples which had been shown to be very susceptible to soft rot, for example, birch and beech. The soft rot fungi may have competed with the bacteria. Nilsson (1982a) reported attack by tunnelling bacteria in birch when the soft rot fungi were suppressed by the antifungal antibiotic natamycin. In addition, birch wood may have been too dry for bacterial attack, Scots pine wood wets up very quickly. A much higher moisture content at w.h.c. may have produced bacterial attack in birch.

2.5.3.2.3 Conclusions

The garden loam was the most suitable soil for producing attack by S_2 degrading bacteria, attack being rapid, within four weeks of soil burial. The moisture content of the soil affected the occurrence of bacterial attack. The use of soil at a moisture content of 150% of its w.h.c. in this test increased the frequency of occurrence of bacterial attack over that recorded in the previous test using a moisture content of 95% of soil w.h.c. In wet soils in New Zealand where free drainage of water is restricted, tunnelling and cavitation bacteria have been more important than soft rot (Butcher, 1984). Even at a moisture content of 150% of soil w.h.c., Rentokil soil was too dry for bacterial attack to occur and was also lacking in the elements analysed. The decay of wood buried in soil appears to be influenced by moisture content, organic carbon, nitrogen and phosphorus content and pH.

The conditions produced in the soil jar were saturated, with

water on the surface of the saturated soil.

Attack by S_2 degrading bacteria was not affected by pretreatment of the sections with hydrofluoric acid, or by the presence of a preservative within the wood.

Birch sections were not found to be susceptible to bacterial attack.

This wet soil system was taken a stage further in the next preliminary test, to determine the range of soil w.h.c.s over which bacterial attack could occur. Only the garden loam was used.

Thin sections only, in preference to wood blocks, were used in the next test as attack of the surface sections of blocks was very similar to that of thin sections but less frequent.

2.5.3.3 Preliminary Test III : Varying The Moisture Content of the Soil

Having established a bacterial soil system using garden loam, a range of soil moisture contents lower than that used in the previous test, was tested.

2.5.3.3.1 Method

Thin sections, 30 μm, were cut from birch and Scots pine 7 mm^3 blocks. Transverse, tangential longitudinal and radial longitudinal sections were used. Some of the sections were pretreated with hydrofluoric acid prior to soil burial (Section 2.5.3.1), others remained untreated.

Sections were also cut from wood blocks which had been treated with a 2% solution of K33 oxide (Section 3.2.3).

Three soil moisture contents were chosen, 95%, 110% and 125% of the soil w.h.c. The amount of water needed to raise the soil in the jars to the required moisture content was calculated (Section 2.4.2).

The thin sections were placed in mesh packets, dipped in distilled water and buried in the garden loam. The sections were removed after 2, 4, 6, 12, 16, 20 and 30 weeks from two sets of replicate soil jars. They were stained in cotton blue in lactophenol and mounted in lactophenol for examination under the light microscope. The presence or absence of attack by S_2 degrading bacteria and soft rot fungi was recorded (Table 7).

2.5.3.3.2 Results and Discussion

Attack by S_2 degrading bacteria was restricted to Scots pine sections as in the previous test.

Table 7 shows that the soil moisture contents used in this test were suitable for producing soft rot attack, this attack was more frequently recorded than bacterial .

At a soil moisture content of 95% of w.h.c. attack was restricted to soft rot in untreated sections whilst in HF pretreated and K33 oxide treated sections, attack by S_2degrading bacteria, together with soft rot fungi, occurred. If the moisture content of the soil was marginal, it would be expected that there would be some soft rot and some bacterial attack. Table 7 shows that apart from untreated sections at a soil moisture content of 110% of the w.h.c., the majority of

observations are of soft rot attack. Looking at the results as a whole, 21 observations of soft rot were recorded, 8 of S_2 degrading bacteria and 8 of soft rot plus bacteria. Clearly soft rot attack predominated at these lower soil moisture contents.

If the results obtained in this test are compared with those from the previous test where a soil moisture content of 150% of w.h.c. was used (Section 2.5.3.2.2), for the garden loam, observations of attack by tunnelling bacteria outnumber those of soft rot, B(10), SR(nil), SR+B(1).

These results would imply that a soil moisture content of at least 150% of w.h.c. is required to preclude fungal attack. Furtado (1978) suggested that the degradation of wood by bacteria could best be appreciated under conditions which preclude growth of other microorganisms e.g. where wood is subjected to waterlogging and low oxygen contents (Boutelje and Bravery, 1968; Greaves, 1968; Holt, Jones and Furtado, 1979) or impregnated with high concentrations of preservatives (Hochman, 1967; Greaves and Foster, 1970; Irvine and Jones, 1975; Eaton and Dickinson, 1976).

Bacterial attack of wood has been shown to occur in a variety of environments but eventually the wood must be very wet (under saturated conditions) e.g. when wood is stored in log ponds or rivers, buried in soil, in marine situations, in mines or when wood is used as a water container or conducting surface as in water cooling towers and ancient troughs and conduits. Fungal growth has rarely been observed in anaerobic muds, confirming reports that they are inactive in conditions of low oxygen concentration (Boutelje and Bravery, 1968; Laurent, 1969; Boutelje and Göransson, 1972; Eslyn and Clarke, 1976). Conditions in the soil used in these preliminary tests must be only

Table 7

Occurrence of S_2 Degrading Bacteria and Soft Rot Fungi in Scots Pine Sections Buried in a Garden Loam at different Soil Moisture Contents.

Soil w.h.c.	Treatment	Incubation Time (Weeks)						
		2	4	6	12	16	20	30
95%	Untreated	-	SR	SR	SR	SR	SR	SR
	HF	-	B	B	B	SR, B	SR	SR
	K33	-	-	-	SR	SR, B	SR	SR
110%	Untreated	B	B	SR, B	SR	SR, B	B	SR, B
	HF	-	SR, B	-	-	SR	SR	SR
	K33	-	-	-	-	B	SR	SR
125%	Untreated	-	-	-	SR	SR	SR	SR
	HF	-	-	-	-	B	-	SR
	K33	-	-	-	-	-	SR	SR, B

B = Bacteria

SR = Soft Rot

- = No attack

partly anaerobic as soft rot attack has been observed.

Pretreatment of the wood sections with hydrofluoric acid did not greatly enhance bacterial attack. The fungi and bacteria were able to attack wood which had been treated with the CCA preservative K33. In New Zealand CCA treated horticultural posts were attacked by soft rot fungi together with tunnelling and cavitation bacteria (Drysdale and Hedley, 1984; Drysdale, 1984; Butcher, 1984; Hedley and Drysdale, 1984).

The omission of predrying of the soils enhanced bacterial attack, both in this test and in the previous test.

2.5.3.3.3 Conclusions

A soil moisture content of 150% of the w.h.c. used in the previous test was the most suitable for producing attack by S_2 degrading bacteria. The soil moisture contents used in this test were too low and allowed fungal attack to predominate. At the higher moisture content fungal attack was virtually eliminated. Saturated conditions proved to be the most effective for studying bacterial attack without interference from soft rot fungi. S_2 degrading bacteria are able to degrade lignin, they attacked wood sections which had been pre-treated with hydrofluoric acid and which had their lignin directly exposed to attack. The bacteria are also able to attack wood which has been treated with a CCA preservative.

2.5.3.4 Conclusions From Preliminary Tests

From the results of this series of preliminary tests the following conclusions were drawn :-

(1) Predrying of the soil prevents or delays bacterial attack. Soils need to be collected wet then the required amount of water added to achieve the desired soil moisture content.

(2) A garden loam produced the most rapid attack by S_2 degrading bacteria. Attack occurred in Scots pine sections after only four weeks soil burial. Soils vary in their ability to promote decay. Soil moisture content, organic carbon, nitrogen and phosphorus content, together with pH all appear to be important.

(3) Soils need to be saturated to eliminate interference from fungi. A soil moisture content of 150% of the w.h.c. provides suitable conditions for bacteria.

(4) No form of pretreatment of the wood e.g. in the form of hydrofluoric acid, is required to produce bacterial attack. Attack occurred as rapidly and as frequently as that in wood that had been pretreated.

(5) The attack of wood which had been pretreated with hydrofluoric acid (a lignin rich substrate), emphasises the ability of S_2 degrading bacteria to degrade lignin.

(6) Treatment with the CCA preservative, K33 oxide, does not prevent the S_2 degrading bacteria or soft rot fungi from attacking the wood.

(7) Birch sections were heavily attacked by soft rot fungi but showed no signs of attack by bacteria. Competition from soft rot fungi is thought

to prevent bacterial attack in timbers very susceptible to soft rot attack e.g. birch (Butcher and Nilsson, 1982; Nilsson and Daniel, 1983a).

(8) The thin section cell wall assay is effective at producing rapid attack by S_2 degrading bacteria as well as soft rot fungi, within the wood sections.

Having established a suitable system for producing bacterial attack in wood in the laboratory, this system was then used to examine the performance of various existing and alternative preservatives and formulations.

Firstly it was necessary to devise a method of quantitatively assessing attack by S_2 degrading bacteria. For this purpose the Bacteria Degree or BD was developed.

2.5.4 Methods of Assessing Attack By S_2 Degrading Bacteria

Bacterial degradation of wood proceeds slowly compared with fungal decay, it often causes extensive surface degradation and may have been ignored by other workers who removed surface layers when examining decayed wood blocks.

The disadvantages associated with weight loss methods of assessment of soft rot attack (Section 2.4.4) apply even more strongly to bacterial studies. Holt and Jones (1983) found very small weight losses in wood blocks attacked by bacteria. Bacterial attack begins at the surface of the wood and initially relatively little weight loss occurs.

Wood attacked by bacteria is often macroscopically sound, although the outer layers are sometimes very much softened. Attack by S_2 degrading bacteria is more difficult to detect macroscopically than other types of decay.

The thin section cell wall assay used in this work, relies on the microscopic examination of buried wood sections. The bacteria are allowed immediate access to the S_2 layer of the wood cell wall. Therefore, the performance of any treatment of the S_2 layer can be directly assessed. The assay acts as a screening technique indicating the effectiveness of formulations against bacterial attack. Information can be gathered as to the relationship of the bacteria to the treated wood, at the cell wall level.

There are no tests existing at the moment to assess preservative performance against bacterial attack. There are a number of advantages associated with using this new thin section technique for bacterial

assessments. These are :-

(1) Bacteria are thought to prefer samples of a small volume but a larger surface area (Holt and Jones, 1978; Schmidt, 1978). Thin sections used here are preferable to wood blocks in this respect.

(2) Bacteria cause extensive surface degradation of wood. The use of thin sections allows the bacteria direct access to the wood cell wall layers and therefore increases the rate of attack. In New Zealand attack by tunnelling and cavitation bacteria was confined to the outer few millimetres of horticultural posts.

(3) Bacterial attack is much slower than fungal attack, but with the thin section assay the attack can be seen as soon as it begins and so the assay can be regarded as an accelerated test. The efficacy of preservatives can be rapidly assessed using the assay, results can be obtained in weeks which would take years to develop in the field.

(4) The use of thin sections allows an examination of the relationship of the bacteria to the treated wood, at the cell wall level. Bacterial attack can be assessed in relation to the microdistribution of the preservative in the cell wall layers.

(5) Attack by soft rot fungi can be assessed at the same time using the assay. An overall degree of attack can be determined. Preservative efficacy can be tested in terms of performance against S_2 degrading bacteria and soft rot fungi.

The stages of degradation of the S_2 layer of the cell wall by bacteria can be classified in a similar manner to those of soft rot attack (Section 2.3.3.2).

2.5.4.1 Visual Assessment of Decay

The pattern of bacterial attack observed in this work in the light microscope was very similar to that described by Nilsson and

Holt (1983), who attributed the attack to S_2 degrading bacteria, without characterising the bacterial type. The most characteristic features of S_2 degrading bacteria are growth within the secondary wall of wood cells and their ability to produce tunnels or cavities through the wood substance.

The pattern of attack observed is characterised by a heavily degraded central area, surrounded by a fringe of radiating branched tunnels (Figure 3). Decay by S_2 degrading bacteria is initiated by a single bacterium eroding through the S_3 into the S_2 layer (Figure 3a). Here it produces a cavity which is enlarged when the organism grows and divides. As the bacteria increase in number they start to tunnel within the S_2 layer, forming narrow channels (Figure 3b). The bacteria in the channels cause branching when new individual tunnels occur in different directions after cell division (Figure 3c). Tunnelling is not restricted to the S_2 layer but can also be seen in the S_1 layer, especially in softwoods and the channels extend through the middle lamella from one fibre to another.

In the advanced stages of attack (Figure 3d) a granular central area can be seen, produced by channels coalescing, surrounded by a radiating fringe of cavities or narrow, branched channels. Continued attack leads to complete destruction of the S_2 layer.

This pattern of bacterial attack has been described by other workers (Nilsson, 1982a; Nilsson and Daniel, 1983a; Daniel and Nilsson, 1985), who have attributed the attack to tunnelling bacteria. Comparing the light micrographs produced here (Plates 11-17, 24-29) with those micrographs obtained by the above authors, it would appear that the patterns observed in this work may be due to tunnelling bacteria. As will be shown later, however, (Section 4.3), scanning electron

micrographs depict patterns attributable to cavitation and erosion bacteria, as well as tunnelling bacteria (Nilsson, 1982a; Holt, 1983). Time did not permit a detailed investigation into the exact nature of the bacteria involved here.

A Bacteria Degree (BD) was determined, as an estimate of decay in terms of loss of cross-sectional area of the secondary cell wall, when viewed with the light microscope, compared with a defined standard (Table 8).

Attack by S_2 degrading bacteria of the hardwood, birch, was not observed, the BD standards have therefore been defined only for softwoods. Attack was confined to Scots pine sections.

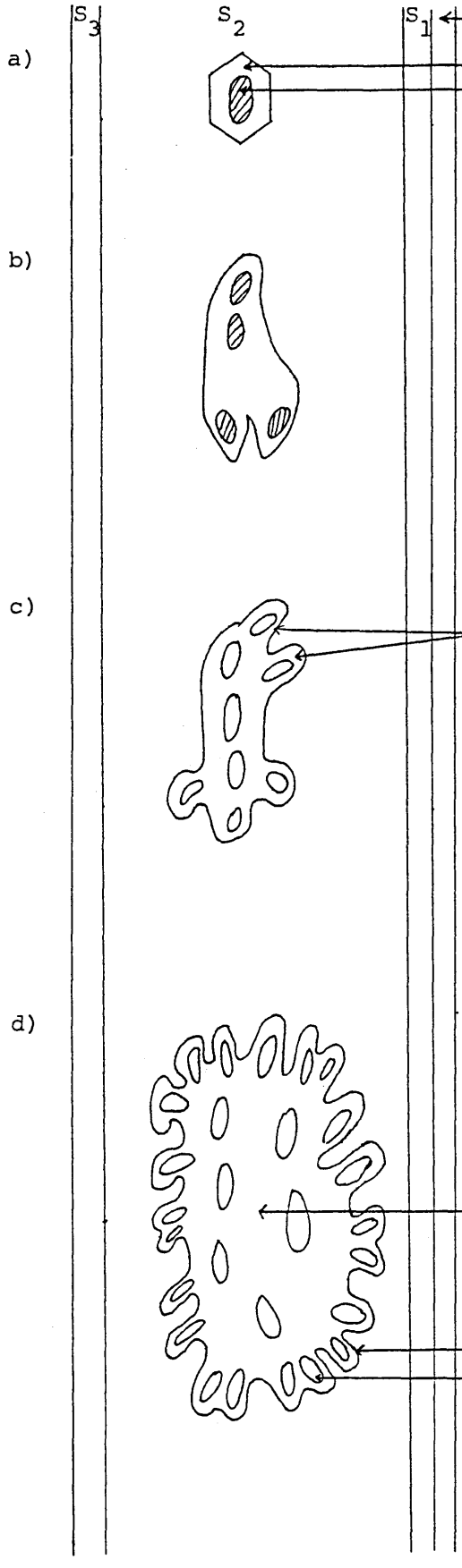
Table 8

Definition of Bacterial Degree

BD Class	Appearance of Longitudinal Thin Section	Area of Disintegrated Secondary Wall (%)
0	No bacterial patterns	0
1	A few bacterial patterns	1 - 10
2	Many bacterial patterns in most tracheids	10 - 40
3	Bacterial patterns abundant, often confluent with each other along S_2 layer of tracheid wall	40 - 90
4	Bacterial patterns confluent, completely degrading S_2	90 - 100

Photomicrographs were taken to illustrate the different S_2 degrading Bacteria Degree classes (Plates 11-15), using a Zeiss Photomicroscope.

Figure 3 Diagram showing the progressive stages of attack by S_2 Degrading Bacteria in the S_2 Layer after gaining access through the S_3 Layer.



A single bacterium initiates the attack by forming a cavity within the S_2 Layer.

As the bacteria increase in number by cell division, some tunnel into the sides of the cavity.

The bacteria produce "secondary" cavities each of which is initially occupied by a bacterium. Branching follows a division by a bacterium at the front end of a tunnel. Two new bacteria produce tunnels in different directions.

Circular or oval patterns result from the bacteria continuously producing short tunnels and cavities.
A heavily degraded central area is surrounded by a fringe of radiating tunnels.

PLATES 11 - 15

Bacterial attack in Scots pine, illustrating the five Bacterial Degree (BD) classes of decay. All specimens are tangential longitudinal sections stained with cotton blue in lactophenol.

PLATE 11. BD = 0.

No bacterial patterns in S_2 layer of cell wall.

PLATE 12. BD = 1.

A few bacterial patterns (BP) in S_2 layer.

PLATE 13. BD = 2.

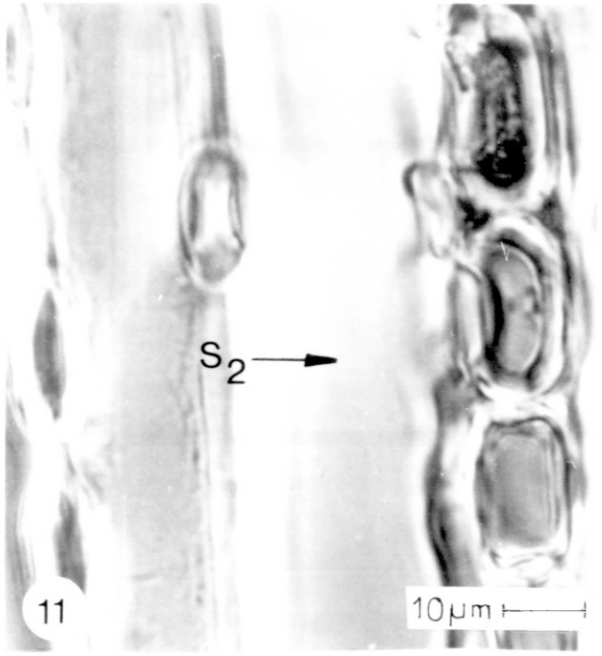
Many bacterial patterns (BP) in most tracheids.

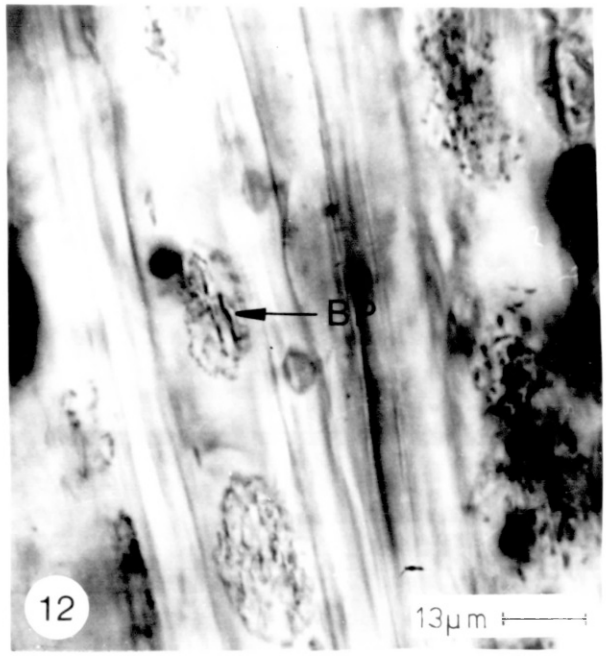
PLATE 14. BD = 3.

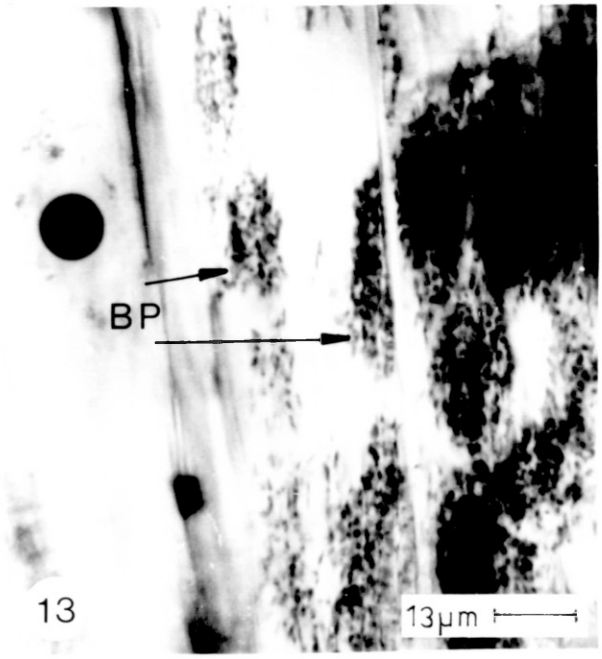
Bacterial patterns abundant, often confluent with each other along the S_2 layer of the tracheid wall. Bacteria (b) seen as dark staining bodies at the ends of channels radiating from a central degraded area (CD).

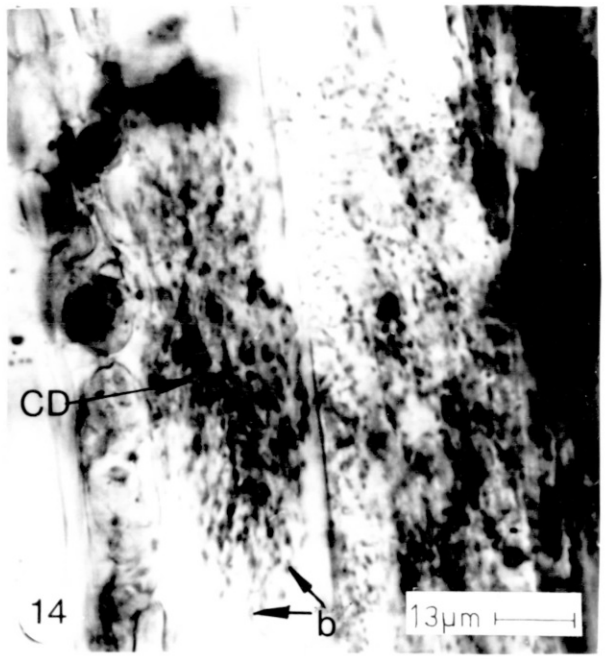
PLATE 15. BD = 4.

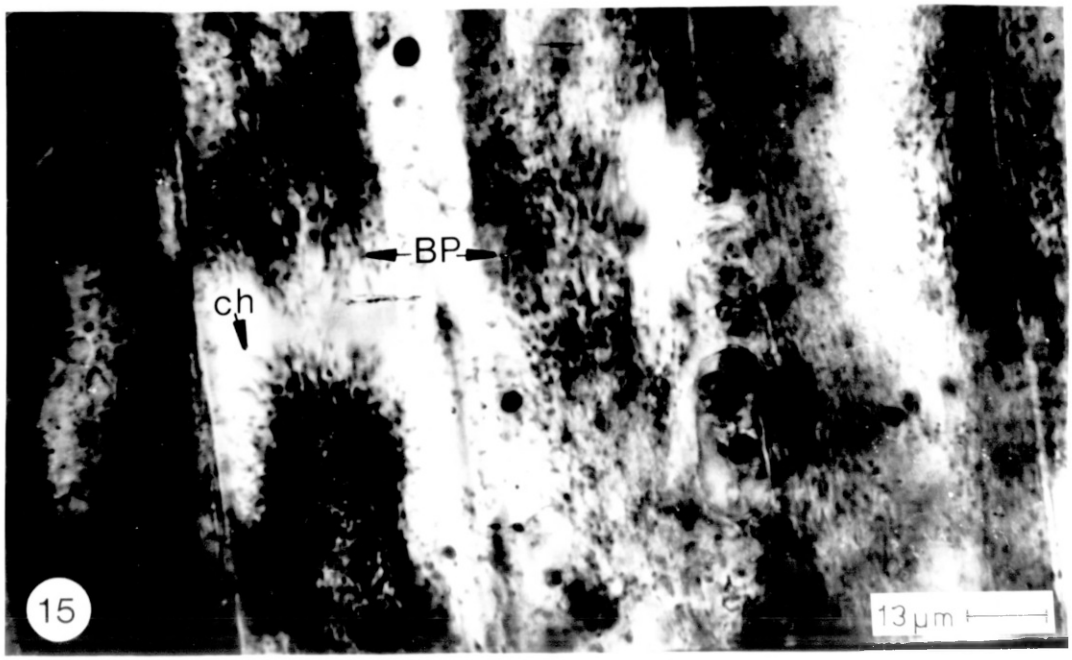
Bacterial patterns (BP) confluent, completely degrading the S_2 layer. Bacteria (b) seen at the ends of channels (ch).


S2
11
10µm


12
13µm


BP
13
13µm


CD
b
14
13µm


BP
ch
15
13µm

2.5.4.2 Method of Visual Assessment of Decay

After removal from unsterile soil, the sections were stored in formalin acetic alcohol, prior to staining and examination under the light microscope.

2.5.4.2.1 Staining

Cotton blue in lactophenol was used as the stain. Sections were placed in this stain for 5 minutes, washed with distilled water, then mounted in lactophenol.

The cotton blue in lactophenol stained the tunnelling bacteria patterns blue and allowed ease of detection of the patterns within the S_2. Any bacteria within the wood were also stained blue.

The sections were examined under a Vickers light microscope for bacterial attack. Any soft rot attack was also recorded.

2.5.4.2.2 Assessment

1. Low Power (x100). Five random fields of view were examined to assess the overall extent of decay. BDs and, where appropriate, SRDs were determined for each field of view. SRDs were adapted for longitudinal sections. Previously in soft rot tests, they had been defined for transverse sections (Section 2.4.4.2). In longitudinal sections, chains of soft rot cavities were produced by the fungi (Plate 28). Sections were scored for frequency of soft rot cavities and S_2 degrading bacteria decay patterns within the S_2 layer.

2. High Power (x450), Ten random fields of view were examined and BDs, together with SRDs recorded. Four sections per treatment were examined, 2 from each of the 2 replicate soil jars, and an overall mean with standard deviation for forty fields of view, determined for each treatment and each time period.

SECTION III: EXAMINATION OF PRESERVATIVE PERFORMANCE USING THE THIN SECTION ASSAY

3. Section III Examination of Preservative Performance Using The Thin Section Assay

3.1 Introduction

Once established as a suitable assay technique for determining attack by soft rot fungi and S_2 degrading bacteria, the thin section assay was used to determine the performance, against these two hazards, of a series of experimental formulations and existing commercially available preservatives.

The thin section assay is suitable for use with both softwoods and hardwoods, with soft rot fungi and S_2 degrading bacteria. The fungi and bacteria both attack the S_2 layer of the wood cell wall, they are allowed immediate access to this layer, without having to come in contact with preservative deposits within the lumen. This allows the effects of the microdistribution of the preservative within the cell wall to be studied.

The assay is more critical than existing tests as it allows an assessment to be made of the performance of preservatives by determining the extent of soft rot and bacterial attack at the cell wall level. The concentration of the preservative within the cell wall controls the fungi and bacteria. Lumen deposits of CCA were described by a number of authors as being important for controlling basidiomycetes which spread through the cell lumina, but unimportant when controlling soft rot, as the fungus bypasses this layer (Dickinson, 1974; Greaves, 1974; Dickinson, Sorkhoh and Levy, 1976; Hulme and Butcher, 1977b; Levy and Greaves, 1978). The thin section assay examining attack at the cell wall level directly assesses the effectiveness of preservatives within

the cell wall.

The assay was used in this work as a rapid screening test for evaluating the performance of experimental formulations and existing preservatives.

3.1.1 Experimental Formulations

Experimental formulations used were modifications of the basic copper chrome arsenic (CCA) preservative, prepared according to BS 4072 Type 2 (1974). (See Section 3.2.2.)

Formulations used were:-

Copper Sulphate	(C)
Copper Chrome	(CC)
Copper Chrome Arsenic	(CCA)
Copper Chrome Boron	(CCB)

The effect of each element within the preservative was therefore examined. The efficacy of CCB as a preservative was tested. Gray (1983) showed that CCB was effective against soft rot in the hardwood, birch. Tillott and Coggins (1981) found that CCB performed better than CCA in hardwoods tested and attributed this to the greater mobility of boron in treated timber. However, they concluded that a high proportion of the active boron components are lost during leaching, rendering CCB treated timber unsuitable in severe leaching situations.

Double treatments were also tested, these were formulations in which the arsenic was added as a second treatment:-

Copper Chrome followed by Arsenic	(CC +A)
Copper Chrome Boron followed by Arsenic	(CCB + A)

Gray (1983) found that formulations in which copper and arsenic were applied separately (CCB; CCB + A) performed better than those in which copper and arsenic were applied together (B + CCA; CCA), in soil burial, soil bed and soft rot monocultures. An attempt was made to confirm this using the thin section assay.

The S_2 layer of the fibres of treated hardwoods is often inadequately treated (Dickinson, 1974; Greaves, 1974; Dickinson et al, 1976; Hulme and Butcher, 1977b; Drysdale, 1979; Drysdale et al, 1980). A certain minimum retention of copper is required to provide effective control of decay. Gray (1983) has shown that by allowing the copper priority with regard to the initial reaction with the wood substance, a better performance can be achieved when the wood is exposed to soft rot assays. The thin section assay assesses the extent of the treatment of the S_2 layer of the wood cell wall and was used here to examine the performance of formulations against soft rot fungi and S_2 degrading bacteria.

3.1.2. Commercially Available Preservatives

Five commercially available CCA preservatives were examined to determine the effect of formulation on preservative performance.

Smith and Williams (1973 a) found that for a wide range of CCA formulations, effectiveness depended more on the copper content than that of the copper-arsenic. They indicated that the most effective formulations for ground contact use would be those with a high chromium, high copper and low arsenic content. Hedley (1984a) confirmed this. After a series of laboratory and field tests he concluded that formulations which approximate to those defined as 'Type C' by the American Wood Preservers'

Association (having a low arsenic and high chromium content) are superior to those defined as 'Type B' (having a high arsenic and low chromium composition).

Type B preservatives e.g. K33 oxide (AWPA Type B), together with a modified Type B, Tanalith NCA and Type C e.g. Tanalith C (BS 4072 Type 2) Celcure A Salt (BS 4072 Type 1), Celcure A Oxide (AWPA Type C) were examined in this work. Type B and Tanalith NCA are in common use in New Zealand.

	Cu	Cr	As
Type B	25%	29%	46%
Type C	24%	40%	36%

Hedley (1984a) concluded that if the proportion of copper is kept constant, a reduction in the chromium to arsenic ratio leads to a reduction in CCA effectiveness.

The CCA preservatives examined here were expressed in terms of their active elements (i.e. Cu, Cr, As). Hedley (1970) and McQuire (1972) showed that if CCA preservatives, within defined limits, were expressed in terms of their active elements and timber was treated to similar retentions of active elements, irrespective of commercial formulation, differences in effectiveness between them only became apparent in the long term. It was hoped that by using the thin section assay, these differences would rapidly become apparent. The preservatives used here were examined for their effectiveness against soft rot fungi and S_2 degrading bacteria. The concentrations used were chosen on the basis of equivalent active elements, irrespective of commercial formulation, to determine the effect of active element composition on performance.

Tunnelling bacteria found in radiata pine posts in New Zealand

(Drysdale and Hedley, 1984) were able to discriminate between formulations. Here we wanted to use S_2 degrading bacteria as a research tool, in conjunction with the thin section assay to evaluate formulations.

It was anticipated that by using a soft rot degree and bacteria degree of attack, the thin section assay would allow a multifacet approach (in terms of soft rot and S_2 degrading bacteria in softwoods and hardwoods), to determine the effect of copper based preservatives at the cell wall level. Woodblocks were treated with experimental formulations and existing preservatives. Thin sections were cut from these blocks for use in soft rot tests and bacterial tests. Results for performance against the two hazards were obtained separately, then discussed together to give an overall determination of the performance of the formulations and preservatives against soft rot fungi and S_2 degrading bacteria.

3.2. Preservative Treatments

3.2.1. Materials

3.2.1.1. Wood Samples

The sapwood of a hardwood, Birch (Betula pendula) and of a softwood, Scots Pine (Pinus sylvestris) were used in the present study. These species were chosen for the following reasons :-

(1) They represent a hardwood and a softwood.

(2) They are both perishable.

(3) Both are permeable to fluids and are therefore effectively treated by vacuum-pressure impregnation. This was important in the present work as it was the preservative and not the method of treatment which was under test.

(4) They have both been widely used in research programmes and there is a variety of relevant data in the literature.

(5) When conventionally treated, they represent a good performer (Scots pine) and a poor performer (birch), against the soft rot hazard.

The timber was collected from the Paddock Hurst Estate, South Hill, Crawley, Sussex. Lengths of wood were sawn into 7 mm^2 strips and planed to thickness before cross cutting to give 7 mm cubes. The wood was kiln dried prior to planing. Only the sapwood of both species was used. This ensured a more uniform test and a more severe one, since sapwood is generally less durable than heartwood.

7 x 7 x 7 mm blocks were used. This optimum size was decided upon from practical considerations. Sorkhoh (1976) found that it was easier to handle and obtain a whole thin section of wood from 7 mm^3

blocks. In addition, for the present work, this size of thin section enabled a large number of sections to be incubated at any one time in jars of soil. The blocks were orientated in the manner described by Corbett (1963), so that the three opposite pairs of faces were in the transverse, tangential longitudinal and radial longitudinal planes. This enabled sectioning of the blocks in each of the three planes without further trimming being necessary.

Blocks with wavy grain, knots, resin pockets , or any other imperfections were rejected. All blocks were lightly sanded with glass paper Grade F2, Grit No. 100, before use.

3.2.1.2 Preservatives

The basic wood preservative used was a water-borne copper chrome arsenic formulation, prepared according to BS 4072 Type 2 (1974). The nominal composition was :-

35% $CuSO_4.5H_2O$ (Cupric sulphate)

45% $K_2Cr_2O_7$ (Potassium dichromate)

20% $As_2O_5.2H_2O$ (Arsenic pentoxide or 17% anhydrous arsenic pentoxide, As_2O_5)

This formulation was used because :-

(1) It is recognised and used worldwide.

(2) There is accumulated data from many sources on its toxicity and susceptibility to soft rot.

(3) There is data on its fixation, permanence and microdistribution.

(4) It has been used by Gray (1983) in tests of efficacy using weight loss techniques,thus results could be compared and an evaluation of the thin section technique could be made.

3.2.1.2.1 Experimental Formulations

The other formulations used were modifications of the basic CCA composition. Their compositions were expressed in terms of CCA equivalents. The composition of the copper chrome boron formulation was:-

35% $CuSO_4.5H_2O$ (Cupric sulphate)

45% $K_2Cr_2O_7$ (Potassium dichromate)

20% H_3BO_3 (Boric acid)

A 2% CCA equivalent solution of CCB contained the same quantities of cupric sulphate and potassium dichromate as a 2% CCA solution. The boric acid replaced the arsenic pentoxide.

The copper sulphate formulation (C) contained 35% cupric sulphate, whilst the copper chrome (CC) had this plus an additional 45% potassium dichromate. Therefore all formulations contained the same quantity of copper sulphate.

Stock solutions of 10% CCA equivalent were made as below:-

C 35 g $CuSO_4.5H_2O$ per litre distilled water

CC 35 g $CuSO_4.5H_2O$
45 g $K_2Cr_2O_7$
} per litre distilled water

CCA 35 g $CuSO_4.5H_2O$
45 g $K_2Cr_2O_7$
20 g $As_2O_5.2H_2O$
} per litre distilled water

CCB 35 g $CuSO_4.5H_2O$
45 g $K_2Cr_2O_7$
20 g H_3BO_3
} per litre distilled water

Serial dilutions were made to give concentrations of 1.8%, 2.6%, and 3.7% CCA equivalents. These concentrations were used in initial tests, to include toxic thresholds for each wood species, as determined by Gray (1983).

In addition, concentrations of

1.6%, 3.25% and 5.7% for birch

0.7%, 2.36% and 4.13% for Scots pine

were used in further tests. This range of concentrations was used by Gray (pers. comm.) in parallel tests of preservative performance using other methods such as weight loss.

In some cases double treatments were used, the treatment schedule was run through twice, with two different solutions. A copper chrome (CC) treatment was followed by an arsenic treatment, of the same strength in terms of CCA equivalents, for CC + A, and a CCB treatment followed by an arsenic treatment for CCB + A.

CC + A:

1st treatment: 35 g $CuSO_4.5H_2O$ + 45 g $K_2Cr_2O_7$ per litre distilled water

↓

Storage

↓

Drying

2nd treatment: 20 g $As_2O_5.2H_2O$ per litre distilled water

CCB + A

1st treatment

35 g $CuSO_4.5H_2O$
45 g $K_2Cr_2O_7$
20 g H_3BO_3
} per litre distilled water

↓

Storage

↓

Drying

2nd treatment 20 g $As_2O_5.2H_2O$ per litre distilled water

A further set of blocks was treated with distilled water to act as control blocks.

3.2.1.2.2 Other Preservatives used

Five commercially available preservatives were used;

(1) Tanalith C Salt (BS 4072 Type 2 described earlier)

Nominal composition: 35% $CuSO_4.5H_2O$
45% $K_2Cr_2O_7$
20% $As_2O_5.2H_2O$

(2) Tanalith NCA

Nominal composition: 29.7% $CuSO_4.5H_2O$
31.7% $Na_2Cr_2O_7$
31.2% $As_2O_5.2H_2O$
7.4% $Na_4As_2O_7$

(3) Celcure A Salt (BS 4072 Type 1)

Nominal composition: 30.42% $CuSO_4.5H_2O$
38.25% $Na_2Cr_2O_7.2H_2O$
24.63% $As_2O_5 2H_2O$
6.7% H_2O

(4) Celcure A Oxide (AWPA Type C)

Nominal composition: 13.32% CuO

34.20% CrO_3

24.48% As_2O_5

28.0% H_2O

(5) K33 Oxide (AWPA Type B)

Nominal composition: 14.8% CuO

26.6% CrO_3

34.0% As_2O_5

24.6% H_2O

The concentration range used for each preservative was based upon (i) % elemental copper

(ii) % active elements i.e. copper and arsenic (See Tables 9,10).

Tanalith C (BS 4072 Type 2) used in initial experiments (Sections 3.3.1 and 3.3.2) was used as a baseline. Ratios of each preservative to Tanalith C were defined, in terms of % elemental copper and % active elements. Results of preservative performance could therefore be explained in terms of % copper and active elements.

Calculation of elemental copper and arsenic

e.g. Tanalith C Proportion of Cu in Formulation;

$CuSO_4.5H_2O$ molecular wt. = 249.68

Cu atomic wt. = 63.55

Therefore Cu = 25.453% of $CuSO_4.5H_2O$

Formulation contains 35% $CuSO_4$

% Cu in formulation = 25.453% of 35% $CuSO_4.5H_2O$

= 8.908% Cu

% Arsenic

e.g. Tanalith C Proportion of As in Formulation;

$As_2O_5.2H_2O$ molecular wt. = 265.82

2 x As atomic wt. = 149.82

Therefore As = 56.361% of $As_2O_5.2H_2O$

Formulation contains 20% $As_2O_5.2H_2O$

% As in formulation = 56.361% of 20% $As_2O_5.2H_2O$

= 11.272% As

% Active elements in Tanalith C = 8.908 + 11.272 = 20.18%

This method was repeated for each preservative.

Table 9 Concentrations of Preservatives used in Birch Blocks

i) Elemental Copper

Preservative	% Cu	Ratio:Tan C	Concentration of Preservative Used (% w/v)		
Tan C	8.908	1.0	1.6	3.25	5.7
Tan NCA	11.821	0.754	1.206	2.451	4.298
Cel A Salt	7.742	1.151	1.842	3.741	6.561
Cel A Oxide	10.641	0.837	1.339	2.720	4.771
K33 Oxide	11.824	0.753	1.205	2.447	4.292

ii) Active Elements

Preservative	% As	Active elements % Cu+As	Ratio:TanC	Concentration of preservative Used (% w/v)		
Tan C	11.272	20.180	1.0	1.6	3.25	5.7
Tan NCA	20.717	32.538	0.620	0.992	2.015	3.534
Cel A Salt	13.881	21.623	0.933	1.493	3.032	5.318
Cel A Oxide	15.959	26.600	0.759	1.214	2.467	4.326
K33 Oxide	22.165	33.989	0.594	0.950	1.931	3.386

Table 10 Concentrations of Preservatives used in Scots Pine blocks

i) Elemental Copper

Preservative	% Cu	Ratio:Tan C	Concentration of Preservative Used (% w/v)		
Tan C	8.908	1.0	0.7	2.36	4.13
Tan NCA	11.821	0.754	0.528	1.779	3.114
Cel A Salt	7.742	1.151	0.806	2.716	4.754
Cel A Oxide	10.641	0.837	0.586	1.975	3.457
K33 Oxide	11.824	0.753	0.527	1.777	3.110

ii) Active Elements

Preservative	% As	Active Elements % Cu+As	Ratio:TanC	Concentration of Preservative Used (% w/v)		
Tan C	11.272	20.180	1.0	0.7	2.36	4.13
Tan NCA	20.717	32.538	0.620	0.434	1.463	2.561
Cel A Salt	13.881	21.623	0.933	0.653	2.202	3.853
Cel A Oxide	15.959	26.600	0.759	0.531	1.791	3.135
K33 Oxide	22.165	33.989	0.594	0.416	1.402	2.453

3.2.2 Methods of Treatment

A vacuum-pressure impregnation treatment was used. This was preferred to vacuum impregnation (EN 113, 1982) because the differences in performance of the preservatives being tested were thought to be subtle differences in detail (e.g. in microdistribution) which may be related to the method of treatment (Dickinson, Sorkhoh and Levy, 1976).

3.2.2.1 Treatment Schedule

The selected wood blocks were oven dried at 105 ^{o}C for 24 hours, cooled in a dessicator, weighed and labelled using waterproof ink. The blocks were loosely arranged in a beaker, ballasted with plastic mesh and glass blocks, and the beaker placed in a dessicator where a vacuum of about 760 mm Hg (depending on atmospheric conditions) was drawn and held for 30 minutes. The treating solution was then drawn into the beaker and the pressure returned to atmospheric pressure. Then the beaker was put into a treatment cylinder where the blocks were subjected to a pressure of 10.2 bar (150 p.s.i) for one hour, followed by a soaking period of one hour at atmospheric pressure. The blocks were removed from the treating solution, blotted and weighed wet to establish their preservative uptakes. Whilst still wet, the blocks were sealed in polythene bags and stored in the dark at room temperature for two weeks for fixation and conditioning.

3.2.2.2 Drying

A process of slow drying of the blocks was adopted to minimise redistribution of the preservative elements in the wood (EN 113, 1982). At the beginning of the third week the blocks were laid out in enamelled trays, whose lids were removed progressively over seven days, and

completely removed for the fourth week. The blocks were turned at intervals throughout this period.

3.2.2.3 Leaching

All wood blocks were cold water leached (EN 84 , 1980 (BS 5761, Part 2)) after the full preservative treatment period. The blocks were impregnated with distilled water under vacuum. After two hours the water was changed, the volume being five times that of the wood. The blocks were kept immersed for fourteen days at room temperature. Nine changes of water were made, after one and two days and at intervals throughout the fourteen days. The blocks were then air dried in the laboratory for two weeks.

3.2.2.4 Determination of Volume

To compare preservatives on a retention basis, the individual volumes of the wood blocks had to be established. Any slight differences in dimensions of the small wood blocks would result in proportionally large differences in volume. Since wood block volumes change with moisture content up to fibre saturation point, a wet volume determination was chosen as the most accurate and simple.

The volume of each block was determined accurately using a method based on Archimedes' principle. A beaker of water was tared on a balance and a block, attached to a mounted needle, was submerged in the water. The block displaced its own volume of water, whose weight in g was equal to its volume in ml. Therefore the reading on the balance was the volume of the block in cm^3. This was carried out during the leaching procedure when the blocks were fully saturated.

3.2.2.5 Calculation of Preservative Retention

$$\text{Uptake} = \text{wet weight after treatment} - \text{dry weight before treatment}$$

$$\text{Net Dry Salt Retention (NDSR) kg/m}^3 = \frac{\text{Uptake}}{\text{Volume}} \times \frac{\text{Treating solution concentration}}{100}$$

The retention expressed as a percentage weight of preservative per weight of wood material was calculated as follows:-

$$\text{Retention} = \frac{\text{Net dry salt retention}}{\text{Wood density}} \times 100 \quad (\% \text{ wt/wt})$$

3.2.2.6 Calculation of Elemental Contents of the Wood

e.g. for BS 4072 Type 2 Formulation

35% $CuSO_4.5H_2O$
45% $K_2Cr_2O_7$
20% $As_2O_5.2H_2O$ or 20% H_3BO_3

Proportion of Cu :-

$CuSO_4.5H_2O$ molecular weight = 249.68
Cu atomic weight = 63.55

Therefore Cu = 25.453% of $CuSO_4.5H_2O$

35% $CuSO_4.5H_2O$ used in formulation,

Therefore % Cu in formulation = 25.453% of $CuSO_4.5H_2O$

= 8.91% Cu

This method was repeated for Cr, As and B. The proportions of Cu, Cr, As and B in the formulations were 8.91%, 15.91%, 11.27% and 3.50% respectively.

The elemental contents of the wood were as follows:

$$\% \text{ w/w Cu} = \frac{8.91}{100} \times \frac{\text{NDSR}}{\text{Density}} \times 100$$

$$\% \text{ w/w Cr} = \frac{15.91}{100} \times \frac{\text{NDSR}}{\text{Density}} \times 100$$

$$\% \text{ w/w As} = \frac{11.27}{100} \times \frac{\text{NDSR}}{\text{Density}} \times 100$$

$$\% \text{ w/w B} = \frac{3.50}{100} \times \frac{\text{NDSR}}{\text{Density}} \times 100$$

Mean preservative retentions and elemental contents of the wood were calculated for each treatment.

3.3 Soft Rot Tests

3.3.1 Performance of CCA and CCB

3.3.1.1 Introduction

The performance of CCA and CCB against soft rot fungi in soil was compared using the thin section assay.

3.3.1.2 Method

30 μm thick transverse sections were cut from CCA and CCB treated wood blocks (Section 3.2). Concentrations of 1.8%, 3.7% and 5.7% CCA equivalent for birch and 1.8%, 3.7% and 4.13% for Scots pine were chosen to include toxic thresholds for each wood species, as determined by Gray (1983). Untreated sections were also used as controls.

Princes Risborough soil (Section 2.4.1) was dried then wetted to achieve a soil moisture content of 95% of the w.h.c (Section 2.4.2).

Thin sections of treated and untreated wood were placed in mesh packets (Section 2.2.2) and buried in the soil. Sections were removed after 1,2,3,4,5,6,8,10,12,16,25 and 30 weeks, examined under the light microscope and a Soft Rot Degree (SRD), determined (Section 2.4.4.2).

3.3.1.3. Results

Mean net dry salt retentions, together with percentage elemental contents of wood blocks were calculated (Section 3.2.2.5) and are recorded in Table 11. The mean SRDs, with 95% confidence limits were calculated (Section 3.3.1.3). A MINITAB statistical computing system

was used to calculate confidence limits (Appendix B) and to determine the significance of any differences between mean SRDs for the treatments using a t test. Results are given in Table 20. Mean SRDs (Tables 12-19) were plotted against time (Figures 4-8).

Untreated Birch (Table 12, Figures 4,5,6)

Initial stages of soft rot decay were observed within the first week of soil burial. Cavities formed in a number of fibre walls and in some there was complete loss of the S_2 layer. After two weeks, many cavities were found and in places only the middle lamella of the wall remained intact. From three to thirty weeks in most fields of view an SRD of 3 or 4 was recorded. Attack was severe in untreated sections.

Areas of attack could easily be identified as those where the S_2 layer no longer showed birefringence, when viewed under polarised light.

1.8% CCA Treated Birch (Table 13, Figure 4)

Onset of cavity formation was delayed until three weeks. After this time a few cavities were found in one or two fibres. Only after sixteen weeks burial was an SRD equal to that found for untreated birch buried for three weeks, recorded.

1.8% CCB Treated Birch (Table 13, Figure 4)

Soft rot attack occurred after three weeks burial. Sections were attacked at a slower rate than untreated but faster than CCA treated. A t test on mean SRDs showed no significant difference between the mean values obtained for CCA and CCB treatments but there was a difference between treated and untreated (Table 20).

3.7% CCA Treated Birch (Table 14, Figure 5)

Rate of attack was reduced with this treatment compared with that for untreated.

3.7% CCB Treated Birch (Table 14, Figure 5)

Attack was reduced with this concentration of CCB after sixteen weeks, compared with that found for a concentration of 1.8%. The difference between the two concentrations was significant (Table 20). CCB performed significantly better than CCA at this concentration after sixteen weeks.

5.7% CCA Treated Birch (Table 15, Figure 6)

Increasing the concentration to this level did not significantly reduce attack.

5.7% CCB Treated Birch (Table 15, Figure 6)

There was no significant difference between the performance of CCA and CCB at this concentration (Table 20).

Untreated Scots Pine (Table 16, Figures 7,8,9)

Attack was delayed until six weeks. After sixteen weeks a mean SRD of 1.65 was recorded compared to that of 3.8 for untreated birch sections buried for this length of time. Only after twenty five weeks were SRDs equal to those for birch found.

1.8% CCA and 1.8% CCB Treated Scots Pine (Table 17, Figure 7)

Attack was delayed until six weeks. A mean SRD of only 0.30 for CCA and of 0.60 for CCB was recorded after thirty weeks.

3.7% CCA and 3.7% CCB Treated Scots Pine (Table 18, Figure 8)

Attack was delayed and minimal even after thirty weeks burial.

Table 11

Mean Preservative Retentions and Elemental Contents

a) Birch

Treatment	Conc (%w/v)	Mean Net Dry Salt Retention (kgm^{-3})	Standard Deviation	% w/w elemental contents of wood			
				Copper	Chromium	Arsenic	Boron
CCA	1.8%	11.973	0.265	0.202	0.360	0.255	
	3.7	24.901	0.531	0.414	0.740	0.524	
	5.7	35.658	0.777	0.561	1.002	0.710	
CCB	1.8	11.392	0.244	0.194	0.346		0.076
	3.7	24.326	0.471	0.401	0.716		0.158
	5.7	32.676	2.309	0.501	0.894		0.197

b) Scots Pine

CCA	1.8	12.866	0.298	0.254	0.454	0.321	
	3.7	27.015	0.507	0.528	0.943	0.668	
	4.13	28.373	1.091	0.552	0.985	0.698	
CCB	1.8	12.920	0.383	0.252	0.451		0.099
	3.7	26.106	0.554	0.510	0.910		0.200
	4.13	28.365	1.000	0.574	1.025		0.226

Table 12

Mean Soft Rot Degree Ratings for Birch Sections

Buried in Princes Risborough Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		1	0.95	0.826	1.335	0.565
		2	2.15	0.988	2.611	1.689
		3	2.90	0.788	3.268	2.532
		4	3.10	0.641	3.399	2.801
		5	3.25	0.716	3.584	2.916
		6	3.30	0.733	3.642	2.958
		8	3.80	0.410	3.991	3.609
		10	3.15	0.671	3.463	2.837
		12	3.80	0.410	3.991	3.609
		16	3.80	0.410	3.991	3.609
		25	3.15	0.366	3.321	2.979
		30	3.45	0.510	3.688	3.212

Table 13

Mean Soft Rot Degree Ratings for Birch Sections Buried in Princes Risborough Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCA	1.8	1	0	0		
		2	0	0		
		3	0.30	0.470	0.519	0.081
		4	1.70	0.571	1.966	1.434
		5	1.95	0.510	2.189	1.712
		6	0.70	1.031	1.181	0.219
		8	1.40	0.754	1.752	1.048
		10	1.10	0.788	1.468	0.732
		12	2.00	0.918	2.428	1.572
		16	2.90	0.718	3.235	2.565
		25	3.10	0.447	3.309	2.891
		30	2.90	0.553	3.158	2.642
CCB	1.8	1	0	0		
		2	0	0		
		3	2.80	1.609	3.551	2.049
		4	1.60	1.847	2.462	0.738
		5	2.80	1.436	3.470	2.130
		6	2.75	0.716	3.084	2.416
		8	2.05	1.234	2.626	1.474
		10	3.30	0.923	3.731	2.869
		12	2.95	1.638	3.714	2.186
		16	3.90	0.308	4.044	3.756
		25	2.75	0.639	3.048	2.452
		30	3.55	0.686	3.870	3.230

Table 14

Mean Soft Rot Degree Ratings for Birch Sections Buried in Princes Risborough Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCA	3.7	1	0	0		
		2	0.30	0.571	0.566	0.034
		3	0.60	0.754	0.952	0.248
		4	1.20	1.010	1.671	0.729
		5	1.70	1.031	2.181	1.219
		6	1.00	0.973	1.454	0.546
		8	1.60	1.230	2.174	1.026
		10	1.70	1.418	2.361	1.039
		12	2.40	0.598	2.679	2.121
		16	3.30	0.571	3.566	3.034
		25	2.80	0.696	3.125	2.475
		30	3.20	0.696	3.525	2.875
CCB	3.7	1	0	0		
		2	0.30	0.657	0.606	- 0.007
		3	1.65	0.671	1.963	1.337
		4	1.45	0.686	1.770	1.130
		5	1.40	1.273	1.994	0.806
		6	0.80	0.768	1.158	0.442
		8	1.45	1.100	1.963	0.937
		10	1.60	0.940	2.039	1.161
		12	2.45	0.686	2.770	2.130
		16	2.30	0.865	2.703	1.897
		25	2.75	0.444	2.957	2.543
		30	2.90	0.308	3.044	2.756

Table 15

Mean Soft Rot Degree Ratings for Birch Sections Buried in Princes Risborough Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCA	5.7	1	0	0		
		2	0.30	0.657	0.606	-0.007
		3	1.75	1.517	2.458	1.042
		4	1.40	1.603	2.148	0.652
		5	1.20	1.281	1.798	0.602
		6	2.35	1.137	2.880	1.820
		8	3.05	0.605	3.332	2.768
		10	2.90	0.718	3.235	2.565
		12	2.05	1.395	2.701	1.399
		16	2.75	1.410	3.408	2.092
		25	3.00	0.562	3.262	2.738
		30	2.50	1.000	2.966	2.034
CCB	5.7	1	0	0		
		2	0	0		
		3	0.90	0.852	1.297	0.503
		4	1.05	1.191	1.606	0.494
		5	0.95	1.050	1.440	0.460
		6	2.95	0.605	3.232	2.668
		8	2.90	0.447	3.109	2.691
		10	1.90	1.021	2.377	1.423
		12	2.90	0.308	3.044	2.756
		16	3.00	0.459	3.214	2.786
		25	2.90	0.788	3.268	2.532
		30	2.90	0.308	3.044	2.756

Figure 4. Performance of Birch in Princes Risborough Soil

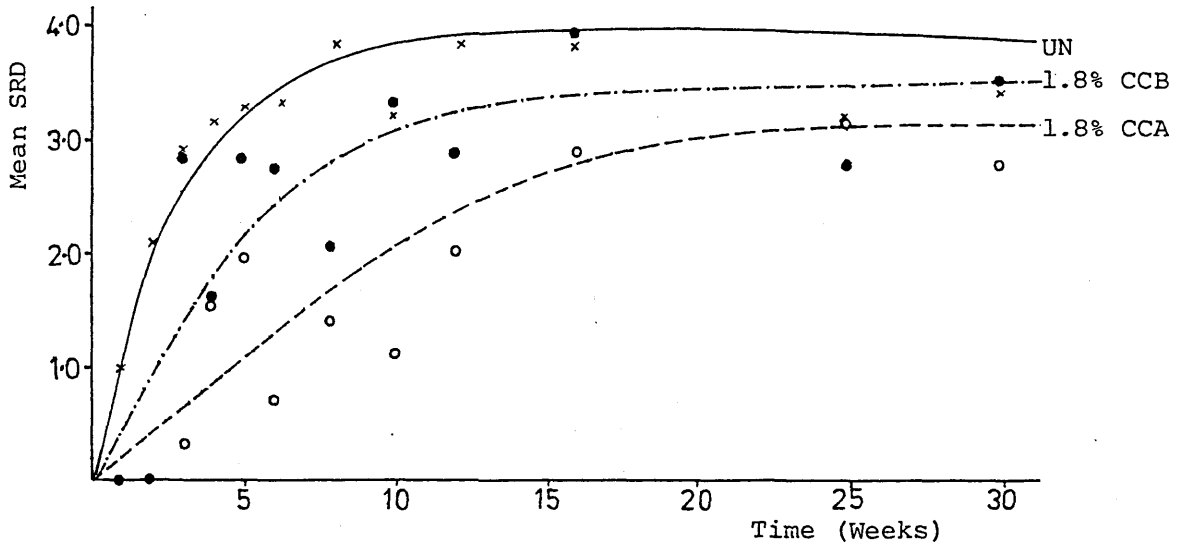


Figure 5. Performance of Birch in Princes Risborough Soil.

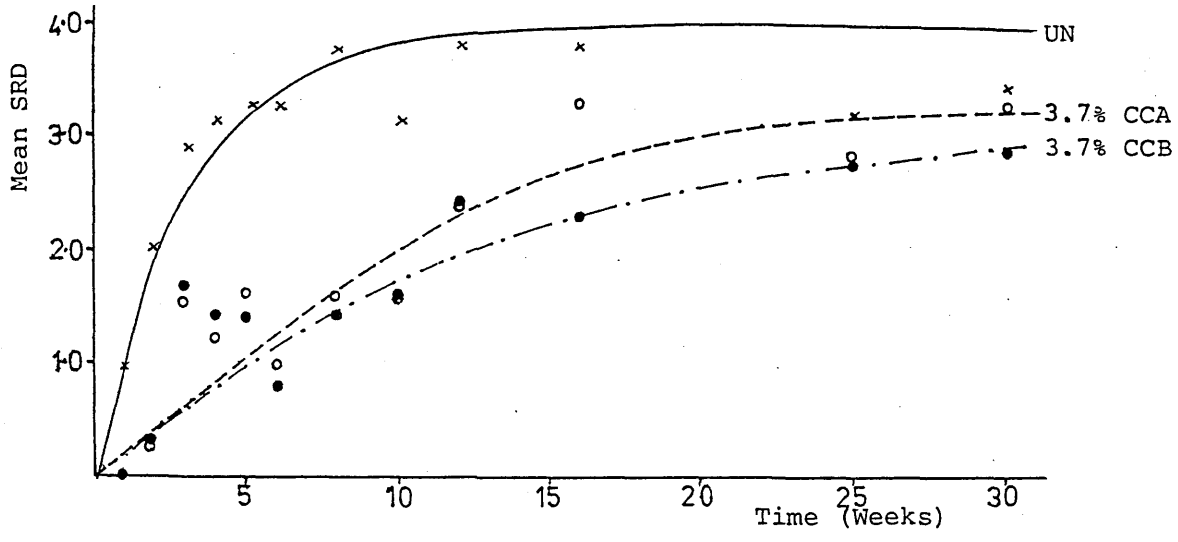


Figure 6. Performance of Birch in Princes Risborough Soil.

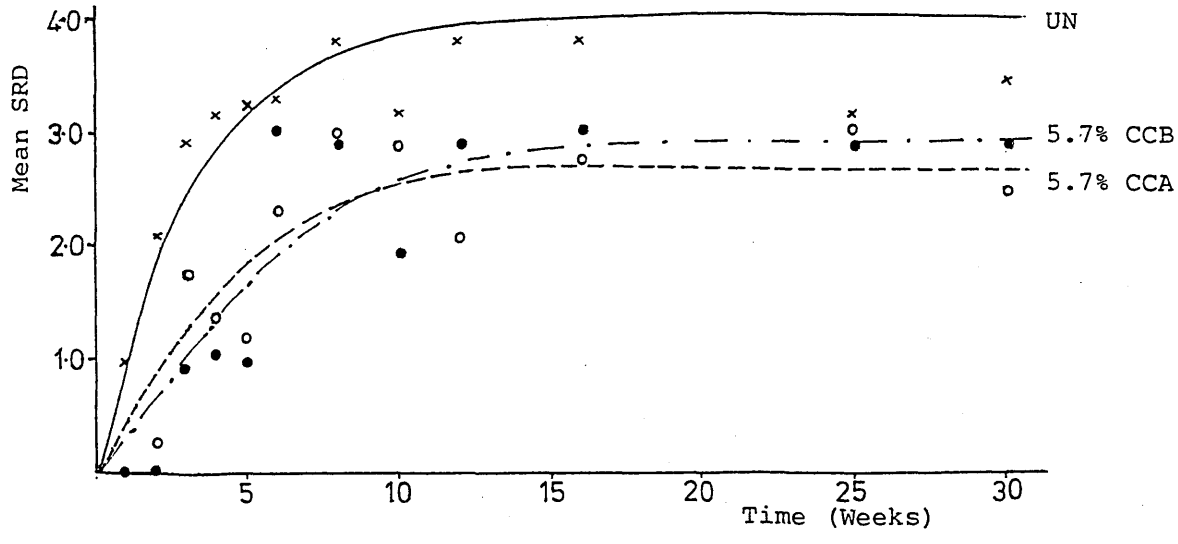


Table 16

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Princes Risborough Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		1	0	0		
		2	0	0		
		3	0	0		
		4	0	0		
		5	0	0		
		6	1.30	0.979	1.757	0.843
		8	1.00	1.026	1.479	0.521
		10	2.05	0.394	2.234	1.866
		12	1.4	1.603	2.148	0.652
		16	1.65	1.040	2.135	1.165
		25	3.55	0.686	3.870	3.230
		30	3.30	0.657	3.606	2.994

Table 17

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Princes Risborough Soil

Treatment	Conc %w/v	Time Weeks	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCA	1.8	1	0	0		
		2	0	0		
		3	0	0		
		4	0	0		
		5	0	0		
		6	0.25	0.444	0.457	0.043
		8	0	0		
		10	0	0		
		12	0.15	0.366	0.321	-0.021
		16	0.25	0.444	0.457	0.043
		25	0.35	0.489	0.578	0.122
		30	0.30	0.470	0.519	0.081
CCB	1.8	1	0	0		
		2	0	0		
		3	0	0		
		4	0	0		
		5	0	0		
		6	0	0		
		8	0.50	0.513	0.739	0.261
		10	0.30	0.470	0.519	0.081
		12	0.85	0.933	1.285	0.415
		16	0.50	0.513	0.739	0.261
		25	0.35	0.489	0.578	0.122
		30	0.60	0.503	0.835	0.365

Table 18

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Princes Risborough Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCA	3.7	1	0	0		
		2	0	0		
		3	0	0		
		4	0	0		
		5	0	0		
		6	0	0		
		8	0.25	0.444	0.457	0.043
		10	0	0		
		12	0.20	0.410	0.391	0.009
		16	0.10	0.308	0.244	-0.044
		25	0.35	0.489	0.578	0.122
		30	0.10	0.308	0.244	-0.044
CCB	3.7	1	0	0		
		2	0	0		
		3	0	0		
		4	0	0		
		5	0	0		
		6	0.05	0.224	0.154	-0.054
		8	0.25	0.444	0.457	0.043
		10	0	0		
		12	0	0		
		16	0.40	0.681	0.718	0.082
		25	0.30	0.470	0.519	0.081
		30	0.30	0.657	0.606	- 0.007

Table 19

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Princes Risborough Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits Upper	Lower
CCA	4.13	1	0	0		
		2	0	0		
		3	0	0		
		4	0	0		
		5	0	0		
		6	0	0		
		8	0	0		
		10	0	0		
		12	0	0		
		16	0	0		
		25	0	0		
		30	0	0		
CCB	4.13	1	0	0		
		2	0	0		
		3	0	0		
		4	0	0		
		5	0	0		
		6	0	0		
		8	0	0		
		10	0	0		
		12	0	0		
		16	0	0		
		25	0	0		
		30	0	0		

Figure 7 Mean SRD for Scots Pine in Princes Risborough Soil

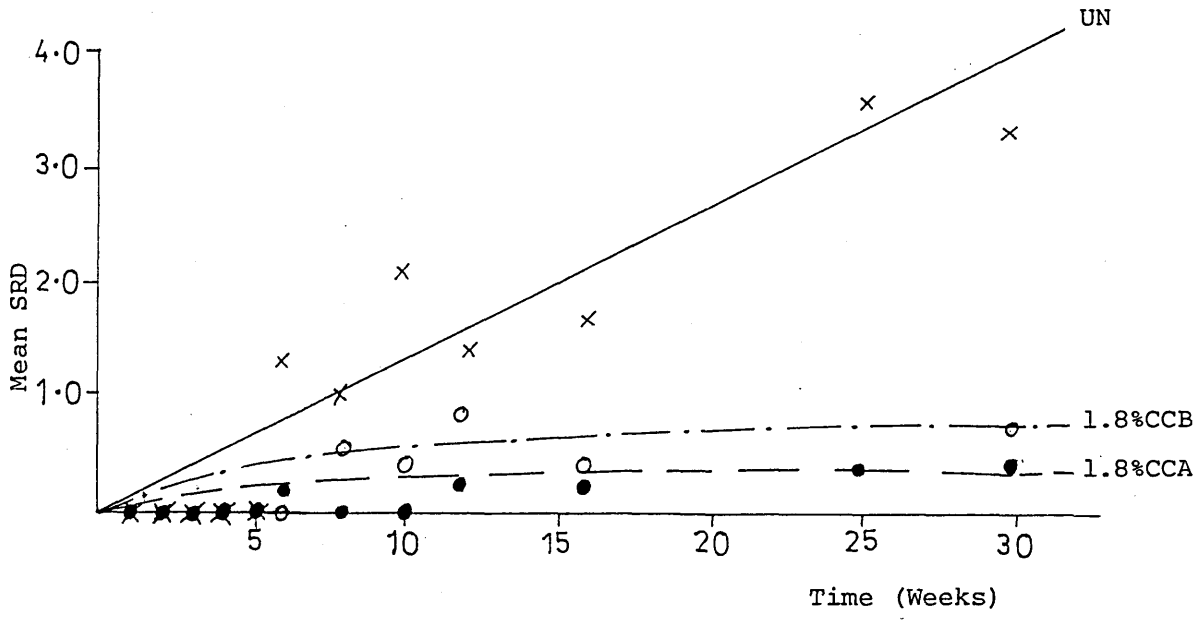


Figure 8 Mean SRD for Scots Pine in Princes Risborough Soil

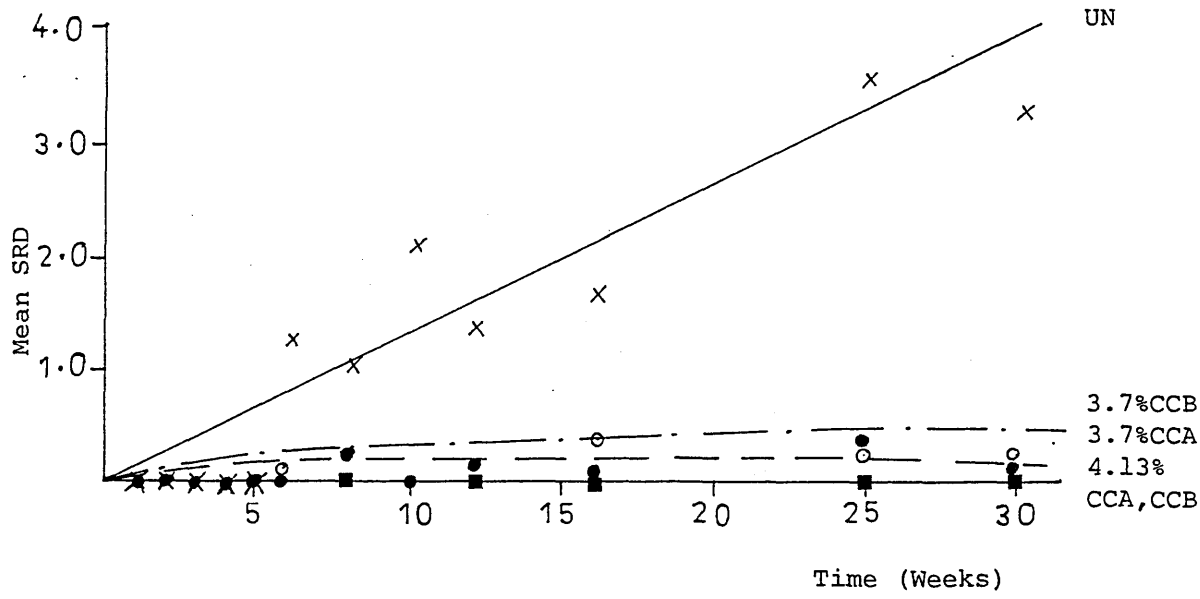


Table 20

A Comparison of Mean SRDs for Formulations.

Birch

4 Weeks

	5.7% CCB	5.7% CCA	3.7% CCB	3.7% CCA	1.8% CCB	1.8% CCA	UT
Untreated	*	*	*	*	*	*	
1.8% CCA	*	-	-	-	-		
1.8% CCB	-	-	-	-			
3.7% CCA	-	-	-				
3.7% CCB	-	-					
5.7% CCA	-						
5.7% CCB							

8 Weeks

	5.7% CCB	5.7% CCA	3.7% CCB	3.7% CCA	1.8% CCB	1.8% CCA	UT
Untreated	*	*	*	*	*	*	
1.8% CCA	*	*	-	-	-		
1.8% CCB	*	*	-	-			
3.7% CCA	*	*	-				
3.7% CCB	*	*					
5.7% CCA	-						
5.7% CCB							

16 Weeks

	5.7% CCB	5.7% CCA	3.7% CCB	3.7% CCA	1.8% CCB	1.8% CCA	UT
Untreated	*	*	*	*	-	*	
1.8% CCA	-	-	-	-	-		
1.8% CCB	*	*	*	*			
3.7% CCA	-	-	*				
3.7% CCB	-	-					
5.7% CCA	-						
5.7% CCB							

* = significant difference between mean values (p = 0.05)

- = no significant difference between mean values

4.13% CCA and 4.13% CCB Treated Scots Pine (Table 19, Figure 8)

No soft rot cavities were found.

3.3.1.4 Discussion

The differences in performance between the low susceptibility softwood, Scots pine and the high susceptibility hardwood, birch, with regard to soft rot attack, have been illustrated here. Untreated Scots pine sections showed minimal attack even after sixteen weeks burial in soil, however, birch sections were severely attacked after only three weeks. Treatment with solutions of CCA and CCB was far more effective at controlling soft rot in Scots pine than in birch. Attack was reduced to a minimum in Scots pine and eliminated altogether at a concentration of 4.13%.

A number of factors have been suggested to cause the difference in performance between hardwoods and softwoods treated with CCA preservatives. These will be discussed in Section 5.

Microanalysis of wood preservative chemicals within the cell wall layers has shown that the S_2 layer of fibres of treated hardwoods is often inadequately treated (Dickinson, Sorkhoh and Levy, 1976; Drysdale et al, 1980) and that a certain minimum retention of chemical is needed to provide effective control of decay. A better cell wall distribution of copper is required. Allowing copper priority with regard to the initial reaction with the wood substance has been shown by Gray (1983) to improve performance when the wood is exposed to biological assay. This therefore means that a modification of the preservative formulation is required. A range of different formulations were examined in later tests, using the thin section assay and an assessment was made of their efficacy for preventing soft rot in hardwoods.

Looking at the soft rot results obtained for CCA and CCB treatments, in Princes Risborough soil, no significant difference could be found throughout the test period between these two treatments. Both reduced the rate and extent of attack of birch. Further tests were required to assess the difference in performance between CCA and CCB. These formulations ,together with others were used in subsequent tests using the thin section assay. A range of different soil types were also used to determine the effect of soil type on formulation performance.

3.3.1.5 Conclusions

(1) Scots pine was relatively resistant to soft rot attack. Treatment with CCA and CCB reduced attack to a minimum.

(2) Birch sections were heavily attacked although treatment did reduce the rate and extent of attack.

(3) No significant difference could be found between the performance of CCA and CCB except for one instance after sixteen weeks when CCB performed better than CCA using a solution concentration of 3.7%.

(4) Increasing the concentration of preservative did not increase the resistance of birch sections to attack by soft rot fungi.

3.3.2 Soft Rot Monoculture Test

3.3.2.1 Introduction

A brief comparison was made between results from the exposure of CCA treated thin sections to soil burial (Section 3.3.1) and results from the exposure of similarly treated sections to a monoculture of the soft rot organism Chaetomium globosum.

Bravery (1968a) concluded that agar pure culture tests may be too severe and too specialised to give a realistic evaluation of preservative performance, but that they may be suitable for comparative evaluations.

The agar medium used for culturing Chaetomium globosum, was one modified after Gersonde and Kerner-Gang (1976) by Gray (1983), who found this to be the most suitable medium. C.globosum has become widely accepted as a convenient laboratory test organism for hardwoods.

A comparison of results obtained using the thin section assay in soil burial and soft rot monoculture tests would allow an appraisal of the versatility of the assay.

3.3.2.2 Method

The mineral nutrient agar medium supporting the monoculture had the following composition :-

6.00 g	NH_4NO_3	Ammonium nitrate
2.56 g	K_2HPO_4	Di-potassium hydrogen phosphate
1.02 g	$MgSO_4$	Magnesium sulphate
0.25 g	KCl	Potassium chloride
0.005g	NaCl	Sodium chloride

(composition cont.)

0.001 g $FeSO_4$ Ferrous sulphate

0.001 g $MnSO_4$ Manganese sulphate

1.000 g Glucose

1 ml Trace element solution

20 g Agar (Per litre of distilled water)

Trace element solution composition (Per ml) :-

570 g Boric acid

310 g Zinc chloride

145 g Ferric chloride

40 g Cobalt chloride

60 g Copper sulphate

30 g Magnesium chloride

20 g Ammonium molybdate

The medium was sterilised by autoclaving at 15 p.s.i. for 20 minutes. Sterile 9 cm Petri dishes (Sterilin Ltd) were charged with 20 ml of medium and inoculated with Chaetomium globosum using a spore suspension. A few drops of sterile distilled water were added to the cultures of C. globosum growing on agar slopes in test tubes. The surface of the culture was scraped with a piece of sterilised wire to liberate the spores. The tube was shaken and the spore suspension filtered through sterile glass wool in a sterile funnel. An atomiser giving a fine spray was used to inoculate the agar plates. The plates were incubated at 30 °C for several days prior to planting of the sterile wood sections.

Thin Sections

30 µm thick sections were cut from birch and Scots pine blocks which had been treated with 3.7% CCA and untreated blocks (Section2.2.2).

The sections were placed in nylon mesh packets (Section 2.2.2), sandwiched between layers of moistened filter paper and then sealed in heavy gauge polythene "layflat" tubing (Transatlantic Plastics Ltd.) using a heat sealer. The sections in the mesh packets were sterilised by gamma irradiation. 2.5 megarad were supplied from a Cobalt 60 source overnight.

The mesh packets each containing six thin sections of wood were placed on the surface of the C. globosum cultures growing on the agar medium (Figure 9). The sections were incubated at 30° C and removed after 1,2,4 and 6 weeks. They were examined in the light microscope for degree of soft rot attack (Section 2.4.4.2).

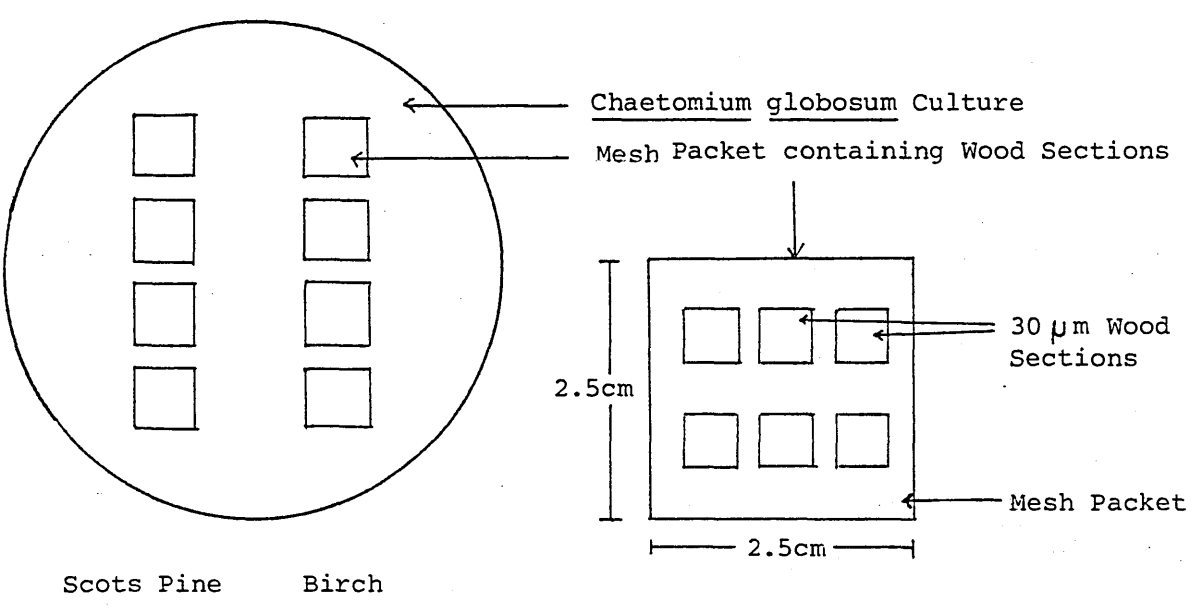


Figure 9 Thin Sections of Wood on a Chaetomium globosum Monoculture

3.3.2.3 Results

Mean SRDs with standard deviations and confidence limits were recorded (Tables 21, 22). A comparison was made between mean SRDs obtained for untreated and 3.7% CCA treated birch and Scots pine sections here in monoculture exposure and from the previous test in soil burial (Section 3.3.1.,Table 23). Mean SRDs were plotted against time (Figure 10).

Exposure of untreated birch sections to a monoculture of C. globosum increased the rate of soft rot attack over that obtained with soil burial. Heavy attack occurred after only one week of exposure to the monoculture. SRDs from two to six weeks exposure, were however, similar for both monoculture and soil burial. Treatment with a 3.7% CCA solution reduced the rate of attack in both cases.

As in the previous test, Scots pine was very resistant to soft rot attack. Attack of untreated sections was postponed until four weeks exposure to a C. globosum monoculture and six weeks exposure to soil burial. For the six weeks of the test no soft rot attack occurred in scots pine sections which had been cut from blocks treated with 3.7% CCA.

3.3.2.4 Discussion

The soft rot monoculture proved to be slightly more efficient for producing soft rot attack of wood sections than soil burial. The effectiveness of CCA against attack has been shown using the monoculture and in soil burial. The thin section assay has been shown to be versatile with regard to the exposure medium.

Table 21

Mean Soft Rot Degree Ratings for Birch Sections on Chaetomium globosum Monocultures

Treatment	Conc (%w/v)	Time (weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		1	2.55	1.146	3.085	2.015
		2	2.65	0.875	3.058	2.242
		4	2.60	0.995	3.064	2.136
		6	3.85	0.366	4.021	3.679
CCA	3.7	1	1.40	0.503	1.635	1.165
		2	1.45	0.510	1.688	1.212
		4	2.00	0		
		6	1.70	0.470	1.919	1.481

Table 22

Mean Soft Rot Degree Ratings for Scots Pine Sections on Chaetomium globosum Monocultures

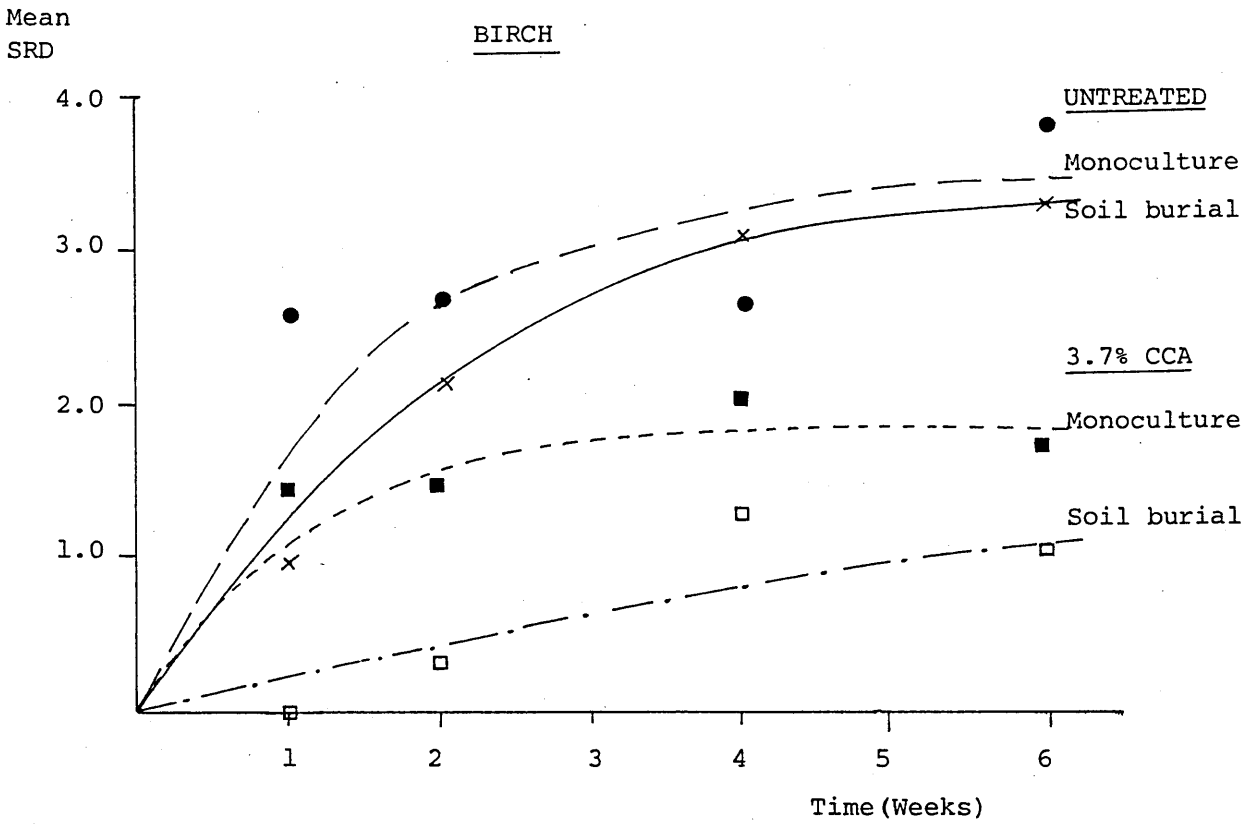
Treatment	Conc (%w/v)	Time (weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		1	0	0		
		2	0	0		
		4	0.20	0.410	0.391	0.009
		6	0.70	0.733	1.042	0.358
CCA	3.7	1	0	0		
		2	0	0		
		4	0	0		
		6	0	0		

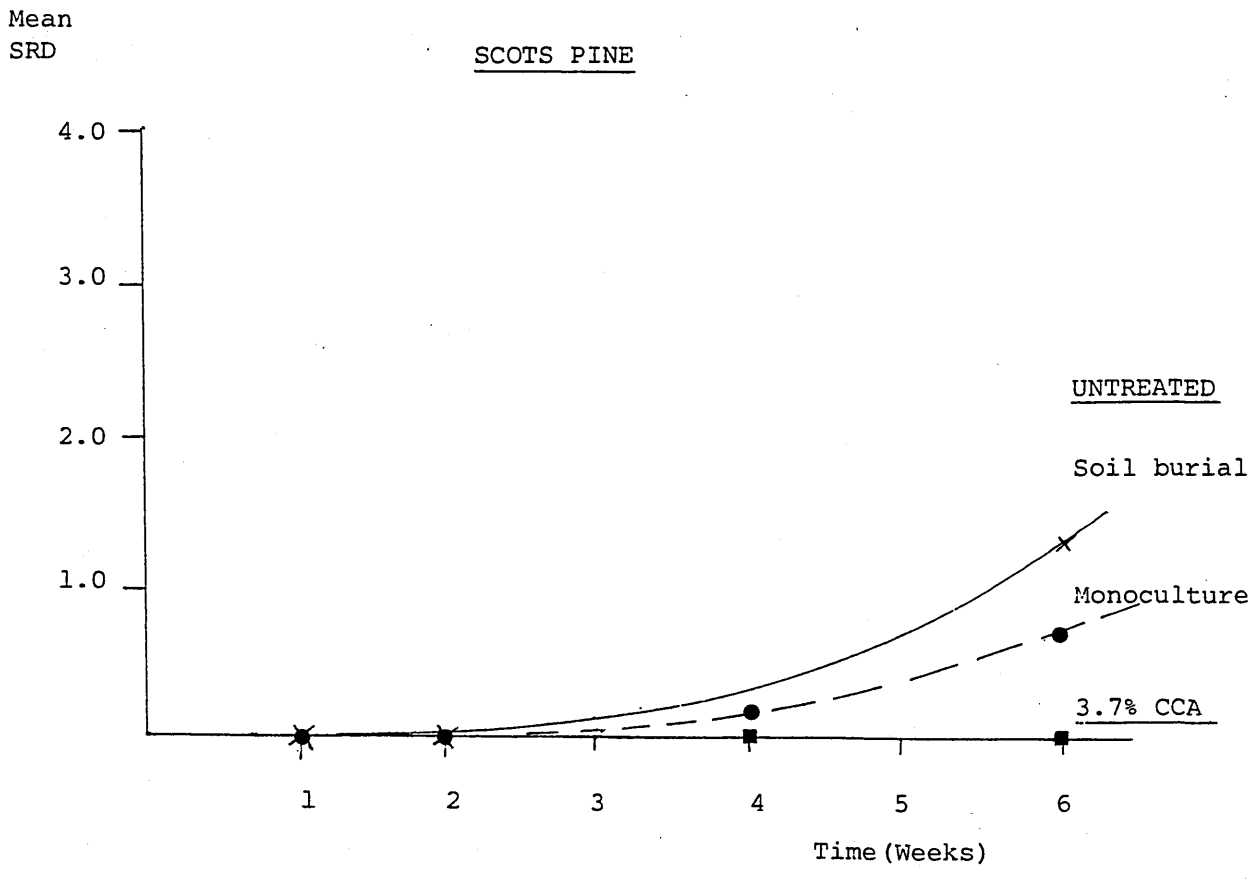
Table 23

Mean Soft Rot Degree Ratings for Birch and Scots Pine Sections buried in soil and exposed to monocultures

a) Birch			Soil Burial		Monocultures	
Treatment	Conc (%w/v)	Time (weeks)	Mean SRD	Standard Deviation	Mean SRD	Standard Deviation
Untreated		1	0.95	0.826	2.55	1.146
		2	2.15	0.988	2.65	0.875
		4	3.10	0.641	2.60	0.995
		6	3.30	0.733	3.85	0.366
CCA	3.7	1	0	0	1.40	0.503
		2	0.30	0.571	1.45	0.510
		4	1.20	1.010	2.00	0
		6	1.00	0.973	1.70	0.470
b) Scots Pine						
Untreated		1	0	0	0	0
		2	0	0	0	0
		4	0	0	0.20	0.410
		6	1.30	0.979	0.70	0.733
CCA	3.7	1	0	0	0	0
		2	0	0	0	0
		4	0	0	0	0
		6	0	0	0	0

Figure 10 Performance of Birch and Scots Pine on Soft Rot Monoculture and buried in Princes Risborough Soil.





Many methods have been proposed for testing wood preservatives against monocultures of soft rot fungi (BS 838, 1961; Schulz and Riewendt, 1962; Duncan, 1965; Bravery, 1968 a,b). These methods rely on the exposure of wood blocks to fungal monocultures on an agar medium and the fungi gain access into the wood block. With the thin section assay the fungi are allowed direct access into the S_2 layer of the wood cell wall. Decay is much quicker than with wood blocks. The effectiveness of the treatment of the S_2 layer can be directly assessed and the relationship of the fungi to the treated wood can be studied at the cell wall level.

Soil burial relies on the natural mixtures of microorganisms in the soil effecting decay of buried wood (Theden, 1961; Becker and Kaune, 1966; Bravery, 1975). This enables the exposure of thin sections to be close to conditions met in practice e.g. poles in ground contact. It was for this reason that unsterile soil was selected as the burial medium for subsequent tests.

3.3.2.5 Conclusions

(1) The thin section assay is effective for determining soft rot attack of untreated and treated thin sections exposed to either soft rot monocultures or soil burial.

(2) Results from monoculture and soil burial tests are similar. Scots pine is more resistant to soft rot attack than birch and treatment with CCA reduces attack of both wood species.

(3) Soil burial represents conditions met in the field, monocultures are more severe.

3.3.3 Performance of Copper-Based Experimental Formulations

3.3.3.1 Introduction

The performance of the copper-based formulations copper sulphate (C), copper chrome (CC), copper chrome arsenic (CCA), copper chrome boron (CCB), CC followed by a second treatment with arsenic (CC + A) and CCB followed by arsenic (CCB + A) (as described in Section 3.3.1), was examined using the thin section assay.

Two different soils were used:

(i) Rentokil soil.

(ii) John Innes No.1 (as described in Section 2.4.1).

An assessment could therefore be made of the effect of soil type on the performance of the formulations.

3.3.3.2 Method

Birch and Scots pine 7 mm^3 blocks treated with C, CC, CCA, CCB, CC + A and CCB + A were used (Section 3.2). The concentrations used for each species were:

Birch 1.6, 3.25, 5.7% w/v

Scots Pine 0.7, 2.36, 4.13% w/v

Transverse sections, 30 μm thick, were cut from treated and untreated blocks, placed in nylon mesh packets (Section 2.2.2) and buried in the two different soils. The soils were kept at a moisture content of 95% of soil water holding capacity as determined in Section 2.4.2.

Sections were removed after 2, 4, 6, 8, 12, 20 and 30 weeks, stained, examined under the light microscope and a Soft Rot Degree (SRD) determined (Section 2.4.4.2). Four sections for each treatment were examined for each time period with ten fields of view for each. A mean SRD was determined.

3.3.3.3 Results

Mean net dry salt retentions, together with percentage elemental contents of the wood blocks were calculated (Section 3.2.2.5) and are recorded in Tables 24 and 25.

The mean SRDs for each treatment, with 95% confidence limits, were calculated (Section 3.3.1.3) and are given in Tables 26-31,39-44. Mean SRDs were plotted against incubation time (Figures 11 - 17) from 0 to 12 weeks only as results showed that after this time SRDs levelled off at a maximum value. An example of this is given in Figure 11 for birch sections buried in Rentokil soil. It was these first twelve weeks that proved to be the most critical for determining differences between the performance of the formulations.

An analysis of variance test was used to determine firstly the effect of preservative type and concentration (Section 3.3.3.3.3) and secondly, soil type (Section 3.3.3.3.4) on SRD values.

The results for the two soils and two wood species will be considered separately.

3.3.3.3.1 Birch in Rentokil Soil

Concentration 1.6% (Table 26, Figures 11 and 12). All treatments reduced

the rate of attack over that found for C and untreated. CCB, CCB+A, CC and CC+A performed better than CCA at this concentration. This agrees with the results found by Gray (1983) that formulations in which arsenic was absent or added as a second treatment performed better than those in which copper and arsenic were added together eg. CCA. The improved performance of CC over C suggests the importance of chromium fixing the copper to the wood cell wall.

Concentration 3.25% (Table 27, Figure 13). Attack was reduced at this concentration. CCA performed the best.

Concentration 5.7% (Table 28, Figure 14). Attack was reduced but eventually all treatments failed.

3.3.3.3. 2 Birch in John Innes No.1 Soil

Concentration 1.6% (Table 29, Figure 15). J.I.No.1 had a greater soft rot potential than Rentokil soil, SRDs were high for all treatments after six to eight weeks and for untreated after only two weeks.

Concentration 3.25% (Table 30, Figure 16). Attack was only slightly reduced at this higher concentration.

Concentration 5.7% (Table 31, Figure 17). Attack was still severe for C and CCB+A treatments. CC and CCA performed the best. After thirty weeks all treatments had been attacked to the same level. A concentration of 5.7% CCA equivalent represents a retention of 41.51 kg/m^3 even at this high retention attack was only delayed and not prevented.

Attack was more severe for birch in J.I.No.1 than in Rentokil soil.

Table 24

Mean Preservative Retentions and Elemental Contents for Birch blocks

Treatment	Conc (% w/v)	Mean Net Dry Salt Retention (Kgm^{-3})	Standard Deviation	% w/w elemental contents of wood			
				Copper	Chromium	Arsenic	Boron
C	1.6%	3.460	0.249	0.053			
	3.25	6.772	0.237	0.110			
	5.7	11.643	0.274	0.189			
CC	1.6%	7.674	0.472	0.120	0.214		
	3.25	15.872	0.491	0.253	0.451		
	5.7	27.965	0.687	0.445	0.794		
CCA	1.6%	9.639	0.246	0.148	0.264	0.187	
	3.25	19.814	0.490	0.319	0.569	0.403	
	5.7	35.658	0.777	0.561	1.002	0.710	
CCB	1.6%	9.221	0.327	0.151	0.269		0.059
	3.25	18.679	0.933	0.289	0.516		0.113
	5.7	32.676	2.309	0.501	0.894		0.197
CC+A	1.6%	8.896	0.446	0.110	0.196	0.037	
	3.25	18.349	0.842	0.235	0.420	0.072	
	5.7	32.261	2.061	0.409	0.730	0.123	
CCB+A	1.6%	10.390	0.818	0.137	0.244	0.037	0.050
	3.25	22.909	1.071	0.305	0.545	0.072	0.111
	5.7	41.514	1.361	0.566	1.011	0.130	0.207

Table 25

Mean Preservative Retentions and Elemental Contents for Scots Pine blocks

Treatment	Conc (%w/v)	Mean Net Dry Salt Retention (kgm^{-3})	Standard Deviation	% w/w elemental contents of wood			
				Copper	Chromium	Arsenic	Boron
C	0.7%	1.638	0.063	0.032			
	2.36	5.207	0.209	0.099			
	4.13	10.011	0.683	0.179			
CC	0.7%	2.984	0.426	0.054	0.097		
	2.36	12.184	0.341	0.224	0.377		
	4.13	22.998	0.818	0.444	0.793		
CCA	0.7%	4.585	0.172	0.087	0.156	0.111	
	2.36	16.168	0.463	0.323	0.577	0.409	
	4.13	28.373	1.091	0.552	0.985	0.698	
CCB	0.7%	4.513	0.239	0.089	0.159		0.035
	2.36	16.161	0.712	0.326	0.581		0.128
	4.13	28.365	1.000	0.574	1.025		0.226
CC+A	0.7%	3.780	0.486	0.057	0.102	0.020	
	2.36	15.293	0.553	0.247	0.440	0.078	
	4.13	26.337	1.442	0.344	0.615	0.102	
CCB+A	0.7%	5.508	0.827	0.086	0.154	0.022	0.032
	2.36	18.044	0.724	0.297	0.531	0.071	0.109
	4.13	30.651	1.664	0.487	0.869	0.116	0.177

Table 26

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		2	2.35	0.483	2.504	2.196
		4	3.28	0.452	3.424	3.136
		6	3.35	0.483	3.504	3.196
		8	3.33	0.474	3.451	3.149
		12	3.23	0.577	3.414	3.046
		20	3.63	0.490	3.787	3.473
		30	4.00	0		
C	1.6	2	2.60	0.955	2.905	2.295
		4	3.20	0.687	3.419	2.981
		6	3.10	0.496	3.258	2.942
		8	3.15	0.362	3.266	3.034
		12	3.00	0		
		20	3.03	0.800	3.144	2.916
		30	3.48	0.600	3.672	3.288
CC	1.6	2	1.63	1.005	1.951	1.309
		4	2.07	1.403	2.518	1.622
		6	1.65	1.424	2.105	1.195
		8	2.63	1.079	2.975	2.285
		12	2.40	1.297	2.814	1.986
		20	3.25	0.494	3.408	3.092
		30	3.35	0.622	3.549	3.151
CCA	1.6	2	1.15	1.051	1.486	0.814
		4	2.63	0.897	2.917	2.343
		6	3.00	0.679	3.217	2.783
		8	3.30	0.564	3.480	3.120
		12	3.00	0		
		20	3.18	0.501	3.340	3.020
		30	3.10	0.304	3.197	3.003

Table 26 cont

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	1.6	2	1.58	1.059	1.918	1.242
		4	2.65	1.099	3.001	2.299
		6	2.40	0.928	2.697	2.103
		8	1.85	1.312	2.269	1.431
		12	3.00	0		
		20	3.00	0		
		30	3.20	0.405	3.329	3.071
CC+A	1.6	2	0	0		
		4	1.18	1.217	1.569	0.791
		6	1.20	1.203	1.584	0.816
		8	1.63	0.868	1.907	1.353
		12	2.65	0.893	2.935	2.365
		20	2.40	0.545	2.574	2.226
		30	3.23	0.423	3.365	3.095
CCB+A	1.6	2	0.58	0.844	0.850	0.310
		4	1.50	1.301	1.916	1.084
		6	2.80	0.516	2.965	2.635
		8	1.90	1.105	2.253	1.547
		12	2.83	0.385	2.953	2.707
		20	3.20	0.516	3.365	3.035
		30	3.38	0.490	3.537	3.223

Table 27

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
C	3.25	2	1.53	0.877	1.810	1.250
		4	2.28	1.601	2.792	1.768
		6	2.63	0.490	2.787	2.473
		8	2.28	1.109	2.634	1.926
		12	3.10	0.304	3.197	3.003
		20	2.88	0.516	3.045	2.715
		30	2.93	0.474	3.081	2.779
CC	3.25	2	0	0		
		4	0.65	0.770	0.896	0.404
		6	0.28	0.640	0.484	0.076
		8	1.33	0.474	1.481	1.179
		12	1.45	0.932	1.748	1.152
		20	3.05	0.221	3.121	2.979
		30	3.18	0.385	3.303	3.057
CCA	3.25	2	0	0		
		4	0	0		
		6	0	0		
		8	0.05	0.221	0.121	-0.021
		12	0.10	0.441	0.241	-0.041
		20	2.68	0.730	2.913	2.447
		30	3.08	0.526	3.248	2.912

Table 27 cont

Mean Soft Rot Degree Ratings for Birch Sections

Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	3.25	2	0.33	0.616	0.527	0.133
		4	0.78	1.187	1.159	0.401
		6	1.60	0.928	1.897	1.303
		8	0.95	1.131	1.311	0.589
		12	0.93	0.797	1.185	0.675
		20	2.45	0.504	2.611	2.289
		30	3.00	0		
CC+A	3.25	2	0.55	0.846	0.820	0.280
		4	0.03	0.158	0.080	-0.020
		6	0.53	1.109	0.884	0.176
		8	1.08	1.047	1.415	0.745
		12	1.90	1.105	2.253	1.547
		20	2.88	0.516	3.045	2.715
		30	2.93	0.267	3.015	2.845
CCB+A	3.25	2	0	0		
		4	1.53	1.664	2.062	0.998
		6	1.30	1.400	1.747	0.853
		8	2.10	1.172	2.474	1.726
		12	2.53	0.506	2.692	2.368
		20	3.00	0		
		30	3.13	0.335	3.237	3.023

Table 28

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
C	5.7	2	1.95	0.986	2.265	1.635
		4	2.33	1.421	2.784	1.876
		6	2.63	0.490	2.787	2.473
		8	2.75	0.439	2.890	2.610
		12	3.00	0.392	3.125	2.875
		20	3.30	0.464	3.448	3.152
		30	3.43	0.501	3.590	3.270
CC	5.7	2	0	0		
		4	0	0		
		6	0.45	0.667	0.663	0.237
		8	1.53	0.877	1.810	1.250
		12	1.20	1.203	1.556	0.844
		20	2.23	1.310	2.649	1.811
		30	2.95	0.221	3.021	2.879
CCA	5.7	2	0	0		
		4	0	0		
		6	0.30	0.464	0.448	0.152
		8	0.05	0.221	0.121	-0.021
		12	0.98	0.768	1.225	0.735
		20	0.65	0.770	0.896	0.404
		30	3.38	0.490	3.537	3.223

Table 28 cont

Mean Soft Rot Degree Ratings for Birch Sections

Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	5.7	2	0.60	0.900	0.888	0.312
		4	1.05	1.260	1.453	0.647
		6	1.63	1.148	1.997	1.263
		8	1.18	1.059	1.518	0.842
		12	1.30	0.883	1.582	1.018
		20	3.00	0		
		30	2.45	0.504	2.611	2.289
CC+A	5.7	2	0	0		
		4	0	0		
		6	0.13	0.404	0.259	0.001
		8	0.33	0.694	0.552	0.108
		12	0.78	0.800	1.036	0.524
		20	2.38	0.586	2.567	2.193
		30	3.10	0.632	3.302	2.898
CCB+A	5.7	2	0.28	0.987	0.595	-0.035
		4	0.33	0.656	0.540	0.120
		6	0.58	0.931	0.877	0.283
		8	0.85	1.252	1.250	0.450
		12	1.85	1.312	2.275	1.425
		20	2.70	0.464	2.848	2.552
		30	3.18	0.385	3.303	3.057

Figure 11. Performance of Birch in Rentokil Soil.

Treating Solution Concentration 1.6%

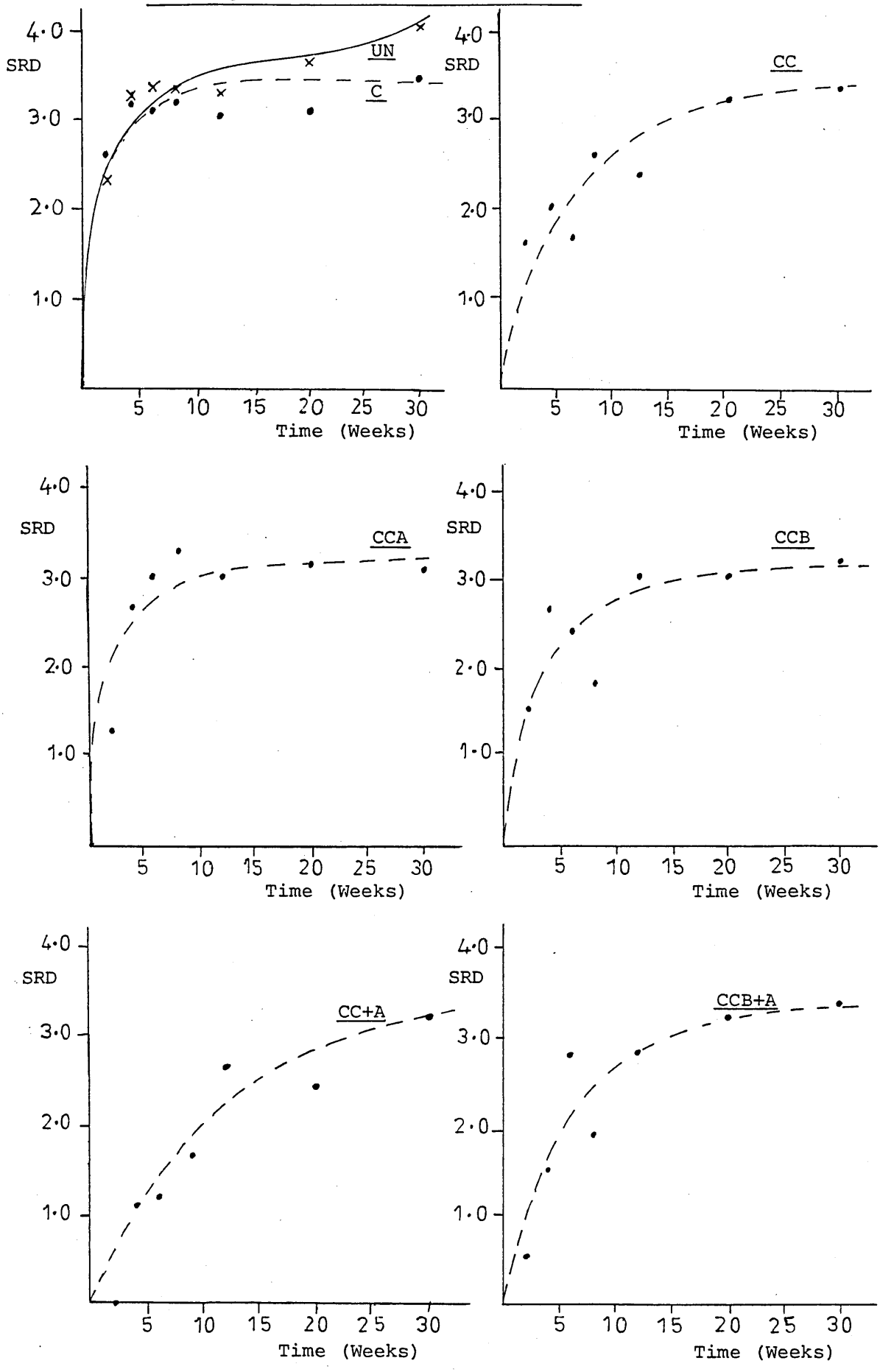


Figure 12 Performance of Birch in Rentokil Soil. Treating Solution 1.6% CCA Equivalent

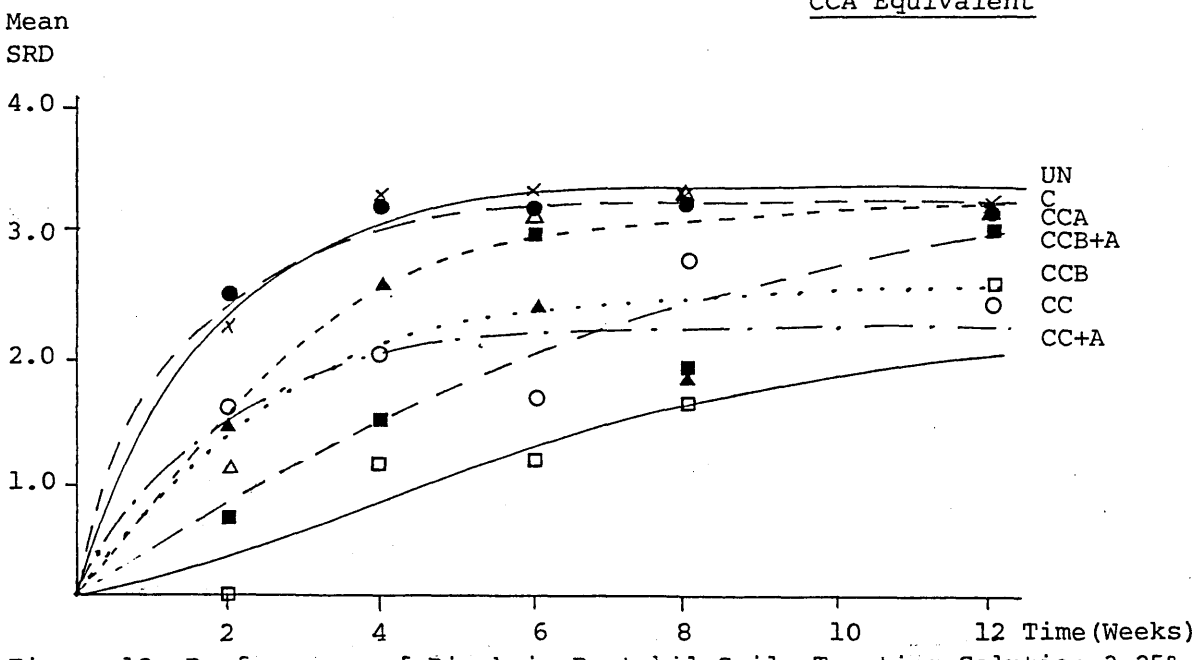


Figure 13 Performance of Birch in Rentokil Soil. Treating Solution 3.25% CCA Equivalent

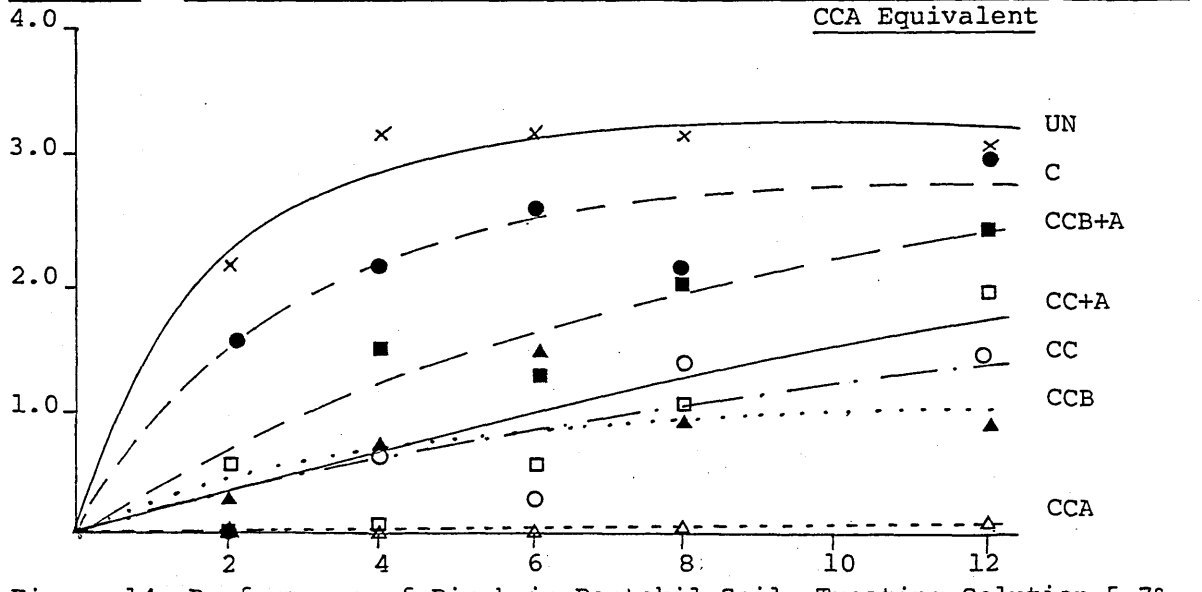


Figure 14 Performance of Birch in Rentokil Soil. Treating Solution 5.7% CCA Equivalent

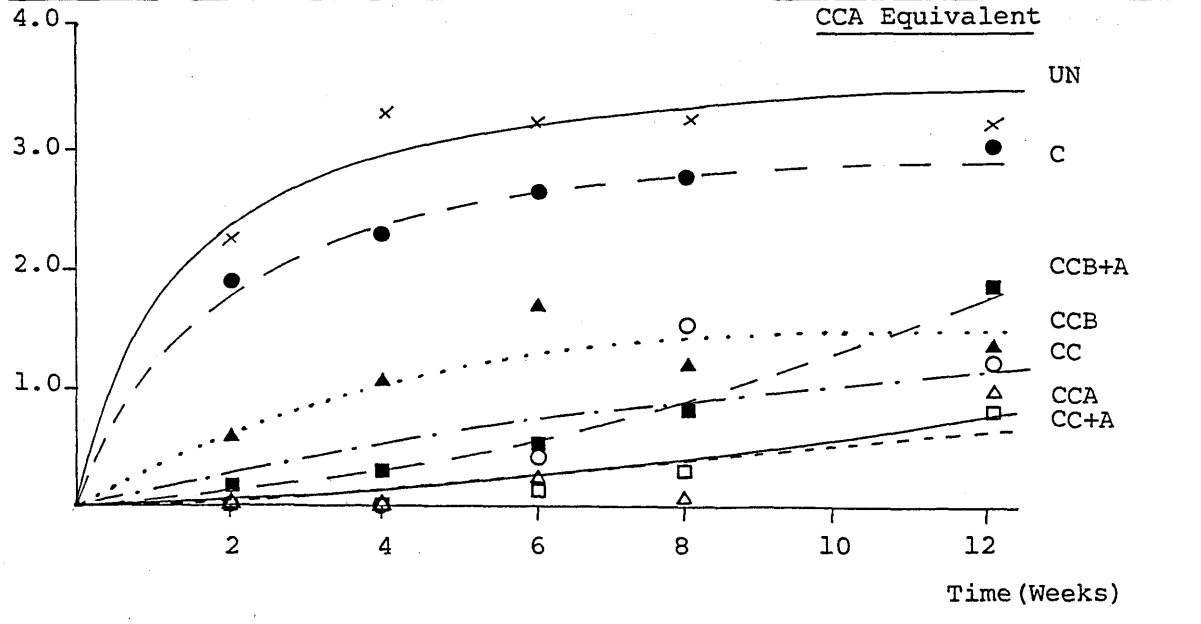


Table 29

Mean Soft Rot Degree Ratings for Birch Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		2	2.95	0.221	3.021	2.879
		4	3.15	0.362	3.266	3.034
		6	3.10	0.304	3.197	3.003
		8	2.55	0.783	2.800	2.300
		12	3.15	0.362	3.266	3.034
		20	3.93	0.267	4.015	3.845
		30	4.00	0		
C	1.6	2	2.45	0.552	2.626	2.274
		4	3.03	0.800	3.286	2.774
		6	2.10	1.057	2.438	1.762
		8	3.00	0		
		12	3.00	0		
		20	3.00	0		
		30	3.18	0.385	3.303	3.057
CC	1.6	2	1.93	0.764	2.174	1.686
		4	2.15	1.099	2.501	1.799
		6	2.68	0.616	2.877	2.483
		8	2.83	0.385	2.953	2.707
		12	3.23	0.423	3.365	3.095
		20	3.05	0.522	3.226	2.874
		30	4.00	0		
CCA	1.6	2	1.93	0.616	2.127	1.733
		4	2.10	1.172	2.474	1.726
		6	2.70	0.516	2.865	2.535
		8	2.95	0.221	3.021	2.879
		12	3.00	0		
		20	2.78	0.698	3.003	2.557
		30	3.23	0.423	3.365	3.095

Table 29 cont.

Mean Soft Rot Degree Ratings for Birch Sections

Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	1.6	2	1.95	0.783	2.200	1.700
		4	2.53	0.905	2.819	2.241
		6	2.95	0.504	3.111	2.789
		8	3.25	0.439	3.390	3.110
		12	3.05	0.316	3.151	2.949
		20	2.95	0.221	3.021	2.879
		30	3.48	0.506	3.642	3.318
CC+A	1.6	2	2.00	0.751	2.240	1.760
		4	3.23	0.530	3.399	3.061
		6	3.05	0.221	3.121	2.979
		8	2.93	0.267	3.015	2.845
		12	3.35	0.483	3.504	3.196
		20	3.25	0.439	3.390	3.110
		30	3.88	0.335	3.987	3.773
CCB+A	1.6	2	2.18	0.446	2.323	2.037
		4	2.73	1.012	3.053	2.407
		6	2.80	0.687	3.020	2.580
		8	3.00	0.320	3.102	2.898
		12	3.00	0		
		20	3.93	0.267	4.015	3.845
		30	4.00	0		

Table 30

Mean Soft Rot Degree Ratings for Birch Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
C	3.25	2	2.65	0.483	2.804	2.496
		4	2.65	0.662	2.862	2.438
		6	2.83	0.446	2.973	2.687
		8	2.45	1.061	2.789	2.111
		12	3.05	0.221	3.121	2.979
		20	2.85	0.483	3.004	2.696
		30	3.13	0.335	3.237	3.023
CC	3.25	2	1.70	0.758	1.942	1.458
		4	2.00	1.414	2.452	1.548
		6	2.95	0.597	3.141	2.759
		8	2.25	1.235	2.645	1.855
		12	2.68	0.616	2.877	2.483
		20	2.73	0.554	2.907	2.553
		30	3.15	0.770	3.396	2.904
CCA	3.25	2	0.75	0.776	0.998	0.502
		4	1.53	1.450	1.993	1.067
		6	2.03	1.368	2.467	1.593
		8	2.25	1.149	2.617	1.883
		12	2.93	0.730	3.163	2.697
		20	2.90	0.304	2.997	2.803
		30	3.83	0.385	3.953	3.707

Table 30 cont

Mean Soft Rot Degree Ratings for Birch Sections

Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	3.25	2	1.38	0.868	1.657	1.103
		4	2.65	0.834	2.916	2.384
		6	2.20	0.823	2.463	1.937
		8	2.85	0.483	3.004	2.696
		12	3.18	0.385	3.303	3.057
		20	3.00	0		
		30	3.68	0.474	3.831	3.529
CC+A	3.25	2	1.45	0.932	1.748	1.152
		4	2.53	0.599	2.721	2.339
		6	2.80	0.608	2.994	2.606
		8	3.00	0		
		12	3.10	0.304	3.197	3.003
		20	3.25	0.439	3.390	3.110
		30	3.73	0.599	3.921	3.539
CCB+A	3.25	2	2.58	0.747	2.819	2.341
		4	2.30	0.939	2.600	2.000
		6	2.65	0.622	2.849	2.451
		8	3.13	0.463	3.278	2.982
		12	3.23	0.480	3.383	3.077
		20	3.15	0.362	3.266	3.034
		30	3.08	0.267	3.165	2.995

Table 31

Mean Soft Rot Degree Ratings for Birch Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
C	5.7	2	2.53	0.506	2.692	2.368
		4	2.53	0.784	2.781	2.279
		6	3.13	0.404	3.259	3.001
		8	2.90	0.304	2.997	2.803
		12	3.03	0.158	3.080	2.980
		20	3.18	0.385	3.303	3.057
		30	3.93	0.267	4.015	3.845
CC	5.7	2	0.65	0.834	0.916	0.384
		4	1.58	1.107	1.934	1.226
		6	1.63	1.030	1.959	1.301
		8	1.40	1.277	1.808	0.992
		12	2.43	0.903	2.719	2.141
		20	2.95	0.221	3.021	2.879
		30	3.10	0.744	3.338	2.862
CCA	5.7	2	0.80	0.791	1.053	0.547
		4	1.30	1.244	1.697	0.903
		6	1.53	1.240	1.926	1.134
		8	1.75	1.127	2.110	1.390
		12	2.50	0.784	2.751	2.249
		20	2.60	0.778	2.849	2.351
		30	3.78	0.530	3.949	3.611

Table 31 cont

Mean Soft Rot Degree Ratings for Birch Sections

Buried in John Innes No.1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	5.7	2	1.13	0.911	1.421	0.839
		4	1.70	0.723	1.931	1.469
		6	2.50	0.906	2.789	2.211
		8	2.83	0.501	2.990	2.670
		12	2.45	0.986	2.765	2.135
		20	2.48	1.012	2.803	2.157
		30	3.48	0.506	3.642	3.318
CC+A	5.7	2	1.33	0.474	1.481	1.179
		4	0.80	0.723	1.031	0.569
		6	2.40	1.033	2.730	2.070
		8	2.85	0.427	2.986	2.714
		12	3.15	0.362	3.266	3.034
		20	3.15	0.362	3.266	3.034
		30	3.68	0.474	3.831	2.714
CCB+A	5.7	2	2.48	0.716	2.709	2.251
		4	2.93	0.267	3.015	2.845
		6	2.05	0.846	2.320	1.780
		8	2.60	0.496	2.758	2.442
		12	3.08	0.267	3.165	2.995
		20	3.00	0.320	3.102	2.898
		30	3.00	0		

Figure 15 Performance of Birch in John Innes No.1 Soil. Treating Solution 1.6% CCA Equivalent

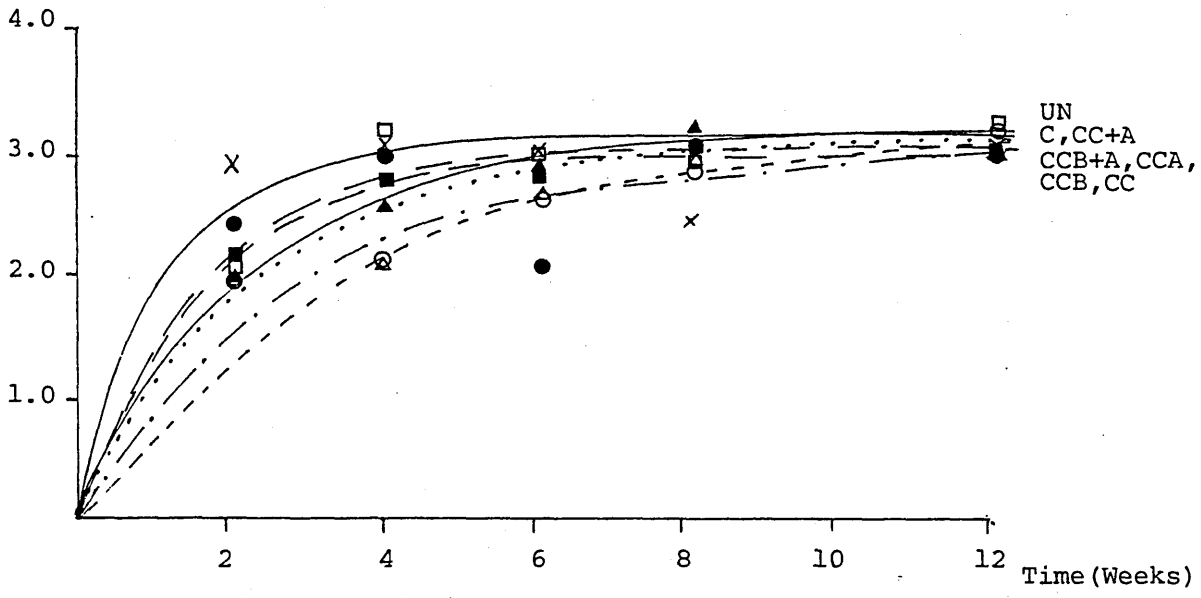


Figure 16 Performance of Birch in John Innes No.1 Soil. Treating Solution 3.25% CCA Equivalent

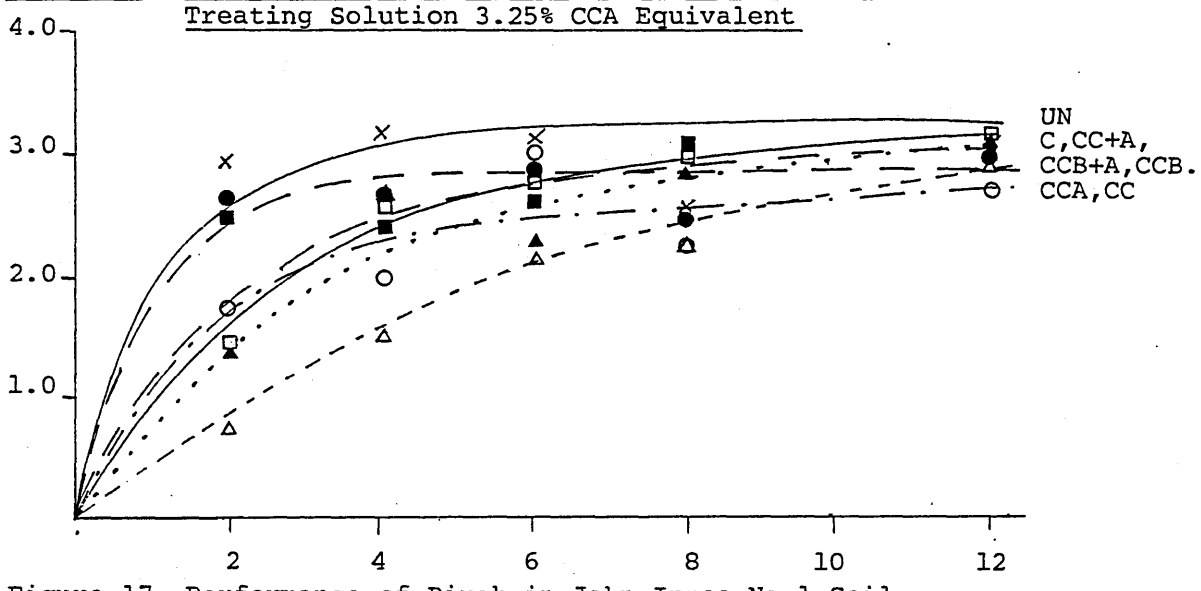
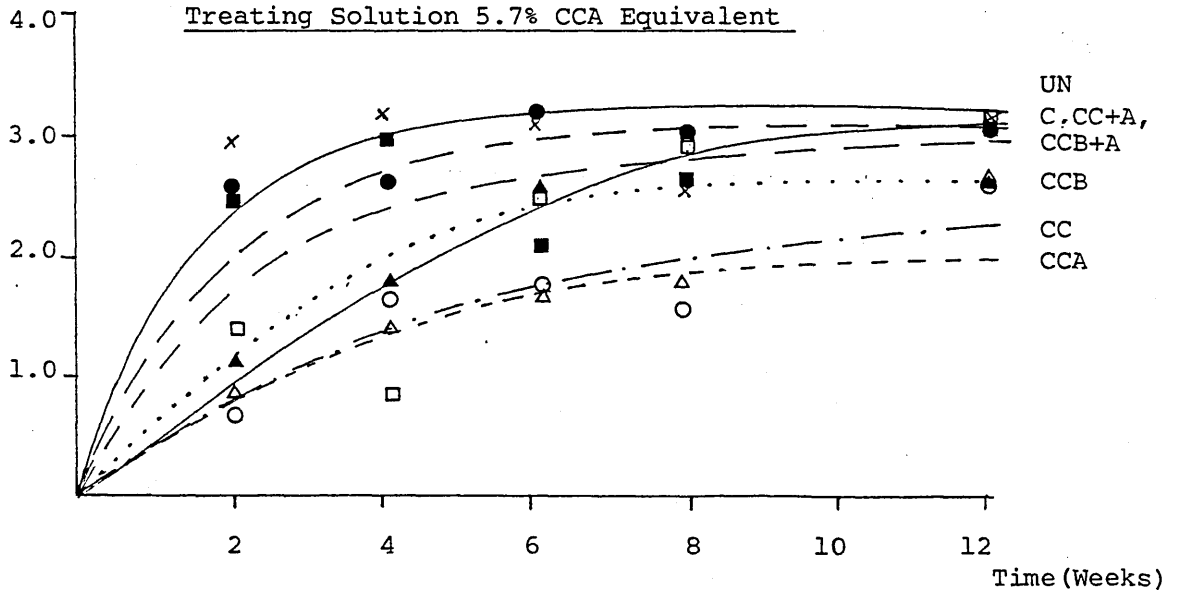


Figure 17 Performance of Birch in John Innes No.1 Soil. Treating Solution 5.7% CCA Equivalent



3.3.3.3.3 An Analysis of the Factors Affecting the Degree of Soft Rot Attack of Birch Sections.

The effect of the type of experimental formulation and concentration used on the degree of soft rot attack was determined by means of an analysis of variance test using the statistical package GENSTAT (Appendix C). The least significant differences (l.s.d) between mean SRDs were calculated for factors having a significant effect on SRDs. The l.s.d was determined:-

$$\text{l.s.d.} = t_{(DF)} \sqrt{\frac{2\ \sigma^2}{n}}$$

where σ^2 = mean square

n = number of observations

t = t value from tables at x degrees of freedom (DF)

Means were compared using the values of least significant difference.

Rentokil Soil

Table 32 shows that for birch sections in Rentokil soil from two to twelve weeks there was a significant difference between mean SRDs. These values after averaging for all concentrations are given in Table 33. After two and four weeks, the performance of C and untreated was significantly worse than that of the other five formulations. After four weeks CC+A performed significantly better than CCB, C and untreated controls. After twenty weeks no significant difference occurred between the formulations. By this time all treatments were failing.

CC+A and CCA had low SRDs at most of the time periods. CCB+A, C treated and untreated sections had highest SRDs after twelve, twenty and thirty weeks.

Table 34 shows that the effect of concentration of formulation used was not significant after twenty and thirty weeks when attack was high.

Table 32

Analysis of Variance On Mean SRD of Treated and Untreated Birch Sections Buried in Rentokil soil For Varying Time Periods.

Time	Unit	DF	F-Ratio	Significance Level
2 Weeks	Preservative	6	12.96	***
	Concentration	2	6.46	*
	Residual	12		
4 Weeks	Preservative	6	10.15	***
	Concentration	2	11.69	**
	Residual	12		
6 Weeks	Preservative	6	9.50	***
	Concentration	2	9.52	**
	Residual	12		
8 Weeks	Preservative	6	4.78	*
	Concentration	2	5.47	*
	Residual	12		
12 Weeks	Preservative	6	3.96	*
	Concentration	2	6.48	*
	Residual	12		
20 Weeks	Preservative	6	2.00	n.s.
	Concentration	2	1.78	n.s.
	Residual	12		
30 Weeks	Preservative	6	8.48	***
	Concentration	2	2.11	n.s.
	Residual	12		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s. = not significant at p level 0.05

Table 33

Mean SRDs From The Analysis of Variance For Birch Sections Buried in Rentokil Soil. (a) Preservative Effect (After Averaging For All Concentrations).

2 Weeks	CC+A	CCB+A	CCA	CC	CCB	C	UN
Mean SRD	0.18	0.29	0.38	0.54	0.84	2.03	2.35
l.s.d. = 0.75	I———	———	———	———	———I	I———	———I

4 Weeks	CC+A	CCA	CC	CCB+A	CCB	C	UN
Mean SRD	0.40	0.88	0.91	1.12	1.49	2.60	3.28
l.s.d. = 1.00	I———	———	———	———I		I———	———I
		I———	———	———	———I		

6 Weeks	CC+A	CC	CCA	CCB+A	CCB	C	UN
Mean SRD	0.62	0.79	1.10	1.56	1.88	2.79	3.35
l.s.d. = 1.02	I———	———	———	———I	I———	———I	
			I———	———	———I	I———	———I

8 Weeks	CC+A	CCA	CCB	CCB+A	CC	C	UN
Mean SRD	1.01	1.13	1.33	1.62	1.83	2.73	3.30
l.s.d. = 1.21	I———	———	———	———	———I	I———	———I
				I———	———	———I	

12 Weeks	CCA	CC	CCB	CC+A	CCB+A	C	UN
Mean SRD	1.36	1.68	1.74	1.78	2.40	3.03	3.23
l.s.d. = 1.12	I———	———	———	———	———I		
					I———	———	———I

20 Weeks	CCA	CC+A	CCB	CC	CCB+A	C	UN
Mean SRD	2.17	2.55	2.82	2.84	2.97	3.07	3.63
	I———	———	———	———	———	———	———I

no significant difference between treatments

30 Weeks	CCB	CC+A	CC	CCA	CCB+A	C	UN
Mean SRD	2.88	3.09	3.16	3.19	3.23	3.28	4.00
l.s.d. = 0.37	I———	———	———	———	———I		
		I———	———	———	———	———I	

I———I = no significant difference

Table 34

Mean SRDs From the analysis of Variance For Birch Sections Buried in Rentokil Soil. (b) Concentration Effect (After Averaging For All Concentrations).

Concentration (% w/v)	Time (Weeks)						
	2	4	6	8	12	20	30
1.6	1.41	2.36	2.50	2.54	2.87	3.10	3.39
3.25	0.68	1.22	1.38	1.58	1.89	2.94	3.18
5.7	0.74	1.00	1.30	1.43	1.76	2.56	3.21
l.s.d.	0.49	0.66	0.67	0.79	0.73	n.s.	n.s.

n.s. = no significant difference at p = 0.05

John Innes No.1 Soil

Table 35 shows that in contrast to the results for birch in Rentokil soil (Table 32), preservative type did not have a significant effect on SRDs at each time period but only after two, four and twenty weeks. Table 36 gives the mean SRDs for each formulation after averaging for all concentrations. After two weeks CCA, CCB, CC and CC+A performed significantly better than CCB+A, C and untreated, after four weeks, CCA performed significantly better than these formulations. After twenty weeks CCA and CCB treated birch sections had SRDs significantly lower than those for CC+A, CCB+A treated or untreated sections.

For six, eight, twelve and thirty weeks there was no significant difference between the performance of the formulations or untreated controls. In Rentokil soil significant differences were more frequent.

Concentration of formulation used had a significant effect only after eight weeks (Tables 35 and 37).

Table 35

Analysis of Variance on Mean SRD of Treated and Untreated Birch Sections Buried In John Innes No.1 Soil For Varying Time Periods.

Time	Unit	DF	F-Ratio	Significance Level
2 Weeks	Preservative	6	10.21	***
	Concentration	2	3.23	n.s.
	Residual	12		
4 Weeks	Preservative	6	3.47	*
	Concentration	2	3.78	n.s.
	Residual			
6 Weeks	Preservative	6	1.51	n.s.
	Concentration	2	1.82	n.s.
	Residual	12		
8 Weeks	Preservative	6	2.99	n,s.
	Concentration	2	4.49	*
	Residual	12		
12 Weeks	Preservative	6	1.89	n.s.
	Concentration	2	3.46	n.s.
	Residual	12		
20 Weeks	Preservative	6	9.49	***
	Concentration	2	1.80	n.s.
	Residual	12		
30 Weeks	Preservative	6	1.11	n.s.
	Concentration	2	0.36	n.s.
	Residual	12		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s.= not significant at p level 0.05

Table 36

Mean SRDs From The Analysis of Variance For Birch Sections Buried in John Innes No.1 Soil. (a) Preservative Effect (After Averaging For All Concentrations).

2 Weeks	CCA	CC	CCB	CC+A	CCB+A	C	UN
Mean SRD	1.16	1.43	1.49	1.59	2.41	2.54	2.95
l.s.d = 0.66	I———	———	———	——I	I———	———	———I
4 Weeks	CCA	CC	CC+A	CCB	CCB+A	C	UN
Mean SRD	1.64	1.91	2.19	2.29	2.65	2.74	3.15
l.s.d. = 0.86	I———	———	———	——I	I———	———	———I
		I———	———	———	———	——I	
6 Weeks	CCA	CC	CCB+A	CCB	C	CC+A	UN
Mean SRD	2.09	2.42	2.50	2.55	2.69	2.75	3.10
	I———	———	———	———	———	———	———I
	no significant difference between treatments						
8 Weeks	CC	CCA	UN	C	CCB+A	CC+A	CCB
Mean SRD	2.16	2.32	2.55	2.78	2.91	2.93	2.98
	I———	———	———	———	———	———	———I
	no significant difference between treatments						
12 Weeks	CC	CCA	CCB	C	CCB+A	UN	CC+A
Mean SRD	2.78	2.81	2.89	3.03	3.10	3.15	3.20
	I———	———	———	———	———	———	———I
	no significant difference between treatments						
20 Weeks	CCA	CCB	CC	C	CC+A	CCB+A	UN
Mean SRD	2.76	2.81	2.91	3.01	3.22	3.36	3.93
l.s.d. = 0.41	I———	———	———	——I			
			I———	———	——I		
				I———	———	——I	
30 Weeks	CCB+A	C	CC	CCB	CCA	CC+A	UN
Mean SRD	3.36	3.41	3.42	3.55	3.61	3.76	4.00
	I———	———	———	———	———	———	———I
	no significant difference between treatments						

I——I = no significant difference

Table 37

Mean SRDs From the Analysis of Variance For Birch Sections Buried in John Innes No.1 Soil. (b) Concentration Effect (After Averaging For All Formulations).

Concentration (% w/v)	Time (Weeks)						
	2	4	6	8	12	20	30
1.6	2.20	2.70	2.77	2.93	3.11	3.27	3.68
3.25	1.92	2.40	2.65	2.64	3.05	3.12	3.51
5.7	1.70	2.00	2.33	2.41	2.83	3.04	3.57
l.s.d.	n.s.	n.s.	n.s.	0.38	n.s.	n.s.	n.s.

n.s. = no significant difference at p = 0.05

In Rentokil soil preservative concentration had a significant effect on mean SRD values for birch sections. This effect was lost in the John Innes No.1 soil.

3.3.3.3.4 An Analysis of The Effect of Soil Type on The Degree of Soft Rot Attack of Birch Sections

A three way analysis of variance test was used to determine the significance of soil type on SRDs. The mean SRDs for each soil were determined after averaging values for all preservatives and concentrations (Table 38).

Table 38

Comparison Between Mean SRD Values for Birch Sections Buried in Rentokil and John Innes No.1 Soils

Time (Weeks)	Soil		l.s.d.	Significance Level
	Rentokil	J.I. No.1		
2	0.94	1.94	0.57	*
4	1.53	2.37	0.36	*
6	1.73	2.59	0.67	*
8	1.85	2.66	0.69	*
12	2.17	3.00	0.53	*
20	2.86	3.14	0.28	*
30	3.26	3.59	0.16	*

* indicates significant at p = 0.05

Results from the analysis of variance show that the performance of the formulations in birch sections depends upon the type of soil used as the burial medium. Higher SRDs were recorded with the more aggressive John Innes No.1 soil.

3.3.3.3.5 Scots Pine in Rentokil Soil

Scots pine is known to be more resistant to soft rot attack than birch and this was found to be the case here.

Concentration 0.7% (Table 39, Figure 18). Attack was very low with SRDs less than 1.0 except for those recorded for C and untreated. Attack was delayed until four weeks for C, CC, CCA and untreated; until six weeks for CCB and CC+A. Treatment with CC+A improved the performance of CCA at this lowest concentration as it had for birch sections using a concentration of 1.6%. CCB+A prevented attack. Even after thirty weeks SRDs were less than 1.0 for scots pine compared with greater than 3.0 for birch sections buried in Rentokil soil.

Concentration 2.36% (Table 40, Figure 19). Attack was delayed until eight weeks for C, twelve weeks for CC and CC+A, twenty weeks for CCA. CCB and CCB+A prevented attack.

Concentration 4.13% (Table 41, Figure 20). Attack at this high concentration was delayed and minimal for each treatment.

3.3.3.3.6 Scots Pine in John Innes No.1 Soil

Concentration 0.7% (Table 42, Figure 21). Treatment reduced attack over that found for untreated controls, but SRDs were higher than those found for Scots pine in Rentokil soil. John Innes No.1 had a higher soft rot decay potential, even the relatively resistant scots pine was attacked. After twelve weeks an SRD of 2.25 was recorded for untreated sections compared to that of only 0.80 in Rentokil soil.

Concentration 2.36% (Table 43, Figure 22). CCA and CCB prevented attack.

Table 39

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		2	0	0		
		4	0.58	0.781	0.830	0.330
		6	0.78	0.800	1.036	0.524
		8	0.75	0.809	1.008	0.492
		12	0.80	0.648	1.007	0.593
		20	0.60	0.709	0.827	0.373
		30	0.85	0.834	1.116	0.584
C	0.7	2	0	0		
		4	0.10	0.304	0.197	0.003
		6	1.03	1.025	1.358	0.702
		8	1.00	0.751	1.240	0.760
		12	1.33	0.888	1.614	1.046
		20	0.63	0.838	0.900	0.362
		30	0.33	0.474	0.481	0.179
CC	0.7	2	0	0		
		4	0.10	0.304	0.197	0.003
		6	0.68	0.572	0.863	0.497
		8	0.50	0.816	0.761	0.239
		12	0.15	0.362	0.266	0.034
		20	0.15	0.483	0.304	-0.004
		30	0.28	0.554	0.457	0.103
CCA	0.7	2	0	0		
		4	0.05	0.221	0.121	-0.021
		6	0.65	0.893	0.935	0.365
		8	0	0		
		12	0.30	0.516	0.465	0.135
		20	0.03	0.158	0.080	-0.020
		30	0.30	0.516	0.465	0.135

Table 39 cont.

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	0.7	2	0	0		
		4	0	0		
		6	0.75	1.127	1.110	0.390
		8	0.33	0.694	0.552	0.108
		12	1.18	0.675	1.396	0.964
		20	0.05	0.221	0.121	-0.021
		30	0.13	0.335	0.237	0.023
CC+A	0.7	2	0	0		
		4	0	0		
		6	0.03	0.158	0.080	-0.020
		8	0	0		
		12	0	0		
		20	0.08	0.267	0.165	-0.005
		30	0.13	0.335	0.237	0.023
CCB+A	0.7	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		

Table 40

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
C	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0.15	0.362	0.266	0.034
		12	0.08	0.267	0.364	-0.204
		20	1.03	0.800	1.286	0.774
		30	0.43	0.636	0.633	0.227
CC	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0.15	0.362	0.266	0.034
		20	0	0		
		30	0.20	0.517	0.365	0.035
CCA	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0.53	0.716	0.759	0.301
		30	0	0		

Table 40 cont.

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		
CC+A	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0.20	0.405	0.329	0.071
		20	0.25	0.439	0.390	0.110
		30	0.20	0.464	0.348	0.052
CCB+A	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		

Table 41

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Rentokil Soil

Treatment	Conc %w/v	Time (Weeks)	Mean (SRD)	Standard Deviation	Confidence Limits	
					Upper	Lower
C	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0.13	0.404	0.259	0.001
		30	1.73	0.679	1.947	1.513
CC	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		
CCA	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0.13	0.335	0.237	0.023
		20	0	0		
		30	0	0		

Table 41 cont.

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Rentokil Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	4.13	2	0	0		
		4	0	0		
		6	0.08	0.267	0.165	-0.005
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		
CC+A	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0.05	0.221	0.121	-0.021
		30	0.33	0.616	0.527	0.133
CCB+A	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		

Figure 18 Performance of Scots Pine in Rentokil Soil. Treating Solution 0.7% CCA Equivalent

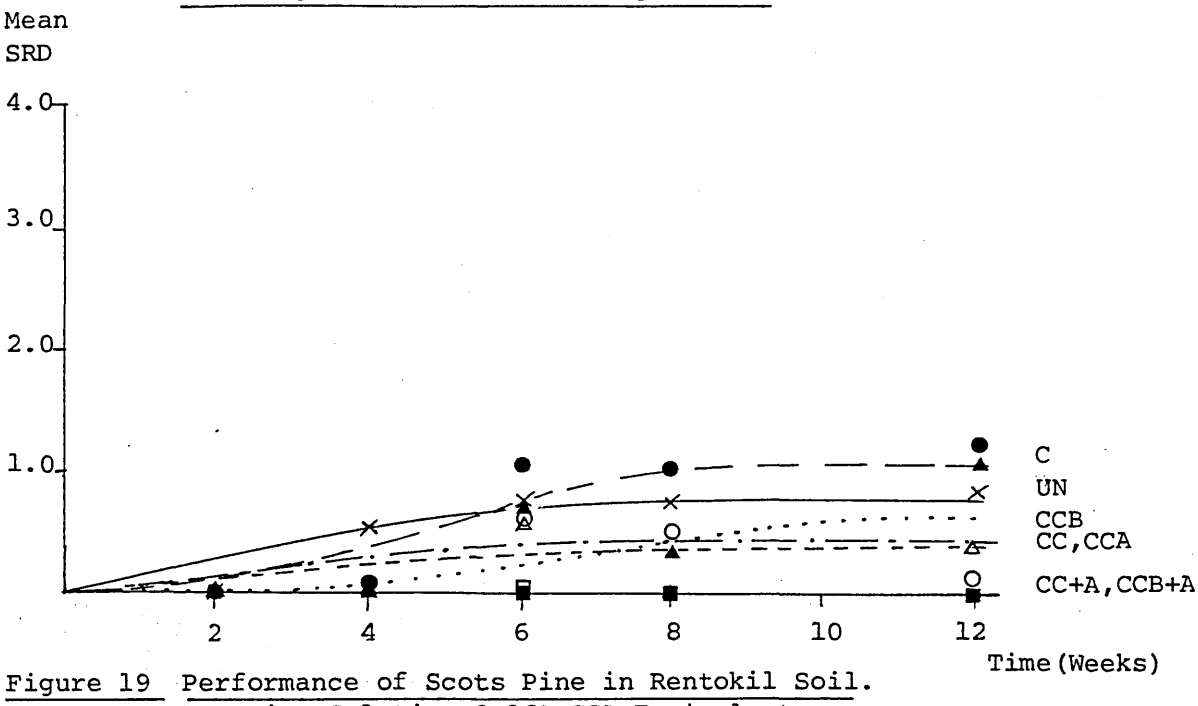


Figure 19 Performance of Scots Pine in Rentokil Soil. Treating Solution 2.36% CCA Equivalent

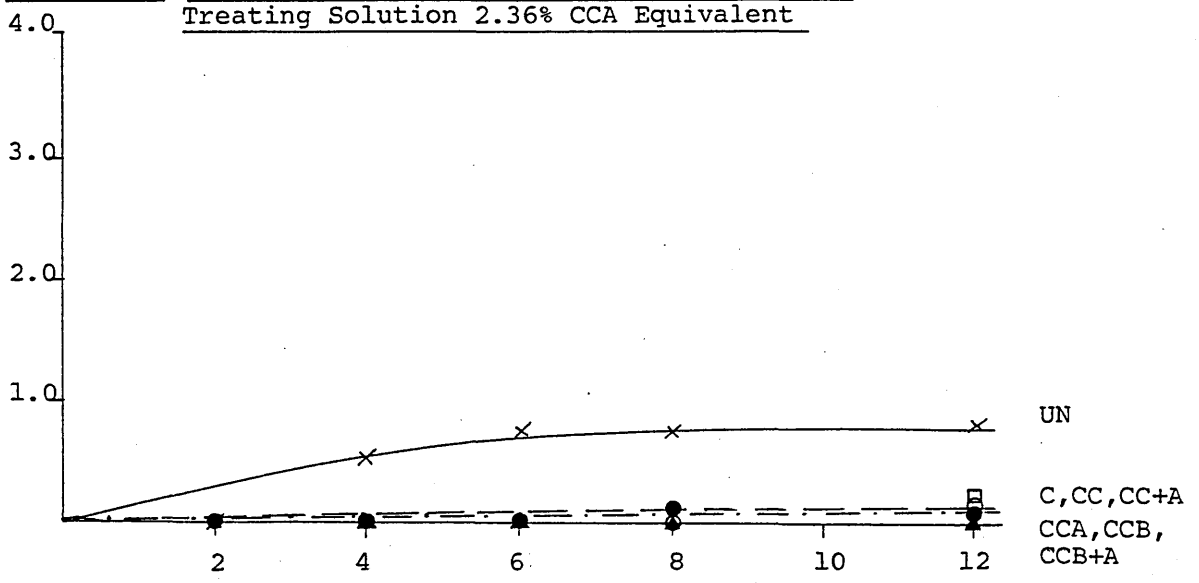


Figure 20 Performance of Scots Pine in Rentokil Soil. Treating Solution 4.13% CCA Equivalent

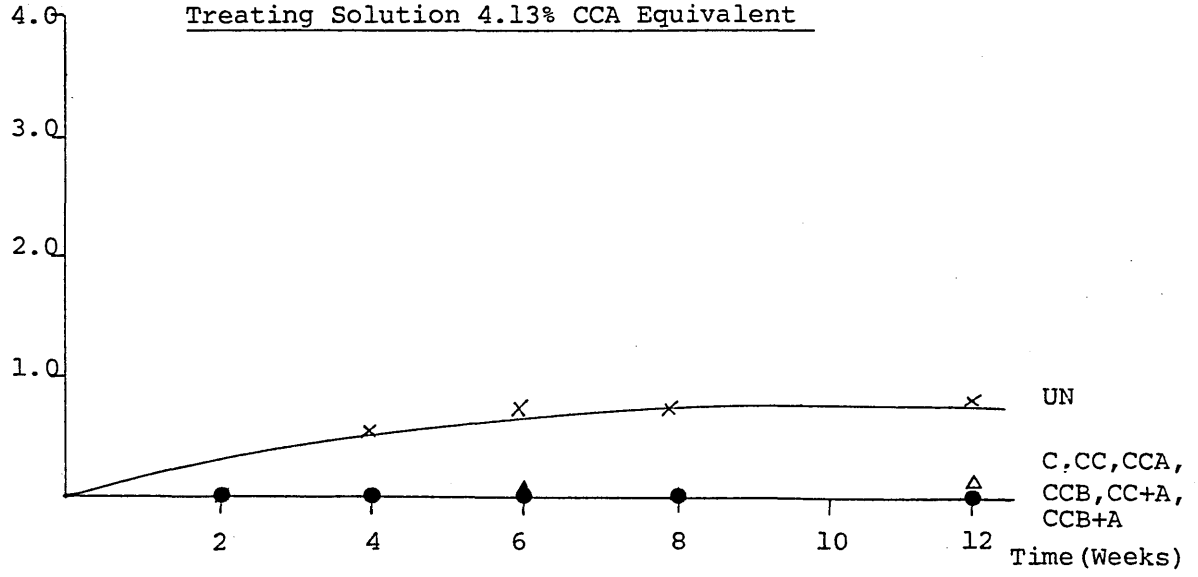


Table 42

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		2	0.85	0.736	1.085	0.615
		4	0.68	0.888	0.964	0.396
		6	1.78	1.291	2.193	1.367
		8	2.10	1.215	2.488	1.712
		12	2.25	0.981	2.563	1.937
		20	3.00	0		
		30	3.65	0.736	3.885	3.415
C	0.7	2	0.45	0.677	0.666	0.234
		4	0.50	0.877	0.780	0.220
		6	0.98	0.832	1.246	0.714
		8	1.30	0.883	1.582	1.018
		12	1.88	1.604	2.393	1.367
		20	1.70	1.381	2.141	1.259
		30	2.00	0.716	2.229	1.771
CC	0.7	2	0	0		
		4	0	0		
		6	0.48	0.640	0.684	0.276
		8	0.18	0.446	0.323	0.037
		12	0.75	1.080	1.095	0.405
		20	1.93	0.888	2.214	1.646
		30	2.98	0.570	3.178	2.782
CCA	0.7	2	0	0		
		4	0	0		
		6	0	0		
		8	1.18	1.059	1.518	0.842
		12	0.93	0.797	1.185	0.675
		20	0.28	0.784	0.531	0.029
		30	0.33	0.572	0.513	0.147

Table 42 cont.

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	0.7	2	0	0		
		4	0	0		
		6	0.20	0.464	0.348	0.052
		8	0.53	0.751	0.770	0.290
		12	1.20	1.114	1.556	0.844
		20	2.23	1.310	2.649	1.811
		30	3.00	1.038	3.332	2.668
CC+A	0.7	2	0	0		
		4	0	0		
		6	0.13	0.404	0.259	0.001
		8	1.38	1.234	1.774	0.986
		12	2.18	0.813	2.440	1.920
		20	3.23	0.423	3.365	3.095
		30	3.83	0.385	3.953	3.707
CCB+A	0.7	2	0.20	0.464	0.348	0.052
		4	0.05	0.221	0.121	-0.021
		6	0.43	0.781	0.680	0.180
		8	0.15	0.362	0.266	0.034
		12	0.45	0.714	0.678	0.222
		20	3.00	0.320	3.102	2.898
		30	4.00	0		

Table 43

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
C	2.36	2	0.28	0.452	0.424	0.136
		4	0.45	0.714	0.678	0.222
		6	1.38	1.234	1.774	0.986
		8	0.98	0.832	1.246	0.714
		12	2.18	0.675	2.396	1.964
		20	3.83	0.385	3.953	3.707
		30	2.53	1.219	2.920	2.140
CC	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0.85	0.893	1.135	0.565
CCA	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		

Table 43 cont.

Mean Soft Rot Degree Ratings for Scots Pine Sections

Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		
CC+A	2.36	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0.40	0.709	0.627	0.173
		20	1.30	0.791	1.553	1.047
		30	3.88	0.335	3.987	3.773
CCB+A	2.36	2	0	0		
		4	0	0		
		6	0.78	0.800	1.036	0.524
		8	0.43	0.712	0.658	0.202
		12	0.83	0.747	1.069	0.591
		20	0.58	0.712	0.808	0.352
		30	0.43	0.675	0.646	0.214

Table 44

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
C	4.13	2	0	0		
		4	0	0		
		6	0.10	0.304	0.197	0.003
		8	0.15	0.427	0.286	0.014
		12	1.18	1.059	1.518	0.842
		20	0.95	1.108	1.304	0.596
		30	3.83	0.385	3.953	3.707
CC	4.13	2	0	0		
		4	0	0		
		6	0.03	0.158	0.080	-0.020
		8	0	0		
		12	0	0		
		20	0	0		
		30	0.18	0.594	0.370	-0.010
CCA	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0.15	0.362	0.266	0.034

Table 44 cont.

Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in John Innes No. 1 Soil

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
CCB	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0	0		
CC+A	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0.10	0.304	0.197	0.003
CCB+A	4.13	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
		20	0	0		
		30	0.20	0.516	0.365	0.035

Figure 21 Performance of Scots Pine in John Innes No.1 Soil. Treating Solution 0.7% CCA Equivalent

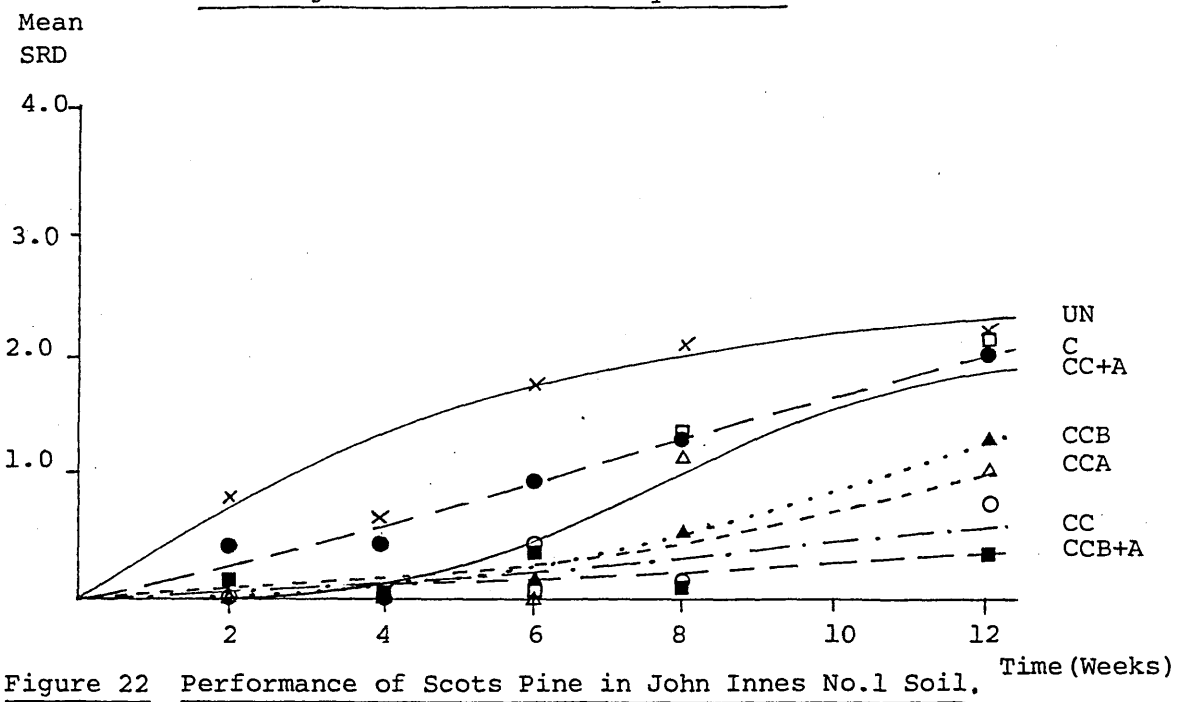


Figure 22 Performance of Scots Pine in John Innes No.1 Soil. Treating Solution 2.36% CCA Equivalent

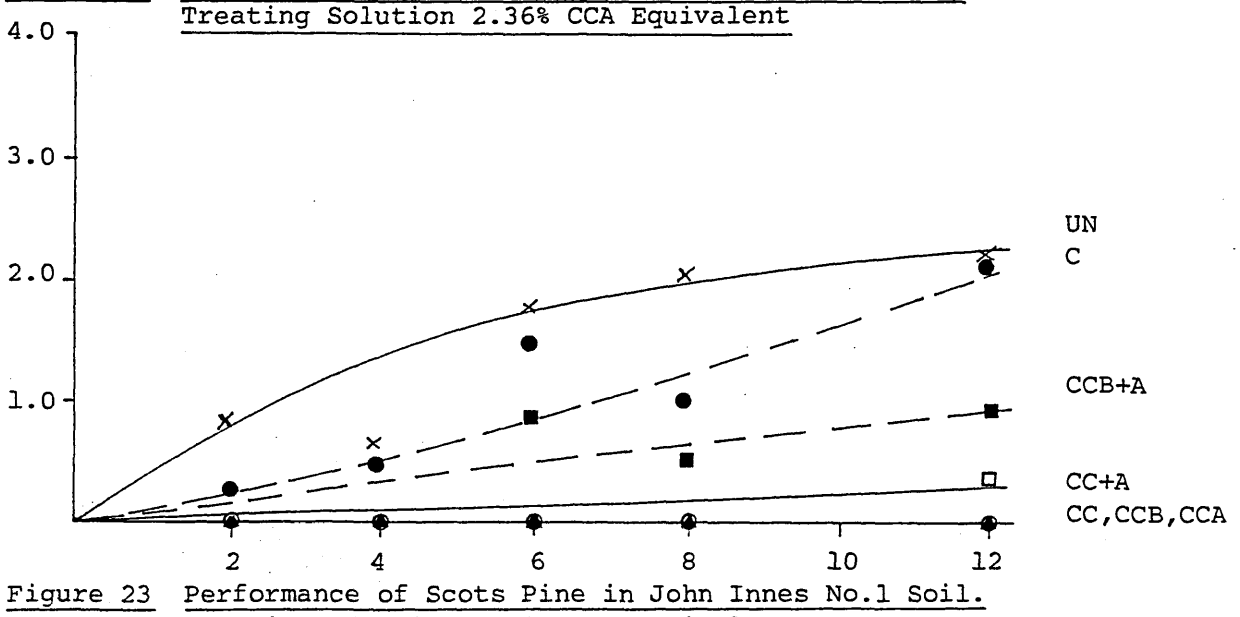
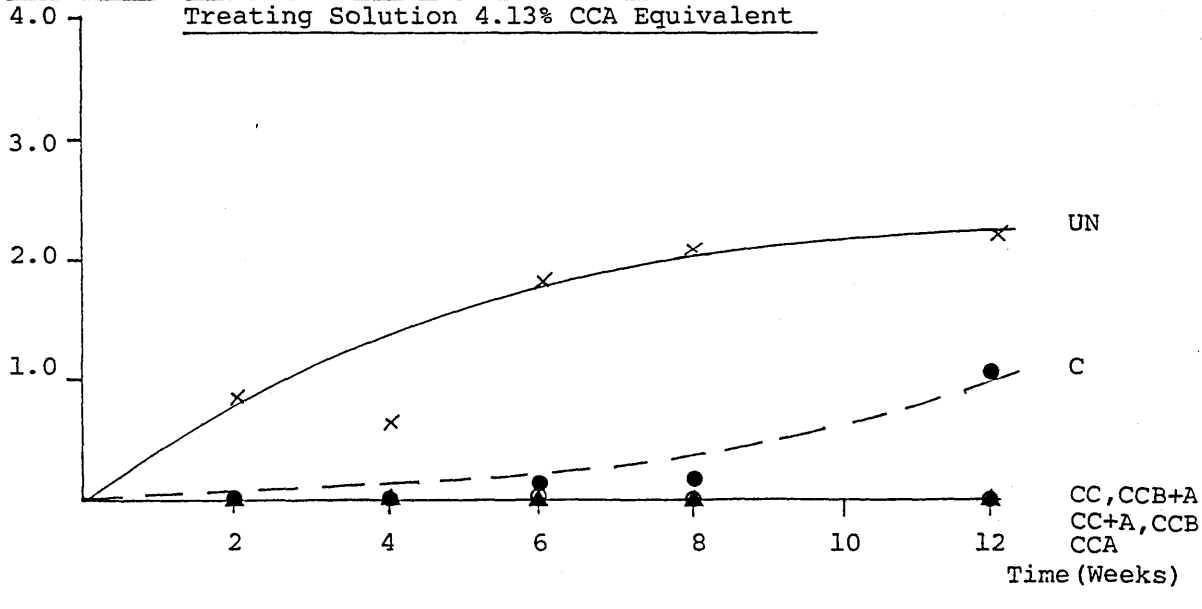


Figure 23 Performance of Scots Pine in John Innes No.1 Soil. Treating Solution 4.13% CCA Equivalent



Attack was minimal for CC, CC+A and CCB+A treated sections. C performed as poorly as untreated controls.

Concentration 4.13% (Table 44, Figure 23). Attack was delayed and reduced. C performed poorly.

3.3.3.3.7 An Analysis of the Factors Affecting the Degree of Soft Rot Attack of Scots Pine. Sections.

Rentokil Soil

Table 45 shows that for Scots pine sections buried in Rentokil soil the type of preservative had a significant effect. Differences, however occurred only between the formulations and untreated after four, six and twelve weeks. Differences between the formulations themselves occurred after eight, twenty and thirty weeks only (Table 46), when CCB+A, CC+A, CCA, CCB and CC performed significantly better than C and untreated. The effect of concentration of formulation used had a significant effect after only six weeks (Table 47).

John Innes No.1 Soil

Table 48 shows that preservative type had a significant effect on SRDs after each time period except thirty weeks when all treatments performed poorly. Differences between the performance of the formulations were more frequent than in Rentokil soil for Scots pine. Table 49 gives the mean SRDs after averaging for all concentrations. After two, four and twelve weeks CCA, CCB, CC+A, CCB+A and CC performed better than C or untreated. After eight weeks CC performed significantly better than C or untreated, after twenty weeks CCA did the same. CCA, CCB and CC had the lowest SRDs for most time periods. The concentration effect was significant after eight, twelve and twenty weeks (Tables 48 and 50).

Table 45

Analysis of Variance on Mean SRD of Treated and Untreated Scots Pine Sections Buried in Rentokil Soil For Varying Time Periods

Time	Unit	DF	F-Ratio	Significance Level
2 Weeks	Preservative	6	0	n.s.
	Concentration	2	0	n.s.
	Residual	12		
4 Weeks	Preservative	6	183.06	***
	Concentration	2	3.95	n.s.
	Residual	12		
6 Weeks	Preservative	6	3.42	*
	Concentration	2	7.62	**
	Residual	12		
8 Weeks	Preservative	6	5.31	**
	Concentration	2	3.36	n.s.
	Residual	12		
12 Weeks	Preservative	6	2.06	n.s.
	Concentration	2	2.88	n.s.
	Residual	12		
20 Weeks	Preservative	6	5.73	**
	Concentration	2	2.63	n.s.
	Residual	12		
30 Weeks	Preservative	6	3.90	*
	Concentration	2	0.56	n.s.
	Residual	12		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s.= not significant at p level 0.05

Table 46

Mean SRDs From the Analysis of Variance For Scots Pine Sections Buried in Rentokil Soil. (a) Preservative Effect (After Averaging For All Concentrations).

2 Weeks							
	CCB+A	CC+A	CCB	CCA	CC	C	UN
Mean SRD	0	0	0	0	0	0	0
	I——— no significant difference between treatments ———I						

4 Weeks							
	CCB+A	CC+A	CCB	CCA	CC	C	UN
Mean SRD	0	0	0	0.02	0.03	0.03	0.58
l.s.d. = 0.05	I———					———I	

6 Weeks							
	CCB+A	CC+A	CCA	CC	CCB	C	UN
Mean SRD	0	0.01	0.22	0.23	0.28	0.34	0.78
l.s.d. = 0.44	I———					———I	

8 Weeks							
	CCB+A	CC+A	CCA	CCB	CC	C	UN
Mean SRD	0	0	0	0.11	0.17	0.38	0.75
l.s.d. = 0.37	I———				———I		
				I———		———I	

12 Weeks							
	CCB+A	CC+A	CC	CCA	CCB	C	UN
Mean SRD	0	0.07	0.10	0.14	0.39	0.47	0.80
	I——— no significant difference between treatments ———I						

20 Weeks							
	CCB+A	CCB	CC	CC+A	CCA	C	UN
Mean SRD	0	0.02	0.05	0.13	0.19	0.60	0.60
l.s.d = 0.34	I———				———I	I———	———I

30 Weeks							
	CCB+A	CCB	CCA	CC	CC+A	C	UN
Mean SRD	0	0.04	0.10	0.16	0.22	0.83	0.85
l.s.d. = 0.57	I———				———I	I———	———I

I——I = no significant difference

Table 47

Mean SRDs From The Analysis of Variance for Scots Pine Sections Buried in Rentokil Soil. (b) Concentration Effect (After Averaging For All Formulations).

Concentration (% w/v)	Time (Weeks)						
	2	4	6	8	12	20	30
0.7	0	0.12	0.56	0.37	0.54	0.22	0.29
2.36	0	0.08	0.11	0.13	0.18	0.34	0.24
4.13	0	0.08	0.12	0.11	0.13	0.11	0.42
l.s.d.	n.s.	n.s.	0.29	n.s.	n.s.	n.s.	n.s.

n.s. = no significant difference at p = 0.05

Table 48

Analysis of Variance on Mean SRD of Treated and Untreated Scots Pine Sections Buried in John Innes No.1 Soil For Varying Time Periods.

Time	Unit	DF	F-Ratio	Significance Level
2 Weeks	Preservative	6	35.79	***
	Concentration	2	1.83	n.s.
	Residual	12		
4 Weeks	Preservative	6	19.39	***
	Concentration	2	1.14	n.s.
	Residual	12		
6 Weeks	Preservative	6	15.93	**
	Concentration	2	2.58	n.s.
	Residual	12		
8 Weeks	Preservative	6	11.12	***
	Concentration	2	5.92	*
	Residual	12		
12 Weeks	Preservative	6	8.57	***
	Concentration	2	6.40	*
	Residual	12		
20 Weeks	Preservative	6	3.13	*
	Concentration	2	5.01	*
	Residual	12		
30 Weeks	Preservative	6	2.56	n.s.
	Concentration	2	3.09	n.s.
	Residual	12		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s. = not significant at p level 0.05

Table 49

Mean SRDs From the Analysis of Variance for Scots Pine Sections Buried in John Innes No.1 Soil (a) Preservative Effect (After Averaging For All Concentrations).

2 Weeks							
	CCA	CCB	CC+A	CC	CCB+A	C	UN
Mean SRD	0	0	0	0	0.07	0.24	0.85
l.s.d. = 0.16	I———				———I		

4 Weeks							
	CCA	CCB	CC+A	CC	CCB+A	C	UN
Mean SRD	0	0	0	0	0.02	0.32	0.68
l.s.d. = 0.18	I———				———I		

6 Weeks							
	CCA	CC+A	CCB	CC	CCB+A	C	UN
Mean SRD	0	0.04	0.07	0.17	0.40	0.82	1.78
l.s.d. = 0.50	I———				———I		
					I———	———I	

8 Weeks							
	CC	CCB	CCB+A	CCA	CC+A	C	UN
Mean SRD	0.06	0.18	0.19	0.39	0.46	0.81	2.10
l.s.d. = 0.65	I———				———I		
		I———				———I	

12 Weeks							
	CC	CCA	CCB	CCB+A	CC+A	C	UN
Mean SRD	0.25	0.31	0.40	0.43	0.86	1.75	2.25
l.s.d. = 0.84	I———				———I	I———	———I

20 Weeks							
	CCA	CC	CCB	CCB+A	CC+A	C	UN
Mean SRD	0.09	0.64	0.74	1.19	1.51	2.16	3.00
l.s.d. = 1.72	I———				———I	I———	———I
		I———				———I	

30 Weeks							
	CCA	CCB	CC	CCB+A	CC+A	C	UN
Mean SRD	0.16	1.00	1.34	1.54	2.60	2.79	3.65
	I———						———I
	no significant difference between treatments						

I——— ———I = no significant difference

Table 50

Mean SRDs from the Analysis of Variance for Scots Pine Sections Buried in John Innes No.1 Soil. (b) Concentration Effect (After Averaging for All Formulations.

Concentration (% w/v)	Time (Weeks)						
	2	4	6	8	12	20	30
0.7	0.21	0.18	0.57	0.97	1.38	2.20	2.83
2.36	0.16	0.16	0.56	0.50	0.81	1.24	1.62
4.13	0.12	0.10	0.27	0.32	0.49	0.56	1.16
l.s.d.	n.s.	n.s.	n.s.	0.43	0.55	1.13	n.s.

n.s. = no significant difference at p = 0.05

3.3.3.3.8 An Analysis of the Effect of Soil Type on The Degree of Soft Rot Attack of Scots Pine Sections.

An analysis of variance test was used to determine the effect of soil type on the degree of soft rot attack of Scots pine sections.

The mean SRDs for each soil were determined after averaging for all formulations and concentrations and are given in Table 51.

Table 51

Comparison Between Mean SRD Values for Scots Pine Sections Buried in Rentokil and John Innes No.1 Soils

Time (Weeks)	Soil		l.s.d.	Significance Level
	Rentokil	J.I. No.1		
2	0	0.17	0.12	*
4	0.10	0.15	0.12	n.s.
6	0.27	0.47	0.42	n.s.
8	0.20	0.60	0.53	n.s.
12	0.28	0.89	0.28	*
20	0.22	1.33	0.74	*
30	0.32	1.87	0.98	*

* indicates significant at p = 0.05
n.s. indicates not significant at p = 0.05

The performance of the formulations was less affected by soil type for Scots pine sections than for birch sections. SRDs were higher in the John Innes No.1 soil but only significantly different from those in Rentokil soil after 2, 12, 20 and 30 Weeks.

3.3.3.3.9 Summary

Birch sections were more susceptible to soft rot attack than

Scots pine sections. Differences between the performance of the formulations were found for birch sections in the less aggressive (in terms of soft rot potential) Rentokil soil. In John Innes No.1 soil all formulations failed to prevent soft rot attack. Differences between the performance of the formulations were found for Scots pine sections in the aggressive John Innes No.1 soil. In the less aggressive Rentokil soil all formulations performed well.

3.3.3.4 Comparison of the Three Soils, Princes Risborough, Rentokil and John Innes No.1 in Terms of Soft Rot Potential

Mean SRDs for sections cut from blocks which had been treated with 5.7% CCA and CCB and those which were untreated, were compared using the results from these soils. In Section 3.3.1 Princes Risborough soil was used, SRDs for birch sections buried in this soil were compared with those for birch sections buried in the two soils used in this test. Mean SRDs were plotted against time in Figures 24, 25, 26. For untreated sections, SRDs were very similar for all three soils. However, for 5.7% CCA and CCB treatments, John Innes No.1 soil and Princes Risborough soil were both more aggressive than Rentokil soil. Attack of thin sections buried in Rentokil soil was reduced. However, after 30 weeks burial, all three soils had recorded the same maximum degree of attack of birch sections.

Figure 24 Performance of Untreated Birch in three soils.

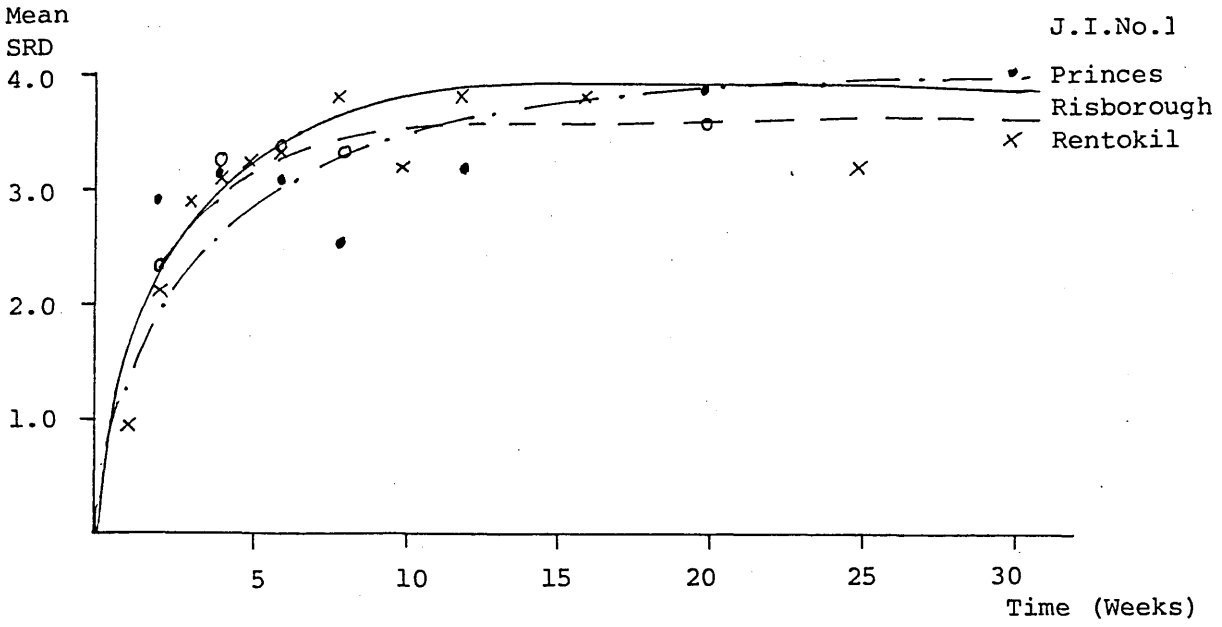


Figure 25 Performance of Birch Treated with 5.7% CCA Equivalent Solution and buried in three soils.

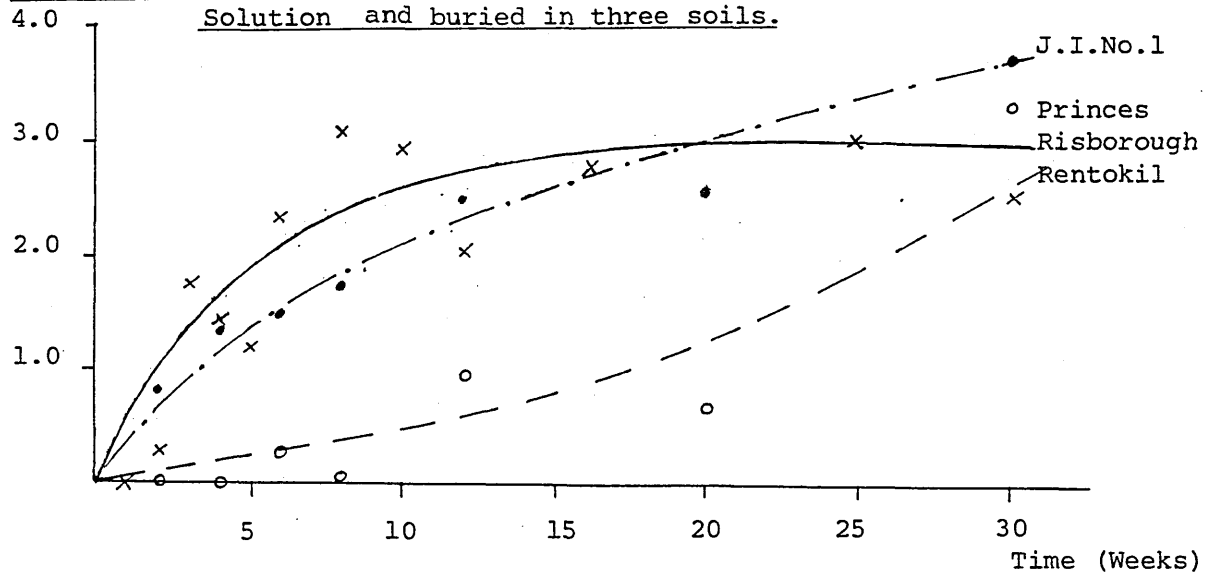
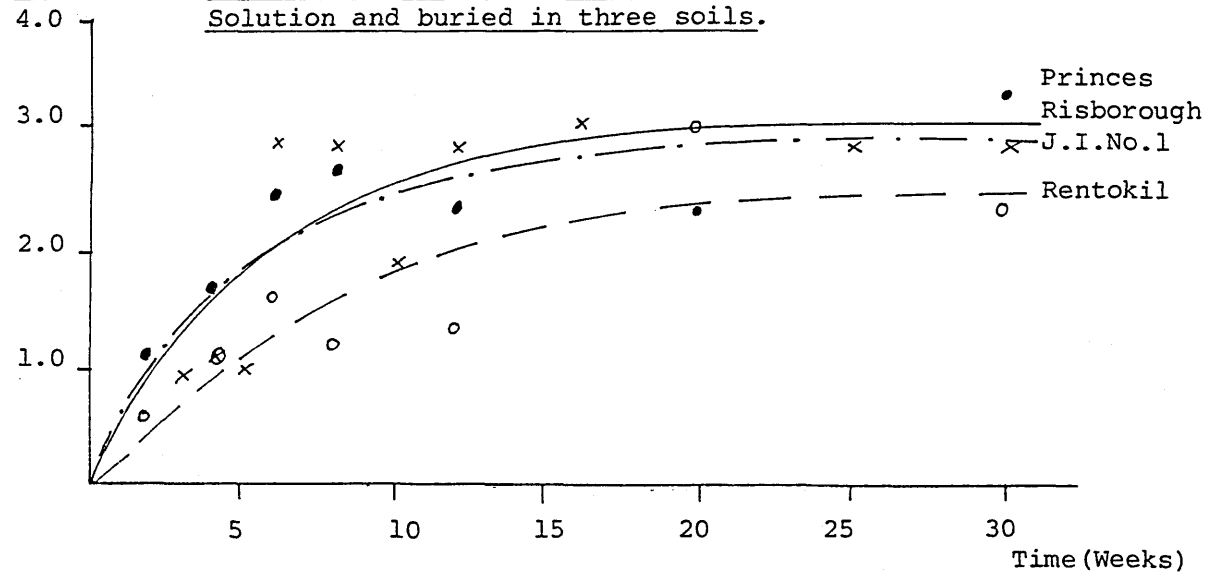


Figure 26 Performance of Birch Treated with 5.7% CCB Equivalent Solution and buried in three soils.



3.3.3.5 Discussion

Results show that the degree of soft rot attack was dependent upon three factors, wood species, preservative type and soil type.

Soft rot attack of Scots pine sections was considerably less than that of birch sections, this was more apparent in Rentokil soil. This agrees with results found in the previous test using Princes Risborough soil (Section 3.3.1) The difference in performance between hardwoods and softwoods treated with CCA preservatives has previously been extensively investigated and will be discussed further in the general discussion (Section 5). Toxic values were only apparent for Scots pine sections, these were concentrations below which attack occurred and above which attack was prevented. For birch sections attack was not prevented at the concentrations of treating solution used.

Differences were found between the performances of the experimental formulations. Gray (1983) suggested that arsenic may interfere with the fixation of copper and reported that those treatments in which arsenic was added separately or absent altogether e.g. CC + A, CCB + A, CC and CCB, performed better than those in which arsenic and copper were added together e.g. CCA. Looking at mean SRDs (Tables 26, 39), this was confirmed here for birch sections treated with 1.6% CCA and Scots pine treated with 0.7%, buried in Rentokil soil. With increasing concentration however, CCA treated sections often had the lowest SRD values. These SRDs were not significantly different from, for example, CCB but this may have been due to the use of the statistical analysis. All mean SRDs were averaged for all concentrations added together. The effect of concentration on SRD values was analysed separately. The lower SRDs for CCA over those for CCB agree with results obtained in the International Research Group on Wood Preservation

collaborative soil burial trial (Carey and Savory, 1975) and those obtained by Kerner-Gang (1975) in beech where CCA was usually more effective than CCB or as effective.

Smith (1970) found that as the concentration of CC increased, it became less effective relative to CCA. This was confirmed in the results obtained here. A treatment of copper sulphate (C) alone, proved to be very ineffective at preventing soft rot attack. For both birch and Scots pine sections buried in the two different soils, treatment with C produced SRDs similar to those found in untreated sections. The copper sulphate treated wood blocks must have suffered severe loss of the preservative during the leaching procedure. Therefore, when sections were cut from these blocks and buried in soil there was probably little of the copper sulphate left in the sections. Chromium is required to fix the copper to the cell wall. Eadie and Wallace (1962) found that with CC there was a greater fixation of copper than with C alone. In the work reported here, CC showed improved performance over C. If only adsorbed copper were effective against soft rot then C would be as effective as CC and CCB. This was not found here.

Soil type proved to be a significant factor affecting the performance of the experimental formulations. Soil composition is very important in determining the aggressiveness of a soil in terms of soft rot potential. Da Costa (1971, 1972) found that the addition of inorganic phosphate to the testing medium or to test blocks decreased the effectiveness of a CCA preservative. The John Innes No.1 and Princes Risborough soils had a higher phosphate content (7.62% and 4.33% respectively) than Rentokil soil (1.12%) (Section 2.4.3). The soils may have reached phosphate contents high enough to decrease the effectiveness of CCA preservatives.

Nitrogen is an important component of soils. Wood decomposition by basidiomycetes and soft rot fungi is stimulated by the addition of nitrogen to the wood or substrate (Savory, 1954 a,b; Duncan, 1965; Becker and Kaune, 1966; Kaune, 1970; Lundstrom, 1973). The nitrogen contents of the soils used here were 0.34% for John Innes No.1, 0.43% for Princes Risborough soil but only 0.16% for Rentokil soil (Section 2.4.3). This illustrates how the nitrogen and as mentioned earlier, phosphate contents of the Rentokil soil were very low in comparison with the other soils, indicating why this soil may have a low soft rot potential. Wälchi (1970, 1972) reported that soft rot decay of wood blocks increased in proportion to the contents of organic matter and nitrogen. The organic carbon composition of Rentokil soil was lower than that of the other two soils (Section 2.4.3).

Differences between the performance of experimental formulations were more apparent for birch sections in the less aggressive Rentokil soil. The performance of CC + A was significantly better than that of CCB, C and untreated after 4 and 6 weeks. The formulations CC + A, CCB + A, CCA, CCB and CC performed significantly better than C and untreated at all time periods except 20 weeks in Rentokil soil. In John Innes No.1, an aggressive soft rotting soil, CCA, CC, CCB and CC + A performed significantly better than CCB + A, C and untreated after 2 weeks. After 6, 8, 12 and 30 weeks however, there was no significant difference, not only between the formulations but also between these and untreated. Differences between the formulations in birch sections were lost in the John Innes No.1 soil.

For *Scots* pine sections differences in the performance of the formulations were not apparent in Rentokil soil but were in John Innes No.1 soil. For the highly susceptible hardwood, birch, differences between formulations were found in the Rentokil soil which had a lower

soft rot potential. However, for the more resistant softwood, Scots pine, differences between the formulations only became apparent in the aggressive John Innes No.1 soil.

Gray (1983) reported significant differences between the performance of CCB+A, CCB and CCA, when examined for each concentration of treating solution. Here these differences may have been lost in the statistical analysis as SRD values for all concentrations were averaged. In addition, Gray (1983) used whole wood blocks, it may be that the entire treated cell wall needs to be present so that fungi attacking the wood encounter the lumen deposits of the preservative. Drysdale et al (1980) stressed the importance of preservative deposits surrounding each individual cell. They suggested that a well treated middle lamella would seal off one cell from the others and effectively reduce the spread of soft rot. With thin sections the soft rot fungus does not have to encounter lumen deposits of the preservative and therefore attack is rapid and severe. With wood blocks there would possibly be cells within that block well treated and the spread of soft rot would be inhibited. The method of soft rot assessment may have been too severe. If one section out of the four had been attacked this may not have been detected within a wood block when assessed on a weight loss basis.

Despite these factors the thin section technique has produced results which agree with other workers for the poor performance of copper sulphate (Eadie and Wallace, 1962), the improved performance of CCA over CCB (Carey and Savory, 1975; Kerner-Gang, 1975) and the improved performance of CCA over CC with increasing solution concentration (Smith, 1970). The technique has also determined the difference in the aggressiveness of soils in terms of soft rot potential.

3.3.3.6 Conclusions

The thin section technique has proved effective in terms of illustrating the importance of soil type and timber species on the degree of soft rot attack of treated wood. There is an interaction between soil type, wood species and preservative type. In the Rentokil soil, having a low soft rot decay potential, the highly susceptible birch, produced differences between the performance of the formulations. Differences were lost in the high decay potential soil, John Innes No.1. For the soft rot resistant species, Scots pine, differences in the performance of the formulations were only significant in the John Innes No.1 soil., in Rentokil soil all formulations performed well.

Soil type and the soft rot susceptibility of wood species affects the performance of experimental formulations against soft rot attack.

3.3.4 Performance of Commercially Available Preservatives

3.3.4.1 Introduction

After laboratory and field tests, Hedley (1984a) found that preservative formulations of Type C were superior to those of Type B. Type C preservatives, Tanalith C, Celcure A salt, Celcure A oxide and Type B, K33 oxide, together with a modified Type B, Tanalith NCA, were used in the thin section assay using soft rot fungi. The results were later compared with those from bacterial tests (Section 3.4.2).

The composition of the preservatives used has been described in Section 3.2.1.2.2. The concentrations used were calculated on an equivalent copper and equivalent active element basis (Section 3.2.1.2.2).

In the previous experiment (Section 3.3.3) John Innes No.1 soil proved to be very aggressive in terms of soft rot potential, differences between the performance of the formulations were more apparent in the Rentokil soil, this was therefore used in this test.

The sections of wood were buried for twelve weeks as it was demonstrated from previous results (Section 3.3.3) that this was the critical time period over which differences between the performance of the preservatives occurred.

3.3.4.2 Method

30 µm thick transverse sections were cut from blocks which had been treated with the five CCA preservatives (Section 3.2.2.2). They were then placed in mesh packets (Section 2.2.2) and buried in Rentokil soil wetted to 95% of its w.h.c. (Section 2.4.2).

The sections were removed after 2, 4, 6, 8 and 12 weeks incubation. SRDs were recorded (Section 2.4.4.2).

3.3.4.3 Results

Mean net dry salt retentions together with percentage elemental contents of the wood blocks were calculated (Section 3.2.2.5) and are given in Tables 52 and 53.

The mean SRDs with 95% confidence intervals are given in Tables 54-57 and 61-63 and plotted against incubation time in Figures 27-32.

Attack of Scots pine sections was minimal. Results are recorded in Table 54.

Table 54
Mean Soft Rot Degree Ratings for Scots Pine Sections Buried in Rentokil Soil.

Treatment	Conc (% w/v)	Time (Weeks)	Mean SRD	Standard Deviation
Untreated		6	1.20	1.114
		8	2.78	0.800
		12	3.00	0
Tan NCA	0.43	8	0.78	0.974
		12	0.75	0.776
	0.53	12	0.45	0.815

Table 52

Mean Preservative Retentions and Elemental Contents

a) Birch

Treatment	Conc (%w/v)	Mean Net Dry Salt Retention (kgm^{-3})	Standard Deviation	% w/w elemental contents of wood		
				Copper	Chromium	Arsenic
Tanalith C	1.6	10.534	0.514	0.163	0.293	0.207
	3.25	21.493	1.056	0.336	0.600	0.425
	5.7	37.414	1.392	0.584	1.042	0.738
Tanalith NCA	0.99	6.297	0.448	0.126	0.134	0.221
	1.21	8.060	0.268	0.166	0.177	0.291
	2.02	12.636	0.634	0.262	0.279	0.459
	2.45	16.007	0.725	0.333	0.355	0.584
	3.53	22.741	0.915	0.469	0.499	0.822
	4.30	28.333	0.923	0.587	0.625	1.029
Celcure A Salt	1.49	9.126	0.579	0.126	0.217	0.226
	1.84	12.077	0.527	0.162	0.279	0.290
	3.03	19.634	0.890	0.258	0.445	0.463
	3.74	23.082	1.050	0.328	0.566	0.589
	5.32	34.892	1.265	0.465	0.802	0.834
	6.56	42.926	1.778	0.575	0.992	1.031
Celcure A Oxide	1.21	7.974	0.520	0.145	0.242	0.218
	1.34	8.269	0.521	0.149	0.249	0.223
	2.47	16.612	1.084	0.301	0.504	0.452
	2.72	18.191	0.526	0.339	0.566	0.508
	4.33	28.796	2.505	0.531	0.886	0.796
	4.77	32.777	0.586	0.622	1.039	0.932
K33 Oxide	0.95	6.189	0.153	0.131	0.153	0.246
	1.21	7.823	0.260	0.164	0.192	0.308
	1.93	12.636	0.664	0.267	0.312	0.501
	2.45	16.930	0.800	0.341	0.400	0.641
	3.39	21.994	1.057	0.448	0.525	0.841
	4.29	29.517	1.222	0.618	0.723	1.159

Table 53

Mean Preservative Retentions and Elemental Contents

b) Scots Pine

Treatment	Conc (%w/v)	Mean Net Dry Salt Retention (kgm^{-3})	Standard Deviation	% w/w elemental contents of wood		
				Copper	Chromium	Arsenic
Tanalith C	0.7	5.192	0.195	0.105	0.188	0.133
	2.36	17.010	0.939	0.350	0.625	0.442
	4.13	30.639	1.656	0.595	1.063	0.753
Tanalith NCA	0.43	3.084	0.150	0.083	0.089	0.146
	0.53	3.887	0.169	0.104	0.110	0.182
	1.46	10.342	0.492	0.281	0.299	0.493
	1.78	13.342	0.676	0.349	0.370	0.611
	2.56	18.461	0.597	0.480	0.511	0.842
	3.11	21.634	1.324	0.600	0.638	1.051
Celcure A Salt	0.65	4.251	0.201	0.068	0.118	0.122
	0.81	5.291	0.218	0.085	0.147	0.152
	2.20	15.080	0.738	0.241	0.415	0.431
	2.72	18.642	0.880	0.292	0.522	0.524
	3.85	26.907	0.973	0.410	0.707	0.735
	4.75	33.340	1.570	0.513	0.886	0.921
Celcure A Oxide	0.53	3.776	0.149	0.079	0.132	0.118
	0.59	4.049	0.151	0.085	0.143	0.128
	1.79	12.255	0.598	0.260	0.435	0.390
	1.98	13.916	0.979	0.289	0.482	0.433
	3.14	23.596	0.875	0.565	0.945	0.848
	3.46	24.130	1.415	0.536	0.896	0.804
K33 Oxide	0.42	2.969	0.142	0.075	0.088	0.142
	0.53	3.855	0.229	0.096	0.113	0.181
	1.40	10.050	0.373	0.257	0.301	0.483
	1.78	12.711	0.654	0.315	0.369	0.591
	2.45	18.140	1.292	0.444	0.520	0.833
	3.11	22.565	0.971	0.548	0.641	1.027

3.3.4.3.1 Birch: Equivalent Copper Comparison

Concentration Equivalent to Tan C 1.6% (Table 55, Figure 27).

The highest SRD was recorded for a treatment of Tanalith NCA. Celcure A salt performed well until twelve weeks when high SRDs were recorded for this treatment. Tanalith C, K33 oxide and Celcure A oxide performed well. All treatments reduced the rate of attack over that of untreated.

Concentration Equivalent to Tan C 3.25% (Table 56, Figure 28).

Tanalith NCA, K33 oxide and Tanalith C performed well.

Concentration Equivalent to Tan C 5.7% (Table 57, Figure 29).

Tanalith C, Celcure A salt, Celcure A oxide and K33 oxide eliminated attack. The maximum SRD for Tanalith NCA was only 0.3.

3.3.4.3.2 An Analysis of the Factors Affecting the Degree of Soft Rot Attack of Birch Sections.

The effect of preservative type and concentration on SRDs was determined by means of an analysis of variance test (Section 3.3.3.3.3 and Appendix C). The significance of the effects of these two factors is given in Table 58.

The effect of preservative type was significant after all time periods, whilst that of concentration, only after two and twelve weeks. The mean SRDs after averaging for all equivalent copper concentrations are given in Table 59. There was a significant difference between the performance of the five preservatives and the untreated controls. Differences between the preservatives were significant after twelve weeks. K33 oxide and Tanalith C performed significantly better than Celcure A salt. SRDs were significantly reduced with increase in concentration after two weeks and again after twelve weeks.

Table 55

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

i) Equivalent Copper Comparison

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Untreated		2	0.78	0.800	1.036	0.524
		4	3.33	0.526	3.498	3.162
		6	3.33	0.474	3.481	3.179
		8	3.30	0.464	3.448	3.152
		12	3.43	0.549	3.605	3.255
Tanalith C	1.6	2	0.68	0.944	0.982	0.378
		4	0.18	0.636	0.383	-0.023
		6	0.78	1.165	1.152	0.408
		8	1.40	1.081	1.745	1.055
		12	1.45	1.131	1.811	1.089
Tanalith NCA	1.21	2	0.55	0.846	0.820	0.280
		4	0.58	0.984	0.894	0.266
		6	3.08	0.694	3.302	2.858
		8	2.53	1.261	2.933	2.127
		12	2.25	1.235	2.645	1.855
Celcure A Salt	1.84	2	0.53	0.905	0.819	0.241
		4	1.40	1.236	1.795	1.005
		6	1.35	1.051	1.686	1.014
		8	1.40	1.105	1.753	1.047
		12	2.90	0.441	3.041	2.759
Celcure A Oxide	1.34	2	0.20	0.516	0.365	0.035
		4	0.20	0.516	0.365	0.035
		6	0.43	0.903	0.719	0.141
		8	0.33	0.797	0.585	0.075
		12	0.38	0.838	0.648	0.112
K33 Oxide	1.21	2	0.55	0.815	0.810	0.290
		4	0.28	0.784	0.530	0.030
		6	0.95	0.986	1.265	0.635
		8	0.58	0.712	0.807	0.353
		12	0.80	1.043	1.133	0.467

Table 56

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

i) Equivalent Copper Comparison

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Tanalith C	3.25	2	0	0		
		4	0	0		
		6	0.15	0.483	0.304	-0.004
		8	0.23	0.577	0.414	0.046
		12	0.28	0.640	0.484	0.076
Tanalith NCA	2.45	2	0.28	0.679	0.497	0.063
		4	0.45	0.876	0.730	0.170
		6	0.28	0.784	0.530	0.030
		8	1.35	1.272	1.756	0.944
		12	0.90	1.008	1.222	0.578
Celcure A Salt	3.74	2	0.68	0.797	0.935	0.425
		4	0.88	1.202	1.264	0.496
		6	1.53	0.933	1.828	1.232
		8	2.18	1.130	2.541	1.819
		12	2.68	0.730	2.913	2.447
Celcure A Oxide	2.72	2	0	0		
		4	1.80	1.224	2.191	1.409
		6	1.68	1.403	2.128	1.232
		8	1.73	1.281	2.139	1.321
		12	1.55	1.239	1.946	1.154
K33 Oxide	2.45	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0.53	0.987	0.845	0.215

Table 57

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

i) Equivalent Copper Comparison

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Tanalith C	5.7	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
Tanalith NCA	4.3	2	0	0		
		4	0.08	0.267	0.165	-0.005
		6	0	0		
		8	0.20	0.564	0.380	0.020
		12	0.30	0.564	0.480	0.120
Celcure A Salt	6.56	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
Celcure A Oxide	4.77	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
K33 Oxide	4.29	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		

Figure 27 Performance of Birch in Rentokil Soil. Treating Solution Concentration Equivalent to 1.6% Tanalith C

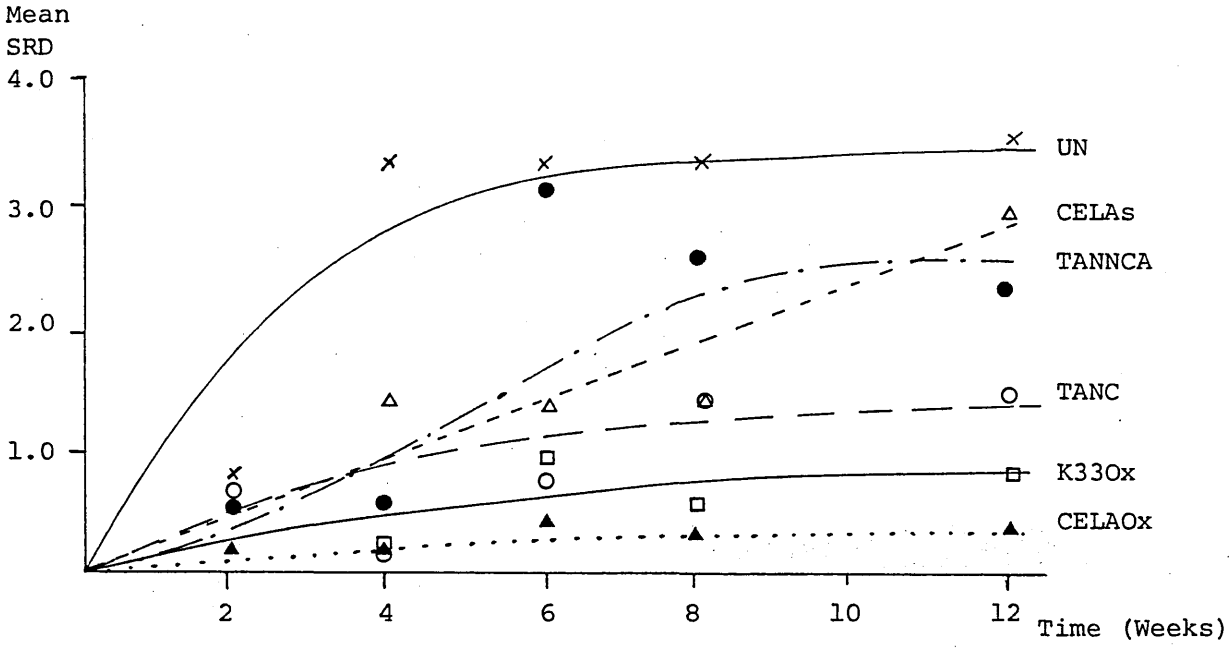


Figure 28 Performance of Birch in Rentokil Soil. Treating Solution Concentration Equivalent to 3.25% Tanalith C

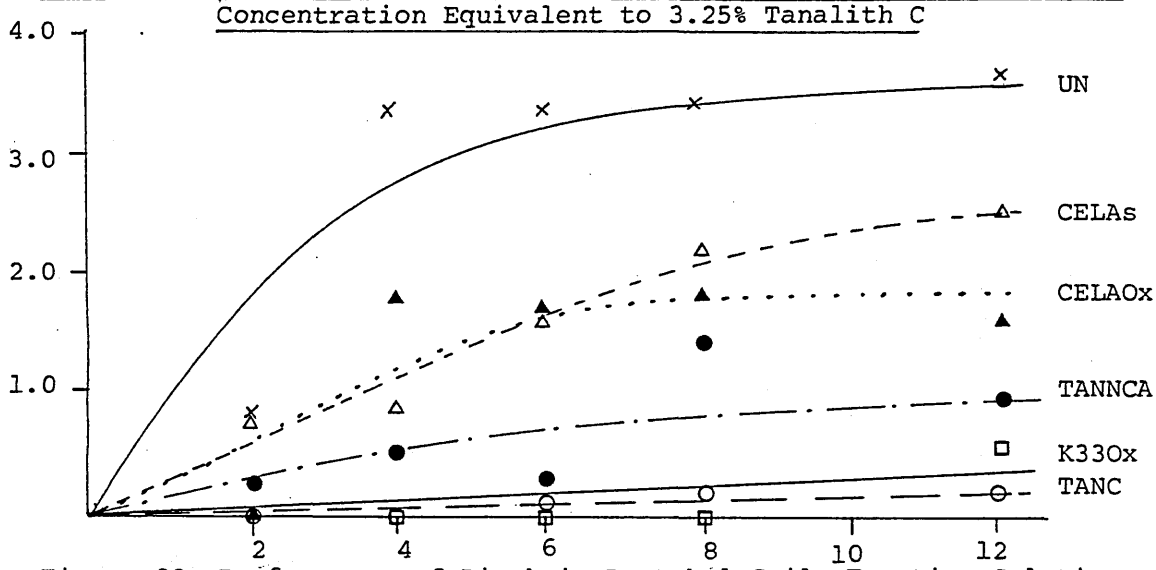


Figure 29 Performance of Birch in Rentokil Soil. Treating Solution Concentration Equivalent to 5.7% Tanalith C

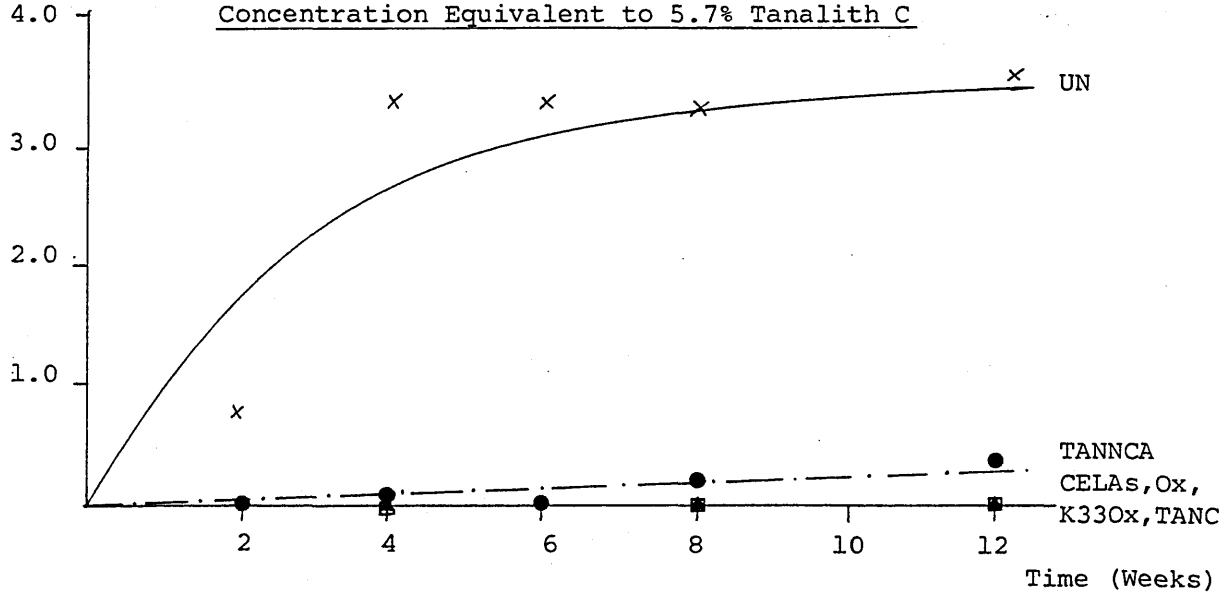


Table 58

Analysis of Variance on Mean SRD of Treated and Untreated Birch Sections buried in Rentokil Soil. Equivalent Copper Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
2 Weeks	Preservative	5	4.58	*
	Concentration	2	6.55	*
	Residual	10		
4 Weeks	Preservative	5	20.19	***
	Concentration	2	1.98	n.s.
	Residual	10		
6 Weeks	Preservative	5	6.35	**
	Concentration	2	3.01	n.s.
	Residual	10		
8 Weeks	Preservative	5	8.05	**
	Concentration	2	3.94	n.s.
	Residual	10		
12 Weeks	Preservative	5	7.91	**
	Concentration	2	5.10	*
	Residual	10		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s. = not significant at p level 0.05

DF = Degrees of Freedom

Table 59

Mean SRDs From The Analysis of Variance for Birch Sections Buried in Rentokil Soil. (a) Preservative Effect (After Averaging for All Equivalent Copper Concentrations).

2 Weeks						
	CELAOx	K33Ox	TANC	TANNCA	CELAs	UN
Mean SRD	0.07	0.18	0.23	0.28	0.40	0.78
l.s.d. = 0.37	I———	———	———	———	———I	
4 Weeks						
	TANC	K33Ox	TANNCA	CELAOx	CELAs	UN
Mean SRD	0.06	0.09	0.37	0.67	0.76	3.33
l.s.d. = 0.86	I———	———	———	———	———I	
6 Weeks						
	TANC	K33Ox	CELAOx	CELAs	TANNCA	UN
Mean SRD	0.31	0.32	0.70	0.96	1.12	3.33
l.s.d. = 1.41	I———	———	———	———	———I	
8 Weeks						
	K33Ox	TANC	CELAOx	CELAs	TANNCA	UN
Mean SRD	0.19	0.54	0.69	1.19	1.36	3.30
l.s.d. = 1.23	I———	———	———	———	———I	
12 Weeks						
	K33Ox	TANC	CELAOx	TANNCA	CELAs	UN
Mean SRD	0.44	0.58	0.64	1.15	1.86	3.43
l.s.d. = 1.28	I———	———	———	———I		
			I———	———	———I	

Table 60

Mean SRDs From The Analysis of Variance for Birch Sections Buried in Rentokil Soil (b) Concentration Effect (After Averaging for All Preservatives).

	Time (Weeks)				
Concentration (%w/v) Equivalent to TAN C	2	4	6	8	12
1.6	0.55	0.99	1.65	1.59	1.87
3.25	0.29	1.08	1.16	1.47	1.56
5.7	0.13	0.57	0.56	0.58	0.62
l.s.d.	0.26	n.s.	n.s.	n.s.	0.91

n.s. = no significant difference

3.3.4.3.3 Birch : Equivalent Active Element Comparison

Concentration Equivalent to Tan C 1.6% (Table 61, Figure 30).

Tanalith C, Celcure A oxide, K33 oxide and Tanalith NCA performed well. Higher SRDs were recorded for Celcure A salt. All treatments reduced the rate and extent of attack compared to that of untreated controls.

Concentration Equivalent to Tan C 3.25% (Table 62, Figure 31).

SRDs were lower, minimal attack occurred with the treatments Tanalith C, Celcure A oxide and K33 oxide.

Concentration Equivalent to Tan C 5.7% (Table 63, Figure 32).

Tanalith C, Celcure A oxide and K33 oxide eliminated attack. After twelve weeks SRDs were less than 1.0 for Tanalith NCA and Celcure A salt treatments.

3.3.4.3.4 An Analysis of the Factors Affecting the Degree of Soft Rot Attack of Birch Sections

An analysis of variance was used as in Section 3.3.4.3.2. Table 64 shows that preservative type had a significant effect on SRDs at all time periods, whilst concentration of preservative affected results at two and six weeks (Table 66).

The mean SRDs for each preservative after averaging for all equivalent active element concentrations are given in Table 65. The five preservative treatments performed significantly better than untreated controls at each time period.

Table 61

Mean Soft Rot Degree Ratings for Birch Sections

Buried in Rentokil Soil

ii) Equivalent Active Element (Cu + As) Comparison

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Tanalith C	1.6	2	0.68	0.944	0.982	0.378
		4	0.18	0.636	0.383	-0.023
		6	0.78	1.165	1.152	0.408
		8	1.40	1.081	1.745	1.055
		12	1.45	1.131	1.811	1.089
Tanalith NCA	0.99	2	0.80	1.203	1.184	0.416
		4	0.30	0.687	0.519	0.081
		6	0.58	0.931	0.877	0.283
		8	0.75	1.006	1.071	0.429
		12	0.85	1.189	1.230	0.470
Celcure A Salt	1.49	2	0.70	0.853	0.973	0.427
		4	0.90	1.128	1.260	0.540
		6	1.78	1.423	2.235	1.325
		8	2.75	0.543	2.923	2.577
		12	3.00	0		
Celcure A Oxide	1.21	2	0	0		
		4	0.50	0.847	0.771	0.229
		6	0.15	0.580	0.335	-0.035
		8	0	0		
		12	0.65	1.051	0.986	0.314
K33 Oxide	0.95	2	0.08	0.350	0.192	-0.032
		4	0.48	0.960	0.787	0.173
		6	0.78	1.074	1.123	0.437
		8	0	0		
		12	0.38	0.705	0.605	0.155

Table 62

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

ii) Equivalent Active Element Comparison

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Tanalith C	3.25	2	0	0		
		4	0	0		
		6	0.15	0.483	0.304	-0.004
		8	0.23	0.577	0.414	0.046
		12	0.28	0.640	0.484	0.076
Tanalith NCA	2.02	2	0.15	0.427	0.286	0.014
		4	0.68	1.071	1.022	0.338
		6	0.73	0.877	1.010	0.450
		8	0.68	0.971	0.990	0.370
		12	1.68	0.971	1.990	1.370
Celcure A Salt	3.03	2	0.30	0.791	0.553	0.047
		4	0.70	1.067	1.041	0.359
		6	1.10	1.150	1.467	0.733
		8	1.18	1.238	1.576	0.784
		12	1.33	1.347	1.760	0.900
Celcure A Oxide	2.47	2	0	0		
		4	0	0		
		6	0	0		
		8	0.08	0.350	0.192	-0.032
		12	0.33	0.694	0.552	0.108
K33 Oxide	1.93	2	0	0		
		4	0	0		
		6	0.20	0.564	0.380	0.020
		8	0.28	0.679	0.497	0.063
		12	0.50	0.961	0.807	0.193

Table 63

Mean Soft Rot Degree Ratings for Birch Sections Buried in Rentokil Soil

ii) Equivalent Active Element Composition

Treatment	Conc (%w/v)	Time (Weeks)	Mean SRD	Standard Deviation	Confidence Limits	
					Upper	Lower
Tanalith C	5.7	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
Tanalith NCA	3.53	2	0.20	0.464	0.348	0.052
		4	0.35	0.736	0.585	0.115
		6	0.68	1.047	1.015	0.345
		8	0.60	0.871	0.878	0.322
		12	0.88	1.042	1.213	0.547
Celcure A Salt	5.32	2	0	0		
		4	0	0		
		6	0	0		
		8	0.25	0.707	0.476	0.024
		12	0.28	0.640	0.484	0.076
Celcure A Oxide	4.33	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		
K33 Oxide	3.39	2	0	0		
		4	0	0		
		6	0	0		
		8	0	0		
		12	0	0		

Figure 30 Performance of Birch in Rentokil Soil. Treating Solution Concentration Equivalent to 1.6% Tanalith C

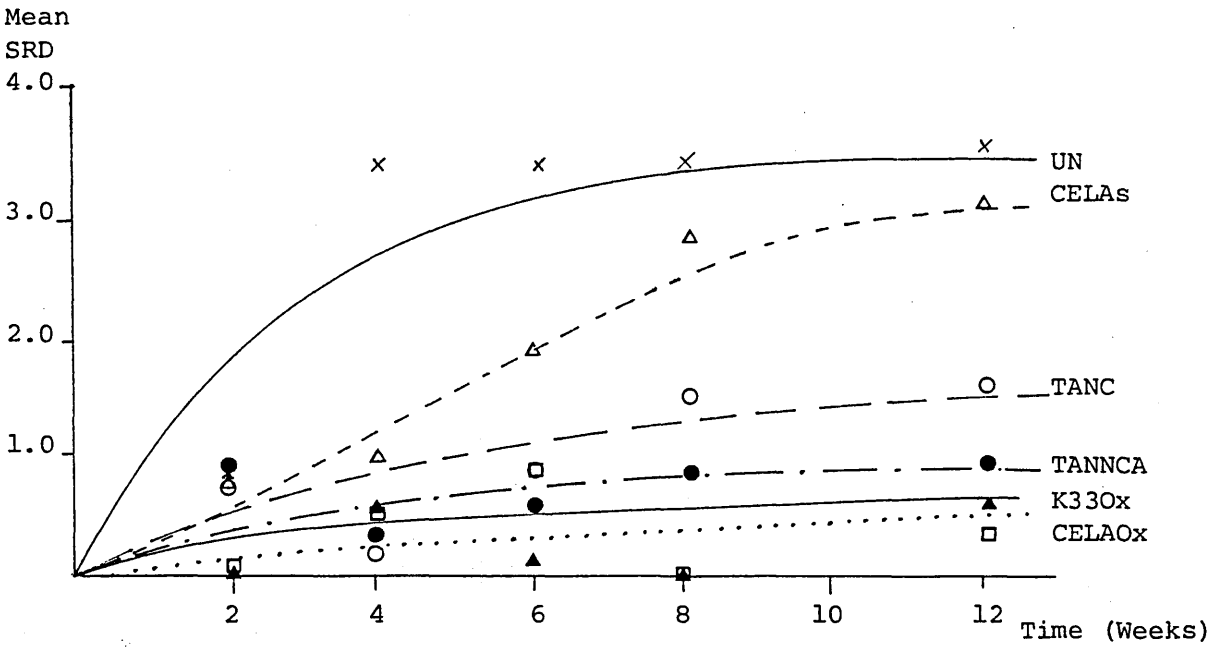


Figure 31 Performance of Birch in Rentokil Soil. Treating Solution Concentration Equivalent to 3.25% Tanalith C

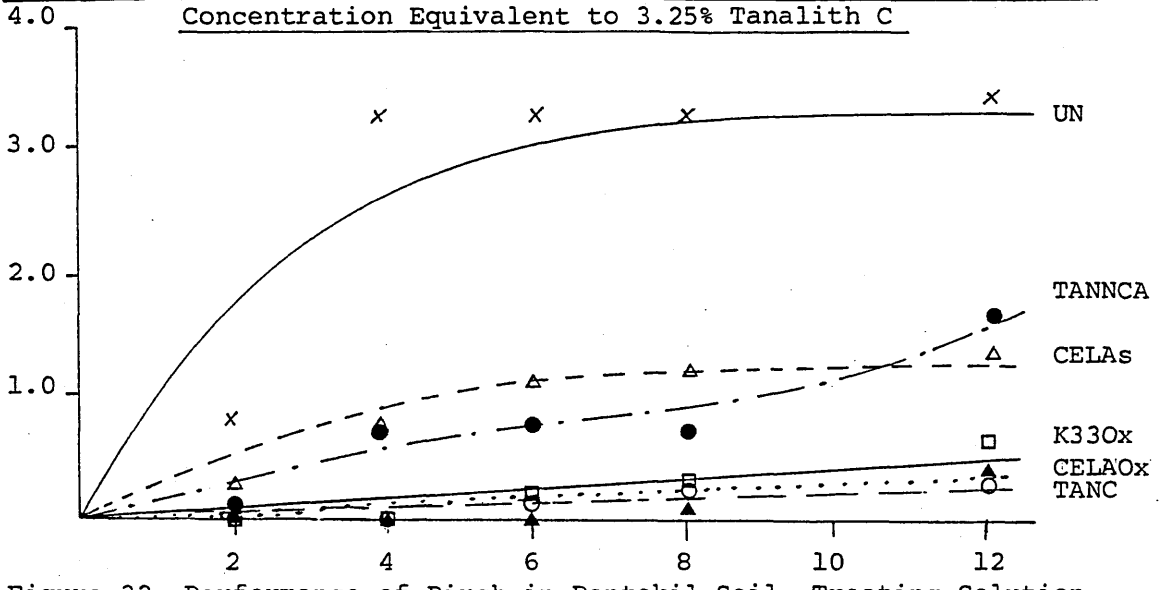


Figure 32 Performance of Birch in Rentokil Soil. Treating Solution Concentration Equivalent to 5.7% Tanalith C

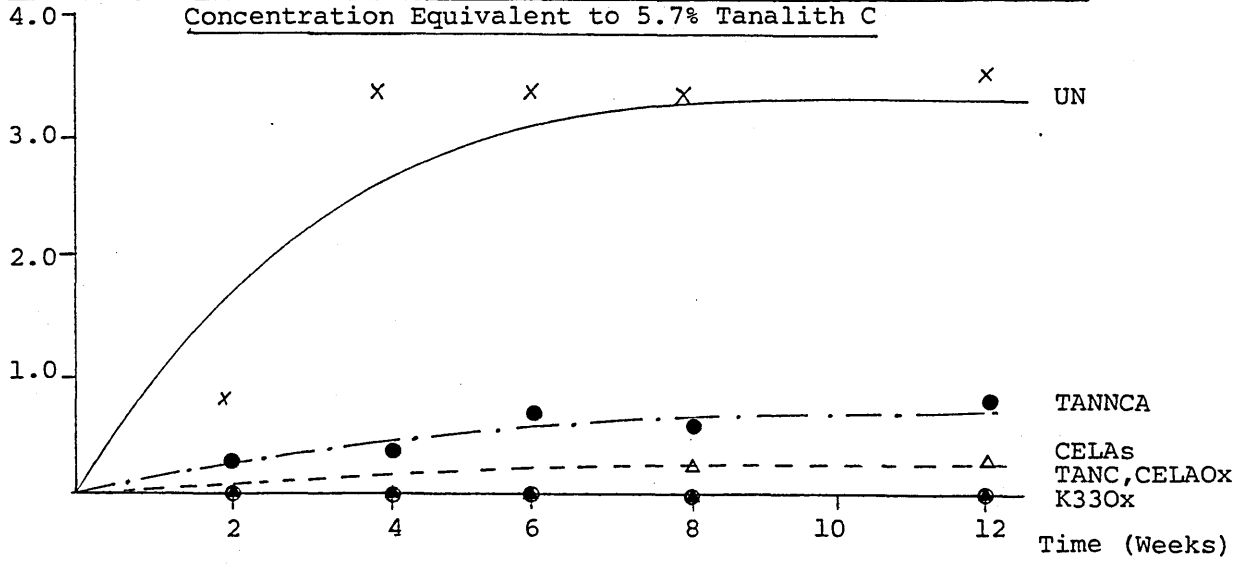


Table 64

Analysis of Variance on Mean SRD of Treated and Untreated Birch Sections Buried in Rentokil Soil. (ii) Equivalent Active Element Concentrations

Time	Unit	DF	F-Ratio	Significance Level
2 Weeks	Preservative	5	6.13	**
	Concentration	2	5.27	*
	Residual	10		
4 Weeks	Preservative	5	88.88	***
	Concentration	2	3.14	n.s.
	Residual	10		
6 Weeks	Preservative	5	32.70	***
	Concentration	2	3.57	*
	Residual	10		
8 Weeks	Preservative	5	15.05	***
	Concentration	2	2.31	n.s.
	Residual	10		
12 Weeks	Preservative	5	12.13	**
	Concentration	2	3.21	n.s.
	Residual	10		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s. = not significant at p level 0.05

Table 65

Mean SRDs From the Analysis of Variance for Birch Sections Buried in Rentokil Soil (a) Preservative Effect (After Averaging For All Equivalent Active Element Concentrations).

2 Weeks	CELAOx	K33Ox	TANC	CELAs	TANNCA	UN
Mean SRD	0	0.03	0.23	0.33	0.38	0.78
l.s.d. = 0.36	I———			———I		
		I———			———I	

4 Weeks	TANC	K33Ox	CELAOx	TANNCA	CELAs	UN
Mean SRD	0.06	0.16	0.17	0.44	0.53	3.33
l.s.d. = 0.42	I———			———I		
		I———			———I	

6 Weeks	CELAOx	TANC	K33Ox	TANNCA	CELAs	UN
Mean SRD	0.05	0.31	0.33	0.66	0.96	3.33
l.s.d. = 0.67	I———			———I		
		I———			———I	

8 Weeks	CELAOx	K33Ox	TANC	TANNCA	CELAs	UN
Mean SRD	0.03	0.09	0.54	0.68	1.39	3.30
l.s.d. = 1.00	I———				———I	

12 Weeks	K33Ox	CELAOx	TANC	TANNCA	CELAs	UN
Mean SRD	0.29	0.33	0.58	1.14	1.54	3.43
l.s.d. = 1.08	I———			———I		
			I———		———I	

Table 66

Mean SRDs From The Analysis of Variance for Birch Sections Buried in Rentokil Soil (b) Concentration Effect (After Averaging for all Preservatives).

	Time (Weeks)				
Concentration (%w/v) Equivalent to TAN C	2	4	6	8	12
1.6	0.51	0.95	1.23	1.37	1.63
3.25	0.21	0.79	0.92	0.96	1.26
5.7	0.16	0.61	0.67	0.69	0.76
l.s.d.	0.26	n.s.	0.47	n.s.	n.s.

I——I
n.s. = no significant difference

Differences between treatments were found. After 2 weeks Celcure A oxide performed significantly better than Tanalith NCA; after 4 weeks Tanalith C performed better than Celcure A salt; after 6 weeks Celcure A oxide performed better than Celcure A salt; after 12 weeks K33 oxide and Celcure A oxide performed better than Celcure A salt.

Differences between the performance of the preservatives were more apparent when treatments were compared on an equivalent active element basis. Results here and for equivalent copper concentration (Table 59) show that Tanalith NCA and Celcure A salt produced the highest SRDs whilst Celcure A oxide, K33 oxide and Tanalith C produced the lowest SRDs.

3.3.4.4 Discussion

Differences in the performance of the five preservatives were statistically significant. Hedley (1970) and McQuire (1972) found that CCA preservatives compared on an equivalent active element basis showed differences in performance in the long term, after years of exposure. It may be that 20 or 30 weeks exposure are required here to produce even finer differences between the preservatives than those already found in this test.

Hedley (1984a,b) found that for CCA treated vineyard posts, AWPA Type C preservatives performed better than those of Type B. In the results reported here, the Type C preservatives Celcure A oxide and Tanalith C performed better than the modified Type B, Tanalith NCA.

Pizzi (1982) found that formulations with a low chromium to arsenic ratio e.g. Type B, fixed at a slower rate than Type C with a higher ratio. He suggested that wood treated with a Type B preservative

should have a greater amount of slowly released fungicidal material than Type C but that Type C should be more water-repellent and therefore less prone to decay. In the short term therefore, Type B preservatives would be more effective than Type C until the released toxic materials had leached out. In the long term however, Type C would be more resistant to decay than Type B. This may explain why here K33 oxide, a Type B preservative performed well with longer incubation, whilst Celcure A salt, a Type C preservative performed poorly.

Hartford et al (1982) looking at stake test data found that differences in the performance of CCA formulations only became apparent after a lengthy testing period. Once they were discernible these differences increased exponentially with time. These authors also recorded the inferiority of Type B formulations.

Differences between the preservatives were more apparent here when the preservatives were compared on an equivalent active element basis.

3.3.4.5 Conclusions

The thin section technique has produced results of preservative performance in weeks which agree with those found in the field after years of exposure. The Type B preservative Tanalith NCA is less effective than the Type C preservative Tanalith C and Celcure A oxide against soft rot attack.

If the incubation time was increased the Type B preservative K33 oxide may start to perform poorly.

3.4 Bacteria Tests

3.4.1 Performance of Copper-Based Experimental Formulations

3.4.1.1 Introduction

The copper-based experimental formulations examined for their ability to prevent soft rot attack of wood (Section 3.3.3) were used here to determine their performance against the S_2 degrading bacteria.

From preliminary tests (Section 2.5.3) a wet soil system using a garden loam at a moisture content of 150% of the water holding capacity was found to be the most suitable for producing bacterial attack of wood. The test was limited to the exposure of tangential longitudinal sections of Scots pine wood. Preliminary tests demonstrated that birch sections were unattacked by the bacteria. A soil moisture content of 95% of the w.h.c. was also used to determine the effect of soil moisture content on the type of decay found.

3.4.1.2 Method

Wood blocks (7 mm^3) were treated with the experimental formulations copper sulphate (C), copper chrome (CC), CCA, CCB, CC+A and CCB+A (Section 3.2.1.2.1, 3.2.2) using the same concentrations as those for soft rot tests (Section 3.3.3). 30μm thick tangential longitudinal sections were cut from treated and untreated blocks, placed in mesh packets (Section 2.2.2) and buried in the garden loam which was used at moisture contents of 95% and 150% w.h.c. (Section 2.4.2). Sections were removed after 4, 8 and 12 weeks, BDs and SRDs were determined using the method in Sections 2.5.4.1 and 2.4.4.2 respectively. SRDs were adapted

for use with tangential longitudinal sections, degree of cavity formation only was recorded. Loss of wall had been included in transverse sections (Section 2.4.4.2).

3.4.1.3 Results

The net dry salt retentions and elemental contents of the Scots pine wood blocks are given in Table 25 (Section 3.3.3.3). Mean BDs and SRDs are recorded in Tables 67 - 69 and plotted against time in Figures 33 - 35. The results were analysed firstly in terms of the percentage frequency of occurrence of each BD class for bacterial results. Secondly, an analysis of variance was carried out on the mean BDs and SRDs. This two way approach was found to be necessary as the bacterial attack was very variable. Mean BDs did not give an indication of the instances of attack in the high decay classes, 3 or 4 which although few in number are of significance. There appeared to be pockets of bacterial populations within the soil jars, sections taken from different areas of the soil jar were attacked to varying degrees.

3.4.1.3.1 Mean Values of Attack and Frequency of Occurrence of each Class

Concentration 0.7% . Soil at 95% of w.h.c. (Table 67, Figure 33)

Copper sulphate (C) and CC+A treatments eliminated bacterial attack. CCA treated sections were severely attacked by the bacteria with BDs in class 4 being recorded (Table 70). CC and CCB+A performed poorly, BDs in class 4 were found after four, eight and twelve weeks.

SRDs were high for sections treated with C and those untreated. The other treatments reduced the rate of attack, however, after twelve weeks SRDs were as high as for untreated sections.

Soil at 150% of w.h.c.

C and CCB+A eliminated bacterial attack. CC, CCA, CCB and CC+A performed well against the bacteria whilst untreated sections were severely attacked with BDs in classes 3 and 4 (Table 73) being recorded.

Concentration 2.36%. Soil at 95% of w.h.c. (Table 68, Figure 34)

CC eliminated bacterial attack. C and CCB+A performed intermediately, whilst CCA, CCB and CC+A performed poorly (Table 71).

Soft rot attack decreased at this higher concentration whilst the occurrence of bacterial attack increased.

Soil at 150% of w.h.c.

CCB and CC+A eliminated attack. CC performed intermediately, whilst C, CCA and CCB+A performed poorly having BDs in class 4 (Table 74). The occurrence of bacterial attack increased at this higher concentration.

Concentration 4.13%. Soil at 95% of w.h.c. (Table 69, Figure 35)

Bacterial attack continued even at this high concentration. CC eliminated attack, C, CCA, CCB and CC+A performed intermediately whilst CCB+A performed poorly. For CCB+A BDs were frequently recorded in classes 3 and 4 (Table 72).

Soft rot attack was eliminated by the treatments CC, CCA and CCB.

Soil at 150% of w.h.c.

CC+A eliminated attack. C and CCB performed well. For CC and CCB+A treated sections BDs were recorded in class 4 (Table 75). CCA performed poorly with BDs in classes 3 and 4 after eight and twelve weeks.

Soft rot attack only occurred in sections treated with C, CC and untreated.

Table 67

Mean BD and SRD for Scots Pine sections buried in a garden loam at two soil moisture contents.

Treating Solution 0.7%

Treatment	Time (weeks)	95% whc				150% whc			
		BD		SRD		BD		SRD	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Untreated	4	1.18	1.412	3.00	0	0.43	1.035	-	-
	8	-	-	3.25	0.494	-	-	-	-
	12	0.58	1.010	4.00	0	0.60	1.172	1.00	0.392
C	4	-	-	3.00	0	-	-	-	-
	8	-	-	3.03	0.276	-	-	0.40	0.709
	12	-	-	3.93	0.367	-	-	-	-
CC	4	0.28	0.716	1.10	1.033	1.05	1.260	-	-
	8	0.93	1.185	3.20	0.687	-	-	-	-
	12	0.20	0.564	3.00	0	-	-	-	-
CCA	4	0.90	1.128	0.20	0.516	-	-	-	-
	8	-	-	1.58	0.636	-	-	-	-
	12	-	-	2.85	0.427	0.75	1.127	2.00	0.751
CCB	4	0.23	0.660	1.08	0.829	-	-	0.58	0.712
	8	-	-	2.00	0	-	-	1.03	0.357
	12	-	-	2.55	0.597	0.88	1.244	1.38	1.123
CC+A	4	-	-	1.85	0.427	-	-	-	-
	8	-	-	2.40	0.496	-	-	-	-
	12	-	-	2.88	0.335	0.50	0.987	-	-
CCB+A	4	0.78	1.291	3.00	0.392	-	-	-	-
	8	1.38	1.295	3.25	0.494	-	-	1.08	0.350
	12	0.53	1.109	3.00	0	-	-	2.00	0

Figure 33 Performance of Scots Pine in a Garden Loam at two whcs
Treating Solution Concentration 0.7% ×—×=SRD, □=BD

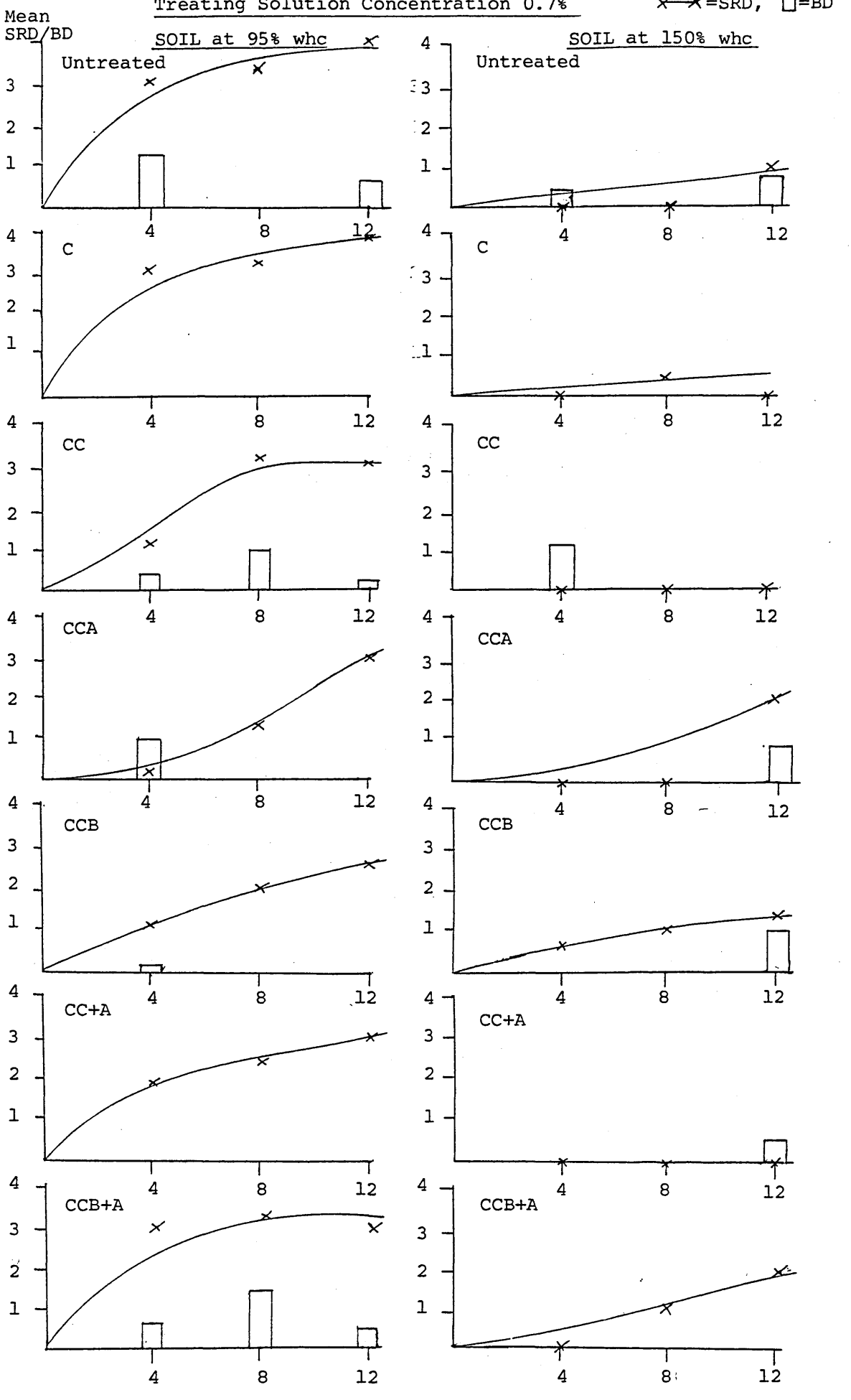


Table 68

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution 2.36%

Treatment	Time (weeks)	95% whc				150% whc			
		BD		SRD		BD		SRD	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	4	2.93	0.572	2.03	0.276	0.30	0.823	-	-
	8	-	-	3.00	0.392	-	-	-	-
	12	-	-	3.83	0.385	0.98	1.405	1.30	0.791
CC	4	-	-	0.25	0.543	0.38	1.030	-	-
	8	-	-	2.85	0.427	-	-	-	-
	12	-	-	3.00	0.226	-	-	-	-
CCA	4	-	-	-	-	0.48	1.086	-	-
	8	0.65	1.027	2.38	0.667	0.38	0.925	-	-
	12	0.78	1.050	2.13	0.686	-	-	-	-
CCB	4	0.08	0.350	-	-	-	-	-	-
	8	-	-	1.23	1.349	-	-	-	-
	12	0.70	1.043	2.13	0.335	-	-	-	-
CC+A	4	-	-	0.98	0.768	-	-	-	-
	8	0.50	0.961	2.18	0.675	-	-	-	-
	12	0.43	0.874	2.53	1.219	-	-	-	-
CCB+A	4	-	-	1.03	0.480	-	-	-	-
	8	0.93	1.207	3.00	0	0.93	1.289	-	-
	12	-	-	2.98	0.357	0.40	0.871	-	-

Figure 34 Performance of Scots Pine in a Garden Loam at two whcs
Treating Solution Concentration 2.36% ×—× = SRD, □ = BD

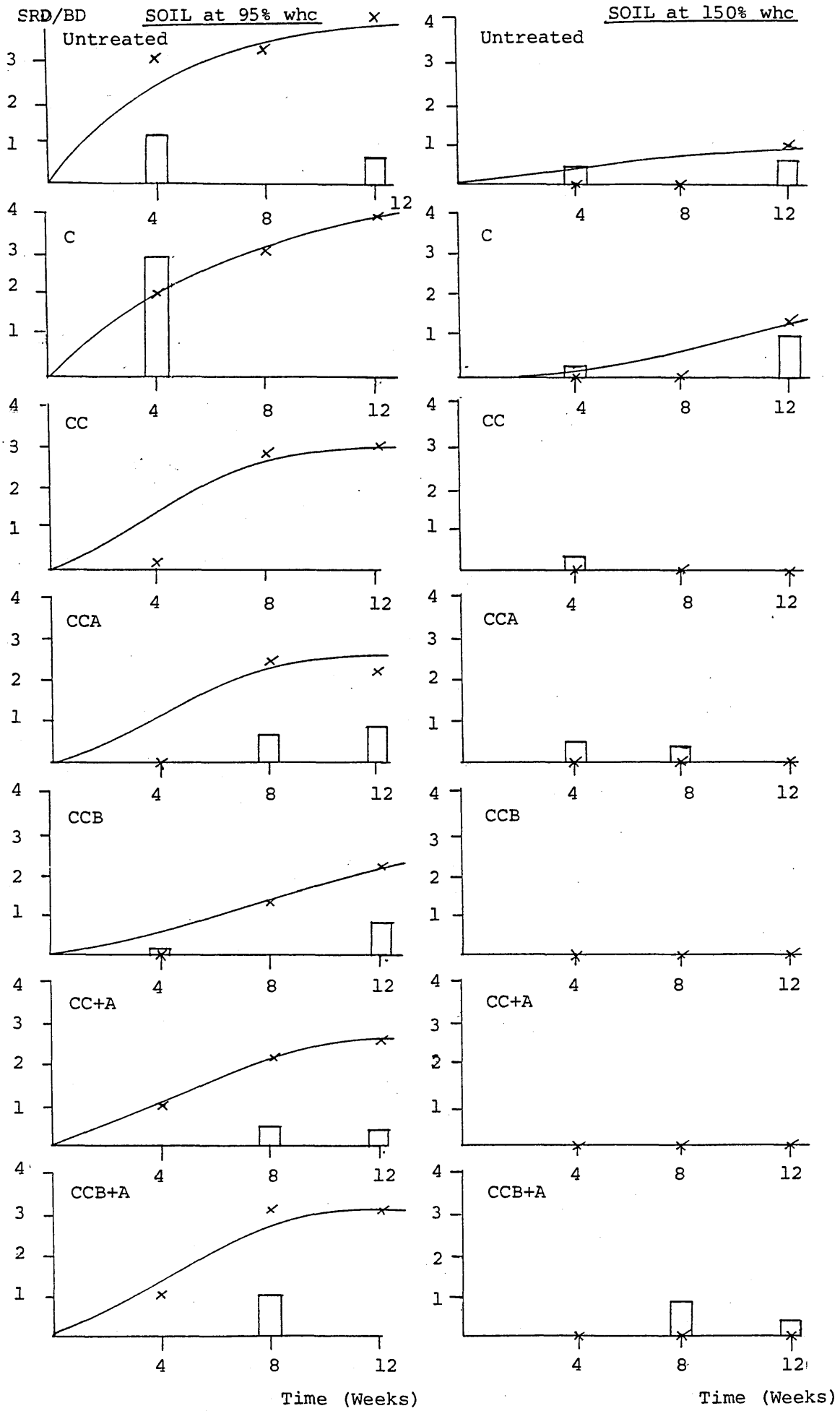


Table 69

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution 4.13%

Treatment	Time (weeks)	95% whc				150% whc			
		BD		SRD		BD		SRD	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	4	1.23	1.349	2.73	0.506	-	-	-	-
	8	-	-	2.75	0.494	-	-	-	-
	12	0.33	0.829	3.00	0	0.38	0.807	0.83	0.747
CC	4	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-
	12	-	-	-	-	1.15	1.477	1.43	0.636
CCA	4	-	-	-	-	-	-	-	-
	8	0.55	0.986	-	-	0.75	1.354	-	-
	12	0.18	0.594	-	-	0.38	0.868	-	-
CCB	4	-	-	-	-	-	-	-	-
	8	0.20	0.648	-	-	0.18	0.549	-	-
	12	0.90	1.128	-	-	-	-	-	-
CC+A	4	-	-	-	-	-	-	-	-
	8	0.73	1.150	-	-	-	-	-	-
	12	0.45	0.876	1.03	0.357	-	-	-	-
CCB+A	4	-	-	-	-	-	-	-	-
	8	0.60	0.955	2.48	0.554	0.60	1.172	-	-
	12	1.25	1.276	2.38	0.540	-	-	-	-

Figure 35 Performance of Scots Pine in a Garden Loam at two whcs Treating Solution Concentration 4.13%. ×—× = SRD, □ = BD

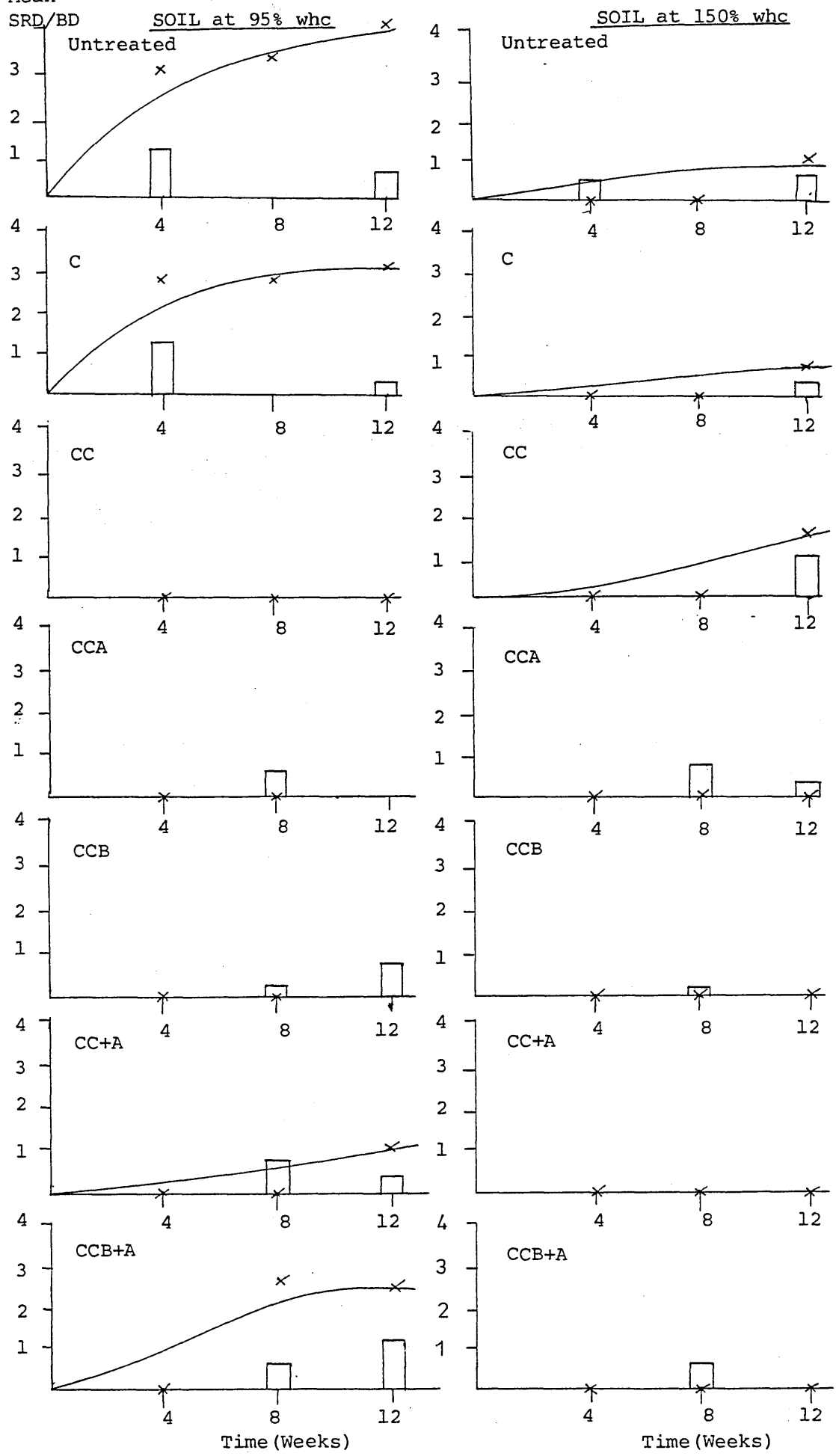


Table 70

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
UN		0	55%	100%	72.5%
		1	2.5	0	5
		2	17.5	0	15
		3	20	0	7.5
		4	5	0	0
C	0.7	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CC	0.7	0	85	60	87.5
		1	5	0	5
		2	7.5	27.5	7.5
		3	2.5	12.5	0
		4	0	0	0
CCA	0.7	0	52.5	100	100
		1	17.5	0	0
		2	20	0	0
		3	7.5	0	0
		4	2.5	0	0
CCB	0.7	0	87.5	100	100
		1	5	0	0
		2	5	0	0
		3	2.5	0	0
		4	0	0	0
CC + A	0.7	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CCB + A	0.7	0	70	40	80
		1	5	10	0
		2	5	25	10
		3	17.5	22.5	7.5
		4	2.5	2.5	2.5

Table 71

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
C	2.36	0	2.5%	100	100
		1	0	0	0
		2	5	0	0
		3	87.5	0	0
		4	5	0	0
CC	2.36	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CCA	2.36	0	100	67.5	60
		1	0	7.5	10
		2	0	17.5	22.5
		3	0	7.5	7.5
		4	0	0	0
CCB	2.36	0	95	100	65
		1	2.5	0	7.5
		2	2.5	0	20
		3	0	0	7.5
		4	0	0	0
CC + A	2.36	0	100	77.5	77.5
		1	0	0	7.5
		2	0	17.5	10
		3	0	5	5
		4	0	0	0
CCB + A	2.36	0	100	60	100
		1	0	2.5	0
		2	0	22.5	0
		3	0	15	0
		4	0	0	0

Table 72

Percentage Frequency of Occurrence of BD Classes (95% w.h.c)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
C	4.13	0	50%	100%	85%
		1	7.5	0	2.5
		2	12.5	0	7.5
		3	30	0	5
		4	0	0	0
CC	4.13	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CCA	4.13	0	100	75	90
		1	0	0	5
		2	0	20	2.5
		3	0	5	2.5
		4	0	0	0
CCB	4.13	0	100	90	55
		1	0	2.5	12.5
		2	0	5	20
		3	0	2.5	12.5
		4	0	0	0
CC + A	4.13	0	100	70	75
		1	0	0	10
		2	0	17.5	10
		3	0	12.5	5
		4	0	0	0
CCB + A	4.13	0	100	67.5	42.5
		1	0	10	15
		2	0	17.5	20
		3	0	5	20
		4	0	0	2.5

Table 73

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
UN		0	85%	100%	77.5
		1	0	0	0
		2	2.5	0	10
		3	12.5	0	10
		4	0	0	2.5
C	0.7	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CC	0.7	0	55	100	100
		1	5	0	0
		2	20	0	0
		3	20	0	0
		4	0	0	0
CCA	0.7	0	100	100	65
		1	0	0	7.5
		2	0	0	15
		3	0	0	12.5
		4	0	0	0
CCB	0.7	0	100	100	65
		1	0	0	0
		2	0	0	17.5
		3	0	0	17.5
		4	0	0	0
CC + A	0.7	0	100	100	77.5
		1	0	0	2.5
		2	0	0	12.5
		3	0	0	7.5
		4	0	0	0
CCB + A	0.7	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0

Table 74

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
C	2.36	0	85%	100%	65%
		1	7.5	0	0
		2	0	0	12.5
		3	7.5	0	17.5
		4	0	0	5
CC	2.36	0	87.5	100	100
		1	0	0	0
		2	2.5	0	0
		3	7.5	0	0
		4	2.5	0	0
CCA	2.36	0	82.5	85	100
		1	0	0	0
		2	7.5	7.5	0
		3	7.5	7.5	0
		4	2.5	0	0
CCB	2.36	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CC + A	2.36	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CCB + A	2.36	0	100	62.5	80
		1	0	2.5	5
		2	0	17.5	10
		3	0	15	5
		4	0	2.5	0

Table 75

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
C	4.13	0	100%	100%	80%
		1	0	0	5
		2	0	0	12.5
		3	0	0	2.5
		4	0	0	0
CC	4.13	0	100	100	60
		1	0	0	0
		2	0	0	10
		3	0	0	25
		4	0	0	5
CCA	4.13	0	100	75	82.5
		1	0	0	2.5
		2	0	5	10
		3	0	15	5
		4	0	5	0
CCB	4.13	0	100	90	100
		1	0	2.5	0
		2	0	7.5	0
		3	0	0	0
		4	0	0	0
CC + A	4.13	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CCB + A	4.13	0	100	77.5	100
		1	0	0	0
		2	0	10	0
		3	0	10	0
		4	0	2.5	0

3.4.1.3.2 An Analysis of the Factors Affecting the Degree of Attack of Scots Pine Sections

The effect of formulation type and concentration on the mean BDs and SRDs was analysed statistically using an analysis of variance test (Section 3.3.3.3.3 and Appendix C). The variability in the occurrence of bacterial attack would be expected to affect the analysis. Four sections with forty fields of view for each mean reading were examined per time period. Often only one section had been attacked therefore mean values were low compared to those for soft rot attack. The bacterial populations appeared to be scattered throughout the soil in isolated areas.

Bacterial Attack. Soil at 95% of w.h.c.

The effect of the type of preservative and concentration used was not significant (Tables 76, 78, 80). No significant differences could be found between the performance of each of the formulations (Table 78).

Soft Rot Attack. Soil at 95% of w.h.c.

Preservative type and concentration significantly affected the degree of soft rot attack (Table 77). CCA performed significantly better than CCB+A, C treated and untreated sections after four, eight and twelve weeks. CCB did so after eight and twelve weeks (Table 79).

CCB+A, C and untreated controls were grouped here as poor performers and also in an earlier soft rot test using Rentokil and John Innes No.1 soils (Section 3.3.3). CCB+A was found to perform poorly against the bacteria with values frequently recorded in classes 3 and 4 (Tables 70, 72).

Table 76

Analysis of Variance on Mean BD of Treated and Untreated Scots Pine Sections Buried in A Garden Loam at a Moisture Content of 95% of its w.h.c.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	6	2.24	n.s.
	Concentration	2	0.27	n.s.
	Residual	12		
8 Weeks	Preservative	6	2.96	n.s.
	Concentration	2	0.02	n.s.
	Residual	12		
12 Weeks	Preservative	6	1.24	n.s.
	Concentration	2	1.75	n.s.
	Residual	12		

Table 77

Analysis of Variance on Mean SRDs of Treated and Untreated Scots Pine Sections Buried in a Garden Loam at a Moisture Content Of 95% of its w.h.c.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	6	12.11	***
	Concentration	2	6.96	**
	Residual	12		
8 Weeks	Preservative	6	4.82	**
	Concentration	2	9.59	**
	Residual	12		
12 Weeks	Preservative	6	6.39	**
	Concentration	2	13.67	***
	Residual	12		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s. = not significant at p level 0.05

Table 78

Mean BDs From The Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c. (a)Preservative Effect(After Averaging For All Concentrations).

4 Weeks	CC+A	CC	CCB	CCB+A	CCA	UN	C
Mean BD	0	0.09	0.10	0.26	0.30	1.18	1.39
	I——— no significant difference between treatments ———I						

8 Weeks	C	UN	CCB	CC	CCA	CC+A	CCB+A
Mean BD	0	0	0.07	0.31	0.40	0.41	0.97
	I——— no significant difference between treatments ———I						

12 Weeks	CC	C	CC+A	CCA	CCB	UN	CCB+A
Mean BD	0.07	0.11	0.29	0.32	0.53	0.58	0.59
	I——— no significant difference between treatments ———I						

Table 79

Mean SRDs From the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c. (a)Preservative Effect (After Averaging For All Concentrations).

4 Weeks	CCA	CCB	CC	CC+A	CCB+A	C	UN
Mean SRD	0.07	0.36	0.45	0.94	1.34	2.59	3.00
l.s.d. = 1.01	I———I					I———I	
		I———I					

8 Weeks	CCB	CCA	CC+A	CC	CCB+A	C	UN
Mean SRD	1.08	1.32	1.53	2.02	2.91	2.93	3.25
l.s.d. = 1.23	I———I				I———I		
				I———I			

12 Weeks	CCB	CCA	CC	CC+A	CCB+A	C	UN
Mean SRD	1.56	1.66	2.00	2.15	2.79	3.59	4.00
l.s.d. = 1.16	I———I				I———I		
		I———I				I———I	

I———I No significant difference

Table 80

Mean BDs From the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam At a Moisture Content of 95% of its w.h.c. (b)Concentration Effect(After Averaging for All Preservatives).

Concentration (%w/v)	Time (Weeks)		
	4	8	12
0.70	0.48	0.33	0.19
2.36	0.60	0.30	0.36
4.13	0.34	0.30	0.53
l.s.d.	n.s.	n.s.	n.s.

Table 81

Mean SRDs From The Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c. (b)Concentration Effect (After Averaging for All Preservatives).

Concentration (%w/v)	Time (Weeks)		
	4	8	12
0.70	1.89	2.67	3.17
2.36	1.04	2.56	2.94
4.13	0.82	1.21	1.49
l.s.d.	0.66	0.81	0.76

Table 82

Analysis of Variance on Mean BD of Treated and Untreated Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	6	2.30	n.s.
	Concentration	2	1.06	n.s.
	Residual	12		
8 Weeks	Preservative	6	3.02	*
	Concentration	2	2.13	n.s.
	Residual	12		
12 Weeks	Preservative	6	0.40	n.s.
	Concentration	2	0.11	n.s.
	Residual	12		

Table 83

Analysis of Variance on Mean SRD of Scots Pine Sections Buried in a Garden Loam at a moisture content of 150% of its w.h.c.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	6	1.00	n.s.
	Concentration	2	1.00	n.s.
	Residual	12		
8 Weeks	Preservative	6	1.00	n.s.
	Concentration	2	3.63	n.s.
	Residual	12		
12 Weeks	Preservative	6	0.45	n.s.
	Concentration	2	1.04	n.s.
	Residual	12		

* = significant at $p < 0.05$

n.s. = not significant at p level 0.05

Table 84

Mean BDs From The Analysis of Variance For Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c. (a)Preservative Effect (After Averaging For All Concentrations.

4 Weeks	CCB	CCB+A	CC+A	C	CCA	UN	CC
Mean BD	0	0	0	0.10	0.16	0.43	0.48

I——————————————————————I

no significant differencebetween treatments

8 Weeks	CC+A	C	CC	UN	CCB	CCA	CCB+A
Mean BD	0	0	0	0	0.06	0.38	0.51
l.s.d. = 0.38	I——————————————————I					I——I	I——I
					I——I		

12 Weeks	CCB+A	CC+A	CCB	CCA	CC	C	UN
Mean BD	0.13	0.17	0.29	0.38	0.38	0.45	0.60

I——————————————————————I

no significant difference between treatments

Table 85

Mean SRDs From the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.

(a) Preservative Effect (After Averaging for All Concentrations.

4 Weeks	CCA	CCB+A	CC+A	CC	C	UN	CCB
Mean SRD	0	0	0	0	0	0	0.19

I——————————————————————I

no significant difference between treatments

8 Weeks	CCA	CCB	CC	UN	C	CCB	CCB+A
Mean SRD	0	0	0	0	0.13	0.34	0.36

I——————————————————————I

no significant difference between treatments

12 Weeks	CC+A	CCB	CC	CCA	CCB+A	C	UN
Mean SRD	0	0.46	0.48	0.67	0.67	0.71	1.00

I——————————————————————I

no significant difference between treatments

I——I No significant difference

Table 86

Mean BDs From The Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.
(b) Concentration Effect (After Averaging for All Preservatives)

Concentration (%w/v)	Time (Weeks)		
	4	8	12
0.70	0.21	0	0.39
2.36	0.23	0.19	0.28
4.13	0.06	0.22	0.36
l.s.d.	n.s.	n.s.	n.s.

Table 87

Mean SRDs from The Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.
(b) Concentration Effect (After Averaging for All Preservatives).

Concentration (%w/v)	Time (Weeks)		
	4	8	12
0.70	0.08	0.36	0.91
2.36	0	0	0.33
4.13	0	0	0.47
l.s.d.	n.s.	n.s.	n.s.

The degree of soft rot attack was significantly reduced with the increase in formulation concentration (Table 81). In contrast bacterial attack was independent of the concentration used.

Bacterial Attack. Soil at 150% of w.h.c.

There was a significant difference between the performance of the experimental formulations after eight weeks (Tables 82, 84). CC+A, C, CC and untreated controls performed significantly better than CCA or CCB+A. These results agree with those obtained when the frequency of occurrence of each BD class was analysed (Tables 74, 75). Concentration did not affect the bacterial results (Table 86).

The variability in the occurrence of bacterial attack was reflected in the results obtained for the untreated sections. After four weeks these were attacked, however, sections removed from another area of the same soil jar after eight weeks, were unattacked. This variability extended to results between soil jars, treated sections from one soil jar were often more attacked than untreated sections from another jar.

Soft Rot Attack. Soil at 150% of w.h.c.

No significant difference was found between the performance of each of the formulations against soft rot attack (Tables 83, 85). Concentration had no effect on SRDs (Table 87). Conditions were unsuitable for soft rot attack.

Summary

Bacteria :- Significant differences between the formulations may have been lost after averaging results for all concentrations. Looking at the results from the analysis of the frequency of occurrence of each BD class at the lower soil moisture content, CC, C and CC+A performed well, CCA and CCB intermediately, whilst CCB+A performed poorly. At the higher soil

moisture content CCA and CCB+A performed poorly.

Soft Rot :- At the lower soil moisture content, CCB+A, C and untreated controls performed poorly. Conditions were unsuitable for soft rot at the higher soil moisture content.

Bacterial attack increased with the increase in soil moisture content and was not affected by high preservative concentrations. Soft rot attack decreased with the increase in soil moisture content and preservative concentration.

3.4.1.4 Discussion

The results show that the soil moisture content affected the type of attack found, soft rot attack was restricted when the soil was saturated. The occurrence of bacterial attack was very variable and this affected the statistical analysis, however, results from the frequency of occurrence of each BD class agreed with those for the performance of the formulations derived statistically.

The type and extent of decay in waterlogged wood depends on the conditions of the surrounding environment. A variation in the results obtained for sections buried in different areas of the same soil jar would be expected. Hedley and Drysdale (1985) reported a great variability in the conditions of posts in horticultural situations in New Zealand, both within and between sites in the same geographical region. The variability within sites may have arisen as a result of the pattern of spread of infection from scattered discrete sources to entire populations (Hedley and Drysdale, 1985). In this thin section test an increase in the incubation time may have allowed the bacteria to grow in numbers and spread throughout the soil jars. The presence of bacterial attack was not

consistent for sections removed from the same jar throughout the test period suggesting that there were isolated pockets of bacterial populations within the same jar. The variability between jars was apparent as untreated sections were occasionally unattacked whilst treated sections from another jar had been attacked.

The variability in the occurrence of bacterial populations found in the field (Hedley and Drysdale, 1985; Butcher, 1984) after years of exposure of posts has been produced using the thin section technique, in weeks in the laboratory.

Soft rot attack was greatly reduced at a soil moisture content of 150% of the w.h.c. Furtado (1978) suggested that the effects of bacteria on wood can be masked by the activities of other wood degrading organisms especially fungi. The bacterial degrade of wood can therefore best be appreciated under conditions which preclude other decay organisms, e.g. low oxygen tension, waterlogging (Boutelje and Bravery, 1968; Greaves, 1968; Holt, Jones and Furtado, 1979) or high preservative concentrations (Hochman, 1967; Greaves and Foster, 1970; Irvine and Jones, 1975). In this thin section test, soft rot attack was restricted under saturated conditions and in the presence of high concentrations of preservative. Bacterial attack, however, was not affected by such harsh conditions.

Soft rot attack was not completely eliminated by the high soil moisture content. This suggests that conditions in the soil jar must have been at least partly aerobic. There may have been pockets of oxygen in areas of the soil allowing the fungi to survive. This would possibly explain how at the high soil moisture content after four and eight weeks, untreated sections were less attacked than some treated sections. More oxygen may have been present in jars which contained sections attacked by soft rot fungi. After twelve weeks SRDs were higher for untreated

sections as conditions may have become more aerobic when the soil began to dry out slightly.

Timber decay proceeds slowly in saturated soil (Scheffer, Duncan and Wilkinson, 1969; Eslyn and Clarke, 1976), however, with the thin section technique decay is more rapid. The bacteria are allowed immediate access to the S_2 layer of the wood cell wall. With whole wood blocks or poles in the ground, bacterial attack is mostly confined to the surface and proceeds very slowly. The thin section technique allows preservatives to be rapidly screened for their performance against bacterial attack.

The presence of the copper-based formulations in the thin sections of wood did not prevent bacterial attack irrespective of the concentration used. Nilsson and Daniel (1983a) found that there was no toxic threshold for tunnelling bacteria, attack occurring in stakes treated with CCA formulations to a retention of 35 kg/m^3. In this work attack occurred in sections cut from blocks which had been treated to retentions of up to 30.65 kg/m^3. Erosion and tunnelling bacteria were resistant to the treatment of Pinus radiata stakes with CCA, in New Zealand (Daniel and Nilsson, 1985). Bacteria may inactivate or destroy preservatives (Scheffer, 1973; Schmidt, Wolf and Liese, 1975). The ability of bacteria to degrade a wide range of preservative treated wood in-service has been recognised under a range of conditions (Greaves, 1968; Greaves and Foster, 1970; Irvine, 1974; Holt, Jones and Furtado, 1979; Nilsson and Daniel, 1983a; Nilsson and Holt, 1983; Nilsson, 1984; Nilsson and Singh, 1984; Daniel and Nilsson, 1985).

Differences between the performance of the formulations were less apparent at the lower soil moisture content. However, when the frequency of occurrence of each BD class was examined, copper sulphate, CC and CC+A performed well, CCA, CCB less so and CCB+A, poorly. This was reflected in

the analysis of variance results at the higher soil moisture content where CCA and CCB+A performed poorly after eight weeks. For soft rot attack, CCA performed the best despite its poor performance against the bacteria. This occurred using both soil moisture contents. Other formulations such as copper sulphate (C) and CCB+A performed poorly against both types of attack.

The SRDs recorded for Scots pine sections were much higher than those found in Rentokil soil used in the test in Section 3.3.3. This may have been due to the higher soft rot potential of the garden loam. The high phosphate content of the garden loam may have reduced the effectiveness of the formulations. Da Costa (1971, 1972) found that the addition of inorganic phosphate to the testing medium or to the blocks reduced the toxicity of CCA preservatives. The garden loam had a similar composition to that of Princes Risborough and John Innes No.1 soils used in earlier tests and showing a high soft rot decay potential.

3.4.1.5 Conclusions

The moisture content of the soil used determines the type of attack found, soft rot attack occurring at the lower soil moisture content whilst bacterial attack predominates at the higher moisture content.

Bacterial attack was variable both within the same soil jar and between jars. There may be discrete loci of infection which spread with time to produce large bacterial populations. Results obtained using the thin section technique agree with those found in the field. Bacterial attack is independent of preservative concentration. CCA performed poorly against the bacteria but well against soft rot. CCB+A performed poorly against both types of attack. Soft rot attack is dependent on soil moisture content and preservative concentration, bacterial attack is independent of these variables.

3.4.2 Performance of Commercially Available Preservatives

3.4.2.1 Introduction

Those commercially available preservatives examined for their ability to prevent soft rot attack (Section 3.3.4) were used to determine their performance against S_2 degrading bacteria. The wet soil system used for testing the performance of copper based experimental formulations in the previous test (Section 3.4.1) was used here.

3.4.2.2 Method

30 μm thick tangential longitudinal sections were cut from untreated scots pine blocks and those treated with Tanalith C, Tanalith NCA, Celcure A salt, Celcure A oxide and K33 oxide (Section 3.2.1.2.2). Concentrations were the same as those used in soft rot tests (Section 3.3.4) based on equivalent copper and equivalent active elements (Section 3.2.1.2.2). The sections were placed in mesh packets (Section 2.2.2) and buried in the garden loam which was moistened to 95% and 150% of the w.h.c. (Section2.4.2). Sections were removed after four, eight and twelve weeks. Mean BDs and SRDs were determined using the method in Sections 2.5.4.1 and 2.4.4.2 respectively.

3.4.2.3 Results

The net dry salt retentions and elemental contents of the wood blocks are given in Table 25, Section 3.3.3.3. Mean BDs and SRDs are recorded in Tables 88-90, 109-111 and plotted against time in Figures 36-38, 39-41. The results were analysed in the same way as those for the experimental formulations in Section 3.4.1. Firstly the frequency of occurrence of each BD class was recorded. Secondly, an analysis of variance

was used to analyse the mean SRDs and BDs. This approach was found to be necessary due to the variability of the bacterial attack. Mean BDs may have concealed occurrences of bacterial attack in the high decay classes of 3 and 4.

Results were considered firstly for equivalent copper then secondly for equivalent active element concentrations.

3.4.2.3.1 Equivalent Copper Concentrations

Concentration equivalent to Tan C 0.7%. Soil at 95% of w.h.c

(Table 88, Figure 36)

Bacterial attack was restricted at this lower soil moisture content. Results from the frequency of occurrence of each BD class (Table 91) show that Celcure A salt prevented attack; Tanalith C, Celcure A oxide and Tanalith NCA performed intermediately, and K33 oxide performed poorly. For this latter treatment, BDs were found in classes 2 and 3. Untreated controls were attacked by the bacteria and soft rot fungi. SRDs were high for all treatments. Celcure A salt and Celcure A oxide performed poorly.

Soil at 150% of w.h.c.

Bacterial attack was increased at this higher soil moisture content. Celcure A salt continued to prevent attack. From the frequency of occurrence of each BD class (Table 94) it can be seen that Celcure A oxide performed intermediately, whilst Tanalith C, Tanalith NCA and K33 oxide performed poorly with BDs in classes 3 and 4. Untreated controls were attacked. Soft rot attack was reduced.

Concentration equivalent to Tan C 2.36%. Soil at 95% of w.h.c.

(Table 89, Figure 37)

Celcure A salt and Celcure A oxide prevented bacterial attack,

whilst Tanalith NCA and K33 oxide performed poorly with a high frequency of BDs in class 4 for Tanalith NCA treated sections (Table 92). Tanalith C performed intermediately. Celcure A salt and oxide prevented soft rot attack.

Soil at 150% of w.h.c.

Bacterial attack was not recorded for Tanalith NCA treated sections. Celcure A salt, Celcure A oxide and K33 oxide performed intermediately, whilst Tanalith C performed poorly (Table 95). Soft rot attack was only found in sections cut from blocks treated with K33 oxide and Tanalith C.

Concentration equivalent to Tan C 4.13%. Soil at 95% and 150% of w.h.c. (Table 90, Figure 38)

Bacterial attack was prevented at this high concentration of preservative at the lower soil moisture content (Table 95). At the higher soil moisture content only K33 oxide treated sections were attacked by the bacteria (Table 98). Soft rot attack was not recorded.

Table 88

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution concentration based on Equivalent Copper Composition.

Treatment	Conc (%w/v)	Time (weeks)	95% whc				150% whc			
			BD		SRD		BD		SRD	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Untreated		4	0.28	0.679	3.00	0	0.55	1.037	-	-
		8	-	-	3.48	0.600	0.48	1.109	-	-
		12	0.15	0.483	4.00	0	-	-	-	-
TAN C	0.7	4	-	-	1.63	1.005	0.13	0.463	-	-
		8	-	-	2.63	1.079	0.55	1.061	1.15	1.167
		12	0.40	0.778	3.03	0.357	0.50	1.132	1.65	1.424
TAN NCA	0.53	4	0.10	0.379	2.07	1.403	0.60	1.172	0.68	0.572
		8	-	-	2.63	1.079	-	-	0.85	0.834
		12	-	-	4.00	0	-	-	-	-
CEL A	0.81	4	-	-	3.10	0.452	-	-	-	-
SALT		8	-	-	3.00	0	-	-	1.63	1.005
		12	-	-	4.00	0	-	-	1.38	1.123
CEL A	0.59	4	-	-	3.30	0.474	-	-	0.13	0.404
OXIDE		8	-	-	3.43	0.549	0.68	1.071	-	-
		12	0.85	1.027	4.00	0	-	-	-	-
K33	0.53	4	-	-	1.55	1.239	-	-	-	-
OXIDE		8	1.18	1.196	1.95	1.176	-	-	1.78	1.423
		12	-	-	2.75	0.543	1.43	1.318	2.88	0.516

Figure 36 Performance of Scots Pine in a Garden Loam at two whcs Treating Solution based on Equivalent Copper.

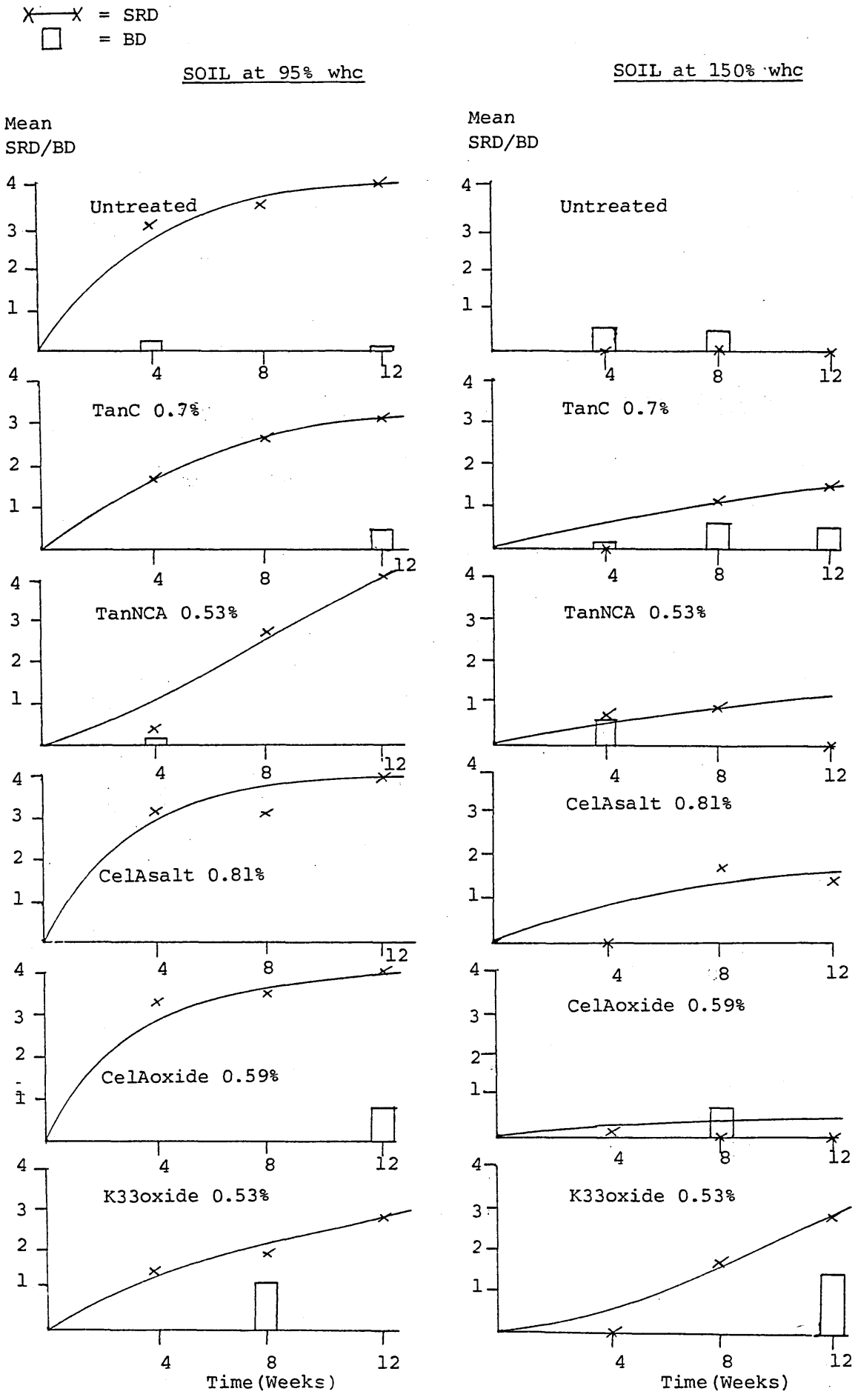


Table 89

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution concentration based on Equivalent Copper Composition.

Treatment	Conc (%w/v)	Time (weeks)	95% whc				150% whc			
			BD		SRD		BD		SRD	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
TAN C	2.36	4	-	-	0.85	0.834	0.13	0.404	0.40	0.982
		8	-	-	3.00	0	0.63	0.952	-	-
		12	0.70	0.911	3.43	0.501	-	-	0.80	0.648
TAN NCA	1.78	4	-	-	0.50	0.816	-	-	-	-
		8	0.40	0.672	1.40	1.464	-	-	-	-
		12	1.30	1.344	2.75	0.439	-	-	-	-
CEL A SALT	2.72	4	-	-	0.65	0.893	-	-	-	-
		8	-	-	1.95	0.986	0.58	1.217	-	-
		12	-	-	2.35	0.483	-	-	-	-
CEL A OXIDE	1.98	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-
		12	-	-	-	-	0.30	0.823	-	-
K33 OXIDE	1.78	4	-	-	-	-	-	-	-	-
		8	0.15	0.533	0.98	1.250	0.35	0.864	-	-
		12	0.25	0.670	1.53	0.877	-	-	0.90	1.128

Figure 37 Performance of Scots Pine in a Garden Loam at two whcs Treating Solution based on Equivalent Copper.

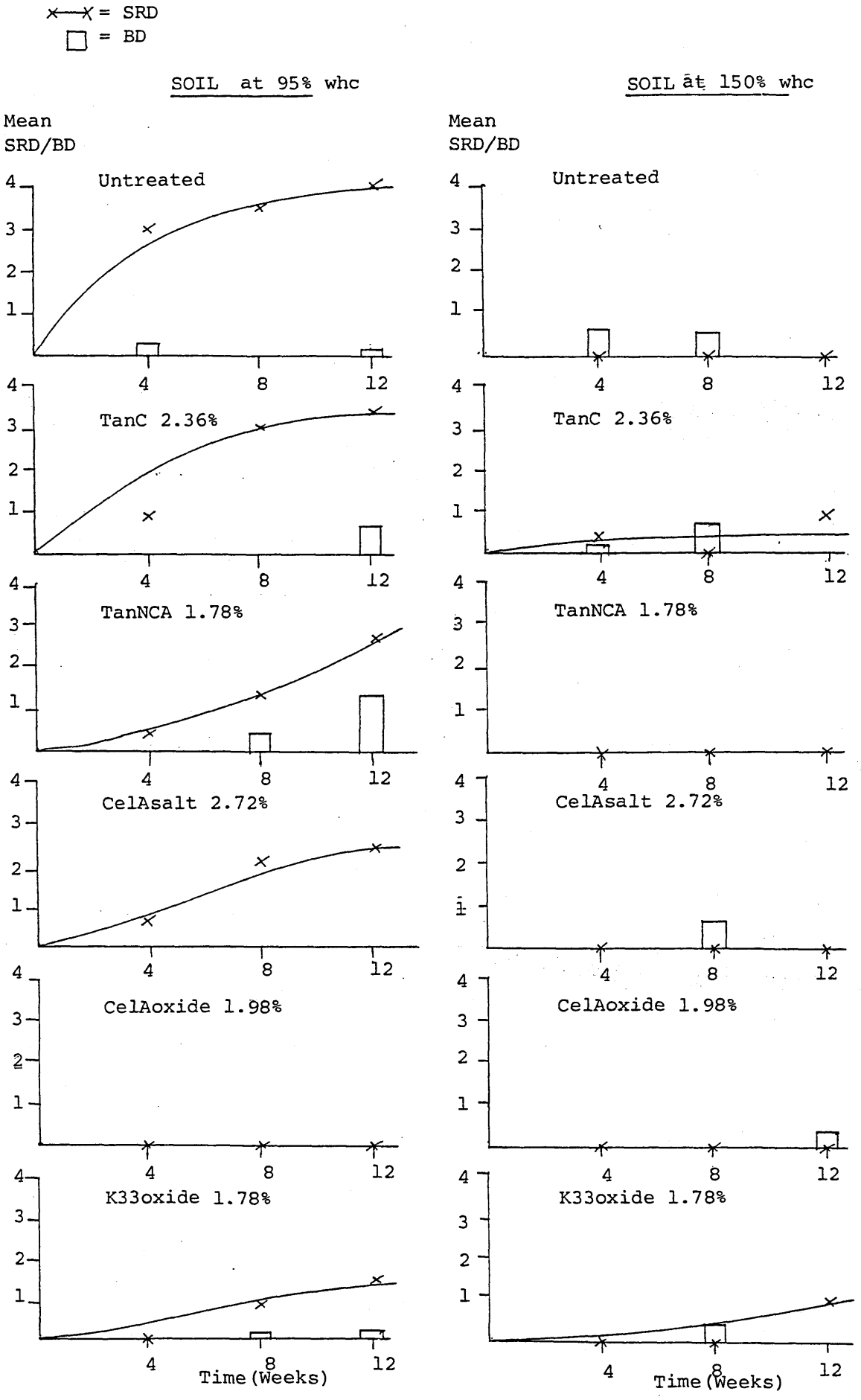


Table 90

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution concentrations based on Equivalent Copper Composition.

Treatment	Conc (%w/v)	Time (weeks)	95% whc				150% whc			
			BD		SRD		BD		SRD	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
TAN C	4.13	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-
TAN NCA	3.11	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-
CEL A SALT	4.75	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-
CEL A OXIDE	3.46	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-
K33 OXIDE	3.11	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	0.13	0.404	-	-
		12	-	-	-	-	0.18	0.501	-	-

Figure 38 Performance of Scots Pine in a garden loam at two whcs

Treating Solution based on Equivalent Copper



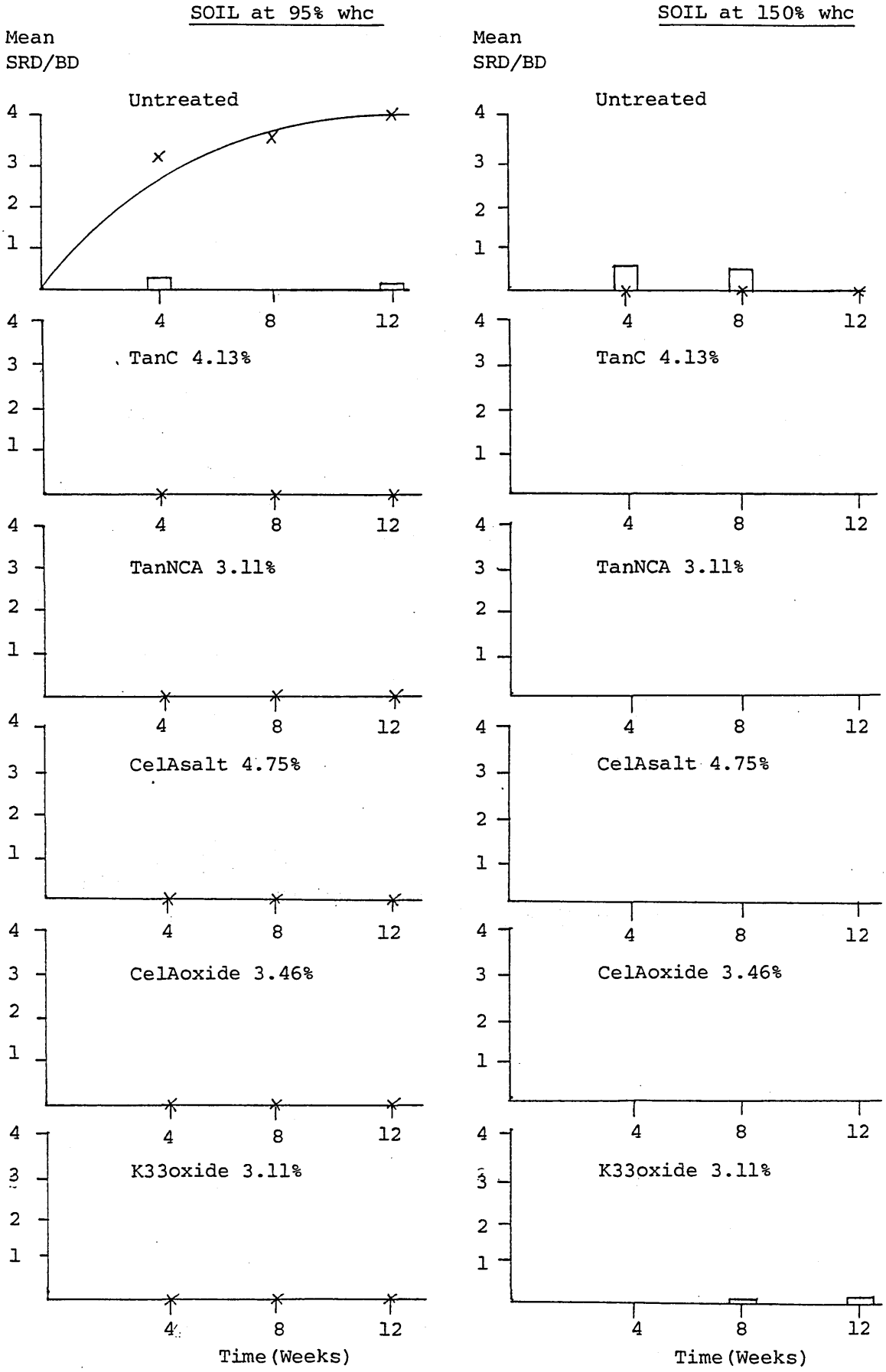


Table 91

Equivalent Copper Concentrations

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
UN		0	82.5%	100%	90%
		1	10	0	5
		2	5	0	5
		3	2.5	0	0
		4	0	0	0
TAN C	0.7	0	100	100	77.5
		1	0	0	5
		2	0	0	17.5
		3	0	0	0
		4	0	0	0
TAN NCA	0.53	0	92.5	100	100
		1	5	0	0
		2	2.5	0	0
		3	0	0	0
		4	0	0	0
CEL A Salt	0.81	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Oxide	0.59	0	100	100	55
		1	0	0	10
		2	0	0	30
		3	0	0	5
		4	0	0	0
K33 Oxide	0.53	0	100	45	100
		1	0	10	0
		2	0	27.5	0
		3	0	17.5	0
		4	0	0	0

Table 92

Equivalent Copper Concentrations

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks		
			4	8	12
TAN C	2.36	0	100%	100%	57.5%
		1	0	0	17.5
		2	0	0	22.5
		3	0	0	2.5
		4	0	0	0
TAN NCA	1.78	0	100	70	42.5
		1	0	20	15
		2	0	10	17.5
		3	0	0	20
		4	0	0	5
CEL A Salt	2.72	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Oxide	1.98	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
K33 Oxide	1.78	0	100	92.5	85
		1	0	0	7.5
		2	0	7.5	5
		3	0	0	2.5
		4	0	0	0

Table 93

Equivalent Copper Concentrations

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
TAN C	4.13	0	100%	100%	100%
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
TAN NCA	3.11	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Salt	4.75	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Oxide	3.46	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
K33 Oxide	3.11	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0

Table 94

Equivalent Copper Concentrations

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
UN		0	75%	82.5%	100%
		1	5	2.5	0
		2	10	2.5	0
		3	10	10	0
		4	0	2.5	0
TAN C	0.7	0	92.5	77.5	82.5
		1	2.5	0	0
		2	5	12.5	5
		3	0	10	10
		4	0	0	2.5
TAN NCA	0.53	0	77.5	100	100
		1	0	0	0
		2	10	0	0
		3	10	0	0
		4	2.5	0	0
CEL A Salt	0.81	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Oxide	0.59	0	100	67.5	100
		1	0	7.5	0
		2	0	15	0
		3	0	10	0
		4	0	0	0
K33 Oxide	0.53	0	100	100	40
		1	0	0	10
		2	0	0	17.5
		3	0	0	32.5
		4	0	0	0

Table 95

Equivalent Copper Concentrations

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
TAN C	2.36	0	90%	62.5%	100%
		1	7.5	20	0
		2	2.5	10	0
		3	0	7.5	0
		4	0	0	0
TAN NCA	1.78	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Salt	2.72	0	100	80	100
		1	0	0	0
		2	0	7.5	0
		3	0	7.5	0
		4	0	5	0
CEL A Oxide	1.98	0	100	100	87.5
		1	0	0	0
		2	0	0	7.5
		3	0	0	5
		4	0	0	0
K33	1.78	0	100	85	100
		1	0	0	0
		2	0	10	0
		3	0	5	0
		4	0	0	0

Table 96

Equivalent Copper Concentrations

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
TAN C	4.13	0	100%	100%	100%
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
TAN NCA	3.11	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Salt	4.75	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Oxide	3.46	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
K33 Oxide	3.11	0	100	90	87.5
		1	0	7.5	7.5
		2	0	2.5	5
		3	0	0	0
		4	0	0	0

3.4.2.3.2 An Analysis of the Factors Affecting the Degree of Bacterial and Soft Rot Attack.

The effect of the type of preservative and concentration used on mean BDs and SRDs was determined using an analysis of variance test (Section 3.3.3.3.3 and Appendix C).

Bacterial Attack. Soil at 95% of w.h.c.

After four weeks, the preservative treatments performed significantly better than untreated controls (Tables 97, 99). Bacterial attack was not affected by the concentration of preservative used (Tables 97, 101). Untreated sections were attacked after four and twelve weeks. The variability in the occurrence of bacterial attack has been discussed in Section 3.4.1.4.

Soft Rot Attack. Soil at 95% of w.h.c.

After four weeks the five preservatives performed significantly better than untreated controls (Tables 98, 100). Concentration of preservative used had a significant effect on SRDs (Tables 98, 102). SRDs decreased with the increase in preservative concentration.

Bacterial Attack. Soil at 150% of w.h.c.

The five preservatives performed significantly better than untreated controls after four weeks (Tables 103, 105). Bacterial attack was independent of preservative concentration (Tables 103, 107). However, at the highest concentration attack was severely restricted.

Soft Rot Attack. Soil at 150% of w.h.c.

There was no significant difference between the performance of the preservatives or between these and untreated controls (Tables 104, 106). Soft rot attack was severly restricted at this high soil moisture content. The preservative concentration used had a significant effect on SRDs (Tables 104, 108).

Table 97

Analysis of Variance on Mean BD of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.(i) Equivalent Copper Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	68.2	***
	Concentration	2	1.00	n.s.
	Residual	10		
8 Weeks	Preservative	5	1.17	n.s.
	Concentration	2	0.71	n.s.
	Residual	10		
12 Weeks	Preservative	5	0.58	n.s.
	Concentration	2	1.44	n.s.
	Residual	10		

Table 98

Analysis of Variance on Mean SRD of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c. (i) Equivalent Copper Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	4.81	*
	Concentration	2	13.11	**
	Residual	10		
8 Weeks	Preservative	5	3.25	n.s.
	Concentration	2	10.14	**
	Residual	10		
12 Weeks	Preservative	5	2.41	n.s.
	Concentration	2	11.56	**
	Residual	10		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

* = significant at $p < 0.05$

n.s. = not significant at p level 0.05

Table 99

Mean BDs From The Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.

(a) Preservative Effect (After Averaging for all Concentrations).

4 Weeks						
	CELAs	CELAOx	TANC	K33Ox	TANNCA	UN
Mean BD	0	0	0	0	0.03	0.28
l.s.d. = 0.04	I———				———I	

8 Weeks						
	CELAs	CELAOx	TANC	UN	TANNCA	K33Ox
Mean BD	0	0	0	0	0.13	0.44
	I———					———I
	no significant difference between treatments					

12 Weeks						
	CELAs	K33Ox	UN	CELAOx	TANC	TANNCA
Mean BD	0	0.08	0.15	0.28	0.37	0.43
	I———					———I
	no significant difference between treatments					

Table 100

Mean SRDs From The Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.

(a) Preservative Effect (After Averaging for All Concentrations).

4 Weeks						
	K33Ox	TANC	TANNCA	CELAOx	CELAs	UN
Mean SRD	0.52	0.83	0.86	1.10	1.25	3.00
l.s.d. = 1.28	I———				———I	

8 Weeks						
	K33Ox	CELAOx	TANNCA	CELAs	TANC	UN
Mean SRD	0.98	1.14	1.34	1.65	1.88	3.48
	I———					———I
	no significant difference between treatments					

12 Weeks						
	CELAOx	K33Ox	CELAs	TANC	TANNCA	UN
Mean SRD	1.33	1.43	2.12	2.15	2.25	4.00
	I———					———I
	no significant difference between treatments					

I———I No significant Difference

Table 101

Mean BDs From the Analysis of Variance For Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.

(b) Concentration Effect (After Averaging for All Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.70	0.06	0.20	0.23
2.36	0.05	0.09	0.40
4.13	0.05	0	0.03
l.s.d.	n.s.	n.s.	n.s.

Table 102

Mean SRDs from The Analysis of Variance For Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.

(b) Concentration Effect (After Averaging for All Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.70	2.44	2.85	3.63
2.36	0.83	1.80	2.34
4.13	0.50	0.58	0.67
l.s.d.	0.90	1.13	1.38

Table 103

Analysis of Variance on Mean BDs of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c. (i)Equivalent Copper Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	6.98	**
	Concentration	2	1.26	n.s.
	Residual	10		
8 Weeks	Preservative	5	1.38	n.s.
	Concentration	2	1.45	n.s.
	Residual	10		
12 Weeks	Preservative	5	1.17	n.s.
	Concentration	2	1.42	n.s.
	Residual	10		

Table 104

Analysis of Variance on Mean SRD of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c. (i)Equivalent Copper Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	0.71	n.s.
	Concentration	2	0.74	n.s.
	Residual	10		
8 Weeks	Preservative	5	1.00	n.s.
	Concentration	2	8.15	**
	Residual	10		
12 Weeks	Preservative	5	2.15	n.s.
	Concentration	2	3.97	n.s.
	Residual	10		

n.s. = not significant at p level 0.05

** = significant at $p < 0.01$

Table 105

Mean BDs From The Analysis of Variance for Scots Pine Sections Buried in A Garden Loam at a Moisture Content Of 150% of its w.h.c.

(a) Preservative Effect (After Averaging For All Concentrations).

4 Weeks						
	CELAs	CELAOx	K33Ox	TANC	TANNCA	UN
Mean BD	0	0	0	0.09	0.20	0.55
l.s.d. = 0.26	I———				———I	

8 Weeks						
	TANNCA	K33Ox	CELAs	CELAOx	TANC	UN
Mean BD	0	0.16	0.19	0.23	0.39	0.48
	I——— no significant difference between treatments ———I					

12 Weeks						
	CELAs	TANNCA	UN	CELAOx	TANC	K33Ox
Mean BD	0	0	0	0.10	0.17	0.54
	I——— no significant difference between treatments ———I					

I———I = no significant difference

Table 106

Mean SRDs From the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.

(a) Preservative Effect (After Averaging For All Concentrations).

4 Weeks						
	CELAs	K33Ox	UN	CELAOx	TANC	TANNCA
Mean SRD	0	0	0	0.04	0.13	0.23
	I——— no significant difference between treatments ———I					

8 Weeks						
	CELAOx	UN	TANNCA	CELAs	TANC	K33Ox
Mean SRD	0	0	0.28	0.38	0.54	0.59
	I——— no significant difference between treatments ———I					

12 Weeks						
	CELAOx	UN	TANNCA	CELAs	TANC	K33Ox
Mean SRD	0	0	0	0.46	0.82	1.26
	I——— no significant difference between treatments ———I					

Table 107

Mean BDs From the Analysis of Variance For Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.
(b) Concentration Effect (After Averaging for all Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.7	0.21	0.29	0.32
2.36	0.11	0.34	0.05
4.13	0.09	0.10	0.03
l.s.d.	n.s.	n.s.	n.s.

Table 108

Mean SRDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.
(b) Concentration Effect (After Averaging for all Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.7	0.14	0.90	0.99
2.36	0.07	0	0.28
4.13	0	0	0
l.s.d.	n.s.	0.57	n.s.

n.s. = not significant

3.4.2.3.3 Equivalent Active Element Concentrations

Concentration equivalent to Tan C 0.7%. Soil at 95% of w.h.c.

(Table 109, Figure 39)

Bacterial attack was variable. Celcure A oxide prevented attack. Tanalith C, Celcure A salt and Tanalith NCA performed similarly. K33 oxide performed poorly with BDs in each of the decay classes from 0 to 3 (Table 112). Untreated sections were severely attacked. Soft rot attack was high for all treated sections and also untreated.

Soil at 150% of w.h.c.

Bacterial attack was increased at this higher soil moisture content. Celcure A salt prevented attack. BDs recorded for K33 oxide were frequently in classes 2,3 and 4 (Table 115). Tanalith NCA and Tanalith C performed poorly with BDs in classes 3 and 4. Untreated sections were severely attacked. Soft rot attack was reduced at this higher soil moisture content.

Concentration equivalent to Tan C 2.36%. Soil at 95% of w.h.c.

(Table 110, Figure 40)

Tanalith C, Celcure A salt and Celcure A oxide performed well against the bacteria. Tanalith NCA and K33 oxide performed poorly (Table 113). Soft rot attack was reduced with increased preservative concentration. A treatment of K33 oxide produced the highest SRDs in wood sections.

Soil at 150% of w.h.c.

Bacterial attack was increased at this higher soil moisture content. Celcure A salt and Celcure A oxide performed well, Tanalith C intermediately against the bacteria and Tanalith NCA, K33 oxide, poorly. BDs for Tanalith NCA were frequently in classes 3 and 4 (Table 116).

Attack of K33 oxide treated sections was consistent over four, eight and twelve weeks. Soft rot attack was reduced at this higher soil moisture content.

Concentration equivalent to Tan C 4.13%. Soil at 95% of w.h.c.

(Table 111, Figure 41)

Only those sections cut from blocks treated with K33 oxide were attacked by the bacteria (Table 114). Tanalith C and Tanalith NCA prevented soft rot attack. K33 oxide performed poorly against soft rot and bacteria.

Soil at 150% of w.h.c.

Bacterial attack only occurred with the Celcure A salt treatment (Table 117). Soft rot attack was eliminated.

Table 109

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution concentration based on Equivalent Active Element Composition.

Treatment	Conc (%w/v)	Time (weeks)	95% whc				150% whc			
			BD		SRD		BD		SRD	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
TAN C	0.7	4	-	-	1.63	1.005	0.13	0.463	-	-
		8	-	-	2.63	1.079	0.55	1.061	1.15	1.167
		12	0.40	0.778	3.03	0.357	0.50	1.132	1.65	1.424
TAN NCA	0.43	4	0.18	0.636	2.58	1.130	0.33	0.730	0.95	0.986
		8	-	-	3.00	0	0.53	1.132	2.53	1.261
		12	-	-	4.00	0	-	-	3.08	0.694
CEL A SALT	0.65	4	-	-	0.58	0.781	-	-	-	-
		8	-	-	2.63	1.079	-	-	0.60	0.709
		12	0.48	1.062	4.00	0	-	-	2.35	0.483
CEL A OXIDE	0.53	4	-	-	2.75	0.439	-	-	0.05	0.221
		8	-	-	3.00	0	0.30	0.791	2.58	0.594
		12	-	-	3.33	0.526	-	-	3.00	0
K33 OXIDE	0.42	4	-	-	1.35	1.051	-	-	1.40	1.105
		8	0.85	1.027	2.90	0.441	0.93	1.309	2.28	1.601
		12	-	-	4.00	0	-	-	3.43	0.501

Figure 39 Performance of Scots Pine in a Garden Loam at two whcs Treating Solution based on Equivalent Active Elements.

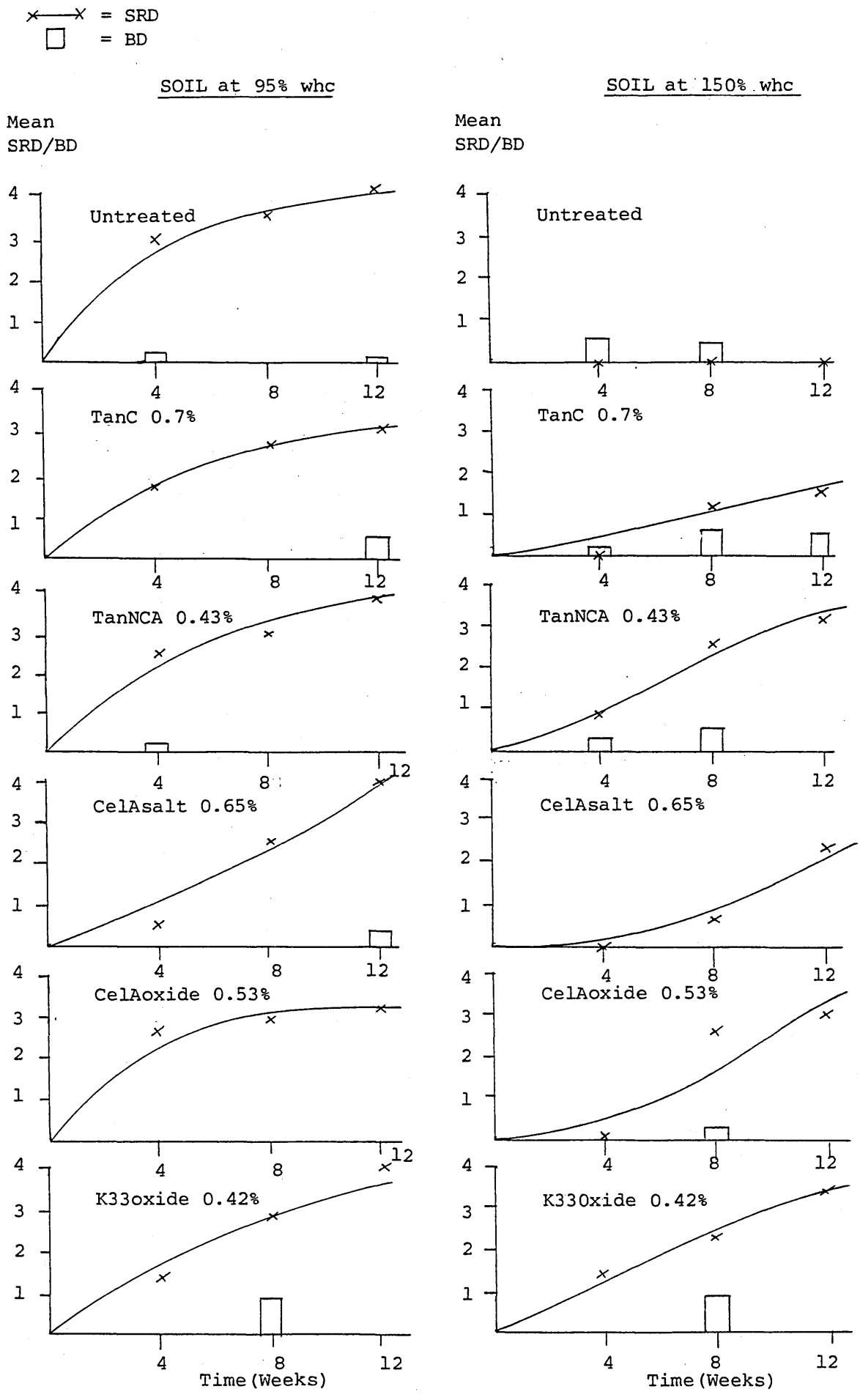


Table 110

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution concentration based on Equivalent Active Element Composition.

Treatment	Conc (%w/v)	Time (weeks)	95% whc				150% whc			
			BD		SRD		BD		SRD	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
TAN C	2.36	4	-	-	0.85	0.834	0.13	0.404	0.40	0.982
		8	-	-	3.00	0	0.63	0.952	-	-
		12	0.70	0.911	3.43	0.501	-	-	0.80	0.648
TAN NCA	1.46	4	-	-	-	-	-	-	-	-
		8	0.10	0.379	2.18	1.130	1.38	1.334	1.30	1.400
		12	0.33	0.829	2.75	0.543	0.50	1.132	1.40	1.355
CEL A SALT	2.20	4	-	-	-	-	-	-	-	-
		8	-	-	0.10	0.304	-	-	-	-
		12	0.38	0.868	2.63	0.897	0.30	0.823	-	-
CEL A OXIDE	1.79	4	-	-	-	-	-	-	-	-
		8	0.35	0.893	1.30	1.344	0.35	0.893	-	-
		12	-	-	2.10	1.172	-	-	-	-
K33 OXIDE	1.40	4	0.43	0.931	2.63	0.490	0.23	0.698	2.33	1.421
		8	0.43	0.844	3.18	0.385	0.20	0.564	1.95	0.986
		12	0.70	1.090	3.30	0.564	0.80	1.091	2.28	1.109

Figure 40 Performance of Scots Pine in a Garden Loam at two whcs Treating Solution based on Eqivalent Active Elements.

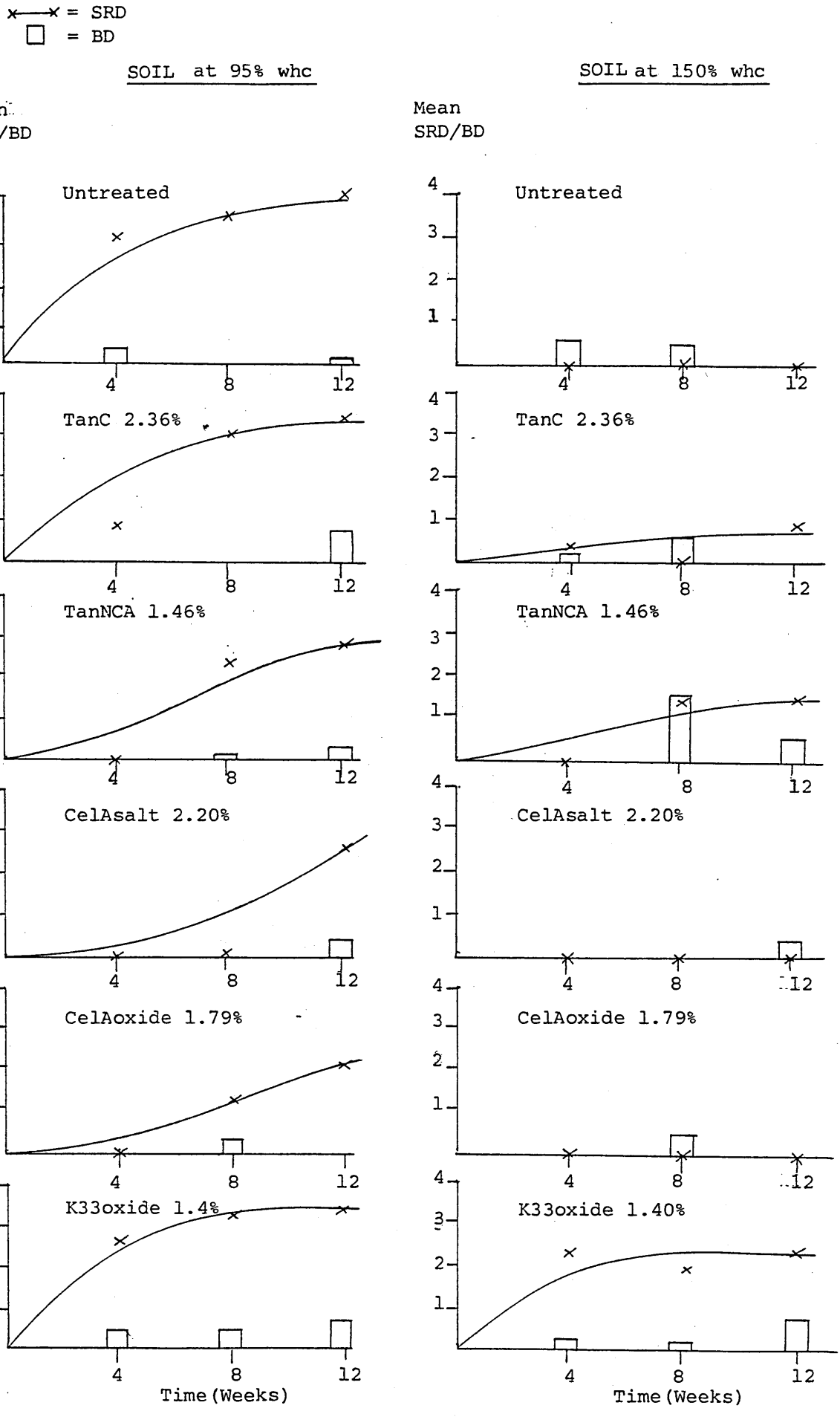


Table 111

Mean BD and SRD for Scots Pine sections buried in a garden loam.

Treating Solution concentration based on Equivalent Active Element Composition.

Treatment	Conc (%w/v)	Time (weeks)	95% whc				150% whc			
			BD		SRD		BD		SRD	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
TAN C	4.13	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-
TAN NCA	2.56	4	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-
CEL A	3.85	4	-	-	-	-	-	-	-	-
SALT		8	-	-	-	-	0.15	0.580	-	-
		12	-	-	1.15	1.051	-	-	-	-
CEL A	3.14	4	-	-	-	-	-	-	-	-
OXIDE		8	-	-	-	-	-	-	-	-
		12	-	-	0.30	0.791	-	-	-	-
K33	2.45	4	0.18	0.594	-	-	-	-	-	-
OXIDE		8	-	-	0.70	0.853	-	-	-	-
		12	0.40	0.871	2.75	0.543	-	-	-	-

Figure 41 Performance of Scots Pine in a Garden Loam at two whcs Treating Solution based on Equivalent Active Elements.

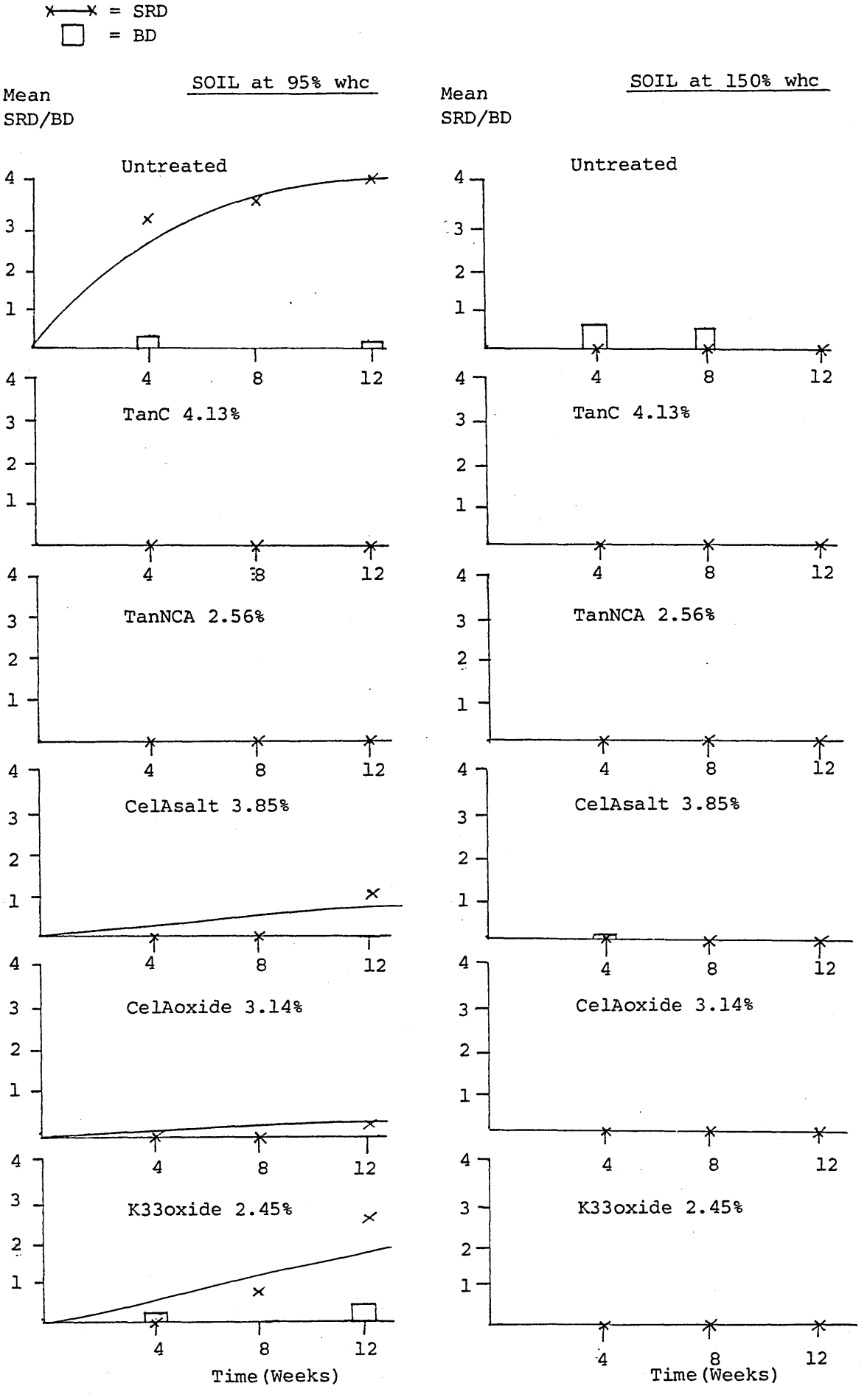


Table 112

Equivalent Active Element Concentrations

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
UN		0	82.5%	100%	90%
		1	10	0	5
		2	5	0	5
		3	2.5	0	0
		4	0	0	0
TAN C	0.7	0	100	100	77.5
		1	0	0	5
		2	0	0	17.5
		3	0	0	0
		4	0	0	0
TAN NCA	0.43	0	92.5	100	100
		1	0	0	0
		2	5	0	0
		3	2.5	0	0
		4	0	0	0
CEL A Salt	0.65	0	100	100	82.5
		1	0	0	0
		2	0	0	5
		3	0	0	12.5
		4	0	0	0
CEL A Oxide	0.53	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
K33 Oxide	0.42	0	100	55	100
		1	0	10	0
		2	0	30	0
		3	0	5	0
		4	0	0	0

Table 113

Equivalent Active Element Concentrations

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
TAN C	2.36	0	100%	100%	57.5%
		1	0	0	17.5
		2	0	0	22.5
		3	0	0	2.5
		4	0	0	0
TAN NCA	1.46	0	100	92.5	85
		1	0	5	2.5
		2	0	2.5	7.5
		3	0	0	5
		4	0	0	0
CEL A Salt	2.20	0	100	100	82.5
		1	0	0	2.5
		2	0	0	10
		3	0	0	5
		4	0	0	0
CEL A Oxide	1.79	0	100	85	100
		1	0	2.5	0
		2	0	5	0
		3	0	7.5	0
		4	0	0	0
K33 Oxide	1.40	0	80	75	67.5
		1	5	12.5	5
		2	7.5	7.5	17.5
		3	7.5	5	10
		4	0	0	0

Table 114

Equivalent Active Element Concentrations

Percentage Frequency of Occurrence of BD Classes (95% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
TAN C	4.13	0	100%	100%	100%
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
TAN NCA	2.56	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Salt	3.85	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Oxide	3.14	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
K33 Oxide	2.45	0	90	100	80
		1	5	0	5
		2	2.5	0	10
		3	2.5	0	5
		4	0	0	0

Table 115

Equivalent Active Element Concentrations

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
UN		0	75%	82.5%	100%
		1	5	2.5	0
		2	10	2.5	0
		3	10	10	0
		4	0	2.5	0
TAN C	0.7	0	92.5	77.5	82.5
		1	2.5	0	0
		2	5	12.5	5
		3	0	10	10
		4	0	0	2.5
TAN NCA	0.43	0	82.5	80	100
		1	2.5	2.5	0
		2	15	5	0
		3	0	10	0
		4	0	2.5	0
CEL A Salt	0.65	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Oxide	0.53	0	100	85	100
		1	0	5	0
		2	0	5	0
		3	0	5	0
		4	0	0	0
K33 Oxide	0.42	0	100	62.5	100
		1	0	2.5	0
		2	0	20	0
		3	0	10	0
		4	0	5	0

Table 116

Equivalent Active Element Concentrations

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
TAN C	2.36	0	90%	62.5%	100%
		1	7.5	20	0
		2	2.5	10	0
		3	0	7.5	0
		4	0	0	0
TAN NCA	1.46	0	100	42.5	82.5
		1	0	7.5	0
		2	0	22.5	5
		3	0	25	10
		4	0	2.5	2.5
CEL A Salt	2.20	0	100	100	87.5
		1	0	0	0
		2	0	0	7.5
		3	0	0	5
		4	0	0	0
CEL A Oxide	1.79	0	100	85	100
		1	0	2.5	0
		2	0	5	0
		3	0	7.5	0
		4	0	0	0
K33 Oxide	1.40	0	90	87.5	60
		1	0	5	10
		2	7.5	7.5	20
		3	2.5	0	10
		4	0	0	0

Table 117

Equivalent Active Element Concentrations

Percentage Frequency of Occurrence of BD Classes (150% w.h.c.)

Treatment	Concentration (%w/v)	Class	Time (Weeks)		
			4	8	12
TAN C	4.13	0	100%	100%	100%
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
TAN NCA	2.56	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
CEL A Salt	3.85	0	100	92.5	100
		1	0	2.5	0
		2	0	2.5	0
		3	0	2.5	0
		4	0	0	0
CEL A Oxide	3.14	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0
K33 Oxide	2.45	0	100	100	100
		1	0	0	0
		2	0	0	0
		3	0	0	0
		4	0	0	0

3.4.2.3.4 An Analysis of the Factors Affecting the Degree of Bacterial and Soft Rot Attack

The effect of preservative type and concentration on BDs and SRDs was determined using an analysis of variance test (Section 3.3.3.3.3 and Appendix C).

Bacterial Attack. Soil at 95% of w.h.c.

Preservative type had a significant effect on BDs (Table 118). Celcure A salt, Celcure A oxide and Tanalith C performed significantly better than K33 oxide and untreated controls. Sections cut from blocks treated with K33 oxide had a high frequency of BDs in classes 3 and 4 (Tables 112-114). After four weeks only sections treated with K33 oxide, Tanalith NCA and untreated controls were attacked by the bacteria. The variability of attack of untreated sections has been discussed in Section 3.4.1.4. Bacterial attack was independent of preservative concentration (Tables 118, 122) although restricted at the highest level.

Soft Rot Attack. Soil at 95% of w.h.c.

The type of preservative used had a significant effect on SRDs after four weeks (Table 119). The five preservatives performed significantly better than untreated controls (Table 121). Concentration affected SRDs (Tables 119 and 123). This agrees with results obtained in soft rot tests (Sections 3.3.4.3.1 and 3.3.4.3.3) and bacterial tests using equivalent copper concentrations (Section 3.4.2.3.2). Soft rot attack was highest for sections cut from blocks treated with K33 oxide.

Table 118

Analysis of Variance on mean BDs of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c. (ii) Equivalent Active Element Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	4.12	*
	Concentration	2	0.32	n.s.
	Residual	10		
8 Weeks	Preservative	5	2.32	n.s.
	Concentration	2	1.14	n.s.
	Residual	10		
12 Weeks	Preservative	5	1.55	n.s.
	Concentration	2	2.98	n.s.
	Residual	10		

Table 119

Analysis of Variance on Mean SRD of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c. (ii) Equivalent Active Element Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	3.54	*
	Concentration	2	4.29	*
	Residual			
8 Weeks	Preservative	5	3.24	n.s.
	Concentration	2	11.03	**
	Residual	10		
12 Weeks	Preservative	5	2.69	n.s.
	Concentration	2	12.20	**
	Residual	10		

n.s. = not significant at p level 0.05

* significant at $p < 0.05$

** significant at $p < 0.01$

Table 120

Mean BDs From the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.

(a) Preservative Effect (After Averaging for all Concentrations).

4 Weeks	CELAs	CELAOx	TANC	TANNCA	K33Ox	UN
BD	0	0	0	0.06	0.20	0.28
l.s.d. = 0.19	I———	———	———	———I	I———	———I
				I———	———I	

8 Weeks	CELAs	TANC	UN	TANNCA	CELAOx	K33Ox
BD	0	0	0	0.03	0.12	0.43
	I———	———	———	———	———	———I
	no significant difference between treatments					

12 Weeks	CELAOx	TANNCA	UN	CELAs	TANC	K33Ox
BD	0	0.11	0.15	0.29	0.37	0.37
	I———	———	———	———	———	———I
	no significant difference between treatments					

Table 121

Mean SRDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.

(a) Preservative Effect (After Averaging for all Concentrations.

4 Weeks	CELAs	TANC	TANNCA	CELAOx	K33Ox	UN
SRD	0.19	0.83	0.86	0.92	1.33	3.00
l.s.d. = 1.61	I———	———	———	———	———I	

8 Weeks	CELAs	CELAOx	TANNCA	TANC	K33Ox	UN
SRD	0.91	1.43	1.73	1.88	2.26	3.48
	I———	———	———	———	———	———I
	no significant difference between treatments					

12 Weeks	CELAOx	TANC	TANNCA	CELAs	K33Ox	UN
SRD	1.91	2.15	2.25	2.59	3.35	4.00
	I———	———	———	———	———	———I
	no significant difference between treatments					

Table 122

Mean BDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.
(b) Concentration Effect (After Averaging for all Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.7	0.08	0.14	0.17
2.36	0.12	0.15	0.38
4.13	0.08	0	0.09
l.s.d.	n.s.	n.s.	n.s.

Table 123

Mean SRDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 95% of its w.h.c.
(b) Concentration Effect (After Averaging for all Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.7	1.98	2.94	3.73
2.36	1.08	2.21	3.03
4.13	0.50	0.70	1.37
l.s.d.	1.14	1.09	1.09

n.s. = not significant

Table 124

Analysis of Variance on Mean BDs of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c. (ii) Equivalent Active Element Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	12.95	***
	Concentration	2	0.98	n.s.
	Residual	10		
8 Weeks	Preservative	5	1.44	n.s.
	Concentration	2	2.47	n.s.
	Residual	10		
12 Weeks	Preservative	5	0.55	n.s.
	Concentration	2	1.87	n.s.
	Residual	10		

Table 125

Analysis of Variance on Mean SRDs of Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c. (ii) Equivalent Active Element Concentrations.

Time	Unit	DF	F-Ratio	Significance Level
4 Weeks	Preservative	5	2.60	n.s.
	Concentration	2	1.36	n.s.
	Residual	10		
8 Weeks	Preservative	5	2.20	n.s.
	Concentration	2	7.71	**
	Residual	10		
12 Weeks	Preservative	5	2.10	n.s.
	Concentration	2	12.92	**
	Residual	10		

*** = significant at $p < 0.001$

** = significant at $p < 0.01$

n.s. = not significant at p level 0.05

Table 126

Mean BDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.

(a) Preservative Effect (After Averaging for All Concentrations)

4 Weeks	CELAs	CELAOx	K33Ox	TANC	TANNCA	UN
BD	0	0	0.08	0.09	0.11	0.55
l.s.d = 0.18	I———				———I	

8 Weeks	CELAs	CELAOx	K33Ox	TANC	UN	TANNCA
BD	0.05	0.22	0.38	0.39	0.48	0.64
	I——— no significant difference between treatments ———I					

12 Weeks	CELAOx	UN	CELAs	TANC	TANNCA	K33Ox
BD	0	0	0.10	0.17	0.17	0.27
	I——— no significant difference between treatments ———I					

Table 127

Mean SRDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.

(a) Preservative Effect (After Averaging for all Concentrations).

4 Weeks	CELAs	UN	CELAOx	TANC	TANNCA	K33Ox
SRD	0	0	0.02	0.13	0.32	1.24
	I——— no significant difference between treatments ———I					

8 Weeks	UN	CELAs	CELAOx	TANC	TANNCA	K33Ox
SRD	0	0.20	0.38	0.86	1.28	1.41
	I——— no significant difference between treatments ———I					

12 Weeks	UN	CELAs	TANC	CELAOx	TANNCA	K33Ox
SRD	0	0.78	0.82	1.00	1.49	1.90
	I——— no significant difference between treatments ———I					

I——I = no significant difference

Table 128

Mean BDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.
(b) Concentration Effect (After Averaging for all Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.7	0.17	0.39	0.08
2.36	0.15	0.43	0.27
4.13	0.09	0.03	0
l.s.d.	n.s.	n.s.	n.s.

Table 129

Mean SRDs from the Analysis of Variance for Scots Pine Sections Buried in a Garden Loam at a Moisture Content of 150% of its w.h.c.
(b) Concentration Effect (After Averaging for all Preservatives).

Concentration (%w/v) Equivalent to TAN C	Time (Weeks)		
	4	8	12
0.7	0.40	1.52	2.25
2.36	0.46	0.54	0.75
4.13	0	0	0
l.s.d.	n.s.	0.88	1.00

n.s. = not significant

Bacterial Attack. Soil at 150% of w.h.c.

The five preservatives performed significantly better than untreated controls (Table 124). Celcure A salt and Celcure A oxide performed well, whilst Tanalith NCA and K33 oxide performed poorly. The bacterial attack was independent of preservative concentration (Tables 124 and 128). This agrees with results in Sections 3.4.1.3.2 and 3.4.2.3.2 for equivalent copper concentrations.

Soft Rot Attack. Soil at 150% of w.h.c.

There was no significant difference between the performance of the five preservatives and untreated controls (Tables 125, 127). Tanalith NCA and K33 oxide treated sections had the highest SRDs. The absence of attack of untreated sections may have been due to the saturating conditions within the soil jar. The soil moisture content was very high, excess water floated on the surface of the soil and the oxygen within the soil may have been reduced in some soil jars. The concentration of preservative used had a significant effect on SRDs (Tables 125, 129).

3.4.2.3.5 Summary of Results

Equivalent Copper Concentrations

At the lower soil moisture content, Celcure A salt and Celcure A oxide performed well against bacterial attack, also against soft rot attack at concentrations of 2.36 and 4.13%. Tanalith NCA and K33 oxide performed poorly against bacterial attack. At the higher soil moisture content, Celcure A salt performed well against bacterial attack, whilst Tanalith NCA and K33 oxide performed poorly. Soft rot attack was highest for sections treated with K33 oxide. The performance of Tanalith C was intermediate between the best performers (Celcure A salt and Celcure A oxide) and the poor performers (Tanalith NCA and K33 oxide).

Equivalent Active Element Concentrations

Celcure A salt, Celcure A oxide and Tanalith C performed the best for both soil moisture contents against soft rot and bacterial attack. Tanalith NCA and K33 oxide performed poorly. The exception to this trend was found with the one incident of bacterial attack for Celcure A salt treated sections at the highest preservative concentration used.

3.4.2.4 Discussion

The moisture content of the soil affected the type of attack found. This agrees with results from the test using the experimental formulations (Section 3.4.1). At the higher soil moisture content soft rot attack was restricted.

The results when combined overall comparing preservatives on an equivalent copper and equivalent active element basis, show that against both soft rot and S_2 degrading bacteria, Celcure A salt, Celcure A oxide and Tanalith C performed better than K33 oxide (a Type B preservative) and Tanalith NCA (a modified Type B). Hedley (1984 a,b) looking at the data from the failure of treated vineyard posts in New Zealand, found that CCA formulations which approximated to Type C performed better than those of Type B. The latter preservatives such as K33 oxide and Tanalith NCA are in common use in New Zealand. Plackett (1984) found that CCA formulations with a high chromium to arsenic ratio were more leach resistant than those with a low ratio. K33 oxide and Tanalith NCA have a low chromium to arsenic ratio. Hedley (1984 a,b) found that if the amount of copper is kept constant, then a reduction in the chromium to arsenic ratio leads to a decrease in the preservative effectiveness and this becomes most marked when the ratio drops below unity. Hartford et al (1982) reported that Type B formulations were inferior to those of Type C in service tests.

The thin section assay has confirmed results obtained in the field. The variability found with the bacterial attack in this test was observed in the field in New Zealand (Hedley and Drysdale, 1985), isolated pockets of decay occurred. This variability of attack made it necessary to examine the attack in terms of frequency of occurrence of each BD class as well as by an analysis of variance test. The presence of only one or two readings in class 4 was very important , however their significance would be lost if mean values only were used. The results from these two types of analyses show that Type C preservatives, Celcure A salt, oxide and Tanalith C performed better than Type B preservatives, K33 oxide and Tanalith NCA.

The S_2 degrading bacteria are non-flagellated bacteria spreading through the soil passively (Nilsson and Daniel, 1983a), they can only vary their position by increasing the size of their colony. Soft rot fungi however, spread by means of the fungus growing through cells, attack increases with time and there is less variability in the occurrence of fungal attack. With increased incubation time the bacteria may have spread through the entire area of the soil jar. The variability in the position of the bacterial colonies in relation to the buried wood sections probably produced the inconsistent results for untreated sections. Such sections may have been buried in an area of soil supporting no bacterial populations. Variability occurred both within the soil jars and between them. This reflects the findings in the field of Hedley and Drysdale (1985) where variability occurred in the condition of posts planted at the same sites and also occurred between sites.

Untreated sections did not always have the highest degree of soft rot attack at the higher soil moisture content, as they did at the lower. This may have been due to the saturating conditions within the soil jars preventing fungal attack. There may have been more oxygen in those jars containing some of the treated sections. Soft rot attack was severely

restricted at the higher soil moisture content. The bacteria were dominant under these conditions and may have affected the soft rot attack. Jacquiot (1968) reported a number of instances where bacteria inhibited the growth and activity of wood rotting fungi. The bacteria and fungi may compete for nutrients. A primary invasion of wood by bacteria may retard fungal growth by depleting valuable carbon reserves in the wood. Bacterial attack of the thin sections may have prevented attack by the soft rot fungi. However, the two types of attack were frequently found together, especially at the lower soil moisture content.

The higher SRDs recorded in Scots pine sections buried in the garden loam compared to those buried in Rentokil soil examined in an earlier test (Section 3.3.3) have already been discussed in Section 3.4.1.4. The garden loam had a higher soft rot potential, but in addition, the presence of the bacteria may have enhanced the attack found over that with soft rot alone (Section 3.3.3). Bacteria make conditions more suitable for soft rot attack by detoxifying preservatives or natural toxic extractives in timber. Wood-degrading bacteria have been shown to precipitate and detoxify metal preservatives in situ in pine treated to high retentions (Daniel and Nilsson, 1985). Associations have often been observed between bacteria and soft rot fungi (Greaves and Levy, 1968; Tranina, 1968; Greaves and Foster, 1970).

3.4.2.5 Conclusions

The thin section technique has successfully reproduced results found by other workers in the field examining the performance of CCA preservatives. Those preservatives approximating to Type B, K33 oxide and the modified Type B, Tanalith NCA are inferior to those of Type C, Tanalith C, Celcure A salt and Celcure A oxide.

The occurrence of bacterial attack in the laboratory agrees with that found in the field. Loci of infection were found in the soil jars and variability occurred in the degree of attack within the same soil jar and between jars. In the field, variability occurs within the same site and between sites.

Soft rot attack is severely restricted in soils at a very high moisture content and also in the presence of high concentrations of preservatives in the wood. The fact that under these conditions bacterial attack is increased may be due to the removal of fungal competitors from the wood sections.

The bacterial degree can be used as an indicator of the long term performance of preservatives.

SECTION IV: TYPES OF BACTERIAL ATTACK

4 Section IV Types of Bacterial Attack

4.1 Introduction

The type of bacterial attack found in this work may be due to a number of different species of bacteria and no attempt has been made to critically characterise the attack. Using the thin section technique the bacteria attack the S_2 layer of the wood cell wall and have been termed S_2 degrading bacteria. An attempt was made to relate the patterns of attack found to those observed by other workers.

The bacteria responsible for the various degradation patterns reported may be the same, or different species or stages in the succession of bacterial wood destroying species. They may be acting independently or synergistically or they may be in a different physiological state caused by local conditions within the wood e.g. oxygen levels, pH etc. Daniel and Nilsson (1986) suggest that within wood there is a succession of bacterial species but that not all species are active in timber decay. A bacterial consortia have been effective in the decay of lignocellulose while single strains are able to degrade only delignified wood (Nilsson, 1982a).

The micromorphology of the forms of attack and degrade have been described using the light microscope, as conical depressions (Harmsen and Nissen, 1965 a,b);erosion zones through the cell wall layers (Greaves, 1969); erosion troughs and lysis zones in the cell wall layers attributed to actinomycetes (Eaton and Dickinson, 1976; Leightley and Eaton, 1977).

Scanning electron microscope studies have described erosion, cavitation and tunnelling patterns (Sieburth, 1975; Cundell and Mitchell, 1977 ; Holt et al, 1979; Holt, 1981, 1983; Nilsson and Holt, 1983;

Nilsson and Singh, 1984).

Investigations at the level of the transmission electron microscope have shown that tunnelling or burrowing by the bacteria is the most important form of bacterial degrade of the different cell wall layers of a variety of treated and untreated timbers (Nilsson and Daniel, 1983a; Daniel and Nilsson, 1985).

The earlier patterns attributed to actinomycetes by Eaton and Dickinson (1976), Leightley and Eaton (1977), are typical of tunnelling bacteria decay patterns described by Nilsson (1982a), Nilsson and Daniel (1983a), Nilsson and Holt (1983) and Mouzouras et al (1986). Tunnelling bacteria penetrate into the wood fibre walls, into the S_2 layer, where they form tunnels and later complete destruction of the cell wall substance.

The patterns of bacterial attack found in the work described here were characteristic of those attributed to tunnelling bacteria, when viewed under the light microscope. However, using the scanning electron microscope other types of bacterial attack were found.

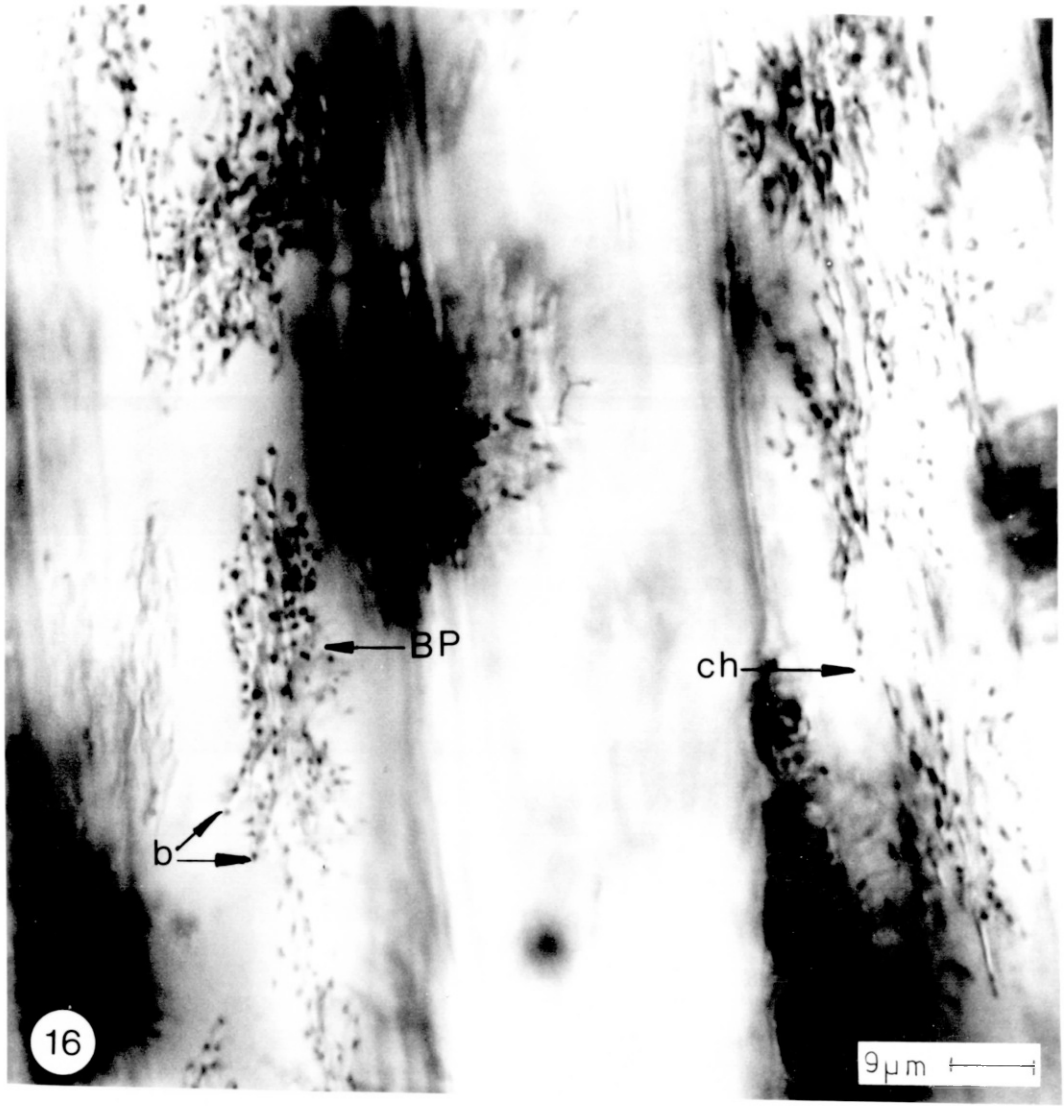
4.2 Light Microscopy

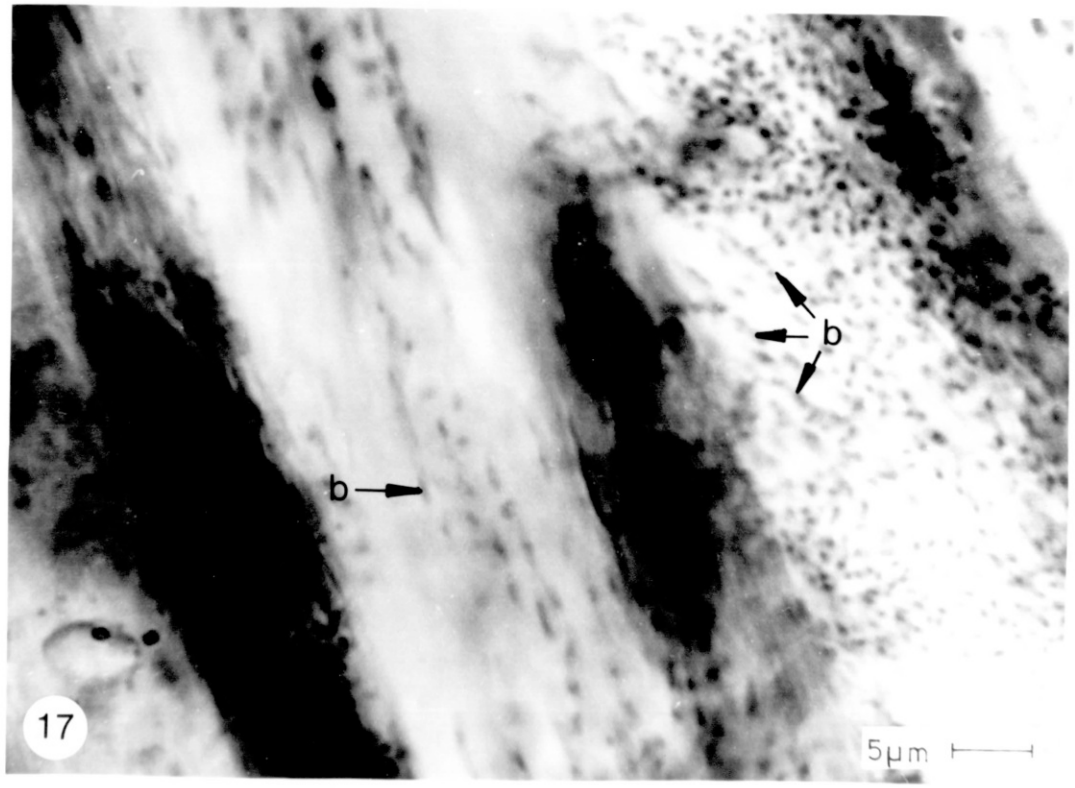
Plates 12, 13, 14, 15 (Section 2.5.4.1) and Plates 16, 17 illustrate the typical pattern of attack found. A heavily degraded area is surrounded by a fringe of radiating branched tunnels. A single bacterium was seen at the front end of each tunnel in Plates 16 and 17. Individual areas of degradation coalesce in the late stages of decay leaving a strongly degraded fibre. This pattern of attack was used to determine a Bacterial Degree or BD of attack for the determination of the effectiveness of the preservatives against S_2 degrading bacteria (Section 2.5.4.1). The frequency of occurrence of these patterns within a tracheid wall was used to describe the extent of attack.

PLATE 16. Tangential longitudinal section cut from an untreated Scots pine block. The section was buried in a garden loam which was at a soil moisture content of 150% of the w.h.c, for four weeks. The section was stained with cotton blue in lactophenol and examined under the light microscope.

Bacterial patterns (BP) caused by the S_2 degrading bacteria can be seen. There is a centrally degraded area from which channels (ch) radiate out. A single bacterium (b) is usually found at the front end of each channel.

PLATE 17. At a higher magnification, the bacteria (b) can be seen within the channels produced in the S_2 layer.


BP
ch
b
16
9µm


b
b
17
5µm

4.3 Scanning Electron Microscopy (S.E.M.)

4.3.1 Introduction

The thin sections of wood examined in the light microscope for degree of bacterial attack were used for S.E.M. studies. With the S.E.M higher magnifications than those used with the light microscope give more detailed information of the decay process. The S.E.M., however, cannot be used to monitor degradation in the S_2 layer whilst the S_3 layer is still intact. The light microscope is best used when examining the initial stages of attack which are mainly confined to the S_2 layer. The two layers, S_2 and S_3 may become separated in the decayed region, by chance during sectioning and preparation of the wood specimens for examination in the S.E.M. Areas of degradation may otherwise remain undetected until the S_3 layer is also affected by the bacteria.

4.3.2 Method

30 μm thick sections were air dried using the method of Findlay (1970). They were laid on filter paper and placed in a dessicator containing silica gel, for four days at room temperature. The dried sections were mounted on aluminium stubs using double-sided adhesive tape. The stubs were marked using a scalpel to allow identification of the sections in the S.E.M. The stubs were coated with a conductive layer of gold 30-50 nm in a Polaron E500 Sputter Coater, to prevent the specimen charging in the S.E.M. The sections were examined in a Philips 500 S.E.M. operating at an accelerating voltage of 12 KV. Photographs were taken on Ilford Pan F film.

4.3.3 Results and Discussion

Channels or tunnels were found in the S_2 layer once the S_3 had been degraded (Plates 18-22). The bacteria first penetrate the S_3 layer. The tunnels found in the S_2 appeared to have cross-walls (Plate 20) as described by Nilsson and Daniel (1983a); Daniel and Nilsson (1985, 1986) and Mouzouras et al (1986). Tunnels were frequently found aligned with each other, separated only by a thin layer of undegraded wood (Plates 19-21). The tunnels may have been caused by tunnelling or erosion bacteria. In the light microscope the patterns found were similar to those described for tunnelling bacteria (Nilsson and Daniel, 1983a). Holt (1981) and Daniel and Nilsson, (1986), described aligned erosion channels often interconnected but lacking the prominent cross-walls formed by the tunnelling bacteria.

No microorganisms were found in the S.E.M. sections although in the light micrographs (Plates 12-17) bacteria were found within the tunnels. Cavalcante (1981) and Holt (1981) have reported the absence of bacteria in their studies. The bacteria may have been lost during the preparation of the wood sections, through air drying or coating of the sections.

Another type of bacterial attack was observed characterised by the presence of large angular cavities (Plates 22 and 23), similar to those described by Leightley (1982) and Willoughby and Leightley (1984). The cavities form ladder-like formations, eventually coalescing and destroying the cell wall.

4.4 Soft Rot and Bacterial Attack Present Together.

Soft rot cavities in longitudinal sections were observed in the same tracheids as bacterial decay patterns (Plates 24-29). Both groups of microorganisms attack the S_2 layer. In Plate 26, the areas attacked by soft

rot fungi and S_2 degrading bacteria can be identified in polarised light (Plate 27) as areas that no longer birefringe. The cellulose structure of the S_2 layer is altered. The two types of attack were frequently found together in soil which had been used at a moisture content of 95% of the w.h.c. At the higher soil moisture content, in bacterial tests, fungal attack was severely restricted (Section 3.4.1.).

Nilsson and Daniel (1983a) showed that fungal attack is not required to precede bacterial attack. The bacteria may make conditions more suitable for soft rot attack by detoxifying preservatives or natural toxic extractives in timber. These authors found that wood species showing an intermediate resistance to soft rot decay e.g. Scots pine were attacked simultaneously by tunnelling bacteria and soft rot fungi in an unsterile soil environment.

Greaves and Levy (1968) found that timber in a disused copper mine had been attacked by bacteria and soft rot fungi, the two had combined to erode completely the secondary cell wall of tracheids and fibres. Willoughby and Leightley (1984) reported bacterial and soft rot decay in CCA treated eucalypt power transmission poles and in CCA treated Pinus elliotti experimental stakes. These authors found that when timber had been completely destroyed throughout the treated zone, it was difficult to identify the causal agent since the final stages of soft rot and bacterial decay are very similar. Drysdale and Hedley (1984) and Nilsson (1984) found that soft rot fungi together with tunnelling and cavitation bacteria were the major causes of the premature decay of CCA treated Pinus radiata horticultural stakes in New Zealand.

4.5 Conclusions

In the light microscope the patterns of attack caused by the S_2

degrading bacteria appear to be very similar to those described for tunnelling bacteria. In the S.E.M., however, other forms of bacterial attack have been found. The patterns of attack have not been defined in this work. The bacteria found, attack the S_2 layer in a similar manner to soft rot fungi and are therefore suitable for use with the thin section technique.

Soft rot fungi produce cavities in the same tracheids as those attacked by S_2 degrading bacteria. Both types of attack can cause extensive degrade of the cell wall. The similarity between the patterns of degrade caused by soft rot fungi and S_2 degrading bacteria may have led earlier workers to overlook the bacterial attack. Both groups of microorganisms penetrate through the S_3 layer and preferentially degrade the less lignified S_2 layer by a process of tunnelling and cavity formation. Both patterns of attack can be detected using the thin section technique and therefore preservatives can be assessed in terms of their performance against the two hazards.

PLATES 18 - 23

Scanning electron micrographs of bacterial patterns produced in tangential longitudinal Scots pine sections which had been cut from a block treated with CCA. The sections were buried in a garden loam at a soil moisture content of 150% of the water holding capacity.

PLATE 18. Channels (CH) were produced in the S_2 layer by the bacteria.

PLATE 19. Parallel channels (CH) found in the S_2 layer

PLATE 20. Plate 19 at a higher magnification shows a cross wall (CW) in one of the channels.

CH
18
6 µm

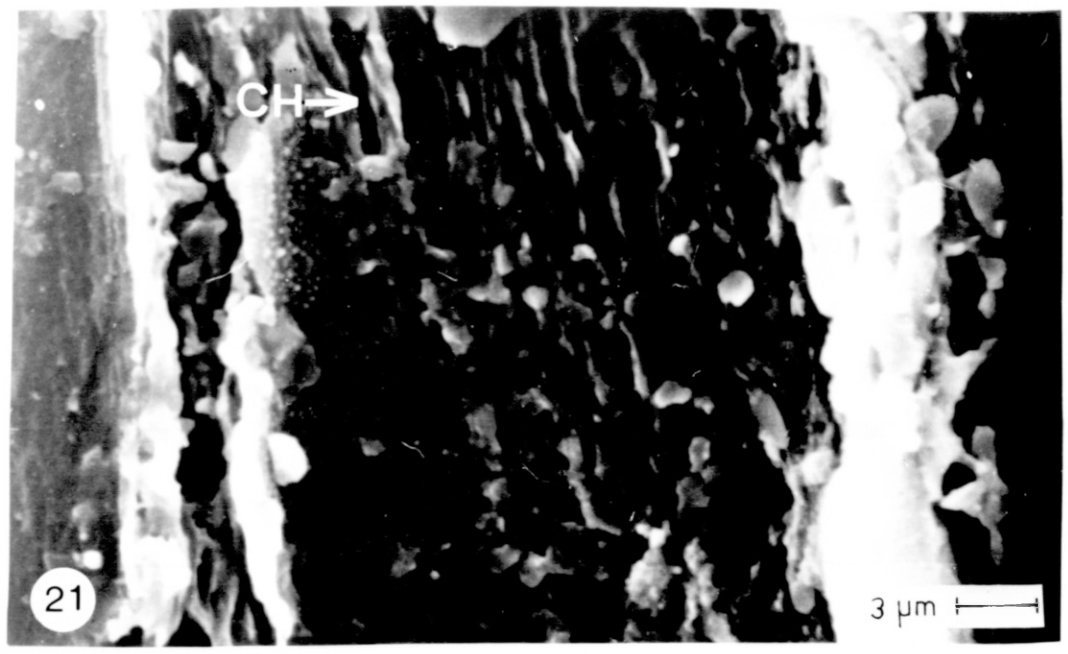
CH
19
4 µm

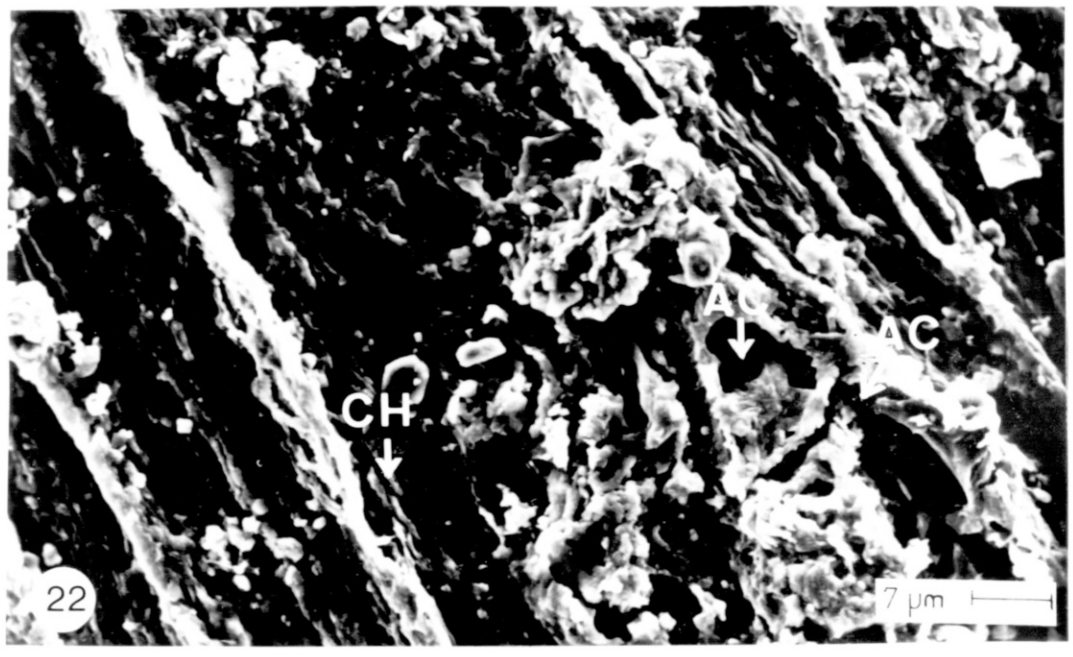
CW
CH
20
2 µm

PLATE 21 Parallel channels (CH) produced by the bacteria degrading the S_2 layer.

PLATE 22. Angular cavities (AC) were produced by the bacteria, together with channels (CH).

PLATE 23. As for plate 22.


CH
21
3 µm


AC
AC
CH
22
7 µm


AC
23
11 µm

PLATES 24 - 29.

Light micrographs of tangential longitudinal Scots pine sections cut from variously treated blocks and buried in a garden loam at a moisture content of 95% of the water holding capacity.

PLATE 24. Scots pine treated with a 2.36% CCB+A solution. A soft rot cavity (SR) can be seen in the same tracheid as a bacterial pattern (BP), both degrade the S_2 layer.

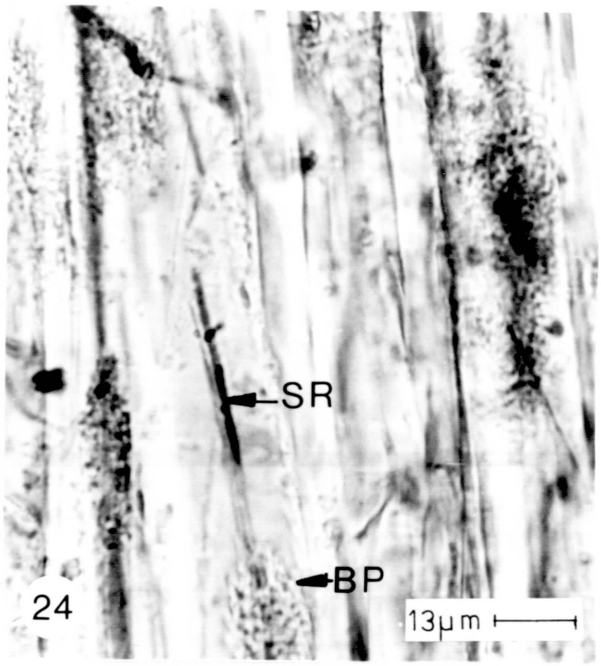
PLATE 25. Plate 24 at a higher magnification.

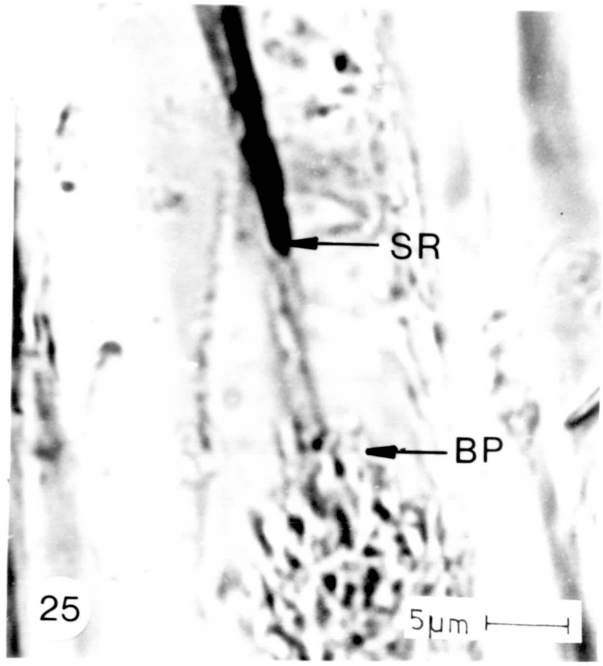
PLATE 26. Scots pine treated with 2.36% CCB+A solution. Soft rot cavities (SR) and bacterial patterns (BP) together degrade the S_2 layer.

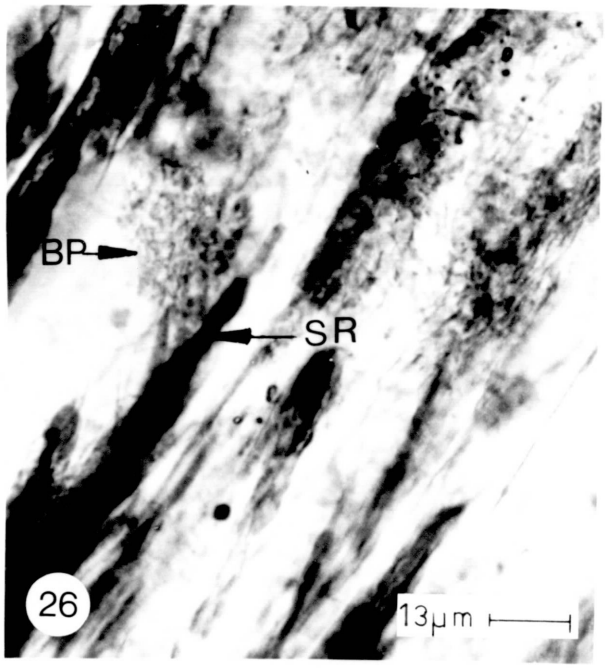
PLATE 27. Plate 26 with polarised light. The soft rot cavities (SR) and bacterial patterns (BP) both cause a loss of birefringence within the S_2.

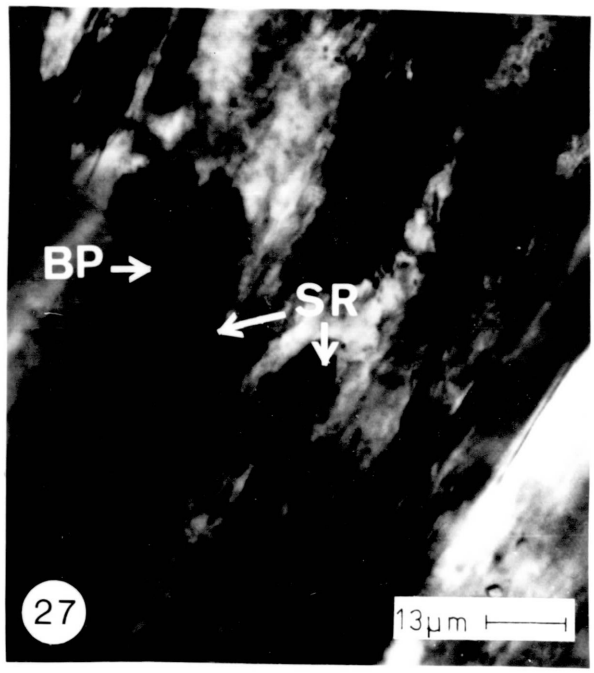
PLATE 28. Scots pine treated with 2.36% CCB+A. The soft rot cavity (SR) and bacterial pattern (BP) are seen in greater detail at this higher magnification.

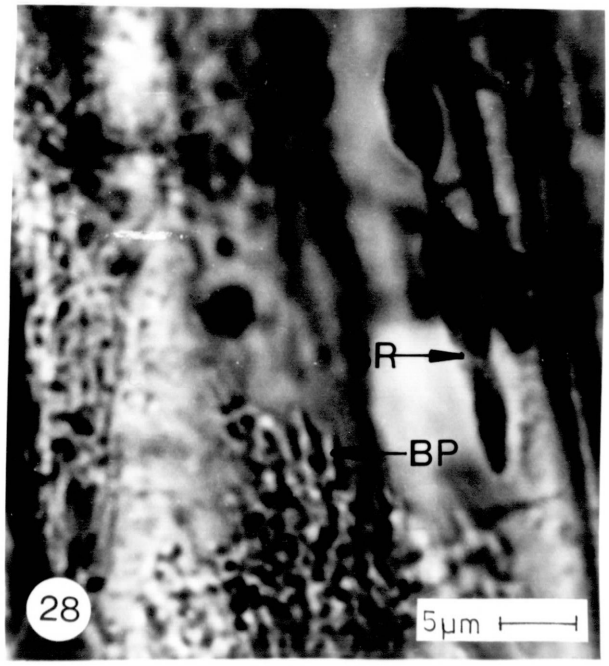
PLATE 29. Scots pine treated with the CCA preservative Celcure A oxide. The soft rot cavities (SR) are seen to be very close to the bacterial patterns (BP).

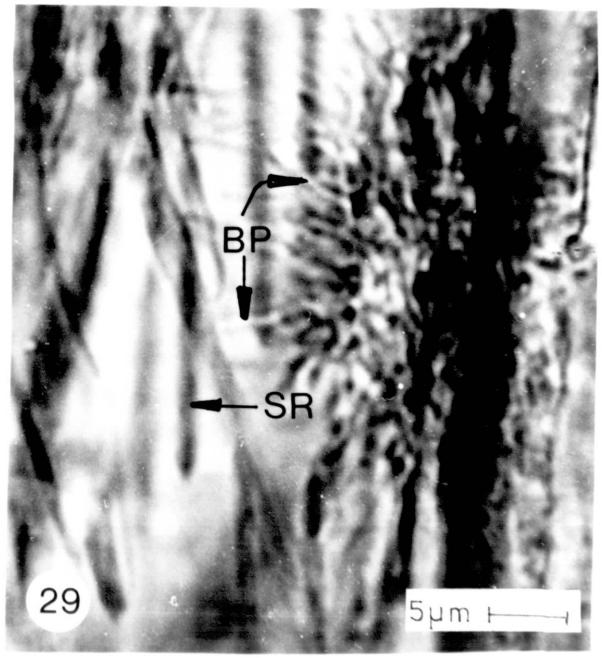

SR
BP
24
13µm


SR
BP
25
5µm


BP
SR
26
13µm


BP
SR
27
13µm


R
BP
28
5µm


BP
SR
29
5µm

SECTION V: GENERAL DISCUSSION

5 Section V General Discussion

5.1 Introduction : Original Plan and Objectives

The original aim of the work described in this thesis was to examine the performance of a wide range of copper based water borne formulations against soft rot attack. Gray (1983) suggested that arsenic interferes with the fixation of copper to the cell wall and that a minimum amount of copper, within the cell wall, is required to prevent decay. The range of formulations used by Gray (1983) was extended in this work in an attempt to examine the effect of each individual preservative element on the overall performance.

An effective preservative is designed to protect timber for many years in practice, therefore, any laboratory test of its long-term performance must be greatly accelerated. For this reason it was decided to expose thin sections of treated wood to the fungal hazard in soil. With thin sections the fungi are allowed immediate access to the cell wall and preservative performance can be determined in weeks which would take years to develop in the field. The effectiveness of the treatment of the S_2 layer of the cell wall which is attacked by soft rot fungi can be directly assessed.

Hedley (1980, 1983) compared the decay rates found in preservative treated stakes buried in a fungal cellar (designed as an intermediary step between laboratory and field tests) (FRI, 1978). He found that untreated stakes decayed nine times faster in the fungal cellar than in the field, whilst the majority of treated stakes showed similar decay rates to that of untreated material. Vinden et al (1983) described three phases in the decay process, namely, colonisation (lag), decay and senescence. They found that the duration of the phases was affected by the fungal cellar conditions

and the techniques used to assess decay. Hedley (1983) found that the lag phase was eliminated in the stakes buried in the fungal cellar. In the field, however, the length of the lag phase before the onset of decay was variable. Once this lag phase had ended, most stakes decayed at a consistent rate irrespective of formulation. Using the thin section test here, once decay was established, all treatments failed, but it was the length of time for failure to occur that determined the performance of each of the preservatives.

Vinden et al (1982) suggested that a correlation was required between the colonisation and decay phases before an assessment of performance in the field could be determined. The thin section technique allows fungal colonisation and the onset of decay to be examined together.

5.2 Development of the Work

Once developed as an effective soft rot assay it became clear that it could be used to determine the activity of other wood-degrading microorganisms. It was decided to extend the work to include a means of assessing the attack of the wood cell wall by bacteria. Recent reports of serious decay of softwoods in horticultural situations in New Zealand (Butcher et al, 1983; Drysdale and Hedley, 1984; Nilsson, 1984) caused by soft rot fungi and bacteria suggest the importance of the bacterial hazard. The bacteria reported in New Zealand are able to attack the S_2 layer of the wood cell wall as can the soft rot fungi. Therefore, with little modification, the technique could be applied to bacterial attack of preservative treated wood.

After a series of preliminary tests, conditions suitable for culturing bacterial attack of treated thin sections of wood, were determined. The same soil jar system used in soft rot tests was found to

be suitable, however, a modification of the soil type and moisture content was required. Bacterial patterns produced in the cell wall were very similar to those found in horticultural stakes attacked by soil bacteria in New Zealand. Once the conditions were right it was decided to test the performance of the copper-based experimental formulations used in soft rot tests, against the bacteria. In addition, commercially available preservatives were used to correlate results of known field performance with those found in the laboratory, using this thin section technique.

5.3 Thin Section Assay

5.3.1 General

The technique developed consisted of cutting thin sections of wood from 7 mm^3 blocks of birch and scots pine, which had been treated with the experimental formulations and commercially available preservatives. The thin sections were placed in nylon mesh packets, buried in soil, then removed after varying time periods. The degree of soft rot and bacterial attack was determined by examining the sections in the light microscope. Attack occurred in weeks. This enabled rapid assessments to be made of the performance of wood preservatives. The attack of the thin sections of wood by both the fungi and bacteria could be readily observed as soon as degradation began. The conditions built up in the soil jar mimicked those known to exist in habitats where such decay occurs in nature, with regard to the distribution of the bacterial and fungal populations. The superior performance of copper chrome arsenic (CCA) Type C wood preservatives over those of Type B, recorded in this work, agrees with that found in the field (Hedley, 1984 a,b).

Using the thin section technique in soft rot tests, the lag

phase was removed in most cases for birch sections buried in soils with a high decay potential e.g. Princes Risborough soil and John Innes No.1. Lag phases were more evident with Scots pine sections. Scots pine is less susceptible to soft rot attack and for some treatments decay was not recorded over the period of the test.

The occurrence of bacterial attack in the soil jars was very variable but this reflected its occurrence in the field. Hedley and Drysdale (1985) reported a great variability in post condition within and between sites in the same region in New Zealand. Butcher (1984) suggested that discrete loci of infection exist which spread to gradualllly encompass the total population. The variability in the occurrence of bacterial attack within and between the soil jars influenced the results of the statistical analysis. Mean bacterial degrees (BD) did not give an indication of the instances of attack in the high decay classes, 3 or 4, which although few in number were of significance. For this reason the percentage frequency of occurrence of each class of decay within the wood sections was recorded for bacterial results in addition to the analysis carried out on mean values. This two way approach was found to be necessary due to the variability of the bacterial attack, (a situation found in the field) in order to determine the performance of the preservatives.

Results from the statistical analysis of variance test of mean soft rot degree for the variously treated wood sections, may not reflect the severity of soft rot attack. Fields of view under the microscope may have been focussed upon fibres of birch which were well treated (due to their close proximity to vessels, the main penetration paths for preservatives). Some hardwoods have been shown to have a poor macro-distribution of CCA preservatives within the wood tissues (Dickinson, 1974; Greaves, 1974; Dickinson et al, 1976; Levy and Greaves, 1978; Greaves

and Levy, 1978). Pockets of fibres may be left untreated. The variability in the occurrence of soft rot cavities may be dependent upon the variability in preservative macrodistribution. Statistical analyses do not take this variability into account. Decay curves (mean soft rot degree, SRD, plotted against time) for each concentration of preservative used, give a better indication of preservative performance than do statistical analyses, which in this work, used results averaged for all concentrations.

The bacteria found degrading the wood sections appear to be similar to those described by Nilsson and Daniel (1983a). The bacteria have not been examined in detail here, they may be non-flagellated, spreading passively through the soil by increasing the size of their colony. Attack would in this case be restricted to certain areas until the population had increased in size and spread to adjacent areas. Alternatively, the bacteria may be flagellate, the excess water in the saturated soil would then act as a pathway for the motile bacteria to spread throughout the soil and colonise it. With soft rot fungi, where the hyphae actively grow through both the wood section and the soil, attack of wood sections within the soil jars was less variable than that found for bacterial attack.

5.3.2 Soil Type

Variability in the occurrence of bacterial attack and the degree of soft rot attack has been found between soil types. The inherent variability of soils (e.g. microflora and nutrients) from different locations has provided a major obstacle in the search for the standardisation of soil burial techniques for use in soft rot tests. Soil burial is favoured over other test methods, such as fungal monocultures (BS 838 Part 2, 1961; Schulz and Riewendt, 1962; Duncan, 1965; Bravery, 1968 a,b) and burial in inoculated vermiculite (Gersonde and Kerner-Gang, 1976). These alternative methods do not use the variable components of

the natural microflora found in the field as soil burial tests do. Conditions in the laboratory should be as close as possible to those found in the field. In this work, the different soils used in soft rot tests, varied in their decay potential, John Innes No.1 and Princes Risborough soils had a higher potential than Rentokil soil. Results obtained simulated the variable decay hazard found under field exposure. Similarly in bacterial tests, the garden loam was found to be the most suitable for producing bacterial attack. This soil contained high amounts of nitrogen, phosphorus and organic carbon as did those soils which in New Zealand were found to produce bacterial decay more readily than those soils low in these elements (Drysdale, 1984).

Hilditch (1978) suggested that the physical, chemical and biological composition of soils would affect the decay of wood by microorganisms and also the performance of wood preservatives in a number of ways. Firstly, directly, by the action on the preservative chemical to increase or decrease its water solubility and hence its resistance to loss of leaching, or through complexing or strong ion adsorption to reduce its availability to poison invading microorganisms, or to cause chemical change to a less toxic form. Secondly, indirectly, in that nutrient salts and particularly nitrogen, can be expected to migrate into wood by chemical diffusion, as well as with invading microorganisms.

Within soil it is difficult to distinguish between chemical, physical and biological effects, especially in relation to the occurrence and abundance of specific microorganisms. In this work, the soils were analysed for their organic carbon, total nitrogen and phosphorus content, cation exchange capacity and pH. Soils having a high soft rot decay potential e.g. John Innes No.1 and Princes Risborough, had higher amounts of carbon, nitrogen and phosphorus than the Rentokil soil which had a lower decay potential. In the international collaborative

field trial of CCA treated wood, tested against soft rot fungi, set up by the International Research Group on Wood Preservation (IRG), the Rentokil soil test site was virulent. The moisture content of this soil used here may have been different to that present in the field trials. The Rentokil soil site is a clay site which is wet most of the year. In the laboratory the soil may have dried out and hence the decay potential may have been reduced.

In bacterial tests, the garden loam produced the most bacterial attack of wood sections. This soil had a similar composition to those with a high soft rot decay potential. The importance of the presence of certain soil constituents for soft rot decay to occur, has been emphasised for organic carbon (Wälchi, 1970, 1972), nitrogen (Savory, 1954 a,b; Duncan, 1965; Becker and Kaune, 1966; Kaune, 1970; Lundstrom, 1973) and phosphorus (Da Costa, 1971, 1972).

In New Zealand, higher incidences of decay were found in posts planted in horticultural soils which were of the heavy silt, or clay loam type and restricted free drainage of water (Hedley and Drysdale, 1985). Soil moisture content was found to be very important in the work reported here. It determined the type of attack found. In drier conditions, at a soil moisture content of 95% of the water holding capacity, attack by soft rot fungi was found together with that caused by S_2 degrading bacteria. When the soil moisture content was increased, however, bacteria became the main decay organisms and attack by soft rot fungi was not evident. The garden loam was suitable for culturing S_2 degrading bacteria. Other soils such as Rentokil and John Innes No.2 were less effective, even at the higher moisture contents. This suggests the importance of determining those soils likely to be capable of supporting bacterial populations and recognising these hazard areas for treated posts.

In New Zealand, horticultural soils represented a severe hazard, whereas in pastoral soils, preservative treatments performed well against both soft rot fungi and S_2 degrading bacteria (Drysdale, 1984).

5.3.3 Bacteria

Bacteria have usually been omitted in tests of preservative performance in ground contact. This may have been due to a number of reasons :

1) Harsh predrying regimes have been employed for soils used in soft rot tests in the laboratory. In the work described here, predrying the soil was shown to greatly reduce bacterial activity. This is an important observation. In soft rot tests the bacteria may have to compete with fungi which have produced spores to overcome drought conditions. The bacteria are clearly affected by the drying of the soil and may thus take longer to recover and are, therefore, at a disadvantage.

2) The high soil moisture contents in which bacterial attack occurs are not usually employed in laboratory tests.

3) Wood blocks or stakes are used in the laboratory tests rather than thin sections of wood. Bacterial attack is slow compared with fungal decay, often causing extensive surface degradation and may, therefore, have been ignored by other workers who removed surface layers when examining decayed wood blocks. Holt and Jones (1978) and Schmidt(1978) reported that bacteria preferred samples of a small volume but large surface area. (The thin sections used here would thus appear to be ideal for providing rapid bacterial attack.)

4) Typical decay patterns caused by the S_2 wall layer degrading bacteria are not easily recognised in the advanced stages and this lack of knowledge may have led workers to overlook them. The final stages of bacterial decay are similar to those of soft rot decay.

Bacteria were previously thought to be unimportant in wood decay since bacterial isolates were only found to decay wood which had been delignified (Greaves, 1965, 1970, 1973; Holt, 1976, 1981; Furtado, 1978; Scmidt, 1978, 1980; Holt and Jones, 1978; Holt, Jones and Furtado, 1979; Scmidt and Liese, 1982). A pretreatment of the wood to produce bacterial attack is no longer thought to be necessary (Nilsson, 1982; Nilsson and Daniel, 1983a; Daniel and Nilsson, 1985, 1986; Mouzouras et al, 1986). The bacteria were found to attack lignin rich substrates in preliminary tests here. After removing the carbohydrates in the wood cell wall, by treatment with hydrofluoric acid, the bacteria were able to attack the lignin portion remaining.

5.4 Differences in the Performance of Hardwoods and Softwoods Treated with the same Preservative System

CCA preservatives have successfully prevented fungal attack in softwoods. In hardwoods, however, the preservative treatment has in many cases proved inadequate (Dickinson, 1974 ; Greaves, 1972, 1977; Tamblyn, 1973, 1975; Dickinson et al, 1976; Hulme and Butcher, 1977b; Levy and Greaves, 1978) . In the work described here, for soft rot tests, treatment of Scots pine sections prevented attack whilst that of birch sections did not. The reasons proposed for the poor performance of treated hardwoods include substrate susceptibility, a hypothesis developed by Hulme and Butcher (1977b,c). These authors found that an increased preservative retention could compensate for the increased susceptibility of a species towards soft rot fungi. Butcher (1979) proposed that decay potential and preservative requirement could be related to the proportion of fibres within the wood tissue of a particular species.

The second hypothesis relates the poor performance of treated

hardwoods against soft rot to the poor macrodistribution of the CCA preservatives in the wood tissues (Dickinson, 1974; Greaves, 1974; Dickinson et al, 1976; Levy and Greaves, 1978; Greaves and Levy, 1978) and poor microdistribution of the CCA preservatives in the cell wall (Dickinson, 1974; Greaves, 1974; Dickinson et al, 1976; Drysdale, 1979; Drysdale et al, 1980). The hypothesis suggests that pockets of fibres may be left inadequately treated and that the penetration of the preservative into the cell wall is not sufficient to give protection against soft rot fungi. This is in contrast to the treatment of softwoods in which an even distribution of preservative is combined with good penetration of the tracheid walls (Petty and Preston, 1968; Dickinson et al, 1976). The thin section technique is an ideal technique to be used to determine the effectiveness of macro and microdistribution of the preservative. Soft rot cavities in fibres could be recorded and their relative position to the main penetration pathways of the preservative solutions, the vessels, noted. This is suggested for future work. Fibres far removed from vessels may be inadequately treated as the preservative had not been distributed to them and they may, therefore, have extensive soft rot decay.

Drysdale et al (1980) noted that the disproportionation of the copper, chromium and arsenic within the cell wall may be of significance in preservative performance. The thin section technique could be used in future work in conjunction with x-ray microanalysis and scanning electron microscopy, to determine the levels of the preservative elements in an area severely attacked by fungi and bacteria, by comparison with unattacked areas. Time did not permit this exercise to be carried out in this work.

Butcher and Nilsson (1982) and Nilsson (1982b) have suggested the importance of lignin in determining the susceptibility of a timber towards soft rot fungi, in association with the role of lignin in offering

fixation sites for copper. Nilsson and Daniel (1983b) and Daniel and Nilsson (1984) have described the presence of concentric layers in ths S_2 layer, alternate layers in which the relative levels of lignin and possibly hemicelluloses are higher with respect to the adjoining layers. These authors suggest that these layers influence the decay processes and the microdistribution of preservatives at the cellular level. The concentric layers are difficult to detect with the light microscope. The fact that the subsidiary concentric layers may represent important sites of decay, having a low lignin content (Nilsson and Daniel, 1983b; Daniel and Nilsson, 1984) supports the hypothesis of species susceptibility to soft rot being dependent upon lignin content.

Butcher and Nilsson (1982) assumed that CCA fixed to lignin is effective against soft rot. Pizzi (1983) has concluded that different treatment conditions are necessary to optimise the durability of treated hardwoods and softwoods. He points out that hardwoods need more CCA fixed to lignin than do softwoods, for preventing soft rot attack. Butcher and Nilsson (1982) suggest that the copper content on lignin correlates to soft rot resistance. Pizzi and Conradie (1986) have implicated the importance of increasing the copper content of CCAs. They suggest that this would overcome tannin interference with CCA, as copper is the fastest reactive element with flavonoid tannins. Tannins protect the cell wall against microbial attack (Zucker, 1983). Interference of CCA with tannin appears, at lower CCA retentions, to destroy the natural durability imparted by tannins (Pizzi and Conradie, 1986). These authors suggest that not only is the copper content of CCAs important but that chromium has an important role in the prevention of decay of treated wood.

Gray (1983) proposed that adsorbed copper was responsible for blocking sites of soft rot attack (TI sites, Nilsson (1982)) in the S_2 layer of the fibre cell wall and that arsenic interferes with this copper

adsorption. In the presence of arsenic, Gray (1983)suggests that only a proportion of the TI sites are masked by copper. Rennie (1986) suggests that the arsenic interference is a pH effect and that the pH differences betweeen copper chrome (CC), CCB and CCAs of all types are large enough to result in significant differences in the level of adsorbed copper. Pizzi (1983) emphasised that it may be possible to improve the resistance to soft rot of wood species of lower lignin content e.g. hardwoods, by decreasing the amount of arsenic in CCA or eliminating it altogether. Gray (1983) has confirmed this. The effect of arsenic was examined in the work reported in this thesis using the thin section assay.

5.5 Performance of Experimental Formulations

In soft rot tests, the five formulations, CC, CCA, CCB, CC+A and CCB+A, performed significantly better than copper sulphate alone (C) and untreated controls, for birch sections buried in Rentokil soil. Looking at the decay curves, CCA performed poorly in comparison to the other formulations, at the lowest concentration used, but performed well at the higher concentrations.

Gray (1983) used weight loss as the criterion of soft rot decay, she found that CCB, CC+A and CCB+A performed better than CCA. Using the thin section technique, CC+A was shown to perform well in birch sections buried in Rentokil soil, as did CCA. Results obtained in the International Research Group on Wood Preservation collaborative soil burial trial (Carey and Savory, 1975) and by Kerner-Gang (1975) for beech, showed that CCA was usually more effective than CCB. Boron is regarded as being unfixed and subject to leaching (Wilkinson, 1979; Becker and Buchmann, 1966; Tillott and Coggins, 1981). In this work, CC+A performed well in birch whilst CCB+A did so in Scots pine, these results agree with Gray (1983), but the fact that CCA performed well in birch and Scots pine, does not. But further work (Gray and Dickinson, 1987) confirms these results.

Differences in the performance of the formulations were less apparent with the thin section technique in soft rot tests. The technique may have been too severe to pick out subtle differences in the performance of preservatives against soft rot fungi. The method of soft rot assessment may also have missed differences in performance. Attack was recorded randomly, hardwoods have an uneven macrodistribution of preservatives, as already discussed. This would affect the distribution of soft rot cavities. In addition, averaging soft rot degrees (SRDs) for all concentrations together may have disregarded differences that existed between the formulations.

As the time of incubation period increased, differences between the performance of the preservatives were less apparent. Bravery (1968a) using pure cultures and sterile soil burial methods demonstrated that toxic values for a given preservative are governed by the period of incubation. With increase in incubation time all preservative treatments fail. The thin section technique allows rapid attack of the wood sections and the period of incubation over which differences in preservative performance appear would be considerably shorter with thin sections than with wood blocks.

In bacterial tests after examining the frequency of occurrence of each decay class for sections treated with the experimental formulations; C, CC and CC+A were found to perform well against the bacteria; CCB and CCA intermediately; CCB+A poorly. A CCB+A treatment produced high degrees of soft rot attack in birch sections in Rentokil soil, but performed well in Scots pine sections.

5.6 Performance of Commercially Available Preservatives

On an equivalent copper basis, differences in the performance of the preservatives against soft rot attack were apparent from the decay curves (Section 3.3. 4).Celcure A salt and Tanalith NCA performed poorly whilst K33 oxide, Celcure A oxide and Tanalith C performed well. These differences in performance were not statistically significant. However, when compared on an equivalent active element basis, taking into account copper and arsenic contents, these differences were significant. This suggests that arsenic has a direct effect on performance. The preservatives at the concentrations used, all contained the same amounts of copper and arsenic, but these elements were in different ratios and this affected performance.

In bacterial tests using Scots pine sections, K33 oxide and Tanalith NCA performed poorly against both soft rot fungi and S_2 degrading bacteria in the garden loam, at the higher soil moisture content. On an equivalent copper basis, K33 oxide did perform well against soft rot at the lower soil moisture content, as it had in soft rot tests using birch in Rentokil soil. The high ratio of arsenic to other active elements in this CCA preservative, may have affected its performance against the bacteria. Hedley (1984a,b) found that in vineyard posts, those CCA preservatives approximating to Type C (with a high chromium to arsenic ratio) performed better than those of Type B (with a low chromium to arsenic ratio). These results agree with those found by Hartford *et al* (1982) and with the results in this work, using the thin section technique. The bacteria were able to discriminate between the high arsenic formulations used in the field (Hedley, 1984a,b) and this has been repeated here under laboratory conditions.

Daniel and Nilsson (1985) found that even *Pinus radiata* stakes treated with CCA preservatives to more than double the retention

recommended for the control of fungal attack, were degraded by the bacteria. In the work reported here, bacterial attack was restricted at the highest concentration used for each preservative. Attack was, however, found in sections cut from blocks treated with K33 oxide. The concentration used was 3.11%, equivalent to a retention of 22.57 kg/m^3. One instance of bacterial attack of sections treated with Celcure A salt at the highest concentration, after eight weeks burial, was recorded. This was an isolated incident. For K33 oxide, however, attack was consistent throughout four, eight and twelve weeks.

5.7 Interaction Between Soil Type, Wood Species and Preservative Type, in Soft Rot Tests

The thin section technique has demonstrated the importance of soil type, wood species and preservative type upon the degree of soft rot attack. In the Rentokil soil, having a low soft rot potential in these tests, the highly susceptible wood species, birch, was attacked and differences between the performance of the experimental formulations (C, CC, CCA, CCB, CC+A and CCB+A) were found. For the relatively resistant Scots pine, all preservatives performed well in Rentokil soil.

In John Innes No.1 soil, having a high soft rot potential, birch sections were attacked, all treatments performed poorly. For Scots pine sections, however, differences were apparent between the performance of the experimental formulations. An aggressive soil, in terms of soft rot potential, was required to produce differences in the performance of these formulations in the less susceptible Scots pine.

It is possible that in a soil having an even higher decay potential than John Innes No.1, differences between the formulations would be lost, when using Scots pine wood. Additionally, in a very poor

soil, treated birch sections may perform as well as treated Scots pine sections did in the Rentokil soil. A model can be drawn up to show the interaction between soil type, wood species and type of preservative used:

		Decay Potential of Soil			
		Poor	Low	High	Very High
Wood Species	Birch	Reduced attack	**	Increased attack	
	Scots Pine		Reduced attack	**	Increased attack

where ** = significant differences between the performance of experimental formulations, derived from an analysis of variance test (Section 3.3.3).

For this model it was assumed that both soils had moisture contents high enough to wet the thin sections of wood sufficiently. Rentokil soil had a moisture content of 34.74% on a dry weight basis, whilst John Innes No 1 had a moisture content of 49.55%. Both soils were wet to a soil moisture content of 95% of the water holding capacity. It is possible that the lower moisture content of the Rentokil soil may have reduced its aggressiveness in terms of soft rot potential.

5.8 Interaction Between Soil Type, Soil Moisture Content, Wood Species, Preservative Type and the Type of Attack found

Soft rot and bacterial attack were frequently found together in the same wood section. The type of attack depended upon the soil type, soil moisture content and wood species.

Birch sections were not attacked by the S_2 degrading bacteria in the preliminary tests but they were by soft rot fungi. Nilsson (1982b) found that hardwoods known to be very susceptible to soft rot e.g. birch, were less susceptible to bacterial attack. This may have been due to fungal competition preventing the bacteria from degrading the wood. Nilsson (1982b) reported that bacterial attack occurred in birch when soft rot fungi were suppressed by the antifungal antibiotic natamycin.

Soil type clearly affected the occurrence of bacterial attack and this was found in the field (Drysdale, 1984). Soil moisture content affected the type of attack found, soft rot together with bacterial attack, occurred at the lower soil moisture content, using the garden loam. Bacterial attack was dominant at the higher moisture content.

Treatment with preservatives did not affect the bacterial attack of wood sections in the same way as it affected soft rot attack. Bacteria were resistant to high concentrations of preservative, as mentioned earlier a retention of 22.57 kg/m^3 of K33 oxide, a high arsenic preservative, did not prevent bacterial attack of scots pine sections. Nilsson and Daniel (1983a) found that there was no toxic threshold for tunnelling bacteria, attack occurred in stakes treated to retentions of CCA of 35 kg/m^3. In this work attack occurred in sections of wood treated with the experimental formulations, C, CC, CCA, CCB and CCB+A, to retentions of 30.65 kg/m^3. Bacterial attack was independent of preservative

concentration whilst soft rot attack was significantly reduced as increased retentions of preservatives were achieved. Erosion and tunnelling bacteria were resistant to the treatment of Pinus radiata stakes with CCA in the field in New Zealand (Daniel and Nilsson, 1985). Bacteria may inactivate or destroy preservatives (Scheffer, 1973; Schmidt, Wolf and Liese, 1975).

5.9 Bacteria as an Important Hazard in Preservative Tests

The results for bacterial attack presented in this thesis, together with those reported in the field (Leightley, 1981, 1982; Nilsson, 1984), suggest the importance of including bacteria in any test of preservative efficacy in the ground contact situation.

Daniel and Nilsson (1985) suggest that bacteria may possess features allowing them to overcome elevated levels of toxic elements in their immediate environment. Even if high CCA levels were used, as the bacteria are slow growing, with degradation occurring over a limited area, then theoretically they may only ever be subjected to low levels of preservative elements at any one time. In this respect, soft rot fungi and S_2 degrading bacteria may have features in common, in contrast to brown and white rot fungi where, by the nature of their more widespread diffusion of the agents of degradation, they may encounter more CCA elements (Daniel and Nilsson, 1985). This is consistent with the known better protection of wood treated with CCA against these latter two types of decay.

Even if most bacterial attack occurs quite superficially, it may be possible to get fairly rapid destruction of large timber structures if the softened outer layers are being continuously removed. Members of the soil fauna, the action of running water (in water cooling towers) or wave

activity in marine environments may also result in the removal of softened outer layers.

Bacteria have never been considered important in tests of preservative performance in ground contact. However, attack occurring in wood in saturated conditions has also been reported in wood in ground contact (Levy, 1975; Clubbe, 1978). Rossell (reported in Levy, 1975) demonstrated that the destruction of the pit membranes, by bacteria, known to occur in the sapwood of logs stored in rivers or lakes, also occurred in sapwood in ground contact. In certain environments such as in fertilised soils with a high moisture content, bacteria represent a severe hazard for the treated timber with which it is in contact (Nilsson, 1984; Drysdale and Hedley, 1984), as has been demonstrated in this work. Bacteria need to be considered as part of the test programme when evaluating preservatives, since a new preservative may select out the bacteria just as the Type B CCA preservatives have in this work and also in the field (Hedley , 1984 a,b). Bacteria need to be included in tests of preservative performance. The thin section technique developed in this work has been shown to be effective for examining bacterial attack. The bacterial degree (BD) derived here has produced results for preservative performance which agree with those found in the field, for the poor performance of CCA Type B preservatives (Hedley, 1984 a,b). This then suggests that the technique could possibly be used to predict the long-term failure of preservatives to bacterial attack.

This use of wood-degrading bacteria, cultured in the laboratory with a view to using them as a research tool for screening wood preservatives for their efficacy, has not previously been reported. Results obtained here in the laboratory in weeks confirm those found in the field after years of exposure of the treated wood. The thin section technique allows the performance of preservatives to be tested against bacteria in softwoods and is also suitable for use with soft rot fungi in hardwoods.

SECTION VI: CONCLUSIONS AND FUTURE WORK

6 Section VI Conclusions and Future Work

6.1 Conclusions

The most important conclusion arising from the work described in this thesis is the valuable assay technique which has been developed, using thin sections cut from treated wood. The following conclusions have all been based on observations made with this technique :

1. The thin section assay allows a rapid determination to be made of preservative performance. There are, however, some limitations with this technique. Differences in the performance of preservatives against soft rot attack may be less apparent than those obtained using weight loss of whole wood blocks as the criterion of decay. Soft rot attack of hardwoods is thought to follow the pattern of preservative macrodistribution within the wood. With thin sections, macrodistribution effects are very important, whereas with whole wood blocks, localised distribution of the preservative is averaged out as results are taken for the whole wood block. The technique could be used to determine the preservative macrodistibution, fibres left untreated would be attacked by the soft rot fungi.

2. The assay can be used successfully in bacterial tests to discriminate between the performance of high and low arsenic content formulations. Results were similar to those found in the field with regard to the poor performance of copper-chrome-arsenic Type B preservatives. In addition, the variability in the occurrence of bacterial attack reported in the field was also found in this work. Conditions can be produced, in the soil jar, in weeks in the laboratory which mimick those found in the field after years of exposure. The rate of bacterial degrade developed here can be used to predict long-term performance

of preservatives.

3. Type B copper-chrome-arsenic preservatives, e.g. K33 oxide and the modified Type B, Tanalith NCA, perform poorly against bacteria degrading the S_2 layer of the wood cell wall. Tanalith NCA treated sections are also prone to heavy soft rot attack.

4. The type of decay hazard found in the soil depends on the type of soil used. Highly fertilised soils readily support soft rot fungi and bacteria degrading the S_2 layer of the cell wall.

5. Soil moisture content also affects the type of decay found. Soils with a high moisture content restrict soft rot attack and enhance bacterial attack.

6. Hardwoods are generally susceptible to soft rot attack and less prone to bacterial attack, probably as a result of fungal competition. Softwoods, however, are less susceptible to soft rot attack. The lack of fungal competition may explain the increased susceptibility to bacterial attack which softwoods exhibit.

7. Differences in the performance of experimental formulations against soft rot are more apparent in soils of low decay potential, when highly susceptible wood species are used.

8. Bacterial attack is independent of preservative concentration.

9. Bacteria are important decay organisms in conditions of high soil moisture content in highly fertilised soils. If treated timber is to be used under such conditions, it should be tested for its performance against both soft rot fungi and bacteria degrading the

S_2 layer of the cell wall.

10. Conditions suitable for the culturing of bacteria may have been removed in earlier laboratory tests. The soil is usually predried (this inhibits bacterial attack) and used at a low moisture content. The surface layer of wood blocks is often removed before the blocks are examined. Bacteria are known to cause slow surface degradation.

11. The thin section assay provides a critical technique for determining the performance of preservatives against bacterial attack. Differences occur in performance which may be lost with other techniques, such as weight loss of wood blocks. The assay may be too severe for soft rot assessments but is suitable for use with S_2 degrading bacteria, which are able to discriminate between preservatives. The need to include bacteria in preservative tests of long-term performance, has been demonstrated. The thin section assay is suitable for this purpose.

6.2 Future Work

The thin section assay technique developed in the course of the work described in this thesis could be a very useful tool to examine the movement, macrodistribution and microdistribution of wood preservative formulations within the wood tissues.

In the case of hardwoods, fibres showing little soft rot attack, may have been well treated because of their close proximity to vessels. Vessels must normally be the main pathway for the movement of wood preservative liquids through hardwoods. The thin section method could well be used to investigate these pathways and also the microdistribution of the preservatives within the layers of the wood cell wall in relation to the action of soft rot fungi.

The work described here has been limited to water-borne copper-based formulations applied by vacuum-pressure treatment. The technique developed, however, could be used equally well with all the other types of wood preservatives applied by any of the wide range of application techniques used in practice.

It would be an extremely valuable method for the screening and evaluation of the performance of new preservatives and preservative systems.

More work is needed on the mode of action of the S_2 degrading bacteria. Their mode of attack of the wood cell wall needs to be examined in detail and the conditions under which they occur more clearly defined. This thin section assay would be a very useful method to carry out these investigations.

Appendix A

Soft Rot Decay Ratings - An example of raw data for 2 - 8 weeks soil burial.

SOFT ROT DECAY RATINGS FOR SECTIONS BURIED IN RENTOKIL SOIL

RENTOKIL	INCUBATION TIME																													
	2 weeks								4 weeks								6 weeks								8 weeks					
Wood Sections	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP
	1	0	2	2	2	2	1	3	0	0	3	3	4	0	3	0	3	3	3	3	2	3	2	2	1	0	3	0	3	3	3	3
	0	0	2	2	2	2	1	2	0	0	0	3	3	0	4	0	3	2	2	3	2	2	3	2	0	0	0	3	3	3	0	2
	0	1	1	1	3	2	0	1	3	3	3	0	0	0	0	3	3	3	3	3	2	2	3	3	3	1	2	2	3	3	2	2
Birch	2	2	0	1	3	2	0	1	4	3	4	0	0	3	0	4	3	3	2	2	3	2	2	3	3	3	3	0	3	2	2	3
3.25%	2	2	0	0	3	3	3	0	4	3	4	0	0	4	0	0	2	2	2	2	2	2	2	2	2	3	0	3	3	3	3	3
C		2		0		2		1		4		4		3		3		3		2		3		3		3		2		3		0
		2		1		2		2		3		4		3		3		2		3		3		2		2		3		3
		2		2		2		3		1		4		3		3		3		2		3		3		1		3		2
		1		2		2		0		1		3		3		4		3		3		3		3		3		3		3
		2		2		2		0		2		3		4		4		3		3		3		3		2		0		3
								$\bar{x}$=1.53 ±0.877								$\bar{x}$=2.28 ±1.601								$\bar{x}$=2.63 ±0.490								$\bar{x}$=2.28 ±1.109
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Scots	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Pine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
2.36%		0		0		0		0		0		0		0		0		0		0		0		0		0		0		1		1
C		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		1
		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0
		0		0		0		0		0		0		0		0		0		0		0		0		0		1		0
		0		0		0		0		0		0		0		0		0		0		0		0		0		0		0
								$\bar{x}$= 0								$\bar{x}$=0								$\bar{x}$= 0								$\bar{x}$=0.15 ±0.362

Soft Rot Decay Ratings

12 - 30 weeks soil burial.

Mean values only were used in statistical analyses.

SOFT ROT DECAY RATINGS FOR SECTIONS BURIED IN RENTOKIL SOIL

Wood Sections	INCUBATION TIME																			
	12 weeks						20 weeks						30 weeks							
	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP
Birch 3.25% C	3	3	3	3	3	3	2	2	3	3	3	3	2	2	2	3	3	3	3	3
	3	3	3	3	3	3	2	3	3	3	3	4	3	3	3	3	3	4	3	3
	3	3	3	3	3	3	3	2	3	3	3	4	3	3	3	2	3	3	3	4
	4	4	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	4	4	4
	3	4	4	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3
		3		3		3		3		3		3		2		3		3		4
		3		4		3		2		3		3		2		3		3		3
		3		3		3		2		3		3		2		3		3		3
		3		3		3		1		3		3		2		3		3		3
		3		3		3		2		3		3		3		3		3		3
						$\bar{x}$=3.10 ± 0.304						$\bar{x}$=2.88 ± 0.516						$\bar{x}$=2.93 ± 0.474		
Scots Pine 2.36% C	0	0	0	0	0	0	0	0	0	0	2	2	1	1	0	0	1	0	1	0
	0	0	0	0	0	0	0	1	2	2	2	2	1	0	0	0	1	1	1	2
	0	0	0	0	0	0	1	1	1	1	2	2	1	1	0	0	2	1	2	0
	0	0	0	0	0	0	2	2	1	1	2	2	0	1	0	0	1	1	0	0
	0	0	0	0	0	0	2	2	2	1	2	2	0	0	0	0	0	1	1	1
		0		0		1		0		1		2		0		0		1		0
		0		0		0		0		2		2		0		0		0		2
		0		0		0		0		0		2		1		0		0		0
		0		0		0		0		1		2		1		0		0		0
						$\bar{x}$=0.075 ± 0.267						$\bar{x}$=1.03 ± 0.800						$\bar{x}$=0.43 ± 0.636		

Appendix B

Calculation of Confidence Limits and t-Test

A MINITAB statistical computing system was used to calculate 95% confidence limits and to determine by means of a t test, the significance of any difference between mean SRDs for the treatments.

1. Confidence Interval for Population Mean, standard deviation not known.

The sample mean for soft rot degree ratings was used as a point estimate of the population mean. A 95% confidence interval is the interval calculated from the sample which will cover the unknown population mean 95% of the time. The standard deviation of the population was calculated from the data, using Student's t, confidence interval procedure and the T Interval command:-

TINTERVAL WITH 95PERCENT CONFIDENCE FOR DATA IN C

This calculates the two-sided t Confidence Interval for the population mean:

$$\bar{x} - t\left(\frac{s}{\sqrt{n}}\right) \text{ to } \bar{x} + t\left(\frac{s}{\sqrt{n}}\right)$$

$\bar{x}$ = sample mean

s = standard deviation

n = sample size

t = value in a table of the student's t distribution with (n - 1) degrees of freedom.

2. Comparing Two Means

The difference between means of the different formulations was calculated using confidence intervals and the t-test. The following command:

TWO SAMPLE T FIRST SAMPLE IN C1, SECOND SAMPLE IN C2

finds a) a two-sided 95% CI for $\mu_2 - \mu_1$, where μ_1 = mean of first pop.
μ_2 = mean of second pop.

b) t-test of $H_0: \mu_1 = \mu_2$ vs $H_1: \mu_1 \neq \mu_2$.

CI in a) goes from

$$\left(\bar{x}_1 - \bar{x}_2\right) - t\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

to

$$\left(\bar{x}_1 - \bar{x}_2\right) + t\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

where t = value from a t table corresponding to 95% confidence and known number of degrees of freedom.

t value for b):

$$t = \frac{\left(\bar{x}_1 - \bar{x}_2\right)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ = sample mean of first column of data

$\bar{x}_2$ = sample mean of second column of data

S_1 and S_2 = sample standard deviations of the two columns

n_1 and n_2 = two sample sizes.

The degrees of freedom are based on the following approximation:

$$\frac{\left(\left(\frac{s_1^2}{n_1}\right) + \left(\frac{s_2^2}{n_2}\right)\right)^2}{\frac{\left(\frac{s_1^2}{n_1}\right)^2}{(n_1 - 1)} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{(n_2 - 1)}}$$

MINITAB prints out n_1, n_2, $\bar{x}_1$, $\bar{x}_2$, S_1, S_2, degrees of freedom, t statistic, attained significance and the confidence interval.

APPENDIX C

Analysis of Variance Test : Example of data output

Preservative vs Concentration : Scots Pine buried in John Innes No.1 Soil

Variate: SRD

Source of Variation	DF	SS	SS%	MS	VR	Sig Level
UNITS STRATUM						
Pres	6	8.9778	73.68	1.4963	11.120	***
Conc	2	1.5918	13.06	0.7959	5.915	*
Residual	12	1.6148	13.25	0.1346		
TOTAL	20	12.1844	100.00	0.6092		
GRAND TOTAL	20	12.1844	100.00			

where *** = significant at p<0.001
* = " at p<0.005

GRAND MEAN 0.599

Total number of observations 21

TABLE OF MEANS

Variate: SRD

GRAND MEAN 0.599

Pres	UN	C	CC	CCA	CCB	CC+A	CCB+A
	2.10	0.81	0.06	0.39	0.18	0.46	0.19

Conc	0.7	2.36	4.13
	0.97	0.50	0.32

STANDARD ERRORS OF DIFFERENCES OF MEANS

TABLE	Pres	Conc
REP	3	7
SED	0.2995	0.1961

STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION

Stratum	DF	SE	CV%
Units	12	0.3668	61.2

L.S.D.	Pres	Conc
	0.65	0.43

The analysis of variance test was repeated using data for each wood species at each of the time periods.

Preservative vs Soil

The effect of soil type on preservative performance was tested using the same analysis of variance test as for preservative vs concentration. Mean SRDs were totalled for all preservatives at all concentrations. A grand mean was determined for each of the two soil types at each time period and used to compare the performance of the preservatives in the soil. Results also gave an indication of the soft rot potential of the soil. (Tables 38 and 51).

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