STUDIES ON WOOD-INHABITING FUNGI IN PRESERVATIVE-TREATED WOOD

A thesis presented in part fulfilment
of the requirements for the Degree of
Doctor of Philosophy
of the
University of London

by

Naser Abdel-Azeez Hasan Sorkhoh, B.Sc., (Kuwait)

Department of Botany and Plant Technology
Imperial College of Science and Technology
London, S.W.7 2BB.

July, 1976.

ABSTRACT

Several authors have indicated a difference in the performance of preservative treated hardwoods and preservative treated softwoods in ground contact. The present investigation was carried out as an attempt to understand the possible reasons for such differences.

0.7cm^3 blocks of two species of wood, a hardwood, birch (Betula sp.) and a softwood, Scots pine (Pinus sylvestris) were used. Some of the blocks of both species of wood were treated with 2.5% and 5% solutions of copper-chrome-arsenic wood preservative and other blocks were left untreated as controls. The blocks were subjected to monocultures of five species of fungi for a period of 12 weeks and a further set were buried in soil for a period of 21 weeks. The species of fungi were chosen to represent a soft-rot stainer (Phialophora fastigiata), a soft-rot (Chaetomium globosum), a white-rot (Coriolus versicolor), a brown-rot (Coniophora puteana) and a stainer (Botryodiplodia theobromae). A comparative study was made under the light microscope after various exposure periods to the test fungi and soil between the 2 species of wood, in the presence and the absence of the wood preservative. These observations showed that all tissues of both species of untreated wood blocks were rapidly colonised and degraded by all species of fungi. In the case of treated wood blocks, colonisation and degradation by the different species of fungi were considerably delayed. The treated blocks of Scots pine showed no signs of decay by all species of fungi even after the last period of exposure, while the treated blocks of birch showed resistance to decay by the white-rot and brown-rot fungi until a late period of exposure, but were decayed by the soft-rot fungi particularly in the fibres. The ray parenchyma in birch showed more

resistance to decay in comparison to the fibres. The two preservative concentrations used (i.e., 2.5% and 5%) showed no differences in the patterns of fungal colonisation or degradation.

These observations clearly indicated that the failure of the treated hardwood is not a matter of preservative retention, but it may be mainly due to the microdistribution of the preservative elements in wood.

A thorough examination of the microdistribution of the preservative elements in the different wood tissues and also through the cell wall layers was carried out using 3 different techniques, namely, an X-ray radiographic technique, an energy dispersive X-ray analyser (EDAX) and an electron microscope microanalyser (EMMA4). The results of such studies have shown marked differences between birch and Scots pine as regards the preservative microdistribution at the tissue level and the cell wall level. The tissues of Scots pine and the individual cell wall layers of the tracheids were very evenly treated. In the case of birch, the vessels and rays had the highest treatment with some penetration of the preservative into the wall in depth but the fibres were very poorly treated with little CCA treatment in depth, particularly the S2 layer. Such differences in microdistribution of the preservative elements between the two species of wood may be one of the reasons for the failure of the treated hardwood but not the treated softwood.

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PART I

INTRODUCTION

1.1. General

The biodeterioration of timber caused by fungal attack is one of the most serious problems facing the wood using industry. The number of observations and experiments carried out in the study of this problem is large, both in the laboratory and in the field. This makes it difficult to review the work conducted in this field. A considerable amount of information is available concerning the different groups of wood-rotting fungi; the colonisation and the cell wall degradation of wood by those organisms; the biochemistry of decay; the ultrastructural details of attack and other aspects of fungal degradation in both preservative treated and untreated timbers.

So far, treating wood with toxic chemicals is considered to be the best practical way of protecting timber against fungal attack. The performance of preservative treated timbers dependson a number of factors, these may be related to the properties of the preservative in use, the timber species and the environmental conditions under which the treated timber will be exposed. Although the wood preservation industry has progressed considerably in the last few years and the preservative treated timber in service has on the whole, shown an excellent durability for many years, nevertheless, certain problems have still to be examined and solved, particularly in wet and hot climates. It has been reported in different parts of the world that certain preservative treated timbers, mainly hardwoods, are susceptible to failure after a comparatively short period of exposure to such conditions. On the International scale, the performance of certain preservative treated hardwoods in ground contact has given rise to special concern, (Minutes I.R.G. 1973, 1974). Field trials have indicated that there can be a substantial difference in performance between treated hardwoods and softwoods. It has been shown

that hardwoods treated commercially with wood preservatives often fail in service, while softwoods treated in the same way and to the same loading remain sound. Savory (1955), made his observations on a range of treated hard- and soft- woods with different preservative treatments, and found that the softwoods showed fewer failures than the hardwoods. More recently, Levy (1971a) tested the effectiveness of various wood preservatives in long term field trials of birch and Scots pine fence posts, and indicated that certain treatments of birch posts were less effective against fungal decay than the same treatment of Scots pine. He also reported (Levy, 1971b), that certain preservatives in fence posts proved to be wholly effective in Scots pine after 12 years exposure, but had completely failed in birch. The failure of preservative treated hardwood, belonging to a number of species of Eucalyptus, compared to treated radiata pine is becoming a major problem in Australia (Tamblyn, 1972). Greaves (1972a) found that the CCA treated pines were more resistant to decay than similarly treated eucalypts. Dickinson (1974) reported the failure of CCA and creosote treated hardwoods in ground contact, where both treatments showed the same type of failure.

The study of the colonisation of untreated wood in ground contact showed that the primary invaders are mainly of the Fungi Imperfecti and Ascomycetes followed by the Basidiomycetes in large numbers as the time of the wood exposure to the soil increases, Levy (1962), Corbett and Levy (1963a, b) Merril and French (1966), Käärik (1967), Butcher (1968) and Banerjee and Levy (1971). These studies established a very useful background for the comparative studies of the colonisation in the presence of wood preservatives.

On the other hand, the studies on the isolation of fungi from preservative-treated wood in ground contact indicated that a large number of soft-rot fungi are present, with no signs of or very few Basidiomycetes colonising the treated wood. Savory (1955) reported that most of the decay of treated zone of preserved timber was due to soft-rot fungi and this group of fungi were the chief cause of failure of the treated hardwoods. Oliver (1959) stated that a high proportion of the failures of graveyard specimens treated with preservatives could be attributed to soft-rot microfungi. Duncan (1960a) found that soft-rot was present in different wood installations that had been treated with creosote, pentachlorophenol, or various water-borne compounds which were sufficient to prevent the attack by Basidiomycetes. Greaves and Savory (1965) isolated fungi from preservative treated timber exposed in contact with the ground for 30 years, using 4 different techniques and 11 different media. They found that most of the attack was due to soft-rot fungi especially in the hardwood. Butcher (1971) in his study of the colonisation of CCA-treated Pinus radiata, found that the succession of fungi was from moulds to soft-rot to secondary moulds and primary Basidiomycetes. Greaves (1972a) found no difference between the microbial populations of 2 species of wood, Pinus radiata and Eucalyptus regnans; the untreated wood of both species was rapidly decayed by soft-rot, while CCA-treated eucalypts were decayed by soft-rot fungi earlier than the treated pines. Dickinson (1974) reported the failure of 2 species of CCA-treated hardwood to be caused by soft-rot attack at the ground line. More recently, Seehann, Liese and Kess (1975) listed over 100 species of soft-rot fungi isolated from preservative treated wood. The field

test results, carried out in the Ivory Coast and France, on the performance of preservative treated hard- and softwoods, indicated that whenever preservative treated timber was concerned, soft-rot attack was dominant, it was shown that over 90% of the failures were caused by soft-rot fungi with no indication of Basidiomycetes, (Fougerousse 1975). Henningsson and Nilsson (1975) working in Sweden, reported that 4-5 million telegraph and electricity transmission poles treated with water-borne salts were seriously attacked by soft-rot fungi. They isolated fungi from a total of 135 telegraph and electricity poles as well as stakes treated with 20 different wood preservatives and they found that soft-rot fungi were dominant and the number of Basidiomycetes isolated was rather small.

Several authors have studied the reasons for the failure of preservative treated timber and they have related this failure to certain important factors, such as, the tolerance of certain micro-organisms to the toxic materials in the preservative, the inadequate availability of the preservative (i.e. the low retention of the preservative in the wood), and the distribution of the preservative materials within the cellular structure, (i.e. the distribution relative to wall layers, or the distribution differences between different cell types and between different wood species). In addition there are some other factors suggested which are of less importance.

An interesting historical review concerning the pioneer work achieved in the study of the toxicity of preservatives against wood destroying fungi was made by Cartwright (1931). Savory (1954a, b) indicated that soft-rot fungi are more tolerant to antiseptics than the Basidiomycetes, and that the preservative treatment adequate to

prevent the Basidiomycetes attack might, in the course of time, be insufficient to protect the wood from attack by soft-rot fungi. He also suggested, (Savory 1955) that failures due to Basidiomycetes usually arose from the imperfections of the treatment rather than failure of the preservative, he showed that the amount of pentachlorophenol needed to reach the toxic limit for Chaetomium globosum in a hardwood was considerably higher than that for Polyporus versicolor which is one of the most phenol-tolerant Basidiomycetes. Oliver (1959) reported that 0.08 lb/cu ft. of pentachlorophenol wood preservative in beech was sufficient to prevent decay by Polystictus versicolor, while Chaetomium globosum tolerates higher concentrations, and 0.6 lb/cu ft. was required to prevent attack by this fungus. Duncan (1960a, b) in her observations of treated woods exposed in the soil and laboratory tests found that most of the soft-rot isolates were more tolerant of sodium fluoride, soldium chromate, sodium arsenate and zinc chloride than the Basidiomycetes tested. The toxicity tests against a wide range of wood-destroying fungi have shown that fungi, in general are not tolerant to both copper and arsenate, the few exceptions were confirmed to be tolerant to CCA, Levi (1969). Butcher (1971) tested the tolerance of the isolated fungi against copper and arsenic and found that the dominant fungi on treated timber were also the most tolerant ones and the degree of their tolerance against these elements was in the same order as that in which they colonise the wood. Bravery (1973) using 3 organic preservatives, tributyltin oxide, copper naphthenate and pentachlorophenol found that the preservative delayed the initiation of colonisation and then reduced the rate of invasion. He showed that by

increasing the retention of the preservative, the period of initial colonisation was delayed and the decay rate reduced. Higher concentrations of preservative caused the destruction of hyphal contents which indicated the absorption of lethal amounts of solubilised preservative. Levi (1973) showed that in laboratory conditions non-wood destroying fungi are able to detoxify pentachlorophenol, he also isolated non-wood destroying fungi tolerant to creosote and CCA from treated wood. Henningsson and Nilsson (1975) found that fungi colonising treated timber were copper, zinc and arsenic tolerant. Stranks and Hulme (1975) studied the biodegradation of most groups of wood preservatives and showed that it was mainly related to the low concentration of the preservative and the detoxification by micro-organisms.

A study of preservative distribution, both in the different types of cells and in the cell-wall layers demonstrated that an important role was played by the preservative distribution in stopping the degradation by wood rotting fungi of preservative treated wood. The application of newly developed techniques for the analysis of the elements in preservative distribution gave reliable results in locating the position of the different elements in wood. Physical methods, electron microscopy and x-ray diffraction analysis, applied by Preston (1959) on treated Pinus radiata have indicated that the preservative in the impregnated wood is located within the cell wall and associated with the structural components. Petty and Preston (1968) found that CCA wood preservative penetrated deeply through the tracheid wall of conifers. Using a different wood preservative, creosote, Bossard (1969) examined the distribution of creosote in hard- and softwoods

under ordinary and UV-microscopy. He found that the fibre and vessel lumina in hardwoods were easily filled with the preservative and that the preservative penetration in the softwoods was through the tracheid wall, he suggested that the rays and resin canals in the conifers are playing an important role for the penetration and distribution of creosote. Chou (1971) showed the deposition of CCA-wood preservative in the lumen and within the tracheid wall of Scots pine with the 3 elements, copper, chrome and arsenic present in every submicroscopic level of wood analysed. Studying the tissue distribution of this preservative, Greaves (1972b) found that the bulk of the preservative in the hardwood was located in the vessels, while closely adjacent fibres were usually poorly treated and the rays contained relatively low levels of CCA. He suggested that there was higher CCA retention in the softwood than in the hardwood and that the cross field pits and tracheid bordered pits acted as filters and were usually well treated by the 3 elements.

Bravery (1972) found that there was a concentration of the preservatives in the ray-tissue of birch, the preservative on the walls of vessels was deposited in the lumen and that some fibres were free of chemicals. In Scots pine, however, the preservatives were concentrated in the ray-tissue, resin canals and the lumina of tracheids immediately adjacent to rays. Chou, Chandler and Preston (1973), indicated that the toxic elements of the preservative are located within the cell wall and the lumen surface was also well protected by a coating of the preservative. Dickinson (1974) analysed the distribution of CCA wood preservative in 2 species of hardwood and showed high levels of preservative on the vessel wall with limited

penetration through the deeper parts of the wall. In the case of the fibre, the cell-wall/lumen interface received a much lower deposition, with no material penetrating into the wall.

1.2. Wood- attacking fungi

Wood, in common with most organic materials, is susceptible to decay by micro-organisms under certain conditions of moisture and aeration in a variety of climates. Wood-attacking fungi can rapidly colonise the wood tissue, decompose its various components and eventually lead to a weak decayed timber. Fungi causing the decay of timber are generally known as the white-rot, brown-rot and soft-rot fungi, in addition, to the staining and mould-fungi which may often be present but have very little or no effect on the reduction of the strength of wood. Each group of wood-rotting fungi is characterised by causing certain types of damage to wood and, therefore, they are generally distinguished from each other according to the patterns of attack caused to wood. Levi (1964) suggested that the differences between the various wood-destroying fungi may be due to the differences in the diffusibility of the enzymes responsible for decay due to size differences, differences in the amounts of enzymes liberated or both factors.

The white-rot fungi cause a bleaching appearance to the wood. They are characterized by the successive decomposition of the various components of the cell walls (i.e., lignin, hemicelluloses and cellulose) through the chemical action of the ectoenzymes produced by the fungus, leading to a considerable thinning of the cell walls which eventually cause the collapse of the wood. This can be considered as a hydrolysis process, where the lignin, cellulose and finally the middle lamella are successively decomposed (Cartwright and Findlay, 1958). The fungal invasion of the wood tissue occurs through the different cell openings, via the

rays or by horizontal penetration of the cell walls, where fine hyphae directly penetrate the cell walls forming bore-holes which, with the progress of the attack, become larger leading to big openings between cells. A fluorescence microscopic technique used by Aufsess et al (1968) showed that the effect of the enzyme is limited to a layer that begins at the lumen and with the progress of the attack it extends towards the middle lamella. The electron-micrographs shown by Liese (1970a) also demonstrated the way in which the wall thinning proceeds, where one layer after another, from the lumen towards the middle lamella, were decomposed. Aufsess et al (1968) noticed that, in certain cases, the effect of the enzymatic action is limited to the immediate surroundings of the hyphae. This was confirmed by the electron-micrographs of the replica technique shown by Liese (1970a) and the scanning electron-micrographs shown by Bravery (1971), where deep grooves in the surface layer of the wall associated with the fungal hyphae were observed. These grooves were related to the localised cell wall erosion by the fungal hyphae due to the limited diffusibility of the ectoenzymes to a comparatively narrow region along the sides of the hyphae. With the progress of decay, the degraded zones unite together leading to the thinning of the wall. Campbell (1930, 1931 and 1932) in his study of the chemical effect of a number of white-rot fungi on wood divided this group of fungi into 3 groups according to the way in which the various components of the cell wall were attacked. The term simultaneous-rot is sometimes used to distinguish the group of white-rot fungi which decompose lignin and cellulose at the same rate (Liese,1970b).

The brown-rot fungi, attack the wood cellulose with little or no change on the lignin fraction. With the progress of decay, the wood develops a brown colour due to the residual lignin content. When dried, the brown-rotted wood shrinks considerably giving rise to brown-coloured cubical pattern of cracks. Hyphae of the brown-

rot fungi are rarely found in the cell wall, Liese (1970a) suggested that the general attack starts from the cell lumen, where the hyphae grow on the surface of what he called the tertiary wall(usually referred to in this thesis as the S_3 layer) and their ectoenzymes diffuse through this wall into the secondary wall to hydrolyse its components. He also suggested that the tertiary wall is resistant to attack. Bravery (1972) found that Coniophora cerebella, a brown-rot fungus, was able to attack the S_3 layer and that the lytic effects were widespread and not localised near the hyphae as in the case of the white-rot fungi. He suggested that the fungus strain, exposure conditions and sample preparation may play a role in the attack of the S_3 layer. The disintegration of the S_2 layer starts at an early stage of attack and cavities of different form and size are formed in the S_2 layer as decay advances. Levy (1969) related the rapidity of decay by this group of fungi to the movement of the active enzyme system through the layers of the cell wall. Most of the active brown-rot fungi penetrate the cell walls forming bore-holes which become wider than the diameter of the hypha itself. The details of this mechanism were early described by Cartwright (1930) and Proctor (1941), where they related the penetration process, mainly to the chemical action of the enzymes produced by the fungus.

It was generally believed for a long period of time that the white-rot and brown-rot fungi, which mainly belong to the wood-rotting Basidiomycetes, were the only groups responsible for the fungal decay of wood. Findlay and Savory (1950) recognised a different type of timber decay in the timber fill of water-cooling towers. This was first thought to be caused by chemical action, but Savory (1954a, b) realized that it was a type of fungal decay and gave it the term "soft-rot" to distinguish it from the white and brown-rots. The type of decay caused

by this group was demonstrated earlier by the drawings of Bailey and Vestal (1937). Since, Savory (1954a, b) recognised the economic importance of soft-rot decay, a considerable amount of work has been carried out to study the different aspects of this type of wood decay. Levy (1965), Thomson (1968), Findlay (1970) and more recently Zainal (1975) comprehensively reviewed the progress in this subject. Species of fungi causing this type of decay were listed by Duncan and Eslyn (1966) and recently by Seehann et al (1975).

Soft-rot fungi are characterised by the attack of the S_2 layer of the cell wall and the formation of characteristic types of cavities in this layer. The initiation of attack, cavity formation and the disintegration of the S_2 layer were described and discussed by Corbett and Levy (1963c), Courtois (1963a,b), Liese (1964, 1970a), Corbett (1965), Levi and Preston (1965), Levy and Stevans (1966), Findlay (1970) and Zainal (1975).

This type of attack has the ability to degrade hardwoods to a greater extent and more rapidly than the softwood species. This has been attributed to the higher lignin content of softwoods and to the different lignin quality in these species of wood. Soft-rot fungi generally invade the wood through the fibres or tracheids and preferably through the rays or vessels. Fine lateral branches from the hyphae in the cell lumen invade the cell wall by penetrating the S_3 layer into the S_2 layer and a T-shaped branching occurs in this layer. The hyphal penetration through the cell wall is generally believed to be due to the enzymatic action of the fungus. After branching, the hypha in the S_2 layer produces characteristic cavities with tapered ends due to the action of its ectoenzymes. The size and shape of these cavities vary

to a great extent, this may be related to many factors such as the fungal species, chemical and morphological structure of the wall layers, species of wood, cell type and the ecological conditions.

The term "soft-rot stainers" is applied to certain species of soft-rot fungi which may behave as stainers, particularly, in the soft-wood species.

The other groups of fungi (i.e., stainers and moulds) are of less economic importance compared to the high loss of timber caused by the white, brown and soft-rot fungi.

The staining fungi (also known as sap-stain or blue-stain fungi) cause the economic loss of timber, mainly, through the discoloration of the sapwood which is considered to be a major defect when using wood for decorative purposes. Karkanis (1966) described and discussed different aspects concerning the staining fungi in wood. This type of attack is common in both softwoods and hardwoods. It has very little effect on the strength properties of wood. The hyphae of the staining fungi heavily colonise the ray cells where they obtain their nutrient and the hyphae become heavily pigmented, which is the cause of the discoloration. They are also found in the cell lumina of fibres, tracheids and vessels, but they do not seem to utilize the components of the cell wall. Hyphae pass from one cell to the other via the simple or bordered pits and also by the characteristic cell wall crossing (Fig.17), where a stainer hyphe can directly penetrate the lignified cell wall layers by making small holes in the wall. Liese (1970a) reported the early observations made by Liese and Schmid (1964) in the mechanism of penetration through the wall, where they found that a stainer hypha penetrated the cell wall by forming a transpressorium. They related the penetration of a cell wall, to a localised enzymatic action at the tip of the transpressorium with an additional mechanical pressure.

The mould-fungi are usually regarded to be the early colonisers of wood in ground contact. They inhabit the surface of the wood with very little or no effect on the basic structure of the wood. Moulds disfigure the wood by means of the pigments in their conidia.

1.3. Aim of the work

It was decided to study the problem of the failure of preservative treated timber caused by fungi so as to establish, in a comparative way, the reasons for such failure in preservative treated hardwoods and not in softwoods treated in the same way.

In order to see what effect the presence of wood preservative chemicals had on the pattern of colonisation and decay of both hardwoods and softwoods, it was first necessary to examine the normal behaviour of a representative selection of organisms in untreated wood. Consequently the first part of the project involved a long and detailed study of five fungi in two species of wood. The normal pathways of entry into and passive penetration through each type of wood were studied until these could be clearly defined. These were then related to the colonisation patterns from wood buried in the soil. It would then be possible to see what immediate effect the presence of a wood preservative might have in changing the normal pattern, quite apart from toxic effects on the organism and on degrade of the cell-wall.

When this had been established it was then necessary to make a thorough examination of the microdistribution of the preservative in the wood and to determine any differences there might be between a hardwood and a softwood.

The methods, materials and results are described in this thesis.

The results are of very great interest since they appear to explain the reason for the different durabilities of a treated hardwood and a treated softwood and also a reason why soft-rot fungi might be able to decay treated hardwood when brown and white-rot fungi are unable to do so.

The Contents of the thesis are divided into 2 main parts:

1. Part II. deals with the Materials and Methods applied to both microbiological and preservative studies.
2. Part III. deals with the experimental observations of both aspects.

Reviews of literature are dealt with in the appropriate sections.

PART II

MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Wood Samples

The sapwoods of two species of wood, namely, birch, Betula sp. and Scots pine, Pinus sylvestris were chosen in the present study for the following reasons:

(1) they are representatives of a hardwood and a softwood respectively,

(2) they are both susceptible to decay (i.e. perishable timbers),

(3) they are both permeable to fluids,

(4) they have both been widely used in research projects.

Small test blocks of one cubic centimetre ($1cm^3$) and $0.7cm^3$ of the 2 species of wood were supplied by PRL*, Penarth Research Centre and Imperial College. These blocks were orientated in the manner described by Corbett (1963) so that the 3 opposite pairs of faces were in the transverse, tangential longitudinal and radial longitudinal planes, so that the specimens could be sectioned in each of the three planes without further trimming being necessary. Two pairs of opposite faces of the $1cm^3$ blocks were sealed with "Araldite" and one pair of faces were left unsealed. A set of unsealed blocks were also included in the investigations. The purpose of sealing the blocks was to study the penetration of the fungus across the block from each of the different planes of the wood structure. This was found to be unnecessary for the present study, so unsealed blocks were used exclusively in the later investigations.

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*) PRL, formerly the Forest Products Research Laboratory, but now the Princes Risborough Laboratory of the Building Research Establishment of the Department of the Environment.

The initial experiments showed certain disadvantages in using the $1cm^3$ blocks, such as,

(1) the large size of the block compared to the depth of the petri-dish in use was causing a pressure on the block when the lid of the petri-dish was put in place. This also interferred with the fungal hyphae underneath the block which appeared to be killed. When using the $0.7cm^3$ blocks, the lids could be put on without touching them and so moisture condensing on the lid did not touch the blocks and increase their moisture content,

(2) the rate of fungal decay was slower in the $1cm^3$ blocks compared to the $0.7cm^3$ blocks (e.g. Phialophora fastigiata was able to form cavities in 4-5 days in the $0.7cm^3$ blocks, while, in the $1cm^3$ blocks it took 10-14 days), this faster rate of decay indicates the presence of an actively growing fungus causing the ultimate decay in a short period of time before growth in the agar medium slows down,

(3) it was easier to handle and obtain a whole section from the $0.7cm^3$ blocks.

These disadvantages were found after the exposure of the untreated blocks to the test fungi, Phialophora fastigiata and Botryodiplodia theobromae, hence $0.7cm^3$ blocks were used for the rest of the test fungi, and for preservative treated and untreated wood. Over 3000 blocks were used, 4 replicates in each experiment at each time period. The initial moisture content of the blocks at the start of the experiments were usually between 5-8% approximately.

2.1.2. Culture Media

Three types of media were used. The composition of each was as follows:-

(1) Malt extract wood agar medium

20g birch sawdust (sieved through 60-80 mesh)

20g malt extract

10g Oxoid agar No. 3

in 1 litre distilled water.

(2) Wood agar medium

35g birch sawdust (sieved through 60-80 mesh)

15g Oxoid agar No. 3

in 1 litre distilled water.

(3) Malt extract agar medium

30g malt extract

15g Oxoid agar No. 3

5g mycological peptone

in 1 litre distilled water.

The media were sterilized by autoclaving at 15 p.s.i. for 20 minutes, left to cool to 45°C and poured, under aseptic conditions, into round sterile disposable plastic petri-dishes, left to cool and then incubated at 25°C for 3 days to ensure the sterility of the dishes before introducing the test fungi inocula to them.

Malt extract agar medium was found to be adequate for the growth of the different test fungi, wood agar and malt extract wood agar were included for the study of sporulation of Phialophora fastigiata in the 2 species of wood.

2.1.3. Test Fungi

2.1.3.1. Monocultures

The following species of fungi were used:-

Phialophora fastigiata (Lagerb and Melin) FPRL No. 56A
a soft-rot of hardwoods and a stainer fungus.

Chaetomium globosum Kunze ex Fr. FPRL No, S708
an active soft-rot fungus.

Botryodiplodia theobromae (Pat.) FPRL No, S22L
a stainer fungus.

Coriolus versicolor (L. ex Fr.) Quel FPRL No. 28A
(Polystictus versicolor (Linn.)Fr.)
a white-rot fungus.

Coniophora puteana (Shum. ex Fr.) P.Karst FPRL No. 11E
(Coniophora cerebella Pers.)
a brown-rot fungus.

These were chosen because:

(1) they were representative of the soft-rot, stainer, white-rot and brown-rot fungi,
(2) widely used in laboratory tests,
(3) active organisms under laboratory conditions,
(4) their patterns of attack have been thoroughly studied by many authors,
(5) certain of them are frequently isolated from preservative treated and untreated timbers,
(6) on the whole they possess a high tolerance of wood preservatives.

2.1.3.2. Soil Burial

Unsterilized soil was considered in this study to represent a natural mixed-culture of different species of micro-organisms normally found. The soil was collected from Silwood Park*, from the Farm site, (Banerjee 1969), and spread out for partial drying on the college roof, it was then sieved through approximately $1cm^2$ mesh to remove the large stones, roots, grass clumps,... etc. and stored in large polythene dust bins, these had previously been thoroughly washed with water and then with 70% methanol to remove any plasticizers present which might be toxic to the micro-organisms in the soil.

The moisture content of the soil at its water holding capacity was measured, using the method of Savory (1971),and found to be about 28%.

The experiments were carried out in thoroughly washed small polythene bread boxes of the size 28cm x 21cm x 10.5cm, (Fig. 1.), in such a way as to allow the soil to be maintained at approximately its water holding capacity by weighing and, when necessary, adding the appropriate amount of water. Sterile soil was also included as a control in this study. The sterilization procedure required a beaker filled with 1:2 epoxypropane to be placed in the middle of the box to maintain the sterile conditions throughout the experiment (Fig. 2).

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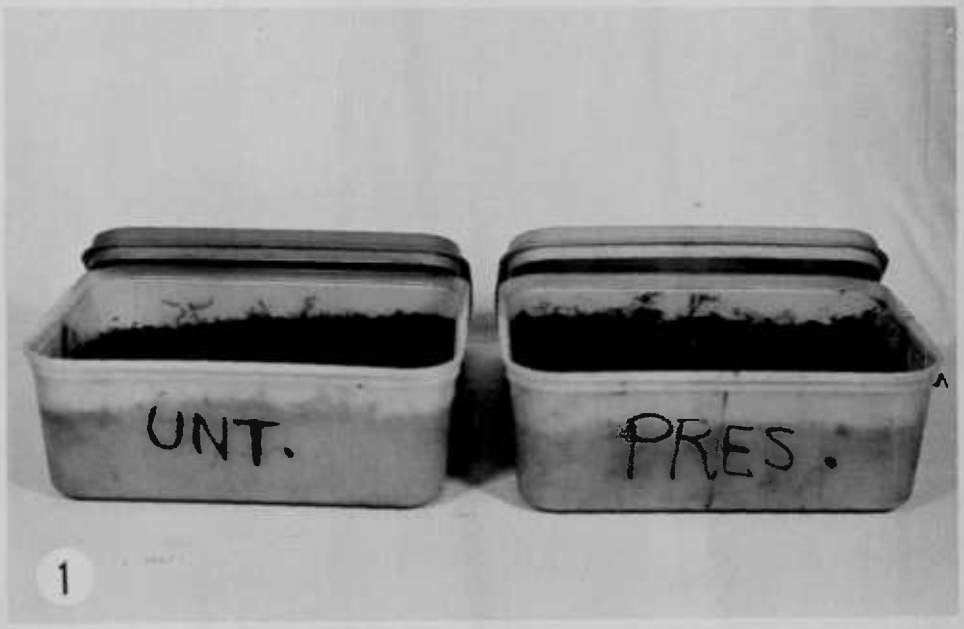
*Silwood Park, Imperial College Field Station, Sunninghill, Ascot, Berks.

Figure 1. Polythene boxes containing unsterile soil for the soil burial tests.

UNT. : untreated blocks of wood buried in the soil.

PRES.: preservative treated blocks of wood buried in soil.

Figure 2. A polythene box containing sterile soil for the control soil burial tests. The beaker in the middle of the box contains 1:2 epoxypropane to maintain the sterile conditions throughout the experiment.



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2.1.4. Preservative

The wood preservative used was a widely used water-borne type, with a formulation of Copper-chrome-Arsenic (CCA), commercially known as Celcure A and supplied by Rentokil Ltd., as a 10% stock solution. The formula of the preservative was as follows:-

32.53g/l $CuSO_4 \cdot 5H_2O$ (Cupric sulphate)

40.74g/l $Na_2Cr_2O_7 \cdot 2H_2O$ (Sodium dichromate)

26.73g/l $As_2O_5 \cdot 2H_2O$ (Arsenic pentoxide)

The preservative was chosen because:

(1) it is widely used commercially throughout the world,

(2) a great deal of research had been carried out in

(a) testing its toxicity against wood rotting fungi,

(b) examining its fixation and permenance in wood,

(c) determining its distribution through wood, both the gross distribution and to some degree, the microdistribution,

(3) field test records showed that CCA preservatives are, in general, one of the best wood preservatives used for the protection of wood against biological hazards.

Four concentrations of the preservative were used for the different studies, these were; 2.5%, 5%, 7.5% and 10%. The last 2 concentrations were used only for the tissue distribution studies of the preservative when a radiographic method was used, where the presence of high concentrations of the preservative in wood was essential for this study.

The two lower concentrations were chosen to give final retentions in the wood at and above commercial levels and were used in the microbiological and distribution studies.

2.1.5. Fixatives

2.1.5.1. Light microscope studies

Formalin acetic alcohol was used as a fixative for the wood blocks after exposure to the fungus. The blocks were stored in FAA until required for preparation prior to examination in the light microscope. The following constituents were used to prepare the fixative:

90ml 70% ethyl alcohol
4ml glacial acetic acid
6ml formalin

Corbett (1963) and Greaves (1966) using the same mixture, showed that it is an extremely good fixative and it did not cause damage to the wood tissue or the fungal hyphae.

2.1.5.2. T.E.M. Studies

The following commonly used fixatives were thought to be adequate to reveal satisfactory fixation results of the fungal hyphae and the wood tissue:

(1) Sorensen's phosphate buffer + Osmium tetroxide

(a) 0.4M buffer solution was freshly prepared by mixing known volumes of molar solutions of disodium hydrogen phosphate ($Na_2 HPO_4 . 12H_2O$) with monosodium-dihydrogen phosphate ($Na H_2 PO_4 . 2H_2O$). The pH of the buffer was adjusted at 7.2.

(b) the fixative was prepared, prior to use, by mixing 2 volumes of the buffer solution with 1 volume of 4% osmium tetroxide and adding 1 volume of distilled water. Zainal (1975) showed excellent details of the fungal hyphae using this fixative.

(2) The other fixative used was a freshly prepared 3% gluteraldehyde solution.

The second fixative was chosen for long periods of specimen storage, where published data had shown its effectiveness (Gay 1974).

These fixatives were only applied to certain preservative treated wood specimens in order to study the fungal behaviour in the presence of the preservative.

2.1.6. Embedding material

"Epon" epoxy resin of medium hardness of the following ingredients was used:

230g Epikote 812

5.5g benzyl dimethyl amine (BDMA)

114g methyl nadiz Anhydride (MNA)

123.6g dodecenyl succinyl anhydride (DDSA)

The embedding material was applied to both, attacked and unattacked preservative treated wood specimens in preparing them to be sectioned with an ultramicrotome for T.E.M. and EMMA4 studies. Zainal (1975) has shown what excellent results can be produced for TEM examination using this embedding medium.

Embedding was not found to be necessary to enable the material to be sectioned with a sledge microtome for the light microscope studies.

2.1.7. Stains

2.1.7.1. Light microscope studies

Two different staining mixtures were used to differentiate between the woody material and the fungal hyphae, these were:

(1) Safranin-Picroaniline blue (Cartwright 1929)

This stain has been used by almost every author studying degradation of wood by fungi when preparing sections for examinations in the light microscope. Greaves (1966) in a comparative study on a range of stains concluded that Safranin-picroaniline blue was the most effective and reliable stain. Safranin-picroaniline blue stains the lignified tissues red and the fungal hyphae blue. The following staining solutions were prepared:

(a) 1% aqueous solution of safranin,

(b) picroaniline blue was prepared by adding 25ml of saturated aqueous aniline blue solution to 100ml of saturated aqueous pictic acid,

(c) a mixture of safranin:picroaniline blue in a ratio of 1:2 was made up in glycerine, which avoided precipitation when the 2 dyes were mixed (Greaves 1966).

(2) Safranin-Fast green (or light green)(Gram and Jørgensen 1953).

A mixture of safranin and fast green was prepared as follows:-

1.5g safranin O

0.5g Fast green FCF

were dissolved in 200ml of 60% ethyl alcohol and 2 drops of concentrated hydrochloric acid were added. The colour of the final solution was dark violet. This stains the woody material red and the fungal hyphae

green. It was found to be an excellent staining mixture because:

(1) it was easy to apply,

(2) it gave excellent differentiation between the wood cells and the fungal hyphae,

(3) no fading of the stain was experienced after long periods of storage and sections were easily photographed.

In these studies, picroaniline blue was found to have certain disadvantages, such as,

(1) precipitation problems,

(2) the blueing of the whole of the section especially when severely decayed,

(3) time consuming,

(4) short lasting stain, which made it difficult to photograph stained sections stored for long periods of time.

Because of the advantages of safranin-fast green stain, it was used for the staining of both, preservative treated and untreated wood sections for the micromorphological studies with the light microscope.

2.1.7.2. T.E.M. Studies

The high contrast obtained from the presence of the preservative in the wood sections was sufficient for the study of the unattacked wood. Double staining with 2% aqueous uranyl acetate and lead citrate was only used with preservative treated attacked specimens, this was carried out to ensure a better contrast of the hyphae present and to compare the results with the unstained attacked specimens.

2.2. METHODS

2.2.1. Physical methods

2.2.1.1. Preservative treatment

The different methods of wood preservation and their application to the different types of preservatives were described in B.S. 1282: 1975.

In the present study, 2 methods of preservation were used with the 0.7 cm^3 wood test blocks, these were:

(1) Vacuum impregnation (B.S, 838: 1961)

Three concentrations of the preservative; 2.5%, 5% and 7.5%, were prepared by diluting the freshly prepared 10% stock solution, supplied by Rentokil Ltd., with water. The wood blocks of both species were first dried in an oven at 105°C for about 18 hours and the dry weights were measured.

The desired number of the test blocks were placed in a number of glass beakers in such a way that enough space between the blocks was maintained and a glass weight was placed over the blocks to prevent floatation of the blocks when applying the preservative solution. Each of the four concentrations were added to the appropriate beakers. At each time, one glass beaker with its contents was placed in a vacuum desiccator and vacuum impregnated with the preservative solution in the way specified in the B.S. 838: 1961. After the completion of the impregnation process, the preservative uptake for each concentration as dry salt was calculated and found to be as follows:-

% Preservative	Preservative uptake	
	Birch	Scots pine
2.5%	13.34 Kg/m^3	14.48 Kg/m^3
5%	25.50 Kg/m^3	31.70 Kg/m^3
7.5%	37.82 Kg/m^3	42.24 Kg/m^3
10%	49.20 Kg/m^3	56.20 Kg/m^3

The blocks were dried by spreading them on aluminium foil in the laboratory for 2 days and then dried for a further 18 hours at 50°C in a ventilated oven provided with a circulating fan. Some of the dry blocks were leached by placing them in a beaker, thoroughly covered with distilled water and left overnight. On the next day the water was changed twice and this was repeated for one week. The blocks were then dried for 3 days at 50°C in a ventilated oven. After this drying period, the blocks were fully impregnated under vacuum with distilled water and left covered with water overnight. The next day the water was changed twice and this was repeated for one week. Finally the blocks were dried again for 3 days at 50°C in a ventilated oven, left aside ready for sterilization and exposure to the test fungi and soil. This procedure of leaching applied to these small blocks was thought to be more than one would expect to occur under the field conditions.

Another set of preservative treated dry blocks were left unleached to study the difference in the behaviour of the test fungi between leached and unleached blocks of each timber species.

(2) Vacuum pressure process (Bethell process)

This method was considered in the study to represent a normal commercial treatment. Two concentrations of the preservative, 2.5% and 5%, were separately applied to both species of wood. The process of impregnation was carried out at the research laboratories of Rentokil Ltd.

and the blocks were treated to refusal. All the blocks were leached in the manner explained previously.

2.2.1.2. Moisture content and weight loss

The moisture content and the weight losses of the preservative treated, leached blocks, and untreated wood blocks exposed to monocultures of fungi or to soil were measured. The initial dry weight (W1) of the blocks, the weight after exposure to fungi or soil (W2) and the final dry weight (W3) were measured. The mean percentage of moisture content and losses of weight of the 4 replicates used were calculated as follows:

$$\% \text{ Moisture content} = \frac{W2 - W3}{W3} \times 100$$

$$\% \text{ Weight loss} = \frac{W1 - W3}{W1} \times 100$$

Control blocks were exposed to sterilised media, with no fungal growth, and to sterilised soil to measure the increase in weight due to mineral uptake and the actual loss of weight caused by the fungal attack was then calculated.

2.2.2. Microbiological methods

2.2.2.1. Infection of wood samples

The wood test blocks of both species of wood were sterilized before introducing them to the fungal cultures or soil. The preservative treated wood blocks were sterilized by placing them in a desiccator, sufficient 1:2 - epoxypropane was introduced to saturate the atmosphere and the blocks were left exposed to the vapour of this compound overnight. The following day, the blocks were transferred to a laminar flow bench where a flow of sterile air was passed over the blocks for 24 hours to remove the 1:2 - epoxypropane vapour. The

untreated blocks were sterilized in an autoclave at 15 p.s.i. for 20 minutes.

2.2.2.1.1. Monocultures

The infection of the wood test blocks with the 5 species of the test fungi was carried out by placing the wood blocks in contact with a mycelial mat on agar as described by Corbett (1963) and Greaves and Levy (1965).

The mycelial mats of the different species of test fungi were prepared by introducing a small inoculum of each test fungus onto malt extract medium in petri-dishes under aseptic conditions in a laminar flow bench. In the same way, inocula of Phialophora fastigiata were introduced to the malt extract wood agar and the wood agar media. The inoculated plates were incubated at 25°C for 7 days to form mycelial mats with the exception of Phialophora fastigiata grown on malt extract medium, where the incubation period was for 14 days because the fungus took a longer time to establish an appropriate mycelial mat.

The 1 cm^3, untreated, sealed and unsealed wood blocks were introduced, under aseptic conditions, onto the growing mycelium of Phialophora fastigiata and Botryodiplodia theobromae with one of the unsealed faces exposed to the fungal mycelium in the case of the sealed blocks and one of the transverse faces in the case of the unsealed blocks. It was found that the fungal colonisation throughout the block was faster through the transverse faces, than through either the radial longitudinal or tangential longitudinal faces. The 0.7cm^3, untreated and the preservative treated wood blocks, treated with the 2.5% and 5% concentrations by applying the 2 methods of preservation and both leached and unleached blocks, were placed in contact with the growing mycelium

in agar. 4 replicates of each species of wood for each treatment were used for each species of fungus and each exposure period. The wood blocks were removed from the petri-dishes after the following incubation periods:

(a) 1 cm^3 wood test blocks: 1, 2, 3, 4, 5 and 6 days
1, 2, 3, 4, 5, and 6 weeks

(b) 0.7cm^3 wood test blocks:
untreated: 1, 3 and 5 days
1, 3, 6, 9 and 12 weeks
different preservative treatments;
1, 3, 6, 9 and 12 weeks.

2.2.2.1.2. Soil burial

The depth of the unsterile soil in the bread boxes (described in section 2.1.3.2.) was 7.5cm. Untreated and preservative treated 0.7cm^3 wood blocks were placed in rows a few m.m. (not exceeding 15mm) below the soil surface. The bread boxes were incubated at 25°C and the different wood test blocks removed from soil after the incubation periods of 1, 3, 6, 9, 12, 15, 18 and 21 weeks.

2.2.2.2. Fixation

2.2.2.2.1. Light microscopy studies

After each incubation period, the different wood test blocks were removed from the culture media or soil and transferred into small bottles with screw caps, where sufficient formalin acetic alcohol was present to completely cover the specimens. The adhering fungus or soil was not removed from the blocks. The blocks were stored in the fixing solution for a period not less than one week before preparing them for sectioning. After the storage period, the blocks were

removed from the fixative, placed in 50% ethyl alcohol for a few minutes and finally washed thoroughly in running water.

2.2.2.2.2. T.E.M. Studies

Prior to fixation, after certain incubation periods, the 2.5% and 5% preservative treated blocks were removed from the culture medium, one replicate block of each treatment immediately placed on a Reichert sledge microtome and sections of 20 µm- 30 µm were cut by using a sharp clean microtome knife. The knife was constantly lubricated with distilled water for easier sectioning.

(1) Sorensen's phosphate buffer + Osmium tetroxide fixative

The 20 µm - 30 µm thick sections were transferred into a clean glass petri-dish and sufficient 0.4M buffer solution was added. The sections were cut into small blocks (approximately 0.2 - 0.5 mm) with a very sharp clean razor blade. These blocks were then transferred into small staining receptacles, commonly known as "embryo cups", where a suitable amount of the fixative was present. The embryo cups were placed in a vacuum desiccator and exposed to a reduced pressure for 20 minutes by connecting the desiccator to a water pump. The desicator with its contents under vacuum were placed in the cold room at $4^{o}C$ for more than 24 hours. Finally the wood blocks were thoroughly washed with the buffer solution on an ice bath for several times to remove all traces of OsO_4 present. The wood blocks after this stage were ready for dehydration.

Due to the toxic nature of the volatile vapours of the OsO_4, the whole procedure of fixation was carried out in a fume cupboard and unwanted fixative was poured into 70% ethyl alcohol in a conical flask.

(2) 3% gluteraldehyde fixative

A whole wood test block or the 20 µm - 30 µm thick sections immediately after removal from the culture medium, or cutting were transferred into the fixative solution in small bottles with the screw caps and stored at $4^{o}C$. Prior to dehydration and embedding, the sections were cut into small blocks (approximately 0.2 - 0.5 mm) in the fixative solution in a clean glass petri-dish.

2.2.2.3. Dehydration

After the fixation of the small wood blocks with the Soreneen's phosphate buffer + OsO_4 or the gluteraldehyde fixatives, the wood specimens were dehydrated by passing them through a series of 20%, 30%, 40%, 50%, 60%, 70% 80% and 90% ethyl alcohol respectively for about 15 - 20 minutes at each concentration. They were then passed through 3 changes of absolute alcohol and left for one hour at each change. The dehydration process was carried out in the embryo cups on an ice bath.

Some blocks of the sound preservative treated unfixed wood were dehydrated in the same way.

2.2.2.4. Embedding

The epoxy propane used is known to be a highly volatile material, so, the whole procedure of embedding was carried out under cold conditions on an ice bath and the embryo cups used were usually covered with a square piece of glass.

The embedding procedure of the small wood blocks with the "Epon" epoxy resin was as follows:-

(1) Immediately after dehydration, the wood specimens were transferred into a freshly prepared mixture of 1:1 absolute ethyl alcohol and epoxy propane, changed twice and left for 15 minutes at each time,

(2) the wood specimens were placed in pure epoxy propane for 30 minutes,

(3) the apecimens were then tranaferred into a mixture of 2:1 epoxy propane: epoxy resin and left for 30 minutes,

(4) the mixture was changed to a 1:2 epoxy propane: epoxy resin and the epecimens placed there for 45 minutes, changed once and atored at 4°C for 18 hours.

The above-mentioned steps were carried out in the same embryo cups and the change of the aolutions was made using clean disposable pipettes,

(5) the wood specimens were then transferred into pure epoxy resin in clean embryo cupa, placed in a vacuum desiccator, exposed to reduced pressure for 20 minutea and then placed overnight, while under vacuum, in the cold room at 4°C,

(6) the next day, the vacuum was released, the epoxy resin was changed, exposed again to reduced pressure for 20 minutes and stored at 4°C for about 5 hours,

(7) the vacuum was releaaed again, the epoxy resin wae changed, no vacuum was applied this time and this procedure was repeated twice a day for 3 days in the caae of the attacked preservative treated specimens (i.e., a total of 9 changes of resin) and 4 daye in the case of sound, unfixed preservative treated specimene (i.e., a total of 11 changes of resin), where it was found that more time was needed for the complete infiltration of the resin into the sound blocks,

(8) about 10 blocks of the embedded specimens were transferred into fresh epoxy resin in small polythene embedding trays and arranged in 2 rows in such a way that the desired face for cutting was facing upwards,

(9) The polythene embedding trays were left at room temperature for about 2-3 hours and finally placed in an ordinary oven or a vacuum oven at 60°C for about 48 hours to obtain a polymerised material.

The changing of resin was achieved by removing the wood blocks from the old resin with a toothpick, drying the specimens very gently on filter paper to remove any adhering old resin from the surface of the blocks and transferring the specimens into the fresh resin. The old resin was disposed of after being polymerised at 60°C for 48 hours to avoid any health hazards which might occur due to the carcinogenic nature of the unpolymerised resin.

2.2.2.5. Mounting and trimming

(1) Mounting

The polymerised discs of the resin were removed from the polythene embedding trays and small blocks (approximately 2-3 mm) of the polymerised resin were cut with a fine saw in such a way that the polymerised wood specimen was in the centre of the block. The resin blocks were then mounted with "Durofix" onto short (approximately 10-15 mm) "Perspex" rods, so that the wood specimen was on the upper side of the resin block, and then placed in an oven at 60°C for 24 hours.

The selection of the different orientations (i.e., T.S. or L.S.) of the embedded wood specimens were made under a dissecting binocular microscope. Small wood blocks of the size 2-3 mm were cut with a sharp clean razor blade from the 2.5% and 5% preservative treated, unfixed, sound 0.7 cm^3 blocks and mounted without embedding to the "Perspex" rods in the same way. These blocks were used in this study to determine any change in the preservative distribution which might have occurred due to the long fixation, dehydration and embedding processes.

(2) Trimming

The shaping of the wood block (i.e., trimming) in order to achieve a suitable size and shape for ultramicrotome sectioning is considered to be one of the most critical stages in electron microscopy studies. At this stage the failure or success of the embedding procedure becomes clear and sectioning depends very much on the quality of trimmed block shape finally produced.

The "Perspex" rod with the mounted material was placed in a special holder and, under a dissecting binocular microscope, the epoxy resin surrounding the specimen was removed very gently by using a fine metal file in such a way that a pyramid shape was formed. Trimming of the wood block was continued using sharp clean razor blades to form a small pyramid with very smooth and shiny faces.

In the same way a pyramid was shaped from the unembedded 2-3 mm wood blocks. The hardness of the block caused difficulty during the trimming process, this was easy to overcome by slightly wetting the wood block with distilled water during the trimming process.

Small pyramids were directly trimmed from the 0.7 cm^3 unfixed, sound 2.5% and 5% preservative treated wood blocks and directly mounted with 6% melted gelatine onto the heads of small clean silver pins. These were then frozen in Freon 12 prior to freeze dry ultra sectioning.

2.2.2.6. Sectioning

2.2.2.6.1. Sledge microtome sectioning

Sections for light microscopy observations, X-ray radiographic studies and EDAX analysis were cut on a Reichert sledge microtome.

Sections of 10-30 µm were cut from more than one replicate of the wood test blocks, preservative treated or untreated, exposed to the different species of fungi or soil at each exposure period for the light microscopy studies. The sections were cut from each face of the block (i.e., T.S., R.L.S, and T.L.S.) and at different depths below surface.

Thick sections, ranging between 50-150 µm, were cut from the sound 2.5%, 5%, 7.5% and 10% preservative treated wood blocks for the X-ray radiographic studies. Sections of the same thickness were cut from sound untreated blocks and included in this study as controls.

Thin sections of 20-60 µm were cut from the sound 2.5% and 5% preservative treated wood blocks for the EDAX-analysis. The rsdisl longitudinal sections were cut in the normal way. It was found that specimen tilt angle had considerable effects on the analysis, so, in order to achieve the best conditions for analysis, the wood blocks were obliquely clamped in the microtome at an angle of 45^{o} and oblique trsnsverse sections of 45^{o} tilt were cut.

Sectioning of the different wood blocks was made easy by constant lubrication of the sharp knife with a mixture of glycerine and 70% alcohol. It was not poseible to obtain a whole section from the severly attscked blocks, but sections of reasonable size were cut from these blocks and embedding was not found to be necessary. The sections were immediately transferred into 60% alcohol, resdy for staining.

Sections from the dry sound wood blocks were easily cut by constant lubrication of the knife with distilled wster or steam by applying the Kisser's steam method, Jane (1970). The sections were dried by placing them on a filter paper at room temperature for about 48 hours.

2.2.2.6.2. Ultramicrotome sectioning

The difficulties in cutting ultrathin sections from wood are numerous and due to the heterogenous nature of wood, the success in obtaining good ultrathin sections depends on many factors, these were fully discussed by Fengel (1967). In this study trials to obtain ultrathin sections from both embedded and unembedded materials are described.

Ultrathin sections were cut on an LKB ultratome type 4802A. A diamond knife was used for cutting sections from the "Epon" embedded material and ribbons of silver or gold sections (600-1500Å, Peachy, 1958) were collected from the water trough on formvar coated copper or palladium grids. Sections from the "Epon" embedded material were also cut with a glass knife but this did not produce satisfactory results especially with sound wood.

Frozen sections were cut from preservative treated, sound, unembedded material on the same ultramicrotome fitted with a special LKB Cryokit freezing attachment. The block prepared for this purpose was placed in the microtome and sections were cut under very cold conditions with a glass knife. The specimen temperature was - 90°C and the knife temperature was - 100°C. Sections were transferred to formvar coated nickel or palladium grids with a fine needle and flattened with the end of a stainless steel rod. The grids were left to sublime in the specimen chamber for 2 hours and then vacuum coated with a thin film of carbon. Liquid nitrogen was constantly used to cool down the knife, specimen and the surroundings of the knife and specimen chamber.

There were many disadvantages in using this technique which led to the failure in obtaining good useful sections, these were:

(1) difficulties in setting and operating the machine since it was not possible for one person to do the work,

(2) it was very difficult to pick up the sections under such cold conditions,

(3) the sections curled into a roll towards the specimen or the edge of the knife and were finally lost,

(4) the sections were damaged when they were flattened with the stainless steel rod,

(5) due to the sublimation of the liquid nitrogen, it was very difficult to see what was going on and to act accordingly.

After the failure in obtaining dry frozen sections, trials were made to cut dry sections under normal conditions from the unembedded dry wood for the preservative microdistribution studies. A glass knife was used without a water trough and few dry sections were cut, transferred to a drop of water in a clean glass petri-dish with an eye-lash and finally collected on formvar coated palladium grids. There were many difficulties involved in using this technique such as transferring the sections to the water in the petri-dish and in maintaining these sections, because the sections tended to curl into rolls towards the specimen or over the edge of the knife and were finally lost. In order to overcome this problem trials were made to gently press over the edge of the first section with an eye-lash, but this proved not to be a practical way. An anti-roll plate, a technique described by Hellstrom and Sjostrom (1974) to collect dry ultrathin frozen sections, was used. This was made by attaching a narrow strip, about 0.2 mm, from a glass cover slip along the edge of the knife in such a way that a narrow gap was formed between the surface of the strip and the trough surface of the knife and the sections can be collected under this gap.

Although this technique was, more or less, successful in avoiding the curling of the sections, it was again very difficult to collect the sections on the grids. Finally it was decided to use a water trough in such a way that the trough was filled with water without reaching the cutting edge of the knife and once the first few sections reach the water level, there was no chance for curling. Sectioning was carried out and the ribbons floating on water were collected on palladium grids. It was essential not to completely fill the water trough, otherwise the wood block would soak up water and it would then be impossible to cut sections. The grids were then vacuum-coated with a thin film of carbon.

2.2.2.7. Staining

2.2.2.7.1. Light microscopy studies

The sections obtained from the different attacked wood specimens for the micromorphological studies were transferred to 60% ethyl alcohol before staining.

The stain mixtures were applied as follows:

(1) Safranin-Picroaniline blue

The sections were:

(i) stained in 1% aqueous safranin for about 15 minutes,

(ii) slightly washed with water,

(iii) transferred to a slide and stained in picroaniline blue by warming under a flame until simmering,

(iv) thoroughly washed with water,

(v) dehydrated with 80%, 90% and absolute alcohol respectively,

(vi) cleared in xylene and mounted in Canada balsam.

Some of the sections were placed directly in a mixture of 1:2, safranin: picroaniline blue and warmed until simmering. Steps (iv), (v) and (vi) were then applied.

(2) Safranin-fast green

This stain mixture was applied as follows:

(i) the sections were stained in the mixture for about 3 minutes,

(ii) dehydrated in 2 or 3 changes of absolute alcohol,

(iii) cleared in xylene and mounted in Canada balsam.

The advantages of safranin-fast green experienced in this study were considered a justification for the use of this stain mixture throughout the study, after it had been demonstrated that the safranin-picroaniline blue stain by comparison showed many disadvantages (c.f. section 2.1.7.1.)

2.2.2.7.2. T.E.M. studies

The double staining with uranyl acetate and lead citrate of certain grids bearing attacked preservative treated sections was carried out in a clean petri-dish as follows:

(i) a few drops of the 2% aqueous uranyl acetate were placed on a thick polythene sheet,

(ii) one grid was submerged into each drop,

(iii) the polythene strip was incubated in an oven at 60°C for 30 minutes,

(iv) the grids were washed in a stream of glass distilled water,

(v) the grids were then transferred to drops of lead citrate placed on another thick polythene sheet in a closed petri-dish with strong NaOH present to keep the atmosphere free from CO_2 which makes the lead solutions become cloudy,

(vi) the petri dishes were left at room temperature for 30 minutes,

(vii) the grids were washed several times in a stream of glass distilled water and finally dried on a filter paper.

The staining procedure was carried out to show the details of the fungus which may be present in the preservative treated sections. Certain grids were left unstained for comparative studies.

2.2.3. Analytical Methods

2.2.3.1. Microbiological studies

A study of the different patterns of colonization and degradation by the species of fungi and the soil population in both species of wood was carried out in order to establish the pattern of behaviour of each fungus in the absence of the preservative (i.e., in untreated blocks). The presence of the preservative in two concentrations, 2.5% and 5% both in leached blocks after preservation and unleached ones might cause changes in this behaviour pattern and the second part of this study was to determine any such changes, and attempt to explain the reasons for their occurrence.

2.2.3.1.1. Visual examinations

Macroscopic observations of the general appearance of the different wood blocks and fungi was carried out after each exposure period. These were made before and after the removal of the wood blocks from the culture media or soil. This involved the observation of the way in which the fungus surrounds the wood blocks and a study of the general condition (i.e., the colour, softness, size ...etc.) of the blocks after the different exposure periods. The differences in

the condition of the untreated and preservative treated wood blocks, when exposed to the same fungus, were also recorded.

2.2.3.1.2. Light microscope observations

The fungal colonization and degradation by the different species of fungi were examined in a large number of transverse, radial longitudinal and tangential longitudinal sections obtained from the untreated and preservative treated blocks after the different exposure periods to monocultures or soil, under a bright field Reichert microscope. Certain sections, especially those attacked by soft-rot fungi, were examined under polarised light to show the typical types of cavities caused by soft-rot fungi. Photographs were taken for the different features observed using a fully automatic Reichert Electronic Camera Kam ES.

2.2.3.1.3. T.E.M. Observations

Stained and unstained ultrathin sections obtained from the preservative treated blocks after six weeks exposure to the 5 species of fungi were examined in an AEI EM6 transmission electron microscope. This period of exposure was chosen because it was found from the light microscope studies that at this stage the fibres of the preservative treated birch blocks started to become succeptible to attack by soft-rot fungi but not by Basidiomycetes. The lengthy procedure involved in the preparation of ultrathin sections and shortage of time, made it impossible to examine sections from each of the different exposure periods. Sections of blocks after six weeks exposure were therefore used to demonstrate the difference in the pattern of soft-rot fungi in the presence of the preservative in birch compared to the behaviour of the Basidiomycetes.

2.2.3.2. Preservative distribution studies

The information obtained from the microbiological studies on the colonization and degradation of the preservative treated wood indicated that the preservative distribution in the different cell types and through the different layers of the cell walls of wood, might have an important role in the protection of timber against fungal attack. Several authors studied the distribution of the different types of preservatives in wood using a considerable number of techniques for the detection of the preservative. Bravery (1972) reviewed the different techniques used, both the destructive analytical techniques and the non-destructive ones, for the detection of the preservative distribution in wood. In the present study, the destructive methods of analysis were avoided and the non-destructive techniques were considered in order to detect the preservative distribution under normal conditions as far as it was possible. Three different techniques were used to study the preservative distribution in the 2 species of wood. The limitations, advantages and disadvantages of the techniques are discussed. The use of these techniques was thought to be appropriate in revealing adequate information on the preservative distribution in wood for the present study.

2.2.3.2.1. X-ray Micro-radiographic technique

The theory of the X-ray micro-radiographic technique and its application to iron ores and sinters was explained by Cohen (1950). Cohen and Schloegl (1959) successfully applied the technique in mineral dressing. Belford (1960) applied this technique in the study of a water borne preservative, "Tanalith" C, distribution in a softwood. He claimed the suitability of this technique for investigations of the heavy metal salts distribution in an organic substrate.

Rudman (1966) showed the presence of CCA wood preservative in cell walls of a hardwood by applying the same technique. More recently, Sharp (1974) studied the distribution of barium salts in 2 hardwoods and one softwood. He concluded that the X-ray technique is a useful tool for determining preservative deposition in wood.

In the present study, the techniques developed by Cohen and Schloegl (1959) and Sharp (1974) using a "Raymax" 60 X-ray machine were applied to study the CCA wood preservative distribution in the 2 species of wood. Transverse sections of 50-150 μm thick were cut in a sledge microtome from untreated wood test blocks and 2.5%, 5%, 7.5% and 10% preservative treated blocks. The sections were attached to adhesive plastic films and fitted on the emulsion side of 5 cm^2 Kodak High Resolution (H-R) photographic plates. The plate was then placed in a "Raymax" 60 X-ray machine and exposed for 3, 5, 7 or 10 minutes to X-rays, obtained from the "Raymax" 60 unit using a chromium or iron target, generated at an accelerating potential of 10, 20, 30, or 40 KeV and tube current of 5 or 10 mA. After exposure, the plate was developed in Kodak D19 developer for 5 minutes, fixed and washed. An image of the wood was formed on the plate of the same size as the specimen. The whole operation was carried out in a dark room using a red safelight. The plates were then examined in an ordinary microscope and enlarged photographs were taken from the images formed on the plates. The contrast in these photographs was highly dependent upon that of the initial contact "H-R" plate. It was found that certain factors were influencing the resolution of these plates, such as:

(1) the thickness of the sections,

(2) the potential (KeV) and current (mA) used for X-ray generation,

(3) the time of specimen exposure to radiation,

(4) the preservative concentration in wood,

(5) the distance between the specimen and the X-ray tube window,

The best results were obtained under the following conditions:

(1) using sections of 50-60 μm thickness,

(2) X-ray generation at 40 KeV and 10 mA,

(3) exposure time of 5 or 7 minutes,

(4) using sections obtained from the higher preservative concentration (i.e., 7.5% or 10%),

(5) the distance between the specimen and the X-ray tube was fixed at 23.5 cm which ensured a uniform beam intensity over the whole area of the plate.

Although the micro-radiographic technique applied in this study revealed certain useful information concerning the distribution of the preservatives in wood, there were also certain disadvantages; these were:

(1) it was not possible to study the micro-distribution of the individual elements in the preservative,

(2) it was difficult to compare the results obtained for one species of wood to the other species,

(3) although the image formed on the "H-R" plates from exposing untreated sections to X-ray was distinguishable from the ones formed from preservative treated sections, the analysis of the image was confusing at certain areas (this will be discussed in detail in a later section in this thesis),

(4) it was not possible to carry out quantitative or semi-quantitative measurements from the results obtained.

2.2.3.2.2. Energy Dispersive Analysis of X-rays (EDAX)

The principles of the energy dispersive X-ray analysis is mainly based on Moseley's discovery that each element in the periodic table is characterised by a relatively simple X-ray spectrum (Cosslett 1969). When an element is bombarded by electrons, they cause the excitation of the shell electrons of the atoms in the element, the atoms lose energy which is emitted in the form of X-ray photons. The energy of this X-ray is characteristic of the element. By the use of an energy dispersion X-ray analyser, an X-ray peak is formed, the location of this peak in the spectrum identifies its energy and hence the element from which the X-rays came.

The various aspects of the energy dispersion X-ray analysis involved in the detection of the different elements were reported and discussed by Russ (1971), Barbi et al (1974), Gullasch and Kaufmann (1974), Dempsey et al (1974) and Yakowitz (1974). Greaves (1972b)applied this method in the detection of CCA wood preservative distribution in 2 species of wood and suggested the suitability of the technique for such studies.

In the present study, a Cambridge Scanning Electron Microscope (SEM) combined with an Energy Dispersive Analyser of X-rays (EDAX), installed in the Electrical Research Association Ltd., Leatherhead, Surrey (ERA), was used to study the preservative distribution in the 2 species of wood. The EDAX system is mainly composed of a detector made of very pure silicon (Si) crystal doped with a small amount of lithium (Li) atoms and an amplifier Fig. (3). The information is displayed on a small television screen Fig. (4) and (5).

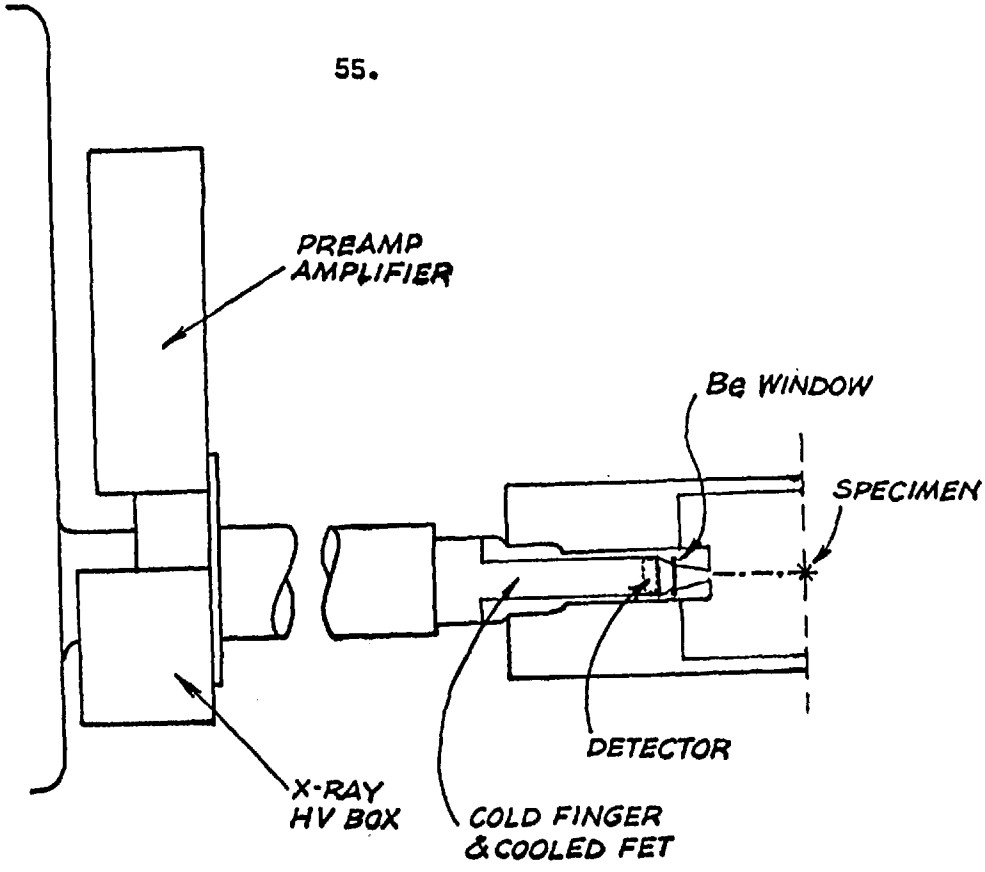


Fig (3)

A diagram showing the detector, specimen position and other main parts of the Edax system.

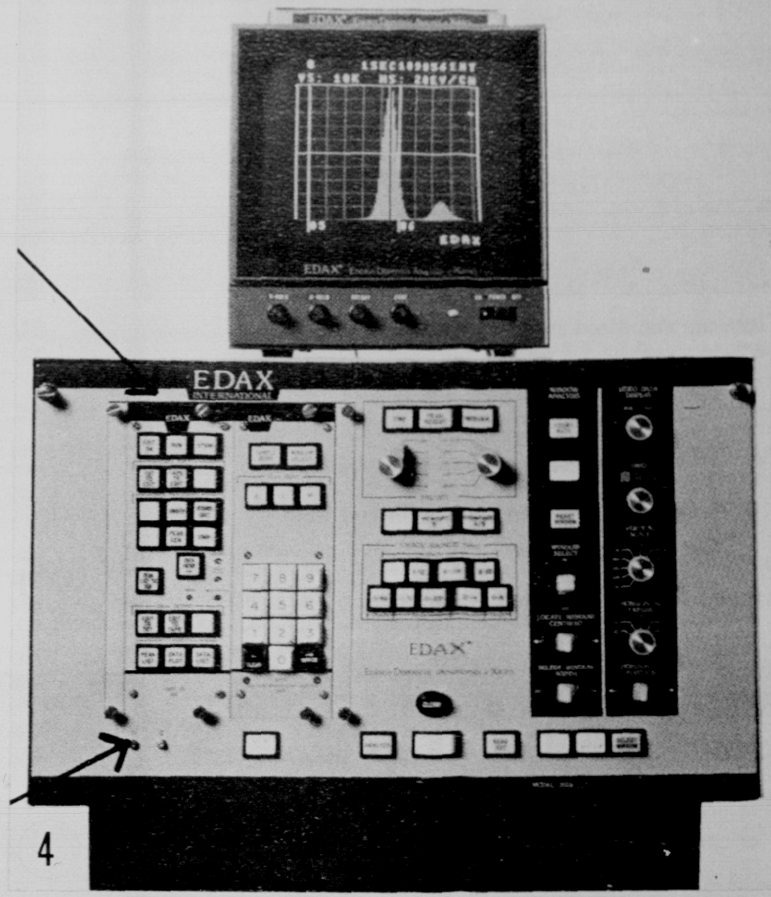
Figure 4. A photograph showing the small T.V. and the control panel of the Edax system.

Figure 5. An Edax spectrum for copper, chromium and arsenic.

T: counting time.

C: number of counts.

X: energy scale in KeV.


EDAX
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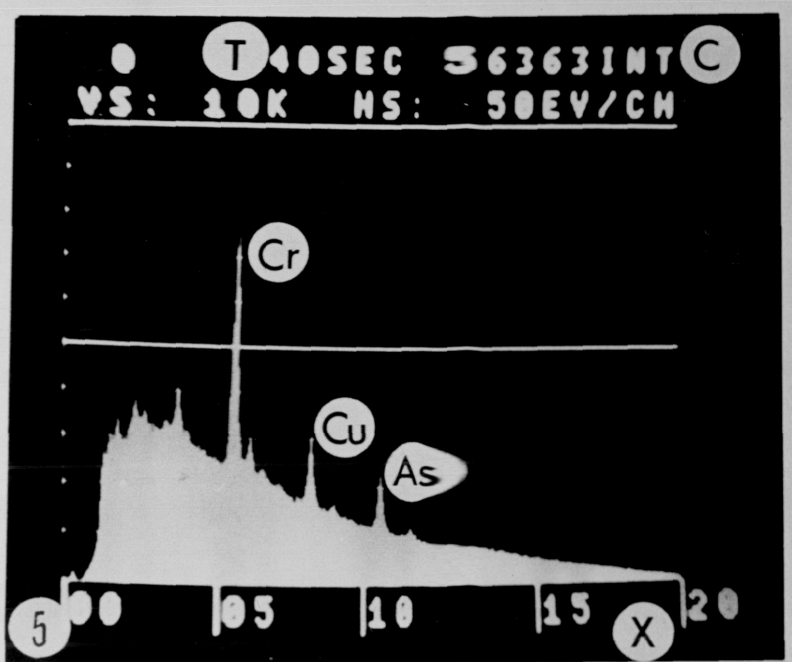
T
40SEC 563631NT
C
VS: 10K MS: 50EV/CH
Cr
Cu
As
5
00
05
10
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X

In the present system (EDAX International Catalogues, Yakowitz, 1974 and Loveland, 1975) the electron beam, generated from the electron gun in the SEM at an accelerating potential of 20 KeV, bombards the specimen causing the excitation of the inner shell electrons of the atoms in the material present in the specimen knocking them out of their orbits. This creates a vacancy in the energy levels of the atom which is filled immediately by an electron from a higher energy level. When this electron drops into the inner shell, the atom loses energy which is emitted in the form of an X-ray photon. The energy of this X-ray is characteristic of the element present. The Si (Li) detector is present above the specimen being bombarded with electrons. The X-rays emitted from the atom pass through a Beryllium window, which acts as a vacuum seal, into the detector which acts as a semi-conductor, each X-ray is absorbed and its energy is used to raise electrons (i.e. charge carriers), the number of these electrons is proportional to the X-ray energy, the detector is ready for the next X-ray to enter within a fraction of a microsecond. The charge carriers are then swept to electrodes by an applied field and the small voltage pulse produced picked up by an amplifier and converted to a digital signal. This signal is processed by a multi-channel analyser to show up as a count on the T.V. screen at a known time and also causes a peak to appear on the screen, each peak at a certain energy represents a specific element. The height of the peak also gives the number of the counts Fig. (6). It is possible to calculate the concentration by weight of the element present in the specimen by measuring the height of the peak (or the number of counts) of the unknown element against the height of the peak of a standard which is a known amount of the element concerned. This was not found to be necessary for the

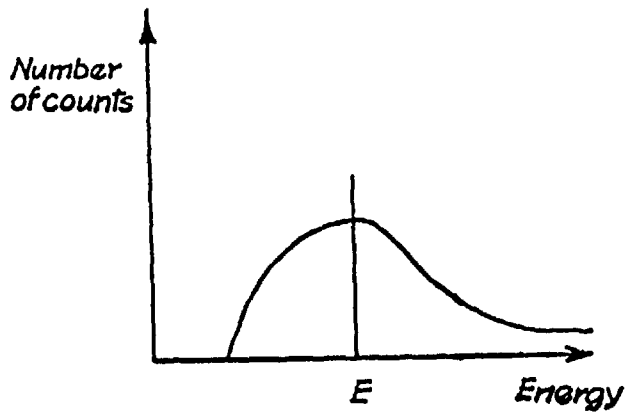

Fig (6)

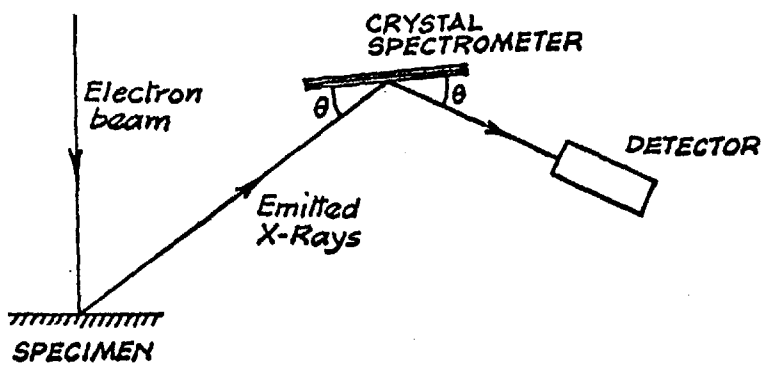

Fig (7)

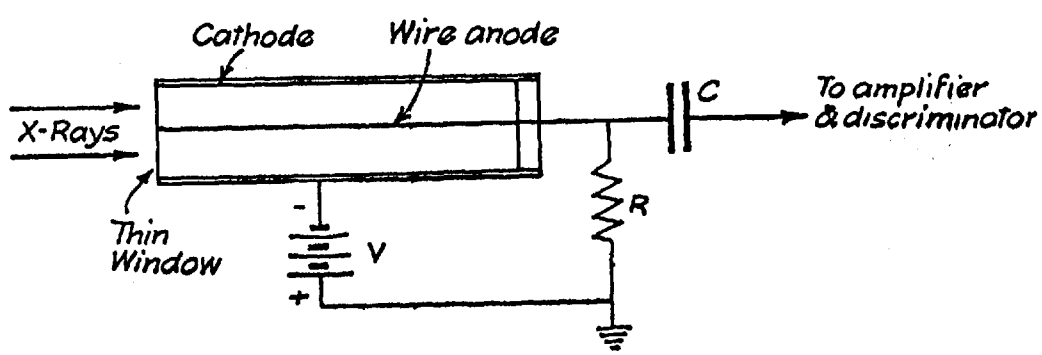

Fig (8)

A diagram of a gas-flow counter

present study and thus the quantitative analysis was not carried out.

Another type of X-ray photon is produced when bombarding the specimen with the electrons generated from the SEM as a result of the high energy electrons being slowed down while passing through any material. This is called the Bremsstrahlung radiation. The energy of this radiation is not characteristic of the elements present and it appears as the background in the spectrum on the T.V. screen.

An elemental mapping for the preservative distribution on a chosen area of wood is possible, where the amplified signal from the energy dispersive detector system is made to modulate the brightness of a cathode-ray tube scanned in synchronism with an electron probe. Thus a picture is obtained on the cathode-ray tube by the variation of X-ray emission from the surface. In a photographic plate, the picture of the scanned area will be white in places where the element is present and black in places where the element is absent. A line scan showing the element distribution across a chosen area in the specimen is also possible with this technique.

Radial longitudinal and 45^{o} tilted transverse sections of 20-60 µm thickness were cut from the 5% CCA treated Scots pine and birch wood blocks and analysed in the EDAX system. The sections were mounted on clean aluminium stubs using double-sided adhesive tape and coated with a thin film of carbon under vacuum to avoid discharging problems when examining them under the SEM. .The analyses were carried out for the individual elements of the preservative in the different tissues of each wood species. The counting time was fixed at 40 seconds in order to be able to compare the results obtained from a certain area of a section or one species of wood with another.

Photographs were taken from the T.V. screen of the EDAX system for the spectrum formed after each analysis on polaroid films.

The following images were also photographed on polaroid and/or 35 mm films from the SEM screen:

(1) an ordinary electron image scan of the area analysed in the wood section,

(2) an element image scan of the X-ray signal for all elements and each individual element which produced a spotty picture representing the preservative distribution in wood,

(3) an image of the X-ray line scan for the individual elements of certain areas.

The limitations, advantages and disadvantages of the technique concerning the present study, will be mentioned later and compared with those of the EMMA4 technique. This will clarify and justify the use of these 2 techniques for the detection of the preservative in wood.

2.2.3.2.3. Electron Microscope Microanalyser (EMMA)

The analysis in the EMMA system is based on the fact that X-rays, like all photons, have a wavelength. As described before (section 2.2.3.2.2.), when electrons strike a specimen Fig. (7), X-ray photons are emitted, with energies characteristic of the elements present. The emitted X-rays strike a crystal, (with a set of atomic planes regularly spaced), which diffracts the X-rays according to Bragg's law

$$n\lambda = 2d \sin\theta$$

where n is an integar, λ is the wavelength of the X-ray photon, d the lattice spacing of the crystal planes involved in the diffraction

and θ is the angle of incidence of the X-rays on the crystal. The diffracted characteristic X-rays enter a gas-flow proportional counter Fig. (8) which gives an electrical pulse from each photon that strikes it. The pulse is fed to an amplifier and then to a discriminator which is a pulse height analyser. The output from the discriminator is fed to a scaler/timer where the X-rays collected are displayed as accumulated counts. The crystal is chosen according to the wavelength of the X-rays and, therefore, the method is called wavelength-dispersive X-ray analysis.

The various aspects concerning the design and application of EMMA were reported and discussed by Cooke and Duncumb (1969), Cosllett (1969) and Hall et al (1974). The same equipment used in this study was used by Chou (1971) and Dickinson (1974) to investigate the distribution of copper, chromium and arsenic in wood.

The electron microscope microanalyser "EMMA" used in this study is a combined AEI EM800 transmission electron microscope and a micro-analyser produced by AEI under the name "EMMA4". The instrument is installed in the Division of Materials Applications at the National Physical Laboratory, Teddington.

The basic features of "EMMA4", (Kent, 1975), is shown in Fig. (9) which is a column cross-section of the transmission electron microscope with the addition of a probe forming objective lens (mini lens) positioned in the column above the specimen. Two crystal spectrometers are positioned either side of the column allowing X-rays to leave the column through 45^{o} take off. The microscope is provided with 2 channels with separate tilts and shift controls, this enables one channel to be used in the normal transmission mode and the other channel can be used for the mini lens or analysis mode.

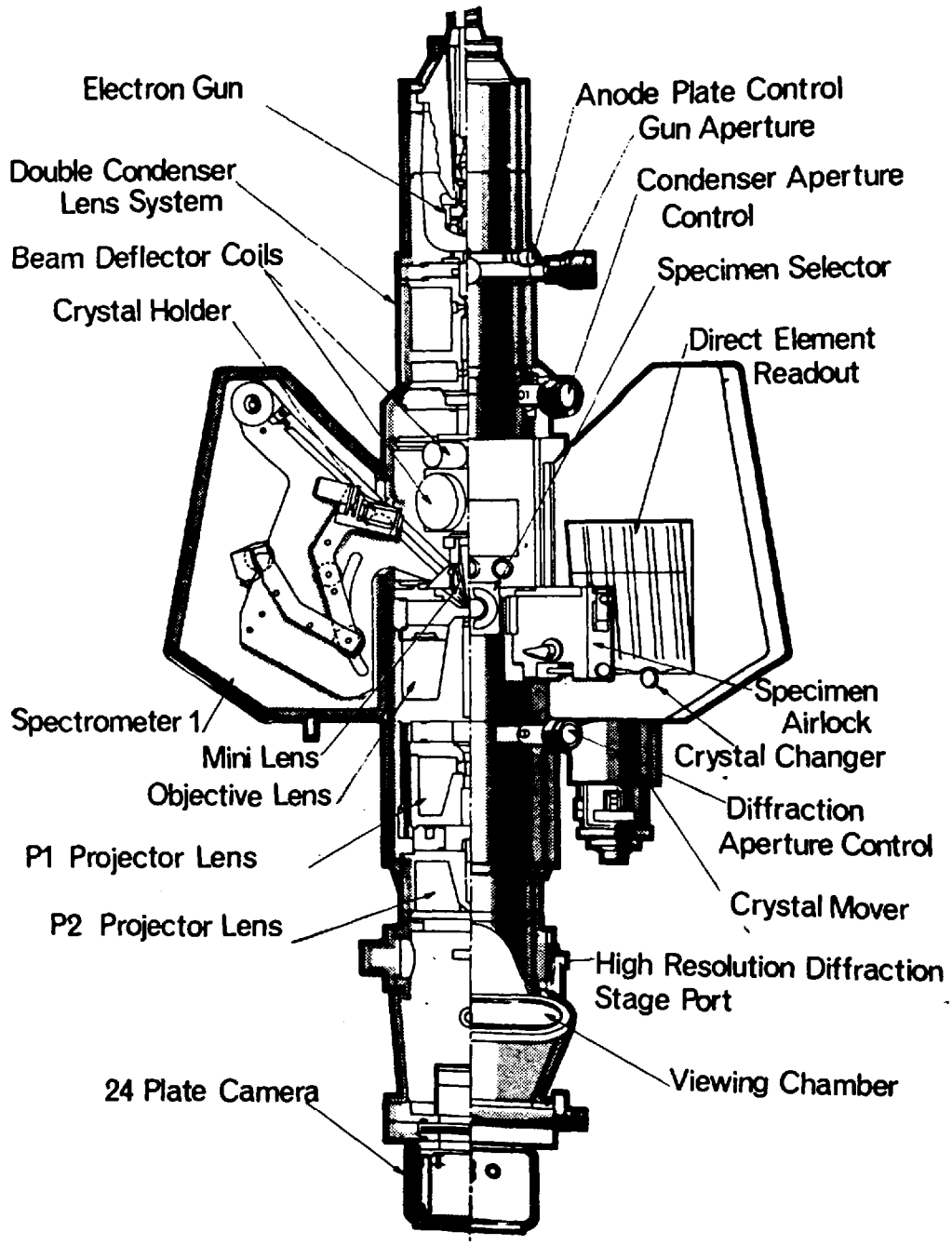

Figure 9 *Diagram of the EMMA-4 instrument.*

The ultrathin sections obtained from the "Epon" embedded material and the unembedded material (as described in section 2.2.2.6.2.) were examined in the college electron microscope to select the best sections for analysis. Detection and analysis of copper, chromium and arsenic in a number of spots throughout the different layers of the cell wall of the different cell types of wood was carried out in the "EMMA4" instrument. This was possible because by focussing the incident electron beam with the mini lens provided, a focussed beam of 2000-4000Å diameter can be produced at the specimen, where the area analysed is directly under the focussed beam. Image magnifications up to 160,000 X was possible but most of the work was carried out at 10,000 X to 25,000 X. The choice of accelerating voltage is between 40-100 KeV.

The X-rays generated by the bombardment of the specimen with the incident electron beam focussed by the mini lens are diffracted, according to Bragg's law, by LiF (420) crystals, the "d" spacing of these crystals enables wavelengths of 1Å to be accepted. The diffracted X-rays characteristic of the element present enter a gas flow counter and are finally fed to a scaler/timer where the X-rays collected are displayed as accumulated counts. The "EMMA4" is also fitted with a non-dispersive detector (NDD) mounted on the front of the column close to the specimen. This is a gas flow proportional counter which receives the complete wavelength spectrum of X-rays generated. The output from the non-dispersive detector is fed to its own scaler/timer. The counts recorded give an indication of the mass thickness of the sample being analysed. This is very useful information for the comparison of a number of characteristic counts for a particular element from similar areas of the same or different specimens where the only variable is the specimen thickness.

The counting time was usually 10 seconds. The results obtained from the non-dispersive detector(NDD), left hand and right hand spectrometer counters were recorded on tables and the relative concentrations of the different elements analysed were calculated. Sketches were drawn for the analysed areas and photographs were also taken.

By applying the last two techniques in the investigations of the preservative distribution in wood, the limitations and disadvantages of one technique were compensated by the advantages of the other technique. The clear images of the analysed areas of the specimens provided by the electron microscopes were among the best advantages of applying such techniques. The spatial resolution of 5-10 $\mathring{A}$ of the specimen image achieved in the T.E.M. is far superior to the resolution achieved in the S.E.M. which is 100-200 $\mathring{A}$ (Kent, 1975). The desired area in the specimen can be located and accurately analysed by focussing the incident electron beam with the mini lens present in the EMMA4. Positioning of the beam, which can be seen on the viewing screen, at the desired area for analysis is direct and positive, while in the S.E.M. the shape or size of the beam cannot be seen (Hall et al, 1974). Therefore this advantage besides the advantage of producing very high image contrast which clearly outlines the cell wall structures of the EMMA4 over the S.E.M. analyser were utilized in the analysis of the different spots throughout the cell wall layers, and analysis of the different types of cells (i.e., tissue distribution) was carried out in the S.E.M. analyser. The preparation of thick sections for the S.E.M. is an advantage over the difficulties involved in the preparation of thin sections for the transmission electron microscope. Thicker specimens can be viewed easily in the S.E.M. and give higher X-ray intensities

(Hall et al, 1974). While specimen thickness in the transmission electron microscope is limited (i.e. up to approximately 3000Å thick)to what can be viewed at 100 KeV. Certain elements such as sodium, potassium, calcium and arsenic volatilise in the electron beam (Kent, 1975). Arsenic was therefore usually analysed first, sometimes by exposing the concerned area to a defocussed beam for about one minute, to build up a thin layer of contamination, before focussing down the beam for analysis. This technique reduced the volatilisation losses.

PART III

EXPERIMENTAL OBSERVATIONS

3.1. Microbiological Observations

The observations made on the general condition of the wood blocks after each exposure period to the 5 species of fungi in agar and soil culture will be briefly described. The different patterns of colonization and degradation caused by the different species of fungi, in each section obtained from the transverse, radial and tangential faces of each block, will also be described. These observations were made for untreated and preservative treated wood blocks of each species of wood. The observations made on the colonization and degradation of the preservative treated wood blocks showed no visual differences between the different methods of the preservative application or the 2 strengths of the preservative solutions, therefore, the results have been grouped together and are regarded as "treated" wood.

3.1.1. Monocultures

3.1.1.1. Phialophora fastigiata

Untreated birch: Within a few weeks of incubation the whole block became grey to black in colour. Towards the later periods of incubation certain parts of the block were slightly soft but fungal decay was easily observed by the fragile nature of the degraded block which, on drying, started to fall apart into small fragments. In the culture media the blocks maintained their general shape throughout the incubation periods.

The 1 cm^3 blocks sealed with "Araldite" showed a slower rate of colonization and degradation by the fungus compared to the rapid colonization and degradation of the unsealed 0.7 cm^3 blocks.

Within the first few days of incubation the margins of the blocks were sparsely colonized by fungal hyphae and spores. At this stage, the fungal spores dominated most of the colonized regions of the blocks

(Fig. 10). They were usually present in the lumina of the fibres and vessels where some of them germinated (Figs. 11 and 12). Fungal hyphae were better established in the ray cells within the first week of incubation but spores were still present in the fibres and vessels (Fig. 13). The growth of the fungus in the 3 different agar media showed the same pattern of sporulation in the wood blocks. In the following weeks the whole block was gradually heavily colonized by the fungal hyphae with no spores present after the 3 weeks exposure period. Ray parenchyma cells and vessels were relatively more colonized than the fibres (Figs.14 and 15). Hyphae were found emerging from the ray cells towards the adjacent fibres (Fig.16). Wall penetration from one cell to the other was a common phenomenon by typical stainer type constrictions. A fungal hypha growing in the cell lumen of one cell forms a transpressorium (Liese, 1970a) close to the cell wall, the hypha penetrates the wall by means of a thin constricted hypha to the adjacent cell lumen where it returns to its normal size. A fungal hypha may penetrate a considerable number of fibre walls (Fig.17) or stop in the S2 layer of a fibre and degrade this layer leading to the formation of diamond-shaped cavities typical of soft rot (Fig.18). The process of wall degradation was clearly demonstrated in the early periods of incubation. The fungal hypha from the cell lumen penetrates the S3 layer horizontally and then turns at right angles and branches at 180^{o} to establish itself in the S2 layer of the cell wall forming a typical soft-rot T-branch (Fig.19). This starts degrading the wall leading to the formation of a diamond-shaped cavity with the hypha inside this cavity (Fig.20). A single hypha forms a chain of cavities and with progress of the decay these cavities become wider leading to the formation of a large continuous cavity in the wall (Fig.21). Ray parenchyma cells were heavily

degraded by the fungus and more rapidly than the fibres. This can be clearly demonstrated by the lack of birefringence in these cells when viewed under polarised light (Figs.21,22,23 and 24). Throughout the exposure periods certain parts of the blocks were only slightly degraded, if at all,especially in the deeper parts of the block.

Treated birch: Towards the later periods of incubation the wood blocks became grey to black in colour. The blocks were, macroscopically, sound even after the 12 weeks incubation period with the exception of certain outer parts which showed slight fragmentation.

The regions towards the surface of the block were slightly colonized by the fungal hyphae within the first few weeks and it was not before 6 weeks of exposure that scattered sparse colonization was observed throughout the block. The fibres and vessels were mostly colonised at the early periods of incubation. Rays, compared to the untreated blocks, were sparsely colonized and then only at a very late stage of incubation. After 9 and 12 weeks of incubation heavier colonization was observed throughout the block. It was still variable from one region to another but a few cells showed no fungal presence especially in the rays of the deeper parts of the block. After 6 weeks slight degradation of the fibres was observed which became heavier in the following weeks of incubation. The ray cells, compared to the treated fibres, showed very much less degradation (Fig.25). This is clearly demonstrated in the pictures obtained with polarised light (Figs.26 and 27), where birefringence is shown in the rays compared to the heavily degraded adjacent fibres. Active wall penetration through the cell walls by thin hyphal protrusions was also common in the treated blocks.

Figure 10. T.L.S. untreated birch, showing spores of *P.fastigiata* after 3 days exposure (x 520)

Figure 11. R.L.S. untreated birch, showing germinated spores of *P.fastigiata* after 3 days exposure (x 800)

Figure 12. T.L.S. untreated birch, as figure 11 (x 800)

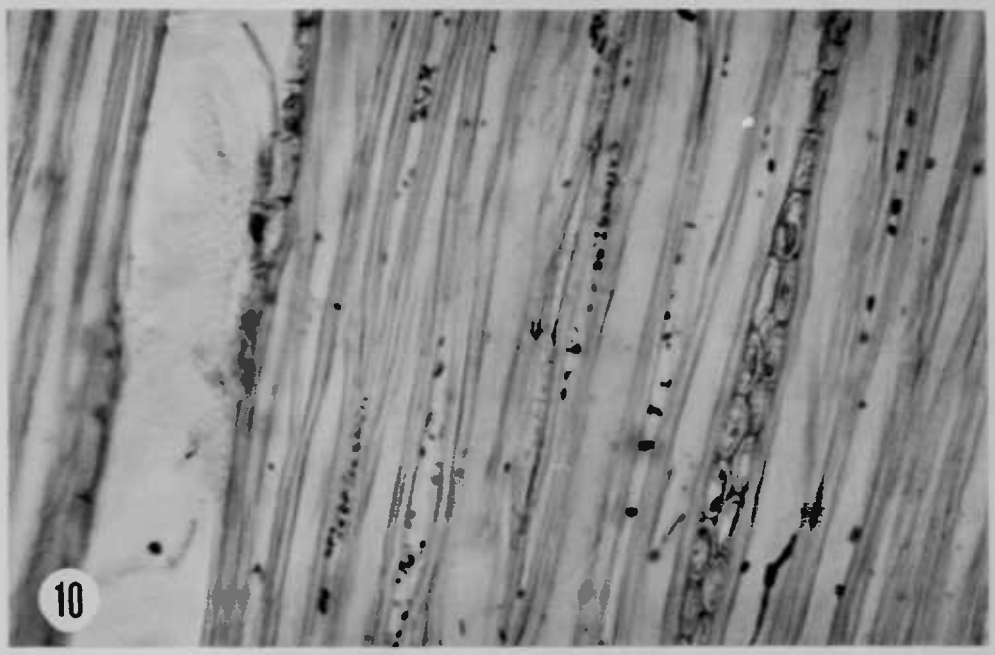
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Figure 13. R.L.S. untreated birch, showing hyphal development in the rays at an earlier stage than in the fibres where there are only spores of P.fastigiata after 5 days exposure (x 860)

Figure 14. T.L.S. untreated birch, showing heavy colonisation of rays and vessels by P.fastigiata after 1 week exposure (x 140)

Figure 15. R.L.S. untreated birch, showing heavy colonisation of all tissues by P.fastigiata after 1 week exposure (x 140)


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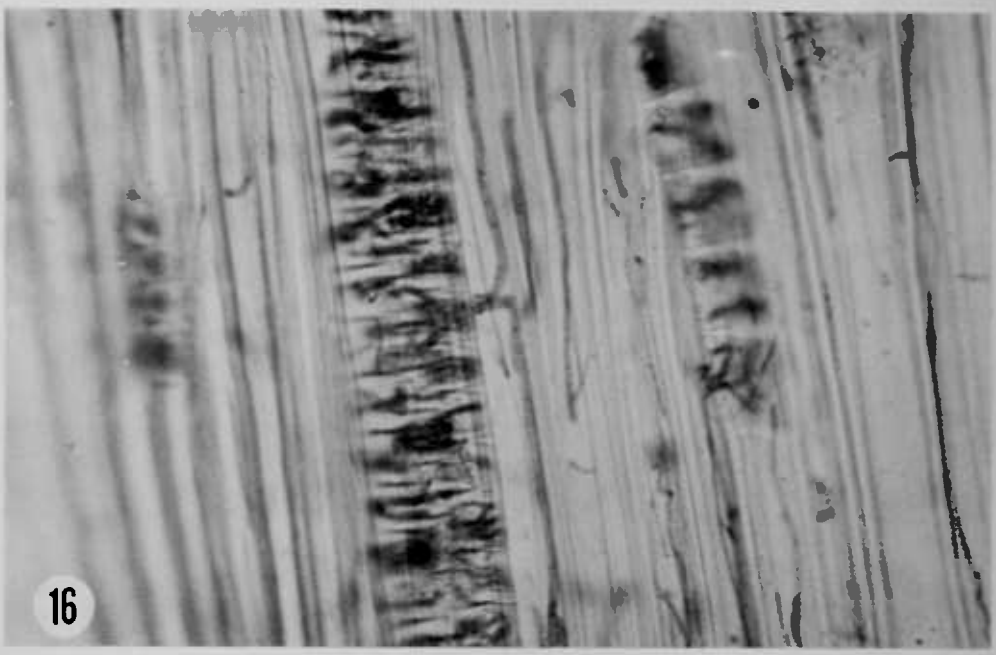
Figure 16. P.fastigiata in untreated birch (R.L.S.) after 2 weeks exposure showing hyphae emerging from the ray cells to the adjacent fibres (x 800)

Figure 17. P.fastigiata in untreated birch (R.L.S.) behaving as a stainer in crossing the fibre walls by thin constricted hyphae (x 520)

Figure 18. P.fastigiata in untreated birch (R.L.S.) showing wall penetration and cavity formation in the same fibre wall. Note the formation of a transpressorium prior to wall penetration by the thin constricted hypha (x 800)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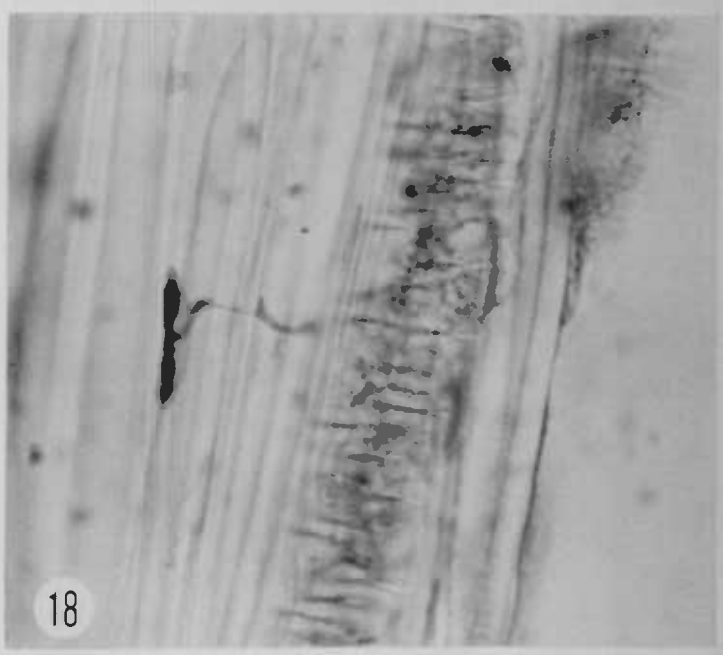
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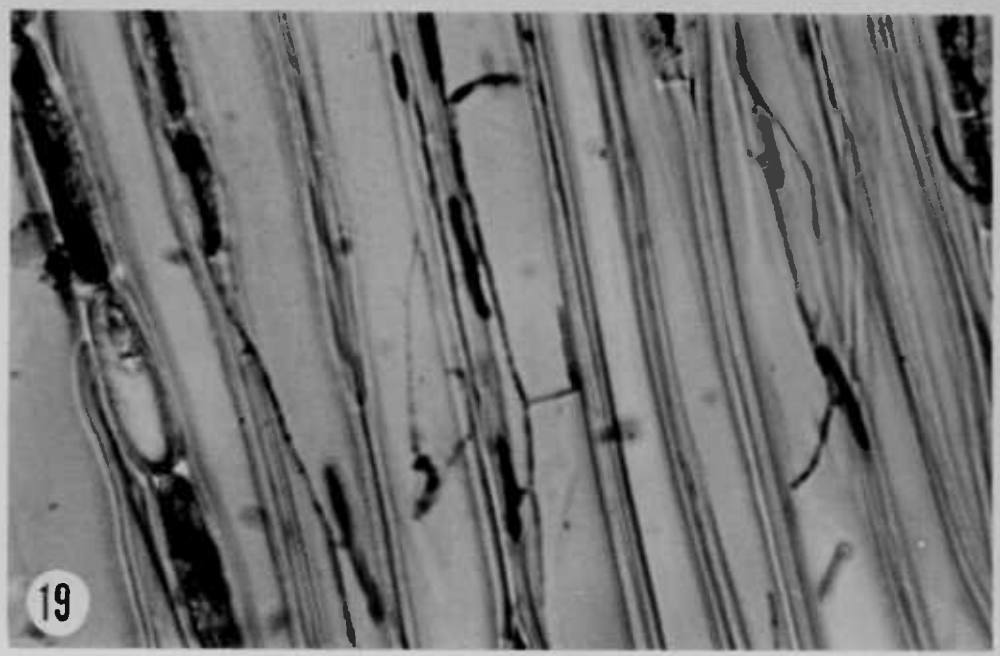
Figure 19. P.fastigiata in untreated birch (R.L.S.) showing typical soft-rot T-branches (x 520)

Figure 20. P.fastigiata in untreated birch (R.L.S.) showing typical soft-rot cavities (x 520)

Figure 21. R.L.S. untreated birch, showing the heavy degradation of fibres, rays and vessels by P.fastigiata after 3 weeks exposure. Polarised light (x 610)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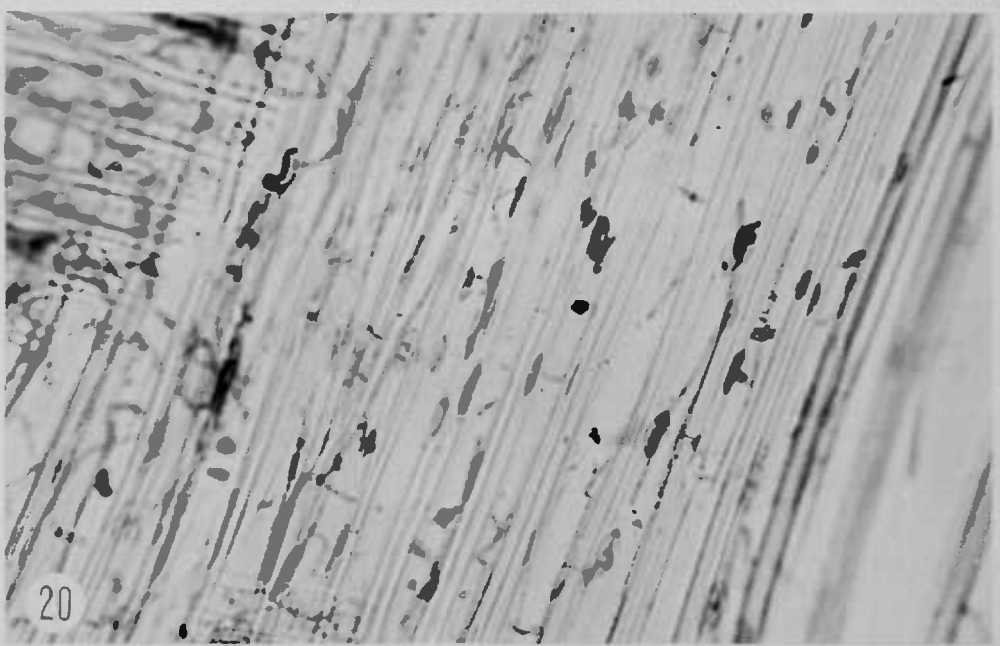
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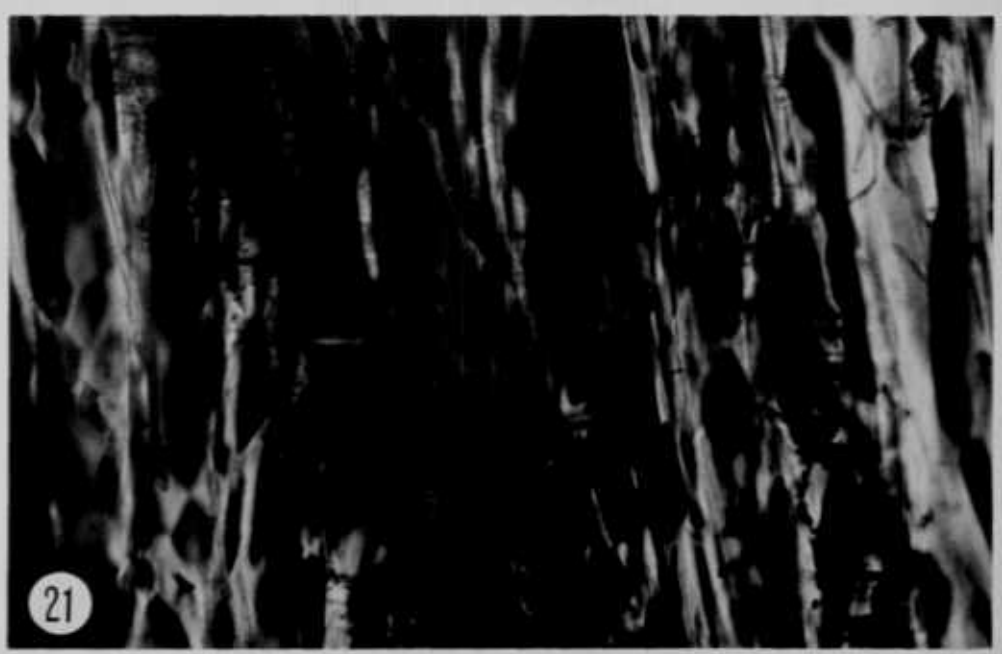
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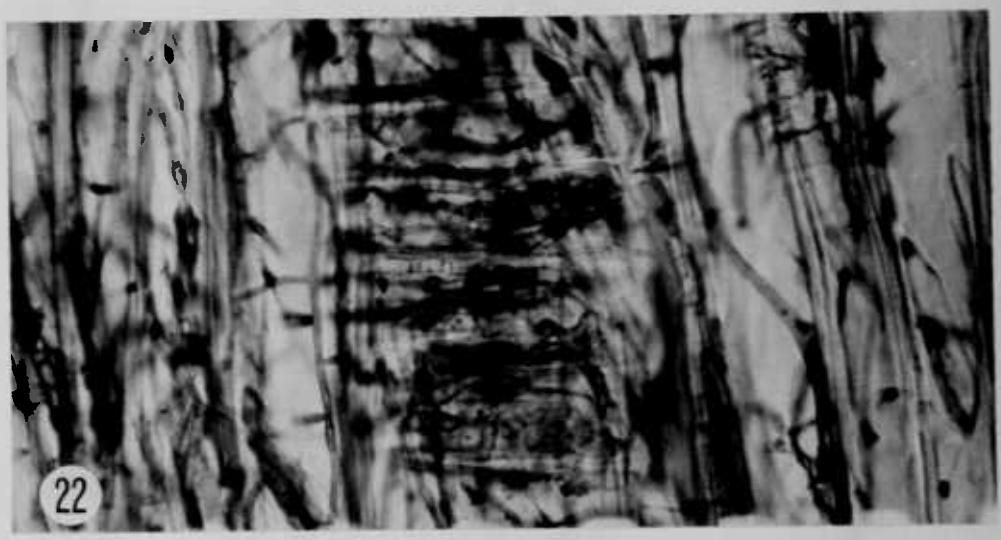
Figure 22. R.L.S. untreated birch, showing the complete destruction of all tissues by P.fastigiata after 3 weeks exposure (x 610)

Figure 23. As figure 22 but under polarised light. Note the complete lack of birefringence in the whole region of the rays (x 610)

Figure 24. R.L.S. untreated birch, showing the earlier destruction of the rays than the fibres by P.fastigiata after 2 weeks exposure. Polarised light (x 160)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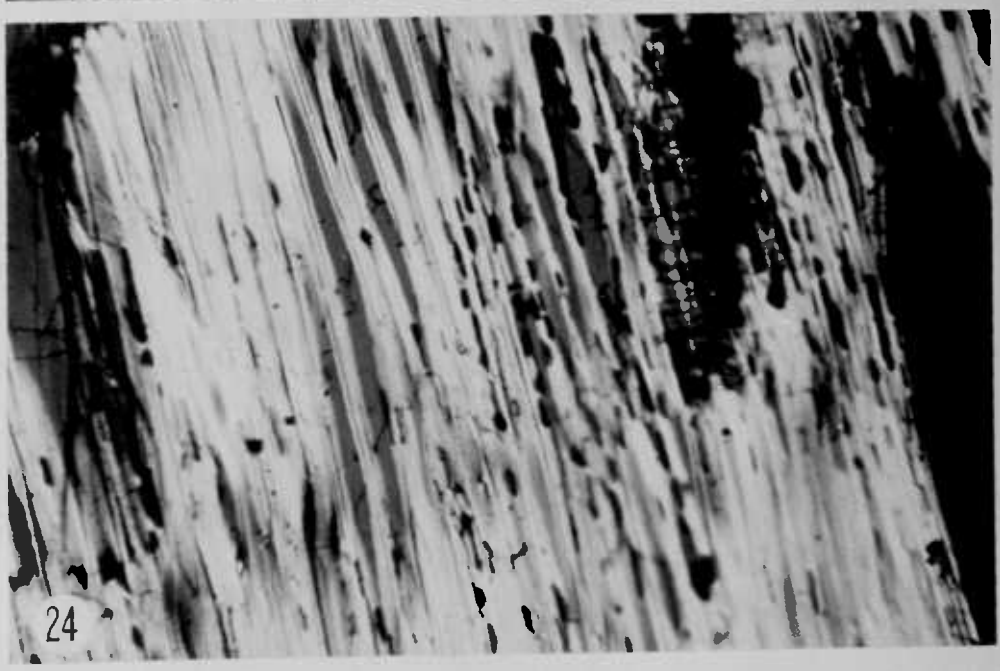
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Figure 25. P.fastigiata in treated birch (R.L.S.) after 9 weeks exposure (x 160)

Figure 26. P.fastigiata in treated birch (R.L.S.) after 9 weeks exposure showing the heavy degradation of fibres but not the rays. Note the birefringence of the rays under polarised light (x 610)

Figure 27. As figure 26 but after 12 weeks exposure (x 610)


25


26


27

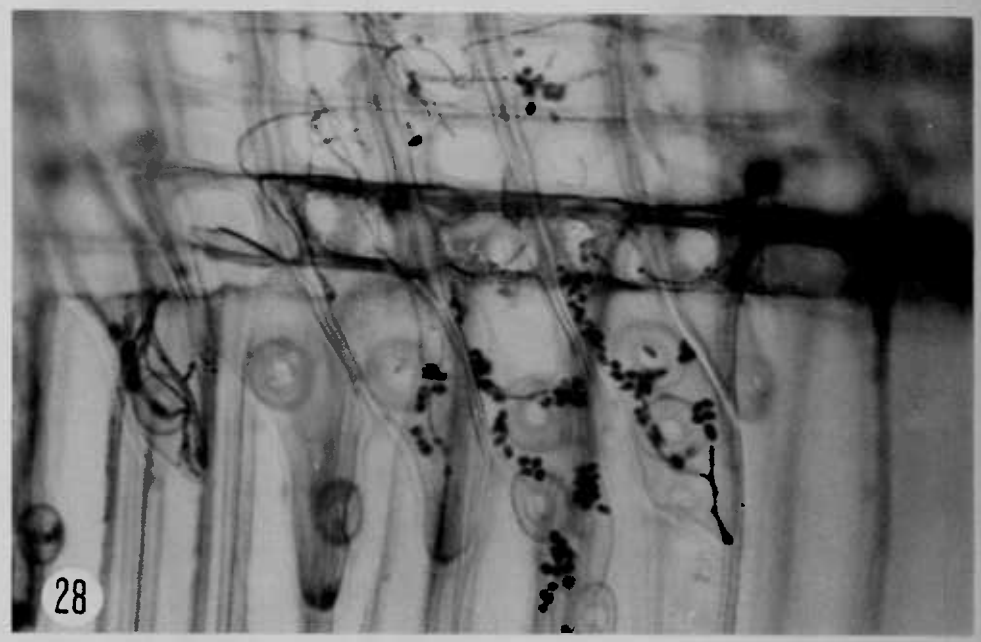
Untreated Scots pine: Towards the later periods of incubation the blocks became slightly pale grey in colour. There was no sign of decay observed by the naked eye and the whole block looked sound.

As in the case of birch, within the first week of incubation spores were mostly present with little sign of fungal hyphae (figs.28 and 29). These were also observed at a later period beside the fungal hyphae (Fig.30). They were present in the ray parenchyma and tracheids and some of them germinated into hyphae. Within the first week colonization was sparse and scattered throughout the block. This became heavier at the later periods of incubation. The rate of colonization in the early-wood was less than the latewood zones. Hyphae were present in the resin canals, rays and tracheids. They were mostly behaving as typical stainer fungi by constricted crossing of the cell walls to adjacent tracheids (Figs.31,32 and 33). This was taking place in such a way that the hypha present in the cell lumen formed a branch which turned towards the wall and formed a thin protrusion which penetrated the wall to the adjacent cell lumen. In this cell the hypha retuned to its normal size, crossed the width of the tracheid towards the next wall where the penetration process was repeated and so on in the next cell (Fig.34). Wall penetration was also observed through the bordered-pits and hyphae were also found emerging from the ray cells to the adjacent tracheids (Fig.35). After 5 weeks certain parts of the blocks were slightly degraded especially in the latewood zones. Ray cells were more heavily degraded than the tracheids. As in the case of the birch fibres, the wall degradation of the tracheids was through the formation of diamond-shaped soft-rot cavities which became continuous and heavier in certain walls leading to the whole destruction of the S2 layer (Fig.36).

Figure 28. R.L.S. untreated Scots pine showing spores of *P.fastigiata* in rays and tracheids after 1 week exposure (x 610)

Figure 29. As figure 28 (x 610)

Figure 30. T.L.S. untreated Scots pine, showing germinated spores of *P.fastigiata* after 2 weeks exposure (x 610)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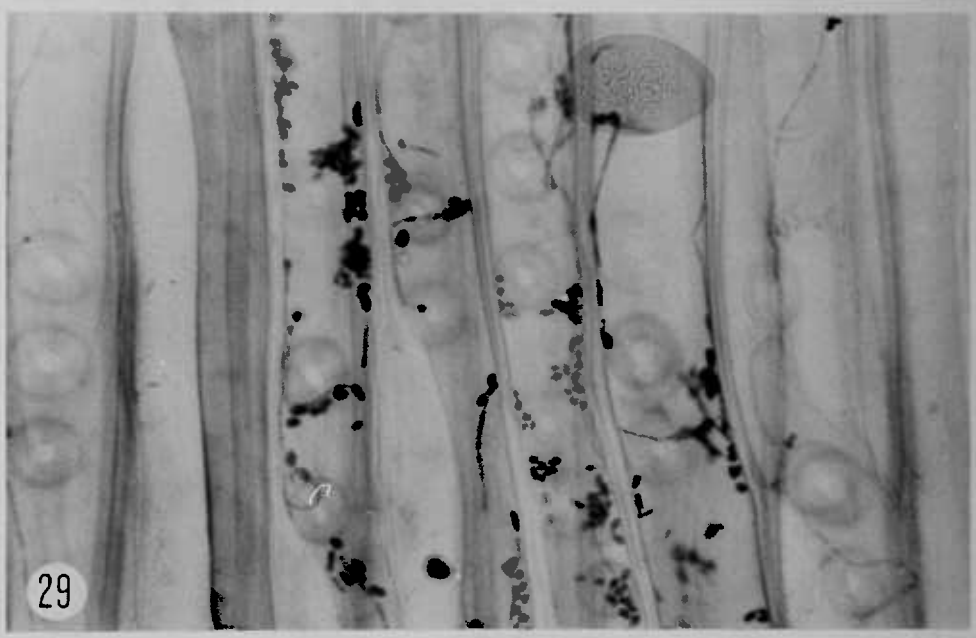
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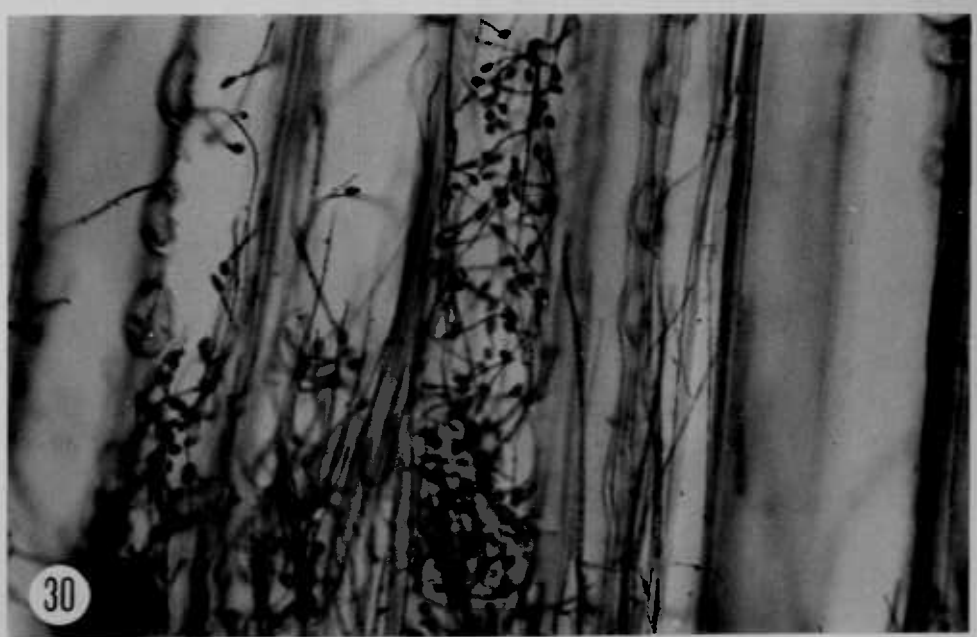
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Figure 31. P.fastigiata in untreated Scots pine (R.L.S.) after 4 weeks exposure showing typical stainer habit in wall crossing (x 520)

Figure 32. As figure 31 (x 520)

Figure 33. As figure 31 (x 800)


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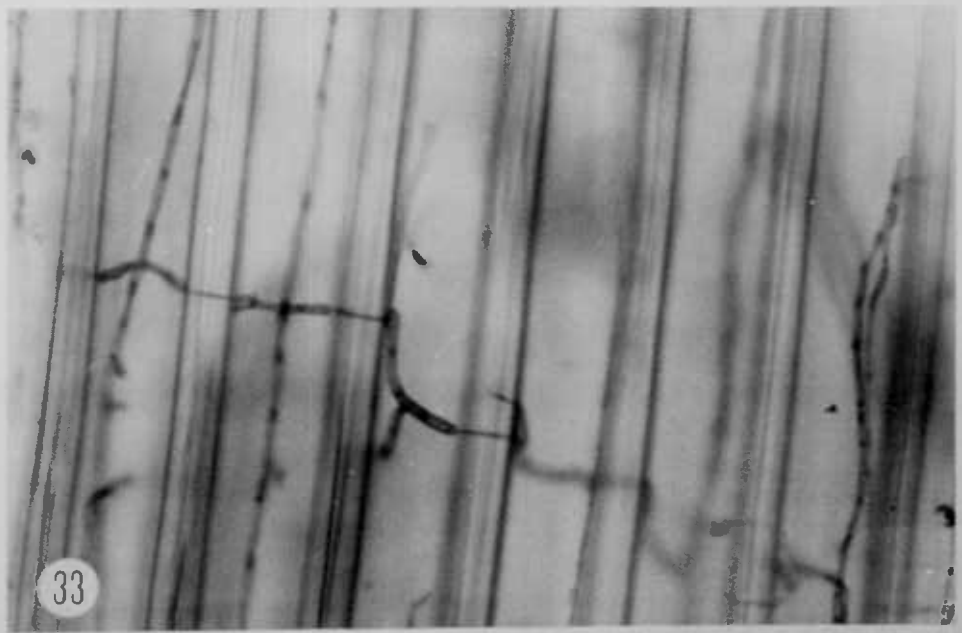
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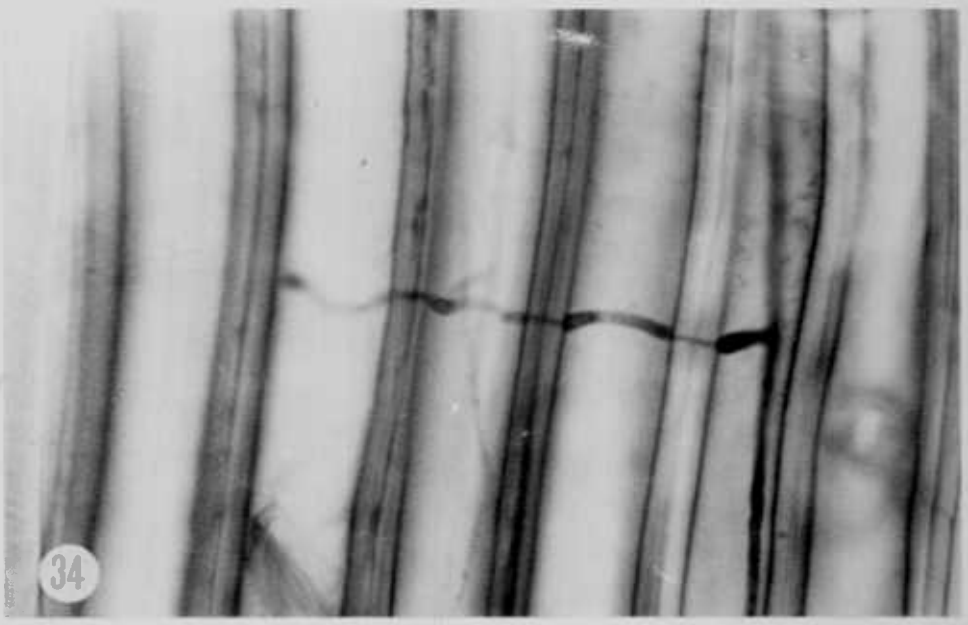
Figure 34. P.fastigiata in untreated Scots pine (R.L.S.) showing a single hypha crossing several tracheid walls as a stainer (x 800)

Figure 35. P.fastigiata in untreated Scots pine (T.L.S.) showing wall penetration via the bordered-pits (x 800)

Figure 36. P.fastigiata in untreated Scots pine (R.L.S.) showing typical soft-rot cavities after 5 weeks exposure (x 610)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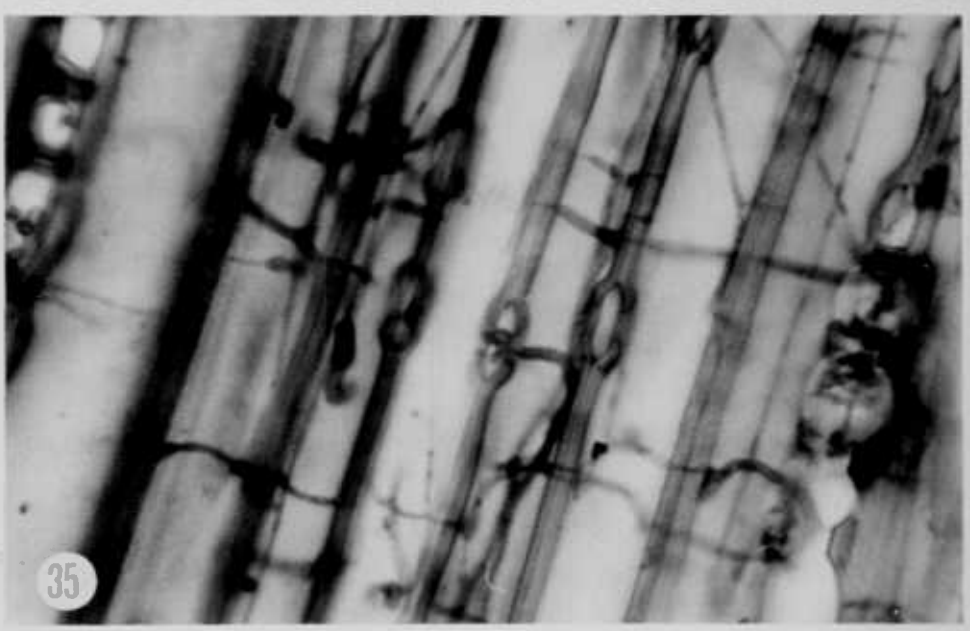
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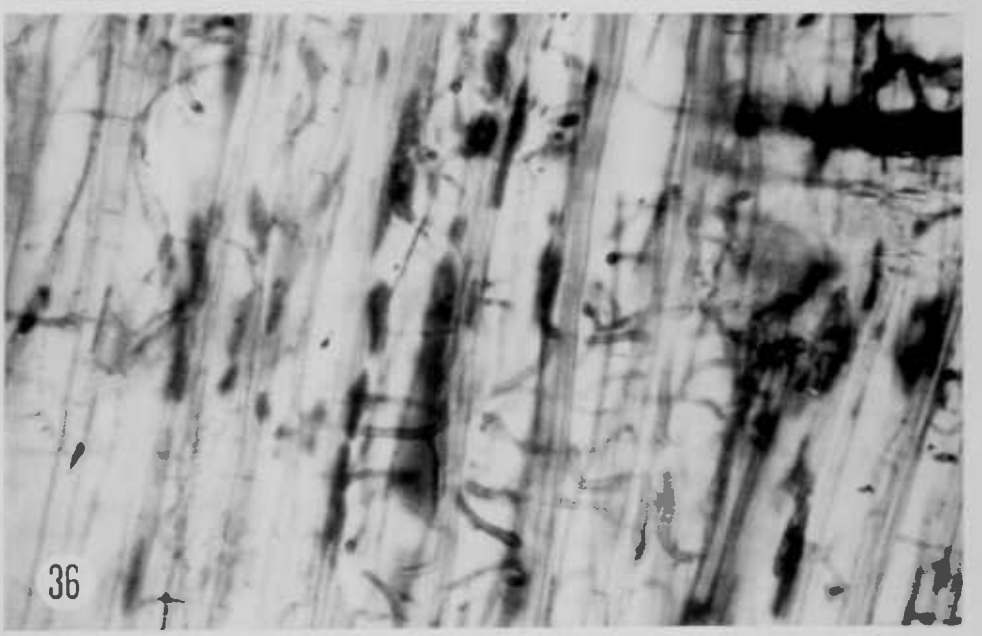
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Figure 37. P.fastigiata in treated Scots pine (R.L.S.) showing slight colonisation mainly by spores but no signs of degradation after 12 weeks exposure (x 610)

Figure 38. As figure 37 (x 610)


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38

Treated Scots pine: The general appearance of the blocks was sound. There was no change in the colour of the blocks even after 12 weeks of incubation.

For the first 3 weeks of exposure the blocks were free from any fungal hyphae or spores. After this period of time a few weak hyphae and clumps of spores were observed randomly colonizing the outer parts of the blocks. This continued to be the case for the remainder of the exposure period (Figs.37 and 38) and was true for both earlywood and latewood zones. Spores were found more often than fungal hyphae but no germination was observed. Wall crossing was not observed, neither directly through the wall nor through the bordered pits. Ray parenchyma cells and tracheids showed no signs of decay.

In general, P.fastigiata mostly behaved as a stainer in the case of untreated Scots pine and as a soft-rot stainer in the case of untreated and treated birch. The presence of the preservative considerably delayed the colonization and degradation of birch blocks. The rays of treated birch showed a much greater resistance to attack than the fibres. The treated blocks of Scots pine were very resistant to both colonization and decay by the fungus.

3.1.1.2. Chaetomium globosum

Untreated birch: Within the first week of exposure the whole block was densely surrounded by the fungus which formed a spongy-like layer on its surface, and became darker in colour. Towards the later periods of incubation the blocks were heavily decayed to the extent of disintegration. They were easily broken down into fragments by applying slight pressure on them. Sections were very difficult to obtain from such blocks.

The fungus rapidly colonized and degraded most parts of the block. After 3 days colonization was sparse throughout the block with more hyphae in the rays than the fibres (Fig.39) or the slightly colonized vessels. Rays and fibres were also slightly decayed in certain parts of the block. After one week fungal colonization was heavy throughout the block (Fig.40). Fungal spores were also present mostly near the margin of the blocks but rarely in the deeper parts of the block (Fig.41). These were also observed after longer exposure periods. The rapid colonization of the block was followed by degradation of certain parts of the block. This was heavier towards the outer and lower parts of the block than nearer the centre. Rays were more heavily decayed than the fibres or vessels (Fig.42). Hyphae were observed degrading the rays by forming cavities which joined together causing the destruction of the whole ray at a later stage (Fig.43). Fibres were degraded by forming typical soft-rot cavities within the S2 layer of the cell walls which can be clearly seen in the transverse sections (Fig.44). The vessel walls were found to be more resistant to attack after the first week of exposure (Fig.45). After 3 weeks of exposure most of the block was heavily degraded. Rays were, more or less, completely destroyed, fibres maintained their shape only by the presence of the highly resistant middle lamella regions. Most vessel walls were also heavily decayed (Fig.46). With the progress of decay the blocks were severely attacked by the fungus and all tissues were considerably decayed (Figs.47 and 48).

Treated birch: Certain parts of the blocks, especially the lower and outer parts, were slightly fragmented towards the end of the period of exposure. They maintained their general shape and gradually become darker in colour. Hyphae did not completely surround the whole block as in the case of the untreated birch, even after 12 weeks of exposure.

Figure 39. C.globosum in untreated birch (R.L.S.) after 3 days exposure showing more hyphae present in the rays than the fibres (x 520)

Figure 40. C.globosum in untreated birch (R.L.S.) after 1 week exposure showing heavy colonisation of all tissues (x 140)

Figure 41. R.L.S. untreated birch, showing spores of C.globosum after 1 week exposure near the margin of the block (x 520)


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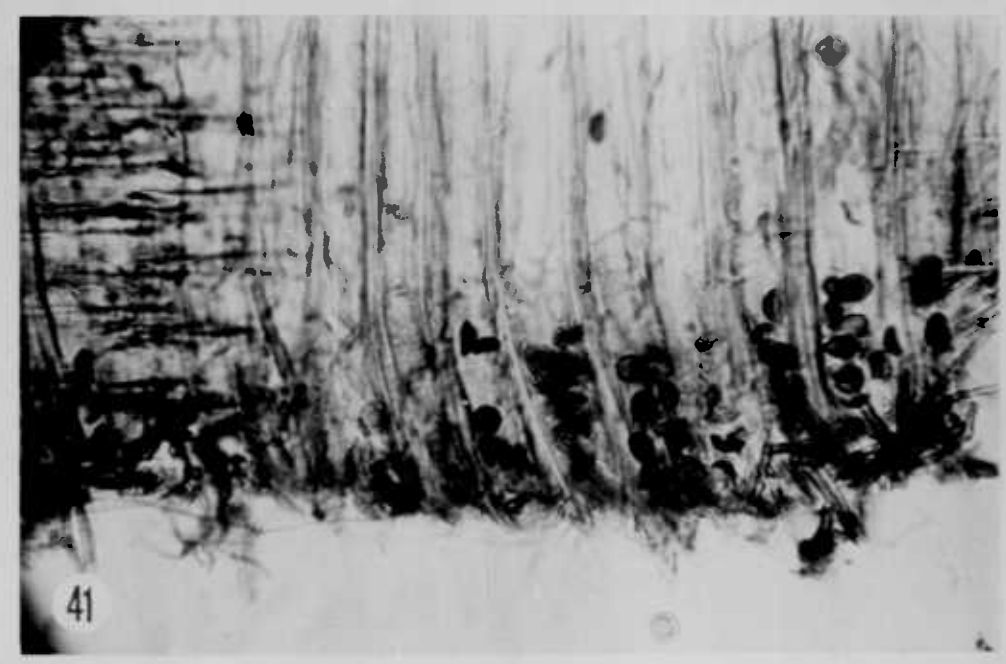
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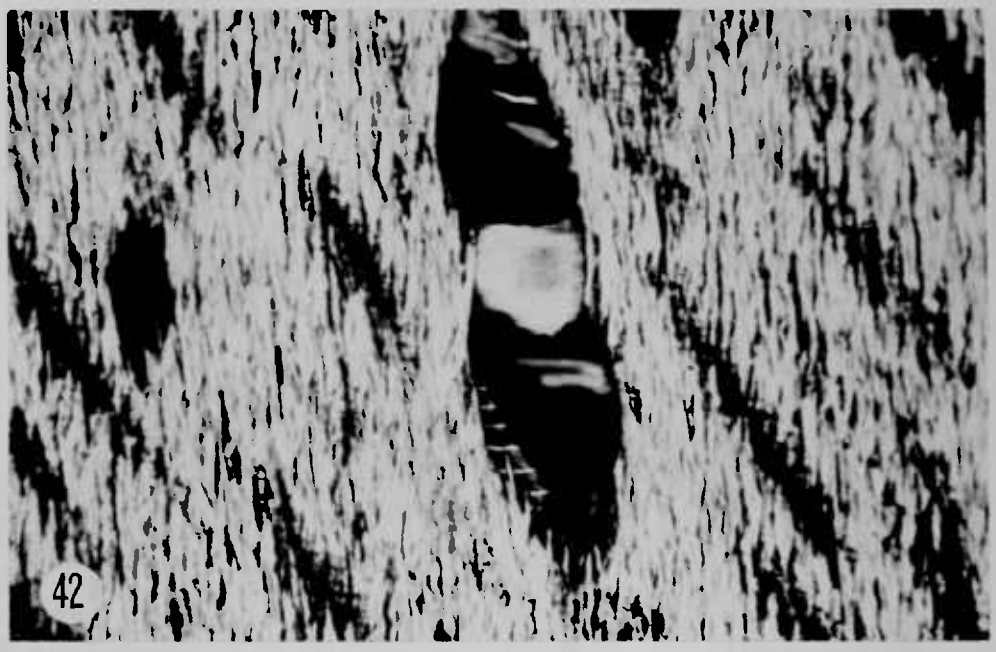
Figure 42. C.globosum in untreated birch (R.L.S.) showing the heavy degradation of rays compared to the less degraded fibres after 1 week exposure. Note the absence of birefringence in the ray regions under polarised light (x 140)

Figure 43. As figure 42. Note the destruction of the rays by cavity formation. Polarised light (x 520)

Figure 44. C.globosum in untreated birch (T.S.) showing cavity formation in the S2 layer of the fibres after 1 week exposure (x 950)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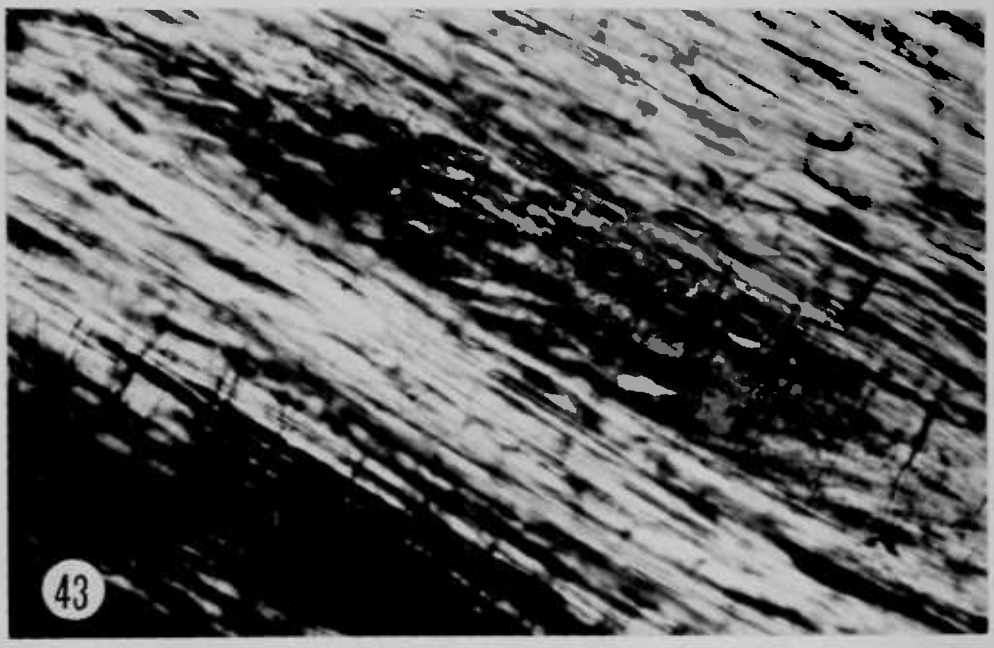
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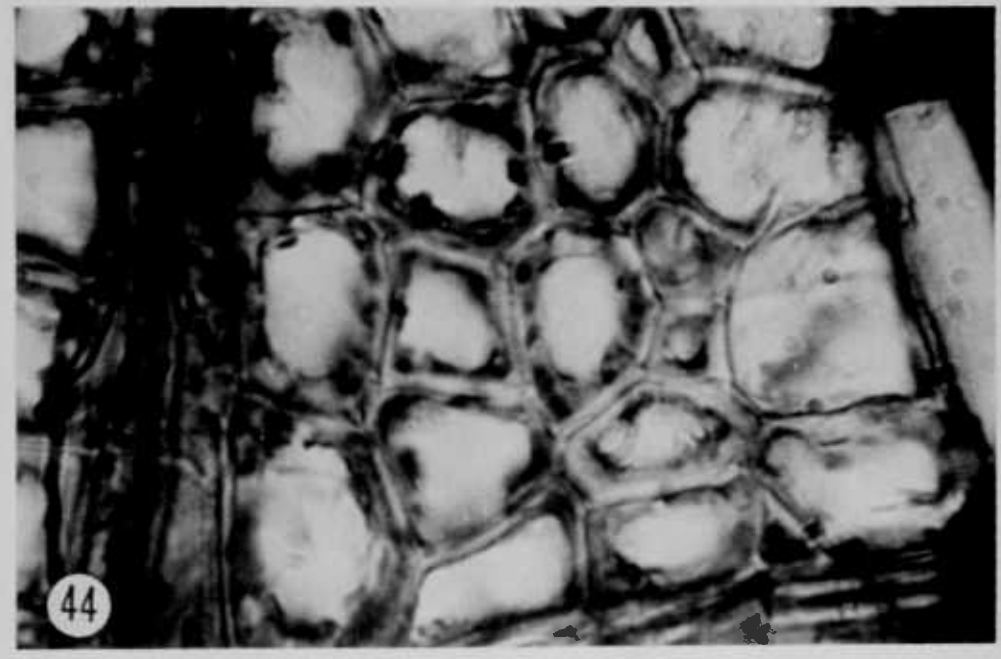
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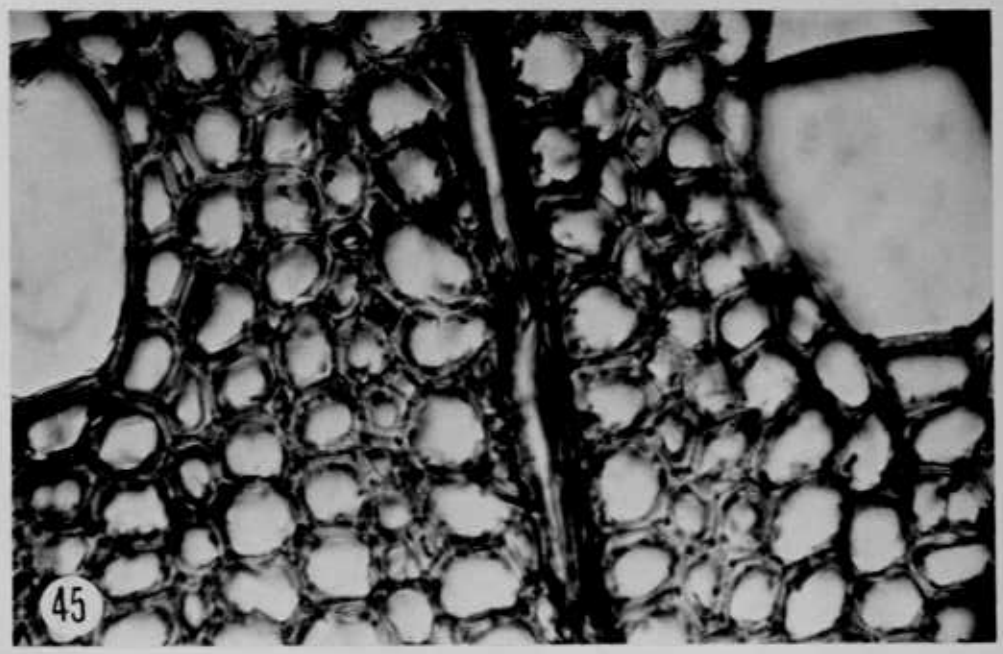
Figure 45. C.globosum in untreated birch (T.S.) showing soft-rot cavities in the S2 layer of the fibre walls after 1 week exposure (x 520)

Figure 46. C.globosum in untreated birch (T.S.) showing heavy degradation of all tissues after 3 weeks exposure. Fibres maintained their shape by the presence of the highly resistant middle lamella regions (x 520)

Figure 47. C.globosum in untreated birch (R.L.S.) showing heavy degradation by cavity formation after 6 weeks exposure. Polarised light (x 520)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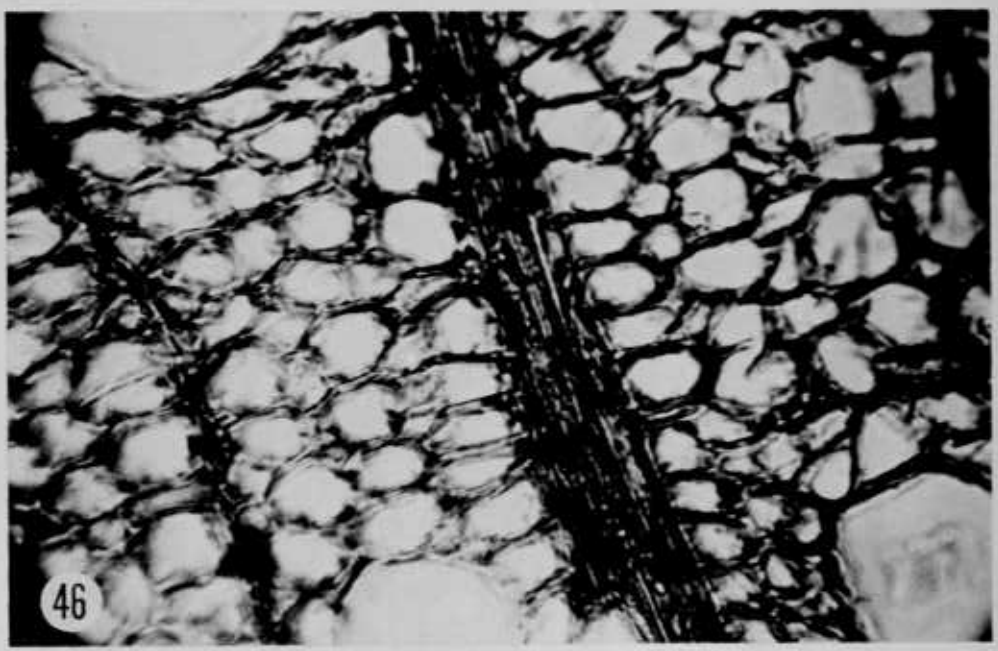
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The presence of the preservative in the blocks delayed the fungal invasion to the third week of exposure where the margins of the blocks were sparsely colonized by the fungal hyphae. This was mostly in the lower parts of the block. Hyphae were very few in scattered cells and usually found in the fibres or vessels but very rare in the ray cells. After 6 weeks exposure colonization had progressed throughout the block. This was sparse in the deeper parts of the block and heavier towards the surface and lower part. Rays and vessels were only slightly colonized by comparison with the heavily colonized adjacent fibres in certain zones of the block. Hyphae in the fibres were observed forming cavities in the S2 layer which led to the destruction of this layer of the wall, but the adjacent rays were not decayed (Figs.49 and 50). After 9 to 12 weeks exposure fungal hyphae progressively increased throughout the block mostly in the fibres but occasionally slightly in the rays or vessels. All tissues in the lower parts of the block were heavily decayed. Towards the centre of the block rays and vessels were more resistant to colonization and decay than the severely degraded adjacent fibres (Fig.51) which suggests that the rays and vessels were colonized and degraded at a later stage than the fibres. Spores were rarely present in the blocks at any time, but when present they were usually near the face exposed to the fungus.

Untreated Scots pine: After a few weeks of exposure the fungus slightly surrounded the whole block. This became heavier with time and the block gradually became darker in colour. With the progress of decay the latewood zones were easily broken down into fragments and separated from the more resistant earlywood zones.

After 3 days exposure fungal colonization was sparsely scattered throughout the block. Hyphae were present in all tissues of the

earlywood and latewood zones with more hyphae in the rays than the resin canals or tracheids (Fig.52). Wall crossing from one cell to the other was through the bordered-pits and hyphae were also found emerging from rays to the adjacent tracheids. Rays were slightly degraded and the attack was initiated in the tracheid wall where the fungal hypha in the cell lumen formed a protrusion which penetrated the S3 layer of the secondary wall to the S2 layer where decay began (Fig.53). Hyphae were not observed crossing the whole width of the wall to the adjacent cell lumen as in the case of P.fastigiata even at the later periods of exposure. Within the first few weeks heavy fungal colonization was building up throughout the block. The latewood tracheids were rapidly and more severely degraded than the earlywood tracheids. The fungus degraded the S2 layer of the tracheid wall through the formation of typical diamond-shaped cavities which at a later etage of attack joined together leading to the complete destruction of this layer (Fig.54). After one weeks' exposure cavities were formed in the S2 layer of the wall which appeared, as small holes in the transverse sections (Fig.55). With the progress of decay these cavities became larger and joined together causing the destruction of the whole S2 layer (Fig.56). After 6 weeks exposure fungal attack became heavier and the highly resistant S3 layer of certain walls could be clearly seen (Figs.57 and 58). Rays of both earlywood and latewood zones were also severely degraded (Fig.59).

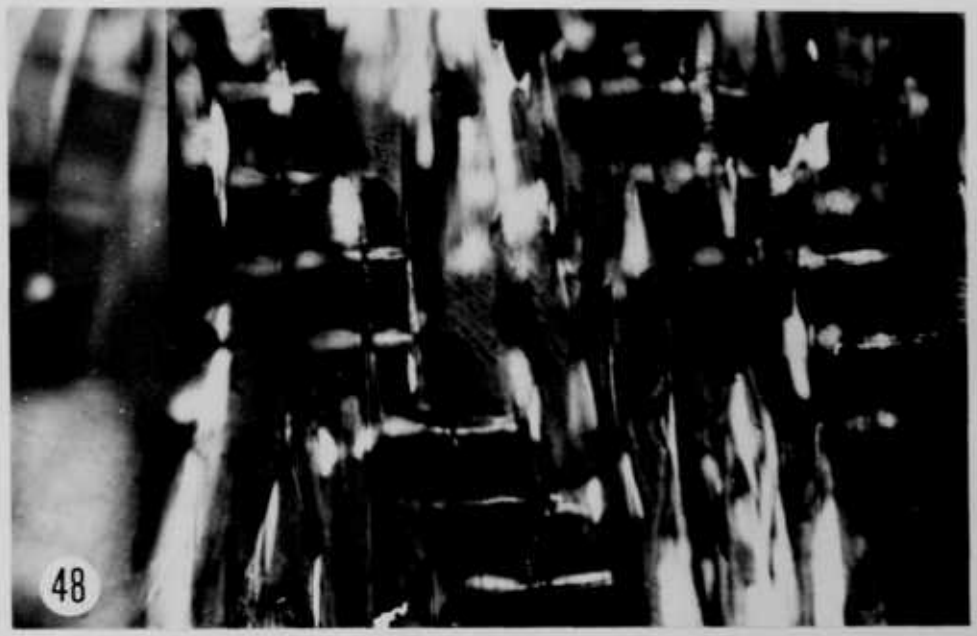
Treated Scots pine: Throughout the exposure period the wood blocks were sound and no change in colour or signs of decay were observed.

The treated blocks were highly resistant to both colonization and degradation even after 12 weeks of incubation. Colonization was sparse and no signs of decay was observed. After 6 weeks exposure the lower parts of the block were slightly colonized by the fungus, but this did

Figure 48. C.globosum in untreated birch (R.L.S.) showing complete destruction of the raye and other tissues after 6 weeks exposure. Polarised light (x 520)

Figure 49. C.globosum in treated birch (T.S.) showing the decay of fibres but not the rays after 6 weeks exposure (x 610)

Figure 50. R.L.S. treated birch showing heavy decay of fibres by C.globosum after 6 weeks exposure but rays showing more resistance to decay (x 610)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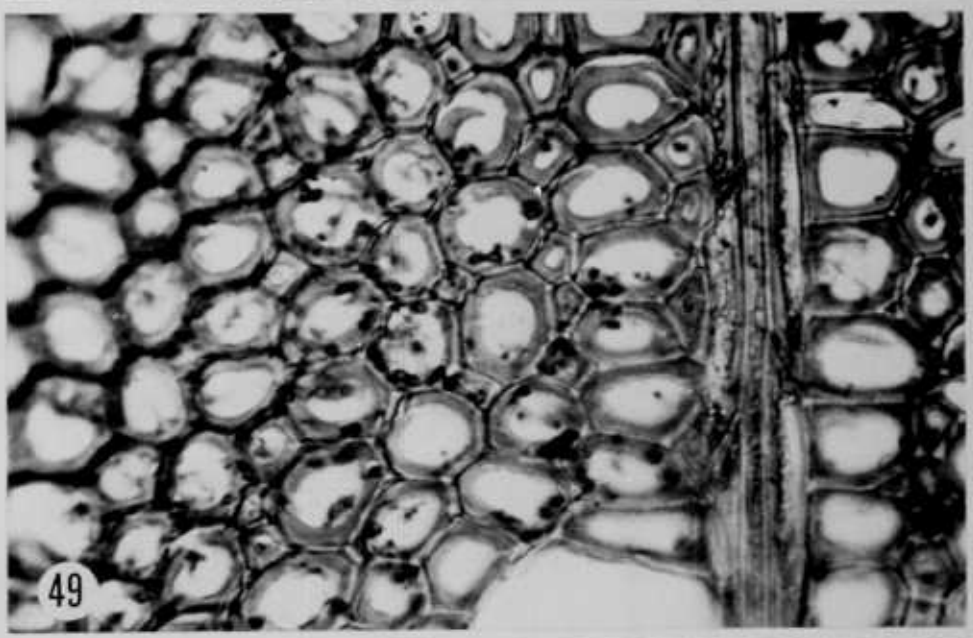
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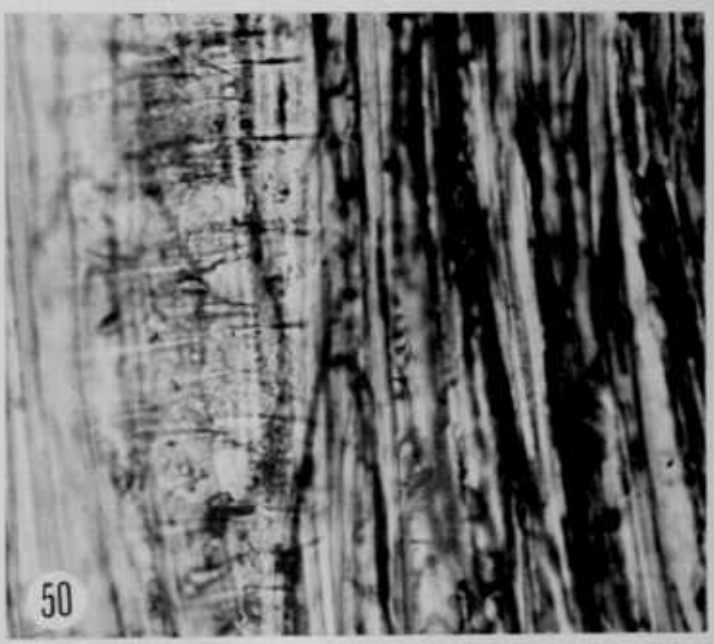
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Figure 51. R.L.S. treated birch showing heavy degradation of fibres compared to the less degraded rays by C.globosum after 12 weeks exposure (x 610)

Figure 52. R.L.S. untreated Scots pine showing colonisation of rays by C.globosum after 3 days exposure (x 520)

Figure 53. C.globosum in untreated Scots pine showing initiation of wall penetration (x 1300)

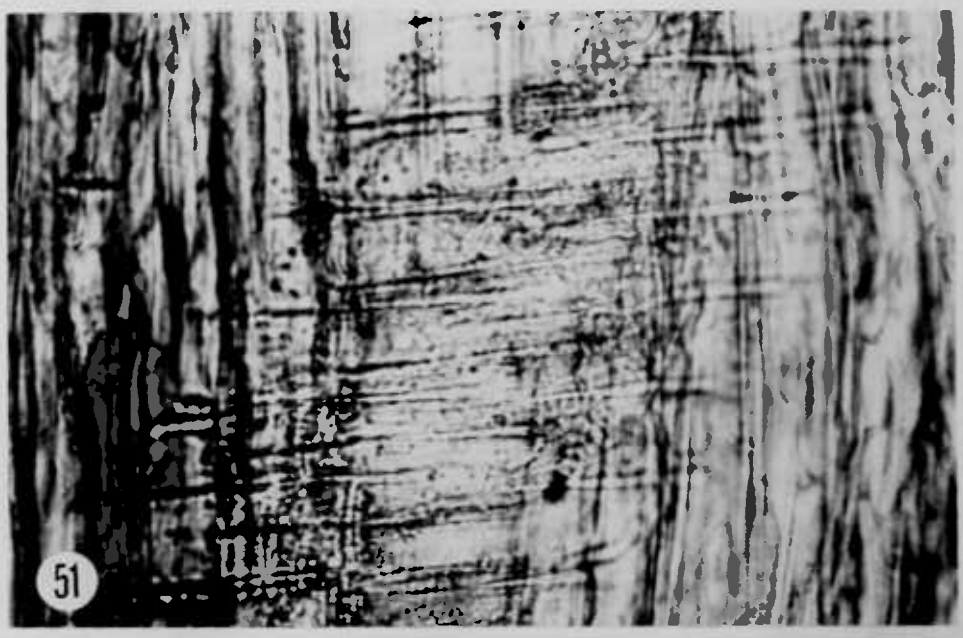
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Figure 54. C.globosum in untreated Scots pine (R.L.S.) showing typical soft-rot cavities (x 610)

Figure 55. C.globosum in untreated Scots pine (T.S.) showing initiation of attack by cavity formation in the S2 layer of the latewood tracheids after 1 week exposure (x 610)

Figure 56. As figure 55 but an advanced stage of attack after 3 weeks exposure (x 610)


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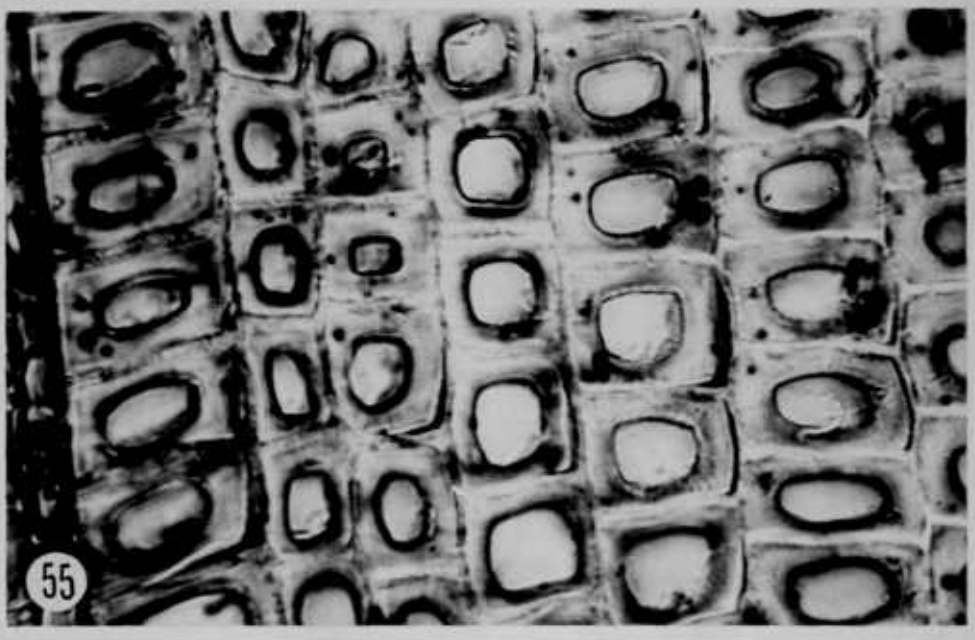
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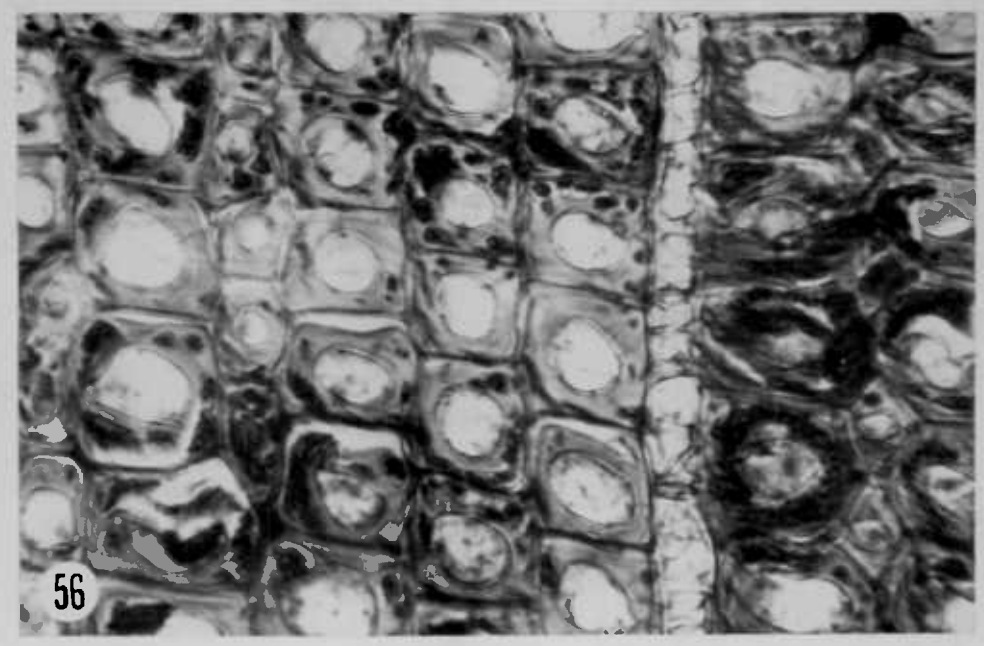
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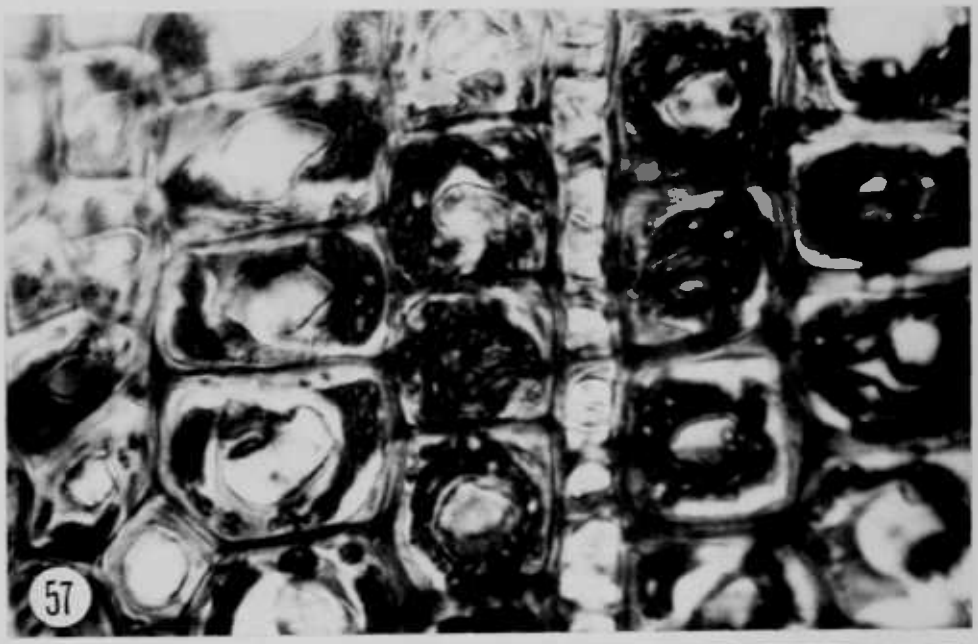
Figure 57. C.globosum in untreated Scots pine (T.S.) showing complete destruction of the S2 layer of the tracheid walls after 6 weeks exposure. Note the presence of the highly resistant S3 layer (x 610)

Figure 58. As figure 57 (x 610)

Figure 59. R.L.S. untreated Scots pine showing heavy degradation of rays and tracheids (x 610)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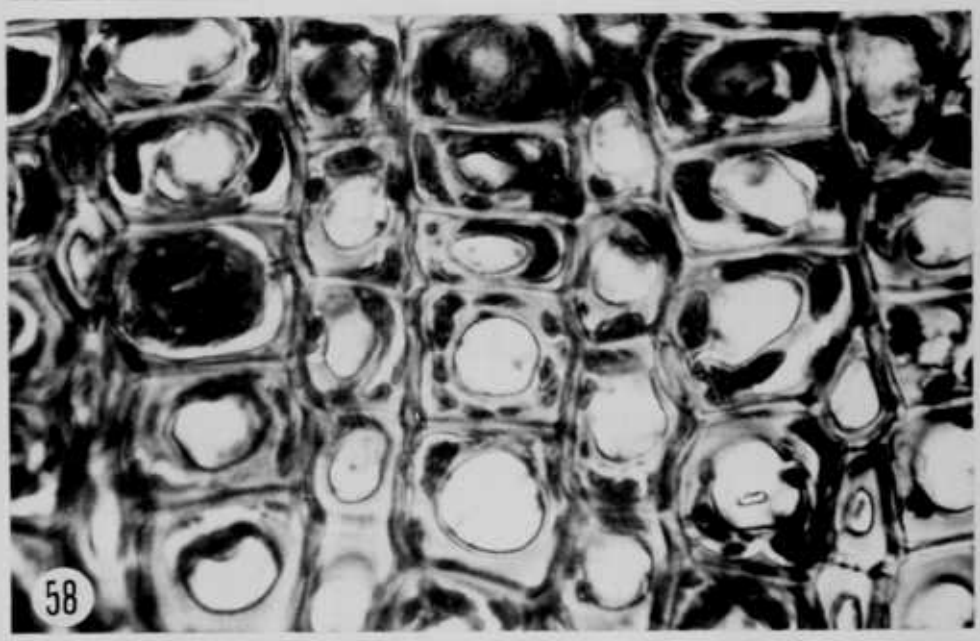
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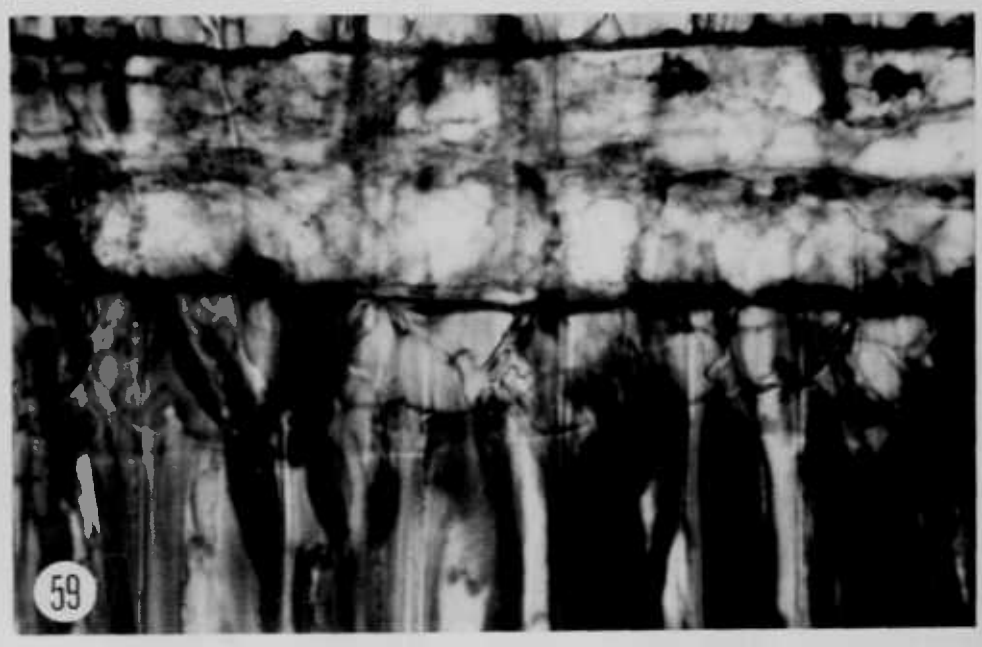
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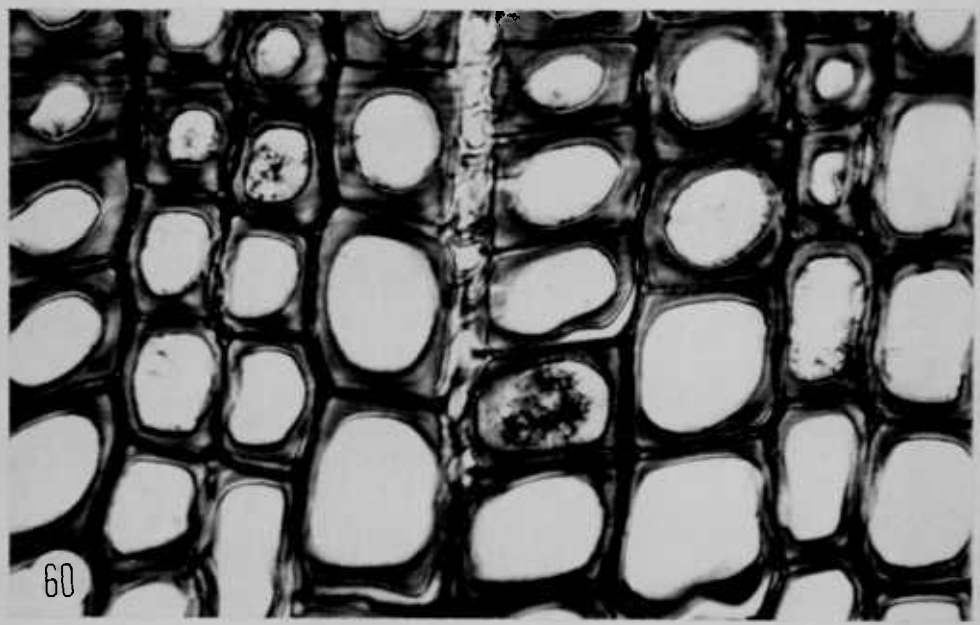
Figure 60. T.S. treated Scots pine showing resistance to decay by C.globosum after 12 weeks exposure (x 610)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not increase or spread for the rest of the period. Most of the cells were free from the fungal hyphae but when present these were always lying in the cell lumina (Fig.60), in both earlywood and latewood zones. The hyphae present were mostly fine and unhealthy looking with very few normal ones present. No signs of decay were observed throughout the exposure periods.

In general C.globosum was a more active soft-rot fungus than P.fastigiata in both rapidly colonizing and degrading the untreated blocks of birch and Scots pine. The presence of the preservative in birch considerably delayed the colonization and degradation by the fungus. As in the case of P.fastigiata, the rays of treated birch were resistant to fungal degrade, whilst the fibres were destroyed. The treated blocks of Scots pine were sound throughout the exposure periods.

3.1.1.3. Coriolus versicolor

Untreated birch: The fungal hyphae heavily surrounded the whole block within the first week of exposure (Fig.61c) and the parts of the block next to the culture were softened due to the fungal decay. With the progress of decay, the wood block became paler in colour, smaller in size, very soft; where it could be squeezed between two fingers, and disintegrated. It was very difficult to separate the block from the surrounding fungal hyphae after 3 weeks of exposure when most of the block was fragmented. Whole sections from the block were easily cut after 3, 5 or 7 days, especially from the longitudinal faces, but this was very difficult after this period and it was only possible to obtain small sections of suitable sizes or fragments at 6 weeks of exposure but even this was not possible after that time.

After 3 days, the colonization was throughout the block, sparse in the middle regions and heavier towards the outer parts. Hyphae were present in the vessels and fibres (Fig.62). The ray cells were heavily colonized and showed some signs of degradation (Fig.63). Hyphae were sometimes found crossing the walls from one cell to the other (Fig.64) and also emerging from the rays to the adjacent fibres. Hyphal colonization became heavier after the first week and most of the block was degraded. The rays showed more decay than the vessels or fibres. Cell wall thinning was obvious at this stage, but the middle lamella region of the heavily degraded walls were still resistant to decay (Figs.65,66 and 67). Ovate-shape cavities of different sizes were frequently observed in the cell walls, these unite together at certain regions forming a large cavity in the wall (Fig.68 and 69). Hyphae were usually found in the cell lumen but not in the degraded cell wall (Fig.70). After 3 weeks, the wood blocks were considerably reduced in size and fragmented. The ray cells were nearly completely destroyed, the fibres and vessels showed considerable wall thinning. This also affected the middle-lamella region causing the destruction of the whole cell wall. Certain cells maintained their shapes by the presence of the more resistant middle-lamella region at certain parts of the block (Fig.71). At the later stages of decay, traces of fragmented wood were left which broke down into small pieces when separated from the surrounding fungal hyphae. It is obvious that the cell wall degradation started from the cell lumen towards the middle lamella region with the hyphae always lying in the cell lumen.

Treated birch: Unlike the untreated wood, the treated blocks were not surrounded by the fungal hyphae even after 12 weeks of exposure (Figs.61a and b) and the macroscopical observations showed

that the hyphae were in contact with the block for only about 1mm above the agar surface. This indicates the prohibition of the fungus by the toxic material present in wood. By the end of the experiment, it was only those parts of the block directly in contact with the culture mycelia which became slightly soft due to the fungal decay, otherwise the block was very sound.

After the first week of exposure, a very few cells near the exposed face of the block were sparsely colonized with no indication of wall lysis. This was also true for the 3 weeks exposure (Fig.72) apart from a few fungal hyphae which colonized a few cells in the deeper parts of the block. The ray cells appeared to be free from fungal colonization. Many hyphae collapsed due, presumably, to the toxic material present and appeared as fragments or fluffy material in the cell lumen. These were found to be more common in the deeper parts of the block than near the margins where normal hyphae were present due, perhaps, to the constant penetration from the surrounding culture. With the progress of the experiment, more collapsed hyphae were present but many cells showed complete absence of fungal colonization. It was also found that the fungal population in the block at the later stages of attack was less than it had been at the earlier periods of exposure. The sections obtained from the outer parts of these blocks, especially, from the face in direct contact with the mycelium mat, showed some wall degradation. Slight wall thinning of the fibres was observed after the 9 weeks exposure but the ray cells and vessels appeared resistant to decay (Fig.73). Both normal hyphae and collapsed ones were present in the cell lumina of the degraded cells. The degradation of the wall by the formation of small cavate-shape cavities were also observed here and the rays

were also slightly degraded after the 12 weeks exposure period (Fig.74). The collapsed hyphae considerably increased and filled the cell lumina (Fig.75).

In general, C.versicolor was a very active fungus in colonising and rapidly causing the complete destruction of the untreated birch blocks. On the other hand, the colonization and degradation of the treated blocks of birch was considerably delayed by the presence of the preservative. The hyphae were seen to be collapsing in the block after showing some resistance to the toxic material in wood and new hyphae were emerging towards the wood block from the culture medium which eventually succeeded in slightly degrading the wood after a long period of time.

Untreated Scots pine: As in the case of birch, the whole block was surrounded by dense fungal hyphae at an early stage of incubation. After 3 weeks, parts of the block became soft due to the fungal decay, especially, the regions in direct contact with the agar medium. The heavily decayed regions were fragmented. The separation of the early-wood zones from the latewood zones due to the attack became obvious after 6 weeks of exposure and handling of the block was very difficult after this stage.

Within 3 days of incubation, the blocks were sparsely colonized. Hyphae were scattered throughout the block with more in the outer regions of the block. This dense colonization was building up through-out the block towards the later stages of incubation. The earlywood zones seemed to be more heavily colonized than the latewood zones (Fig.76). The ray cells and resin canals were, more or less, colonized to the same degree as the tracheids. The fungal hyphae travelled from one cell to the other by crossing the cell walls through the bordered-

pits (Fig.77). Ray cells and the tori of the pits were easily destroyed at an early stage of attack leading to the formation of large voids. With the progress of decay, the degraded pit voids joined together through the dissolution of the wall leading to the formation of larger holes in the wall (Fig. 78 and 79). The different parts of the block were not degraded at the same rate. The earlywood zones were degraded before the latewood zones and even the decay capacity was variable for the same zone where certain cell walls decayed sooner and to a greater extent than others. By the end of the experiment the wood blocks were heavily degraded and considerably reduced in size. The fractions obtained from the soft macerated material were not easily identifiable under the microscope.

Treated Scots pine: The growing mycelia in the agar plates were not able to surround the block even after the 12 weeks incubation period. The wood blocks were sound throughout the exposure periods and no indications of softness or decay were observed.

The treated blocks were very resistant to colonization and decay even after 12 weeks of exposure. Fungal hyphae were not found at depth and the only colonized regions were those towards the surface of the block particularly the ones nearer to the mycelial mat in the plate. No wall crossing from one cell to the other was observed. Few fungal hyphae at the early stages of colonization were found to be normal, while, towards the later periods of incubation, most of the hyphae present were abnormal and collapsed. The collapsed hyphae appeared as fragments or as accumulated fluffy material. These were present in both tracheids and rays (Figs.80 and 81). No signs of wall thinning or pit degradation was observed and the fungal population seemed to be decreasing towards the later expoaure periods.

Figure 61. CCA treated and untreated control blocks of birch and Scots pine in contact with C.versicolor. Note overgrowth of control specimens.

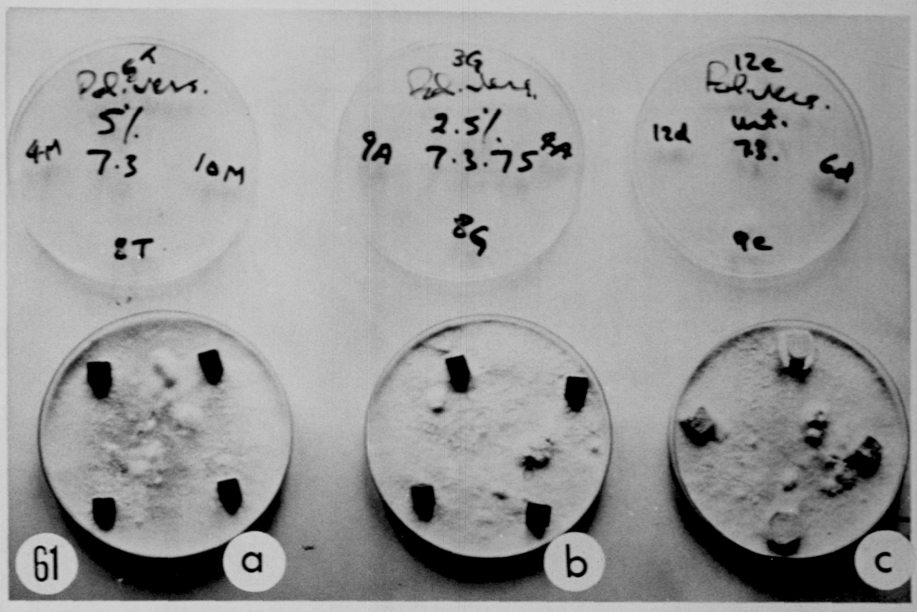
(a) 5% CCA treated blocks.

(b) 2.5% CCA treated blocks.

(c) Untreated control blocks.

Figure 62. T.S. untreated birch, showing colonisation by C.versicolor after 3 days exposure (x 610)

Figure 63. R.L.S. untreated birch, showing heavy colonisation and degradation by C.versicolor after 3 days exposure (x 610)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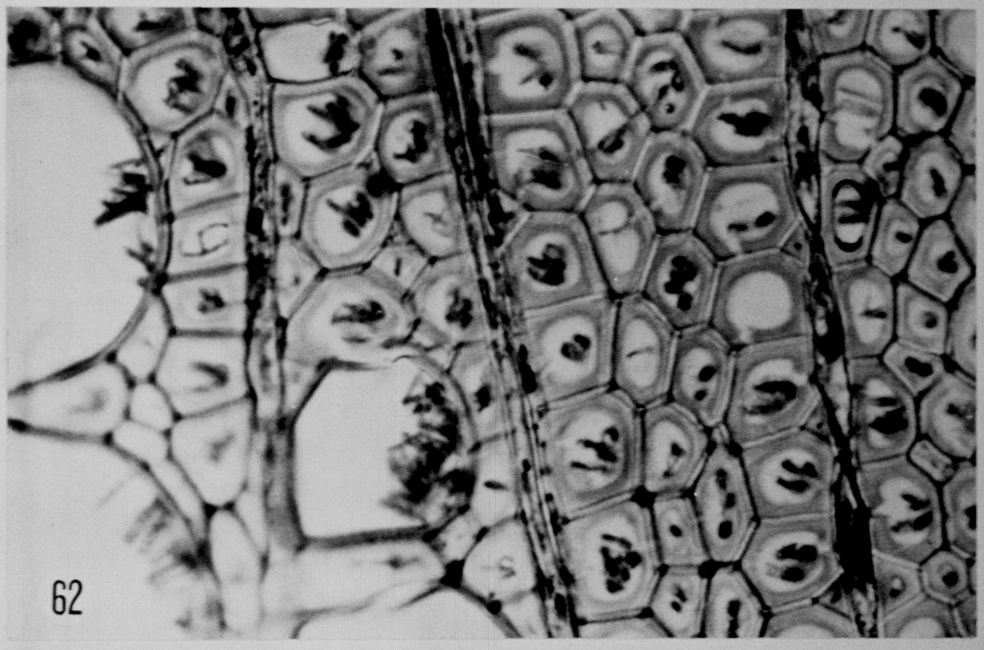
a
b
c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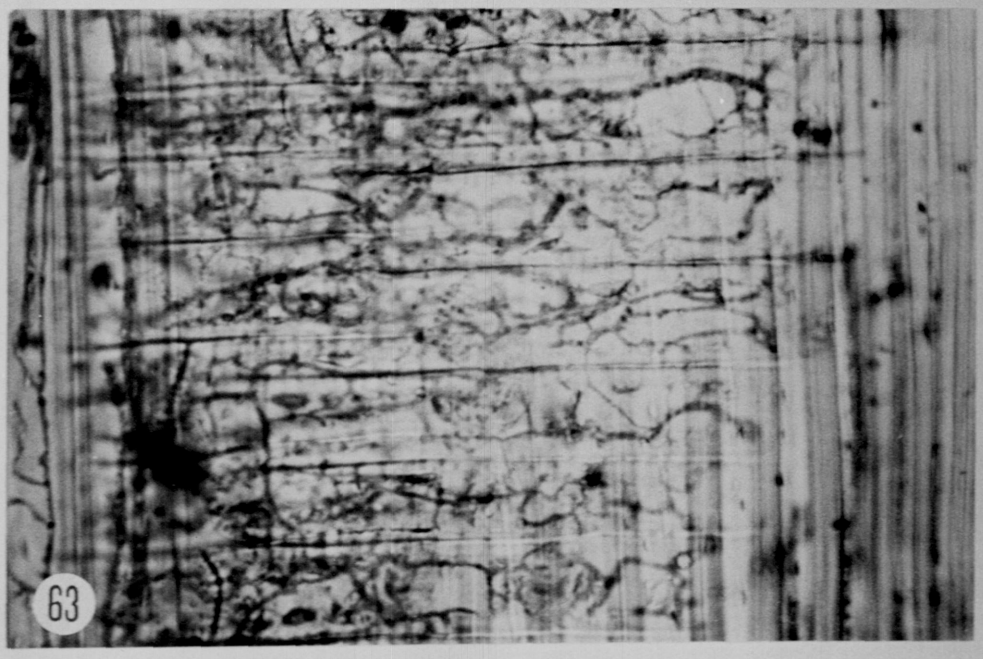
63

Figure 64. C.versicolor in untreated birch (R.L.S.)
showing fibre wall crossing (x 950)

Figure 65. C.versicolor in untreated birch (T.S.)
showing colonisation and degradation
of all tissues after 1 week exposure (x 160)

Figure 66. C.versicolor in untreated birch (T.S.)
showing the hyphae lying in the cell lumen
causing cell wall thinning towards the
middle lamella regions (x 950)

64

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66

Figure 67. As figure 66 (x 610), but under polarized light.

Figure 68. C.versicolor in untreated birch (R.L.S.) showing ovate-shape cavities in the cell walls after 1 week exposure (x 610)

Figure 69. As figure 68, but at another region showing the cavities joining together forming large voids in the walls (x 610) Polarized light.


67

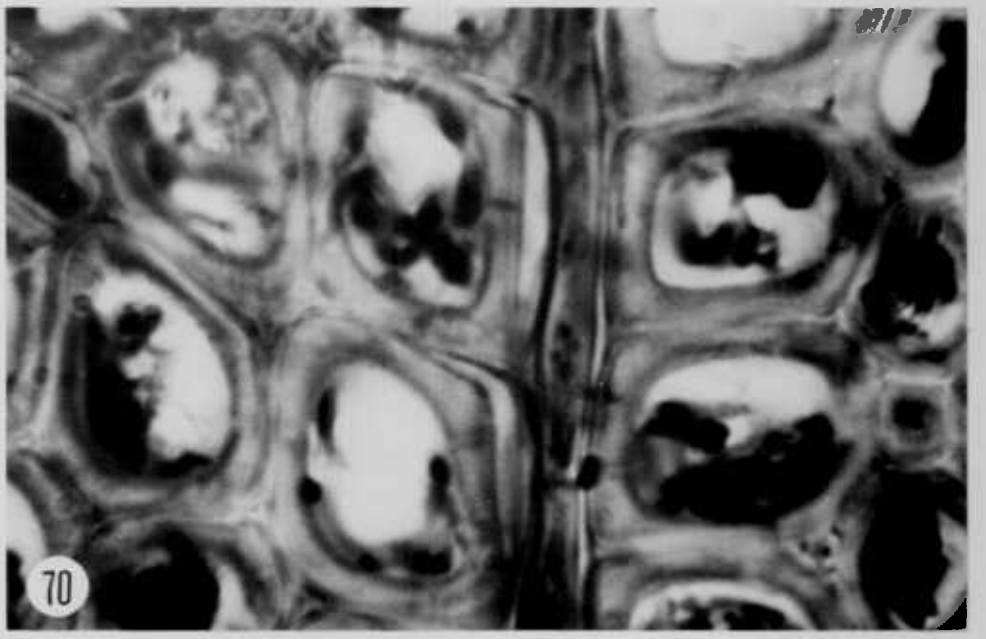

68


69

Figure 70. High magnification of untreated birch (T.S.) showing the hyphae of C.versicolor lying in the fibres cell lumina (x 1600)

Figure 71. C.versicolor in untreated birch (T.S.) showing heavy degradation of fibres and rays after 3 weeks exposure (x 610)

Figure 72. T.S. treated birch after 6 weeks exposure to C.versicolor showing slight colonisation but no degradation (x 610)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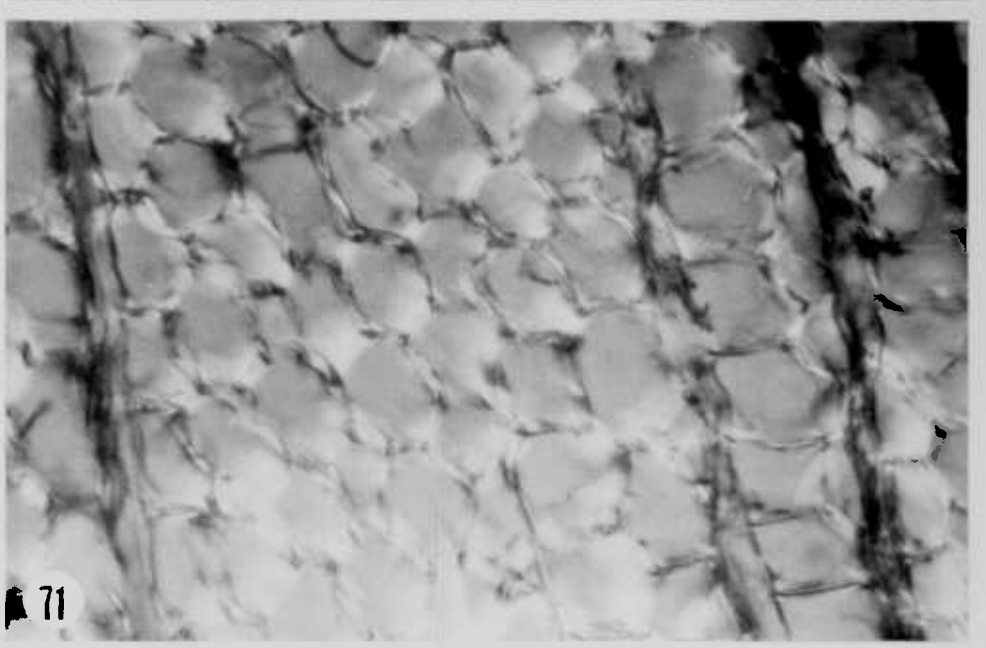
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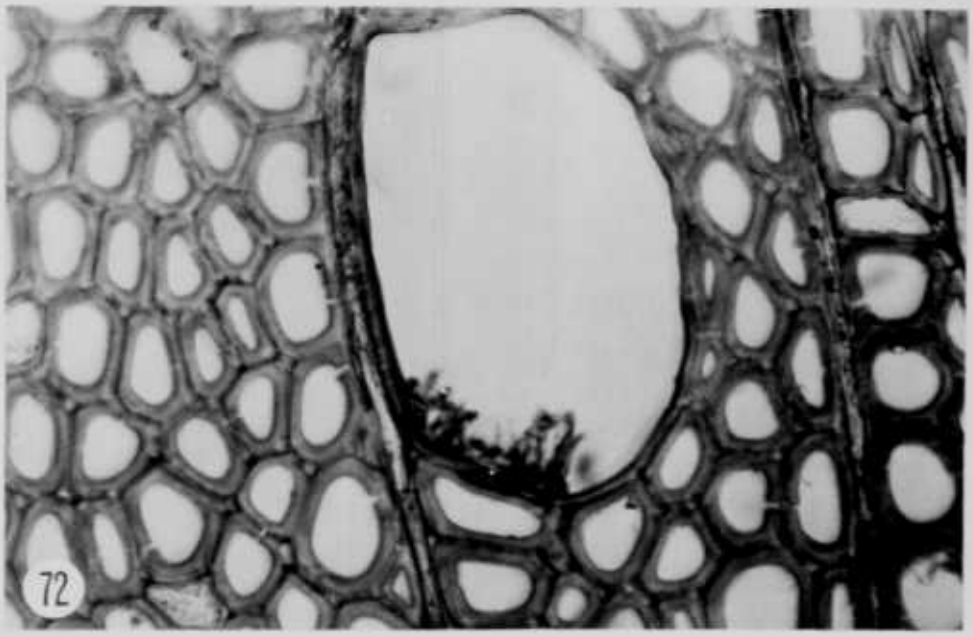
72

Figure 73. T.S. treated birch, showing slight colonisation and fibres wall thinning after 9 weeks exposure to C.versicolor (x 610)

Figure 74. R.L.S. treated birch, showing slight degradation of fibres and to a lesser extent the rays by C.versicolor after 12 weeks exposure (x 610)

Figure 75. R.L.S. treated birch, showing fluffy-like collapsed hyphae of C.versicolor after 12 weeks exposure (x 610)

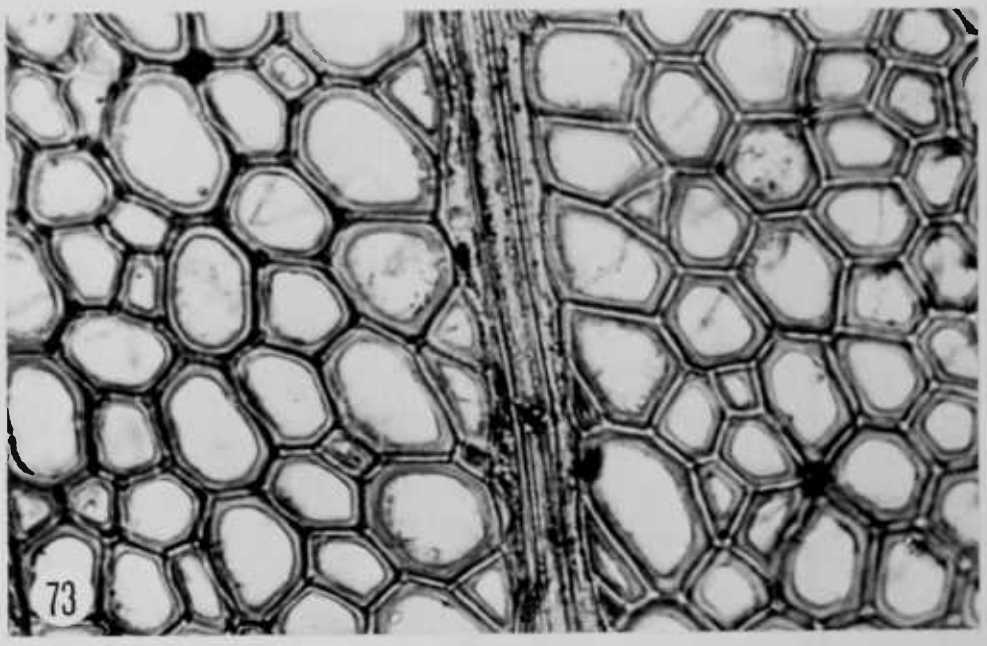
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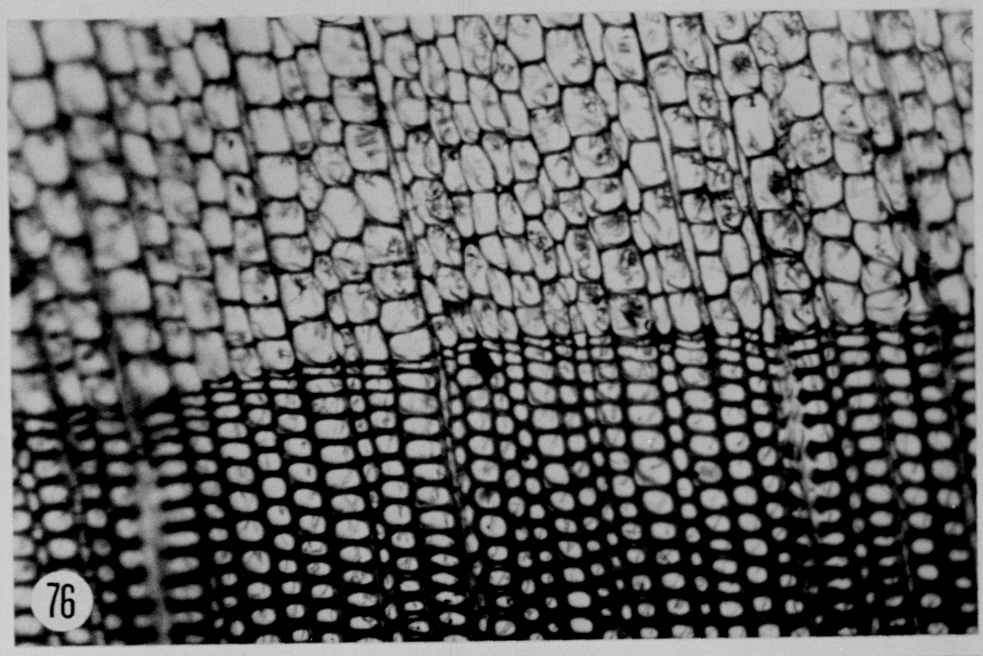
Figure 76. T.S. untreated Scots pine showing colonisation by C.versicolor after 3 days exposure. Note the more heavily colonised earlywood zones than the latewood zones (x 160)

Figure 77. C.versicolor in untreated Scots pine (T.L.S.) showing wall crossing via the bordered-pits (x 950)

Figure 78. C.versicolor in untreated Scots pine (R.L.S.) showing the destruction of the pit-tori and the formation of large voids in the walls due to the dissolution of the walls (x 610)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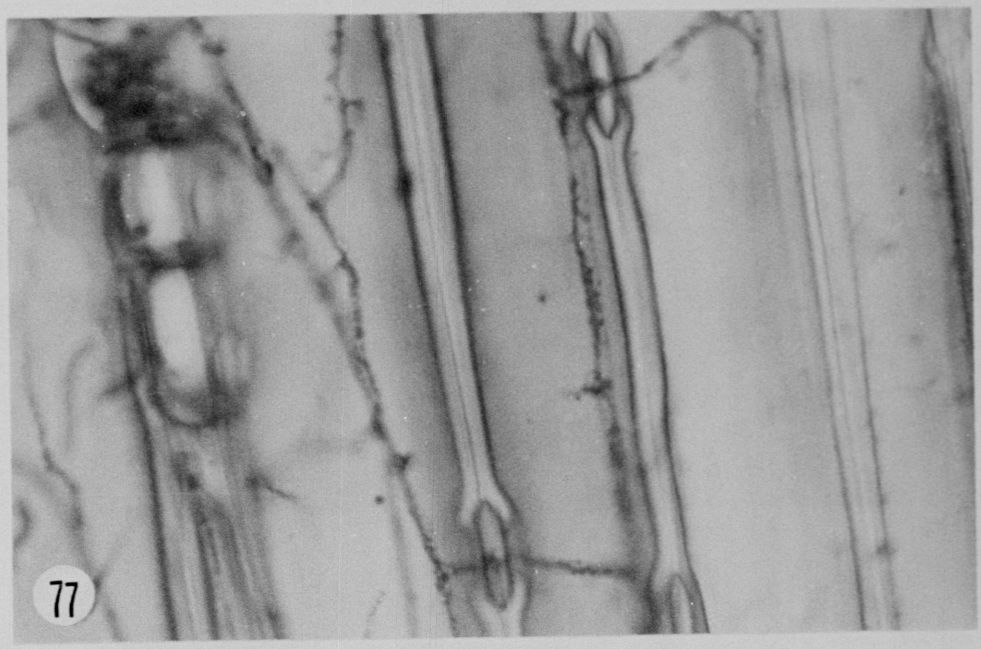
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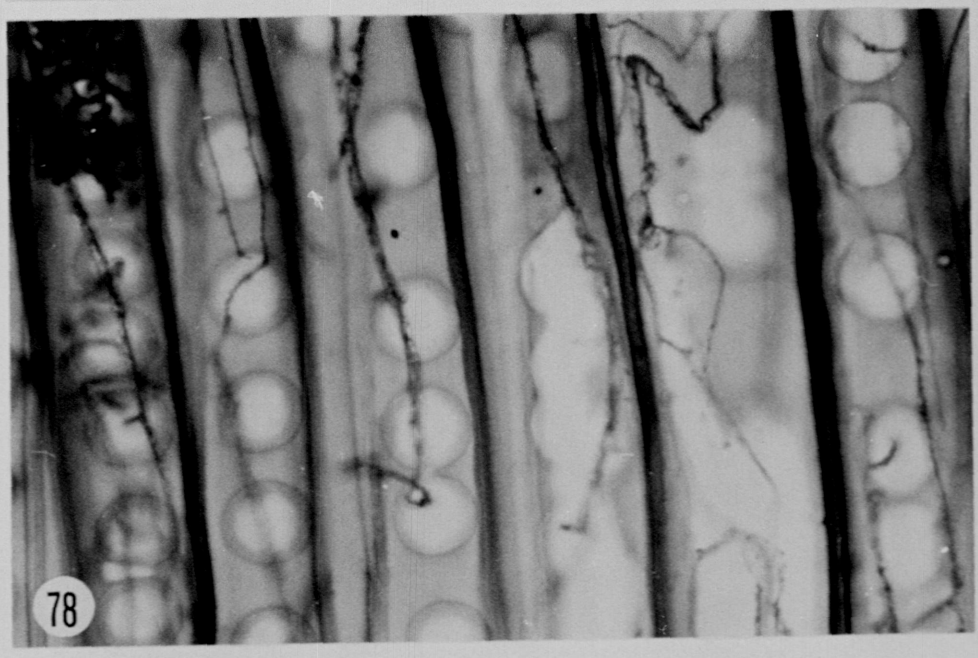
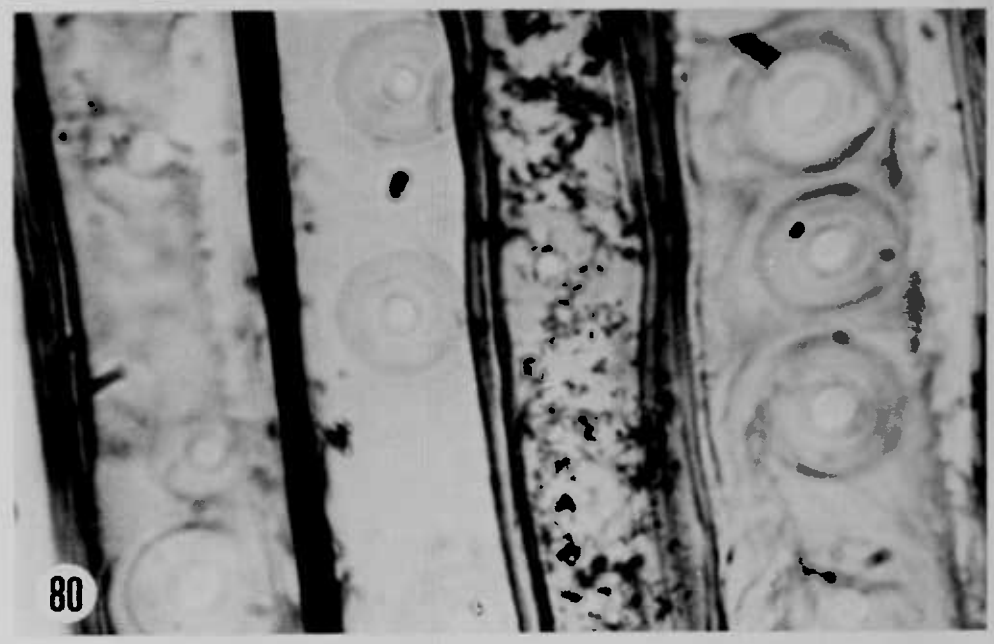

78

Figure 79. As figure 78 (x 610)

Figure 80. R.L.S. treated Scots pine showing collapsed hyphae of C.versicolor after 6 weeks exposure. Note the resistance of the pit-tori to the fungal decay (x 950)

Figure 81. R.L.S. treated Scots pine showing collapsed hyphae of C.versicolor after 12 weeks exposure (x 610)


79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In general, the degradation of the untreated blocks of Scots pine by C. versicolor was slow compared to that of the birch blocks. On the other hand, the treated blocks of Scots pine were very resistant to both colonization and decay by the fungus even after long periods of exposure. Most of the hyphae present were unhealthy and failed to penetrate the deeper parts of the block.

3.1.1.4. Coniophora puteana

Untreated birch: The blocks were completely surrounded by the fungal hyphae within the first week of incubation (Fig.82c). After 3 weeks, parts of the block, especially those in direct contact with the culture medium, became very soft and brittle due to the fungal attack. Towards the later periods of incubation the blocks were heavily attacked by the fungus. They were considerably reduced in size, softened and fragmented. At this stage of attack it was extremely difficult to handle the blocks and separate them from the surrounding fungal hyphae.

After 3 days, sparse fungal colonization was observed. This was randomly scattered throughout the block with the parts of the block next to the invading mycelia being more evenly colonized. The ray cells and vessels showed a greater extent of fungal colonization than the fibres. The fungal hyphae continued to build up and colonized the wood blocks densely in the following weeks of exposure. Throughout the exposure periods, hyphae were always found lying in the cell lumen and no wall crossing from one cell to the other was observed. With the progress of decay the walls were heavily degraded. In the early stages of attack, the fungus rapidly destroyed most of the cell wall leaving thin disrupted layers towards the middle lamella region. After each exposure period certain parts of the block showed less degradation than other heavily degraded parts where no cell structure could be identified.

The less degraded regions, decreased to a large extent at the later exposure periods and it was very difficult to identify the remaining fragments of the block. After one week, slight degradation was observed in the heavily colonized regions. This was well advanced after the 3 weeks incubation period where parts of the block were completely disintegrated, other parts showed heavy wall degradation (Fig.83) and certain cells maintained their shapes by the presence of the more resistant middle lamella regions (Fig.84). It was observed that the ray cells were more heavily degraded than the fibres (Fig.85). This was clearly demonstrated by the lack of birefringence in rays when the same area of figure (85) was viewed with polarised light (Fig.86).

Treated birch: Within 6 weeks of incubation, the fungal hyphae were not found to be able to surround the blocks and even these parts in direct contact with the culture medium were, more or less, free from fungal hyphae. It was after 9 weeks before the lower parts of the blocks were surrounded by a thin sheath of fungal hyphae which extended to a greater extent after the 12 weeks exposure (Fig.82a and b). The blocks were sound throughout the exposure periods with the exception of those parts in direct contact with the agar medium which towards the later periods of exposure, showed slight degradation due to the fungal attack.

The presence of the preservative considerably delayed the colonization and degradation of the blocks. Within the early periods of incubation, only the outer parts of the block especially those in direct contact with the culture mycelium, were sparsely colonized. After 6 weeks, sparse colonization of the deeper parts of the block was achieved by the fungus but most of the hyphae present were abnormal. Towards the end of the experiment the margins of the blocks were more heavily

colonized with normal healthy hyphae which caused some degradation of these areas (Fig.87). The ray cells showed more resistance to colonization and decay than the other cells.

Untreated Scots pine: The wood blocks were completely surrounded by the fungal hyphae within the first week of incubation. Scattered parts of the block were heavily decayed due to the fungal attack within 3 weeks of incubation. The decayed regions were very soft and brittle. Later on, the blocks were considerably reduced in size due to the complete disintegration of most parts of the block. The remaining wood material was fragmented and very difficult to isolate from the attacking hyphae.

The fungal hyphae sparsely colonized the wood blocks within 3 days of incubation with more hyphae in the rays than the other cell lumina. The colonization was scattered throughout the block with relatively more hyphae present in the earlywood regions than the latewood ones. Colonization was followed by gradual degradation and the complete disintegration of certain regions was building up towards the later periods of incubation. The cell wall decomposition seems to start in the S2 layer and then expands towards the other wall layers (Fig.88) with the fungal hyphae always lying in the cell lumen. Ray cells were decomposed at a faster rate than the tracheids. Wall crossing from one cell to the other was through the bordered-pits. These were easily destroyed at an early stage of attack leading to large openings in the wall.

Treated Scots pine: The wood blocks were completely sound even after 12 weeks of incubation and only the lower parts of the blocks were slightly surrounded by the fungal hyphae.

Figure 82. CCA treated and untreated control blocks of birch and Scots pine in contact with C.puteana. Note overgrowth of control specimens.

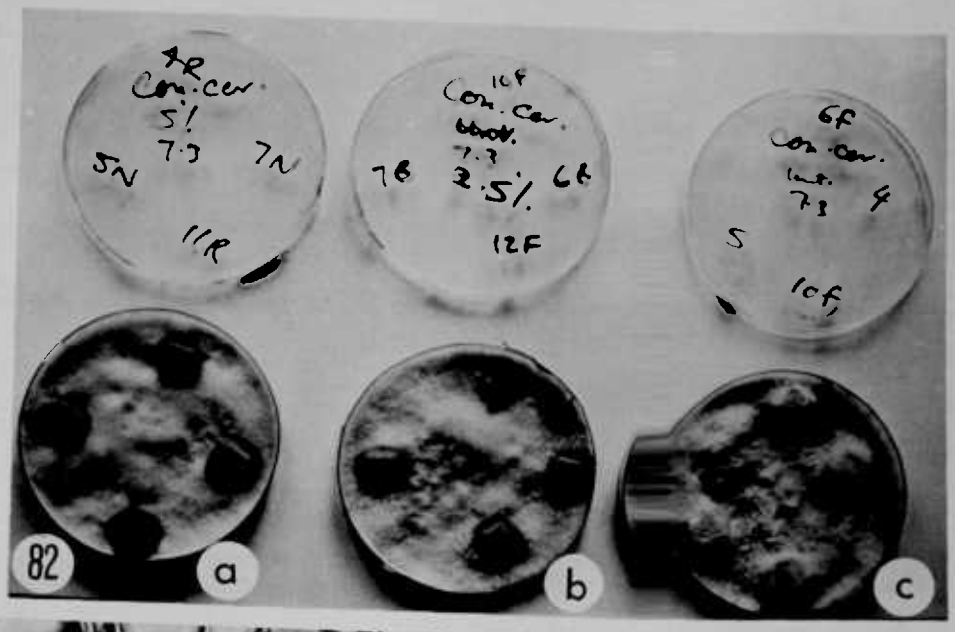
(a) 5% CCA treated blocks.

(b) 2.5% CCA treated blocks.

(c) Untreated control blocks.

Figure 83. T.S. untreated birch showing heavy degradation by C.puteana after 3 weeks exposure (x 610)

Figure 84. As figure 83 (x 610)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a
b
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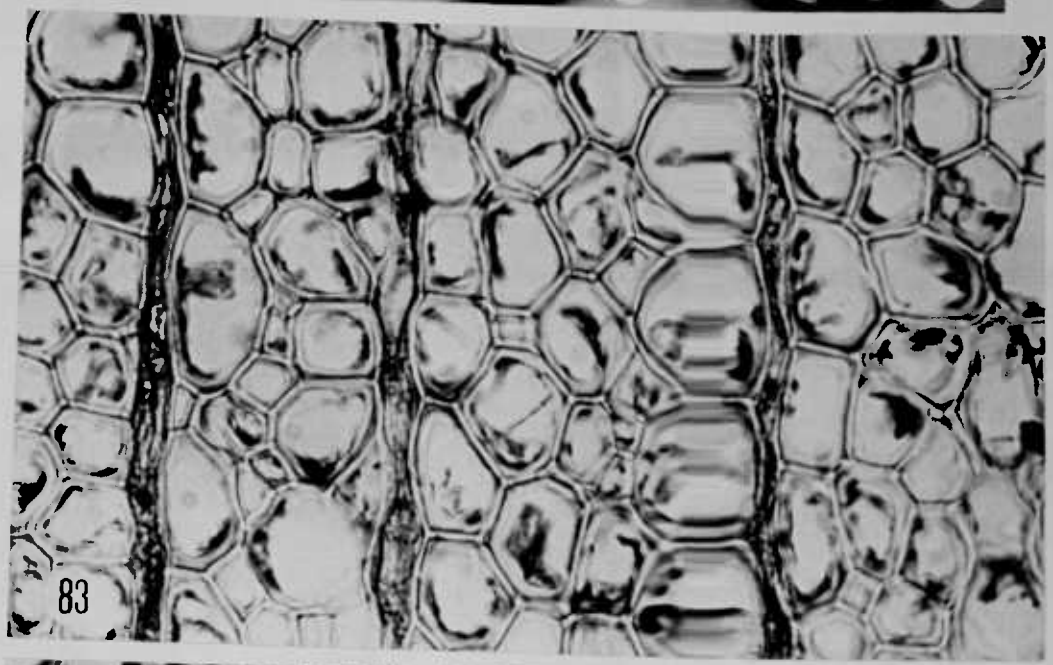
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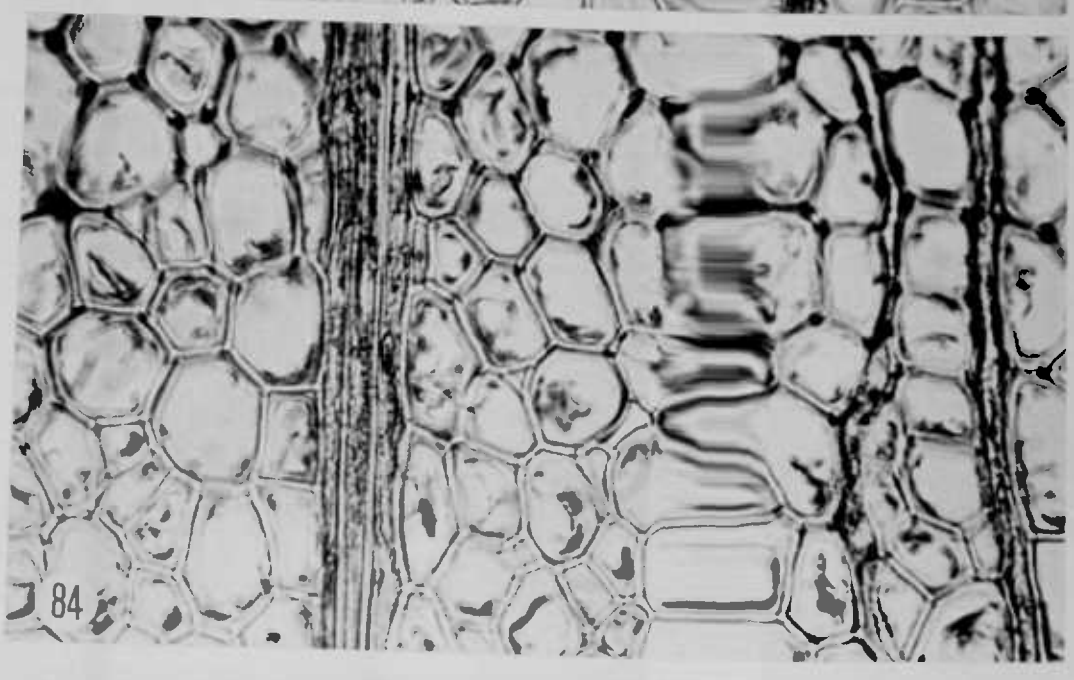
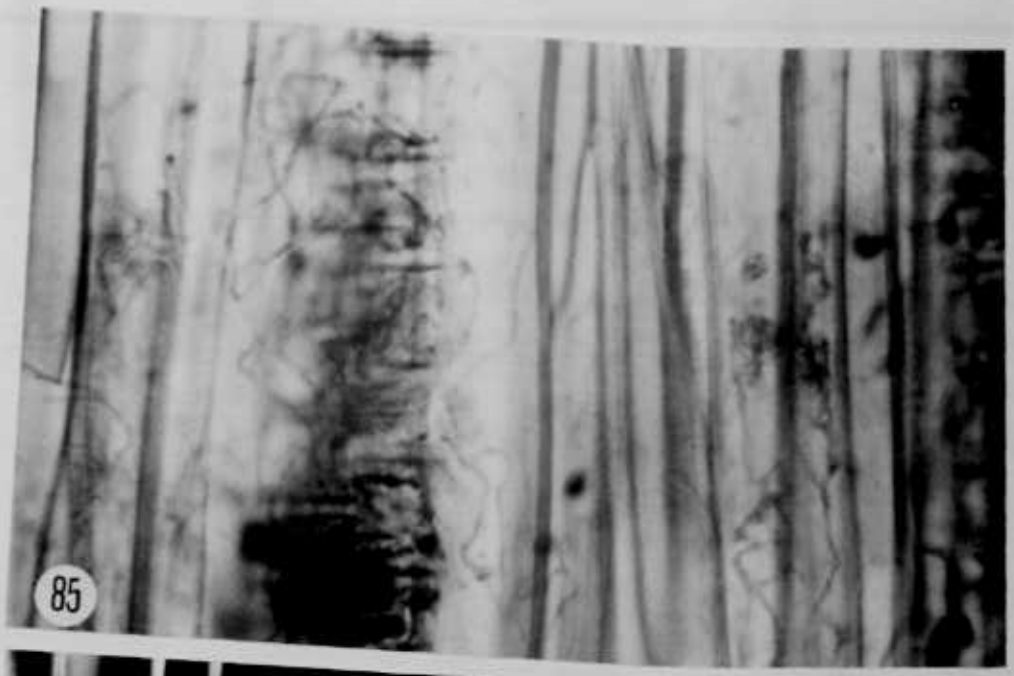

84

Figure 85. C.puteana in untreated birch (R.L.S.) showing heavy degradation of fibres and rays after 6 weeks exposure (x 610)

Figure 86. As figure 85 but under polarised light. Note the complete lack of birefringence in the ray cells region (x 610)

Figure 87. C.puteana in treated birch (T.S.) showing slight degradation after 9 weeks exposure (x 610)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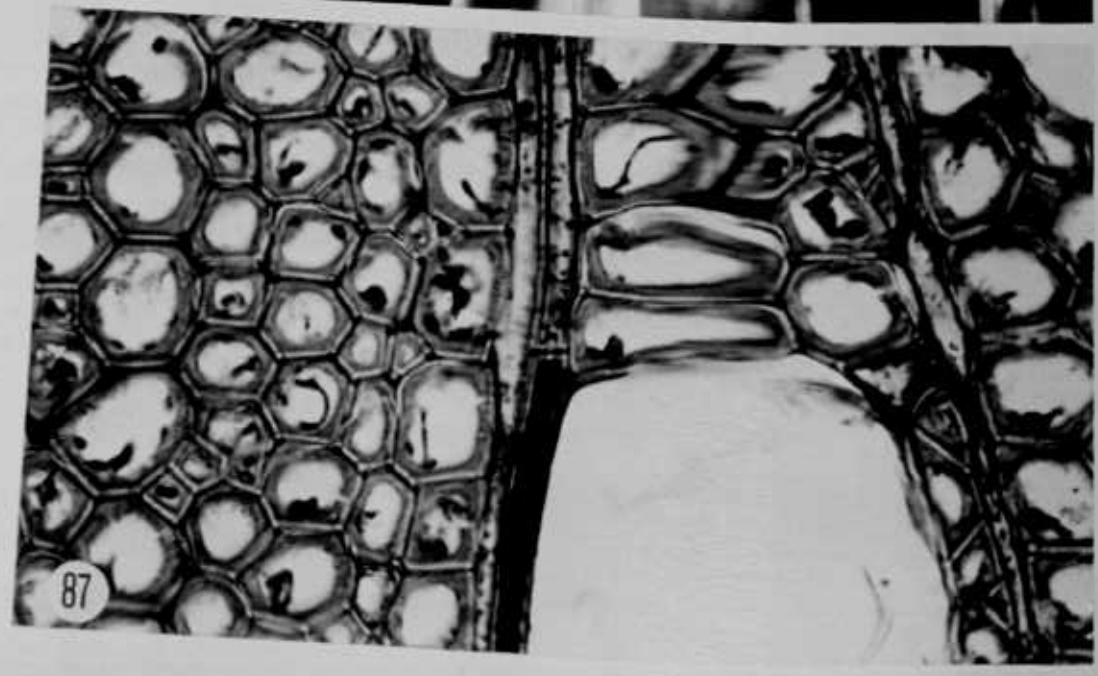
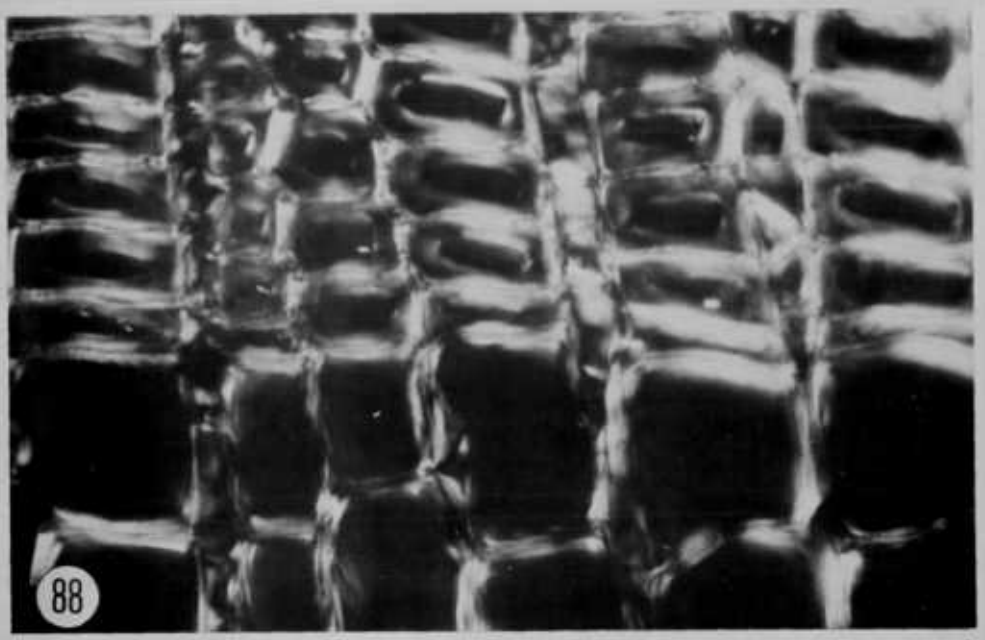

87

Figure 88. C.puteana in untreated Scots pine (T.S.) showing the degradation of the tracheid walls after 3 weeks exposure, Polarised light (x 610)

Figure 89. R.L.S. treated Scots pine showing unhealthy hyphae of C.puteana in the tracheids after 6 weeks exposure (x 610)

Figure 90. R.L.S. treated Scots pine showing unhealthy hyphae of C.puteana in the ray cells after 12 weeks exposure but no signs of decay (x 610)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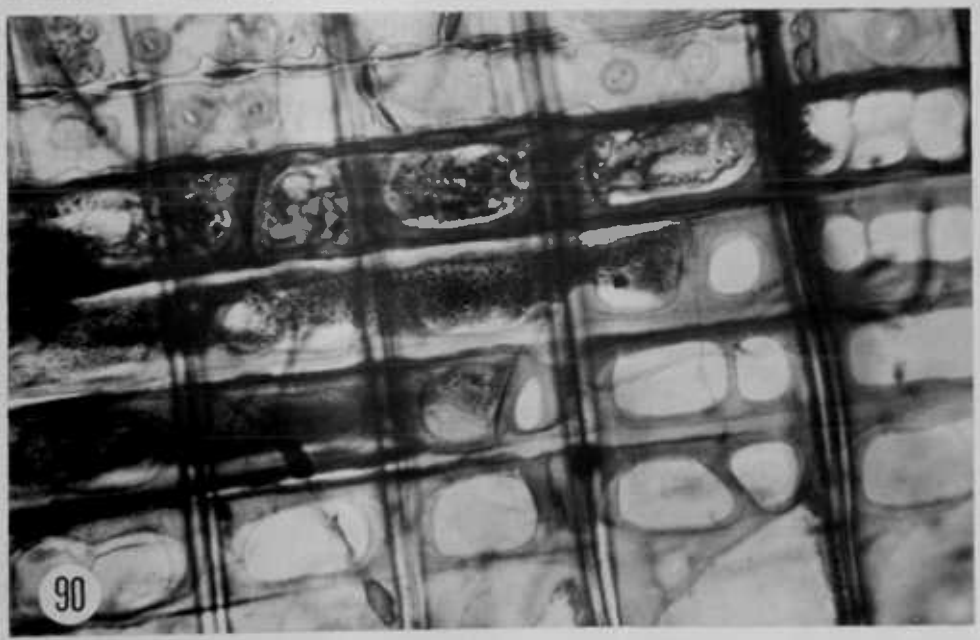
90

Fungal colonization was sparse and scattered throughout the exposure periods with less hyphae present at the later periods of incubation. Most of the cell lumina were free from the fungal hyphae and when present they were collapsed into fragments or accumulated fluffy material (Fig.89 and 90). Normal hyphae were also present especially towards the margins of the block, but they were usually less than the collapsed ones. No wall penetration or signs of degradation was observed throughout the incubation periods.

In general, untreated birch and Scots pine were heavily degraded by C.puteana within 3 weeks of exposure. The treated blocks of birch were slightly decayed at the later exposure periods, while the treated blocks of Scots pine were highly resistant to both colonization and degradation even after 12 weeks of exposure.

3.1.1.5. Botryodiplodia theobromae

Untreated birch: Within the first week of exposure the whole block was surrounded by the fungal mycelium which towards the later periods of incubation formed a thick hairy cushion around the block. The fungal mycelium was firmly associated with the block and not easily removed from the surface as in the case of C.vesicolor, C.globosum and C.puteana. Throughout the exposure periods the block maintained its general structure in the culture medium but at a later stage when the surrounding hyphae were removed, the outer regions of the block were easily peeled off as fragments from the rest of the block. The colour of the whole block rapidly changed to black due to the presence of the dark coloured fungal hyphae.

The wood blocks were mostly colonized by 3 types of fungal hyphae. These were best described as thick, medium and thin types of hyphae.

The thick type were usually found nearly filling the lumina of the cells and can be clearly seen under the microscope without any further staining due to their brown colour. The thin hyphae observed were of the type commonly seen with the previous 4 species of fungi while the medium type of hyphae were thicker than these and seemed to be extensions and therefore possibly a younger form of the thicker brown types of hyphae.

The blocks were rapidly colonized by the fungus. Within 3 days of exposure colonization was throughout the block. It was heavier towards the lower and outer parts than the centre of the blocks. Rays were more heavily colonized than the fibres or vessels. In the following weeks colonization continued to build-up throughout the block and became dense in all tissues. Masses of hyphae of different thicknesses were present in the rays and to a lesser extent in the fibres and vessels. Hyphae were observed emerging from the rays to the adjacent fibres or vessels. They were also found directly crossing the cell wall from one cell lumen to the other. The thinner hyphae were crossing the walls as a typical stainer fungus similar to that described in the case of P.fastigiata (Section 3.1.1.1. page 67). When a thicker hypha came in contact with a cell wall it bulged to form a relatively thin extension which penetrated the wall to the adjacent cell lumen where it returned to its normal size (Figs.91 and 92). Rays were rapidly degraded and at an earlier stage than the fibres or vessels. Within 3 weeks of exposure fibre and vessel walls were also heavily degraded. The fungus seemed to be behaving as a white-rot in gradually causing the thinning of the cell wall from the cell lumen towards the middle lamella region which maintained the shape of the cell. Degradation was usually caused by the

thicker type of the fungal hyphae which filled the spaces made in the different degraded zones (Fige.93 and 94).

Treated birch: The whole block was surrounded by sparse fungal hyphae towards the later periods of exposure. There were no signs of decay but the whole block became black in colour which indicated the presence of the fungal hyphae throughout the block.

Up to the third week of exposure colonization was sparse and mostly in the lower parts of the block. Rays were slightly colonized compared to the more colonized fibres and vessels. After 6 weeks exposure colonization was throughout the block, heavier in the lower and outer parts of the block but sparse in the deeper parts of the block. Most of the hyphae present were unhealthy especially in the deeper parts of the block and looked very similar to unhealthy hyphae of other species. Hyphae present were mostly of the thinner type beside the thicker ones, this was the case throughout the exposure periods. After 9 and 12 weeks of exposure colonization became heavier throughout the block and less unhealthy hyphae were present than the previous weeks. After these periods wall crossing from one cell to the other was also more common than the earlier periods of exposure. All tissues were found to be resistant to decay even after 12 weeks of exposure.

Untreated Scots pine: As in the case of birch, the blocks were surrounded by the fungus and gradually became black in colour but at a slower rate. There were no apparent signs of decay observed after the different exposure periods.

In the early periods of exposure fungal colonization was sparse and scattered throughout the block. The fungus mostly colonized the ray cells and to a less extent the tracheids and resin canals.

Towards the later periods of exposure colonization proceeded throughout the block and mainly in the rays and latewood zones which were filled with masses of fungal hyphae. Wall penetration was either through the bordered-pits or by directly crossing the cell walls in the same manner described in the case of birch (Figs.95 and 96). Hyphae were also found emerging from the ray cells to the adjacent tracheids. Hyphae according to their thickness, were mainly of 3 types, thin, medium and thick. Ray parenchyma cells were the main cells degraded but no degradation of the tracheids was observed (Fig.97).

Treated Scots pine: The blocks were slightly black in colour at a later period of exposure. They were sparsely surrounded by the fungus which could be easily removed from the surface of the block. No sign of decay was observed.

After one week's exposure most of the block was free from the fungus with the exception of the lower parts which were sparsely colonized by all types of fungal hyphae. After 3 weeks exposure colonization was throughout the block. It was heavy in the lower parts, sparse and scattered in the outer parts. All tissues were colonized to the same extent. This was also the case after the 6 and 9 weeks exposure with more hyphae in the deeper parts than the previous weeks but they were mostly unhealthy ones. It was only after the 12 weeks exposure that healthy hyphae were seen to be in the majority and present beside the fewer unhealthy hyphae. Hyphae were also found more actively penetrating the cell walls than in early weeks. All tissues were found to be resistant to degradation.

Figure 91. B.theobromae in untreated birch (T.L.S.) showing different types of hyphae and wall crossing (x 520)

Figure 92. As figure 91 (x 800)

Figure 93. R.L.S. untreated birch showing degraded rays by B.theobromae after 3 weeks exposure (x 610)


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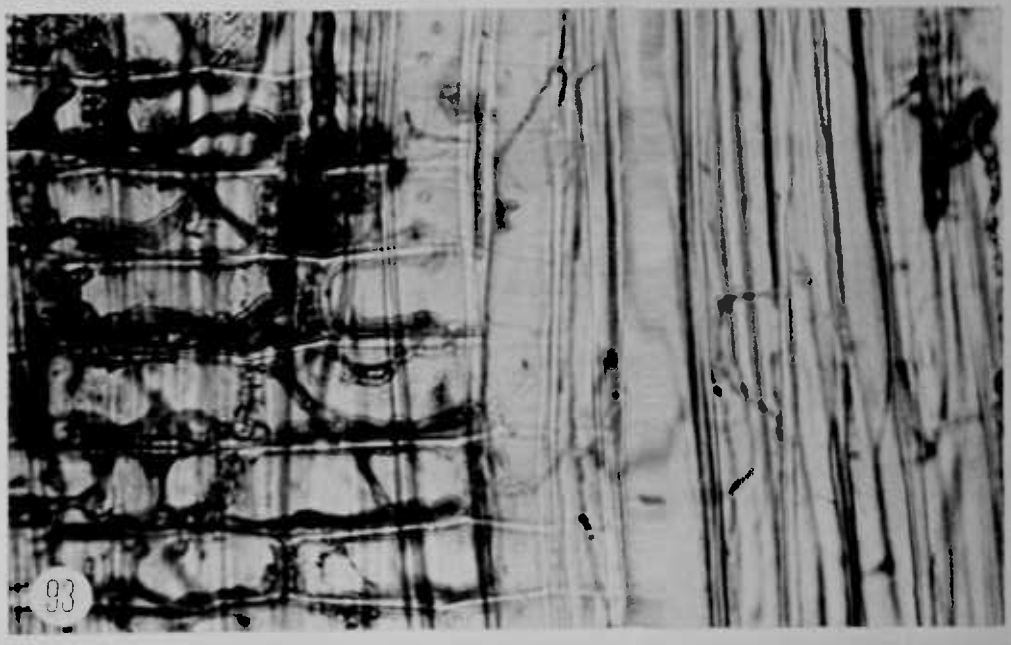
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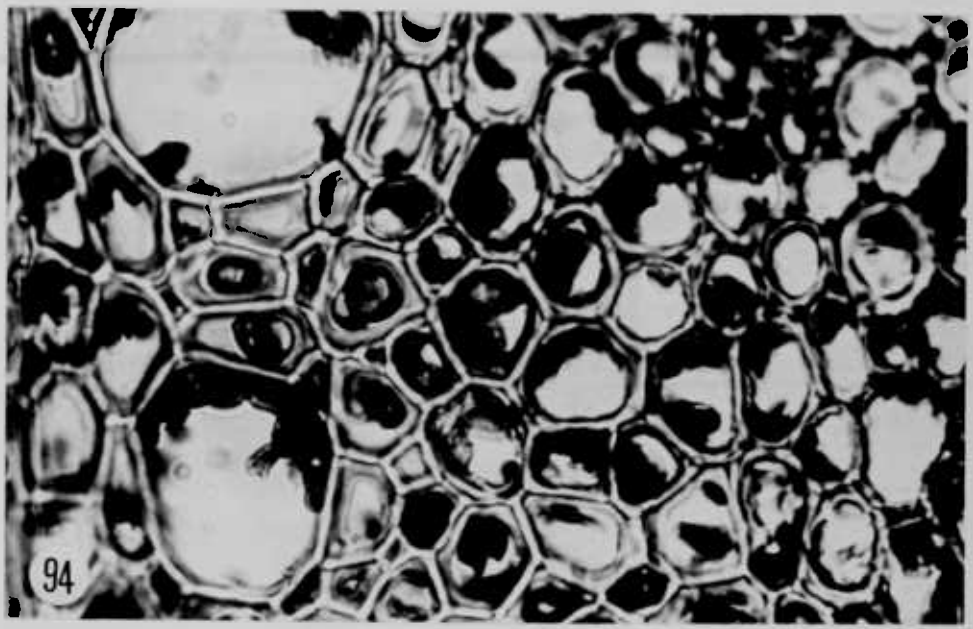
Figure 94. T.S. untreated birch, showing degradation of vessels and fibres by B.theobromae after 3 weeks exposure (x 610)

Figure 95. B.theobromae in untreated Scots pine (T.L.S.) showing wall crossing via the bordered pits (x 610)

Figure 96. B.theobromae in untreated Scots pine (T.L.S.) showing direct wall crossing by thin constricted hyphae (x 950)

Figure 97. T.S. untreated Scots pine showing hyphae of B.theobromae colonising the cell lumina but no degradation after 6 weeks exposure (x 950)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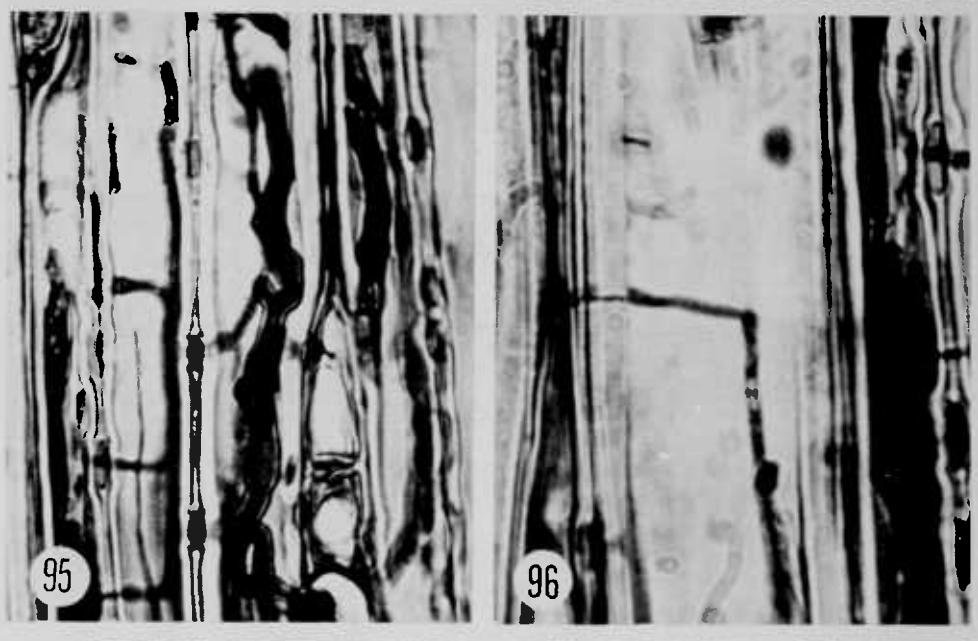
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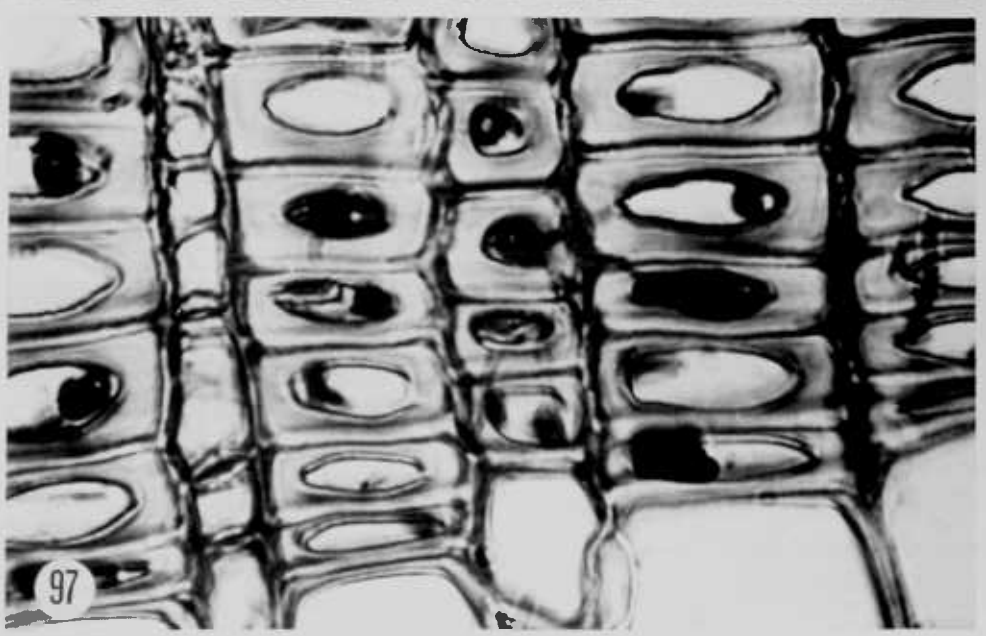

97

In general, B.theobromae rapidly colonized and degraded the rays of untreated blocks of birch. Fibres and vessels were also heavily degraded but at a later period of exposure. The presence of the preservative in birch considerably delayed the fungal colonization of all tissues, especially the rays and protected the blocks against decay. The rate of colonization in untreated Scots pine was slower than that of birch and rays were also found to be the primary tissue colonized and the only one degraded. Colonization of the treated blocks of Scots pine was also delayed and all tissues were found to be resistant to decay.

3.1.2. T.E.M. Observations

The main purpose of the TEM work was to investigate the micro-distribution of the elements in the wood and any possible movement of these elements into or away from the fungal hyphae. At the same time it was hoped that the sections obtained might have thrown new light on the behaviour of the different fungi and their interactions with the cell walls. As the investigation developed it became more and more important to concentrate on the microanalytical side of electron-microscopy and although material had been prepared for micromorphological studies time did not allow development of this aspect.

3.1.3. Soil burial observations

A characteristic of the untreated blocks buried in the soil in this experiment was the apparent encrustation of soil which adhered to the blocks after the colonization by fungal hyphae was well advanced. The reason for this encrustation was not examined in detail but is believed to be a function of the enclosure of soil particles in the

mycelial mat surrounding the blocks. This phenomenon is referred to throughout this section as "soil encrustation".

Untreated birch: Within 3 weeks of exposure the whole block was heavily and firmly encrusted with soil. The blocks became darker in colour and certain parts were observed to be soft or fragile as a result of heavy decay. When the surrounding soil was removed fragments of the block were usually peeled off with it. After 9 and 12 weeks exposure the blocks were considerably reduced in size and sections were not easily obtained due to heavy degradation.

After one week's exposure, colonization was throughout the block. This was randomly heavy or sparse in different regions of the block. Hyphae colonizing the wood were of different thickness. Groups of cells in certain regions were colonized with one type of hyphae while others with more than one type. Rays and vessels were relatively more colonized than the adjacent fibres in the same region (Fig.98). Slight degradation was observed in the rays but not in other cells. After 3 and 6 weeks exposure fungal colonization was heavier throughout the block. Many regions of the block were also severely decayed. Rays seemed to be more heavily degraded than the adjacent fibres or vessels (Fig.99). Certain few areas of the block were heavily colonized but less degraded than the neighbouring areas (Fig.100). Towards the later periods of exposure all tissues were heavily degraded throughout the block and hyphae were mixed up with the remaining parts of the severely degraded blocks (Fig.101). The more recognisable type of decay was the soft-rot type due to the formation of cavities in the cell wall.

Treated birch: The blocks were apparently sound and not encrusted with soil as in the case of untreated birch. They remained in this condition even after 21 weeks of exposure.

Up to 6 weeks of exposure most parts of the blocks were neither colonized nor decayed (Fig.102). It was only after 9 weeks exposure that decay was observed in the block. The colonization appeared to be random and occurred in isolated patches. The full extent was not easy to estimate, but only amounted to a small percentage of the total volume. Apart from these patches there was neither colonization nor decay. Within these patches the fibres, and to a lesser extent the rays were colonized. The fibres became heavily decayed, but the rays were only decayed when adjacent to such decayed fibres, (Figs.103 and 104). The degree of destruction of the fibres can be easily seen in figure (104), and although some decay of the ray cell walls is apparent, many walls can be clearly seen to be undecayed.

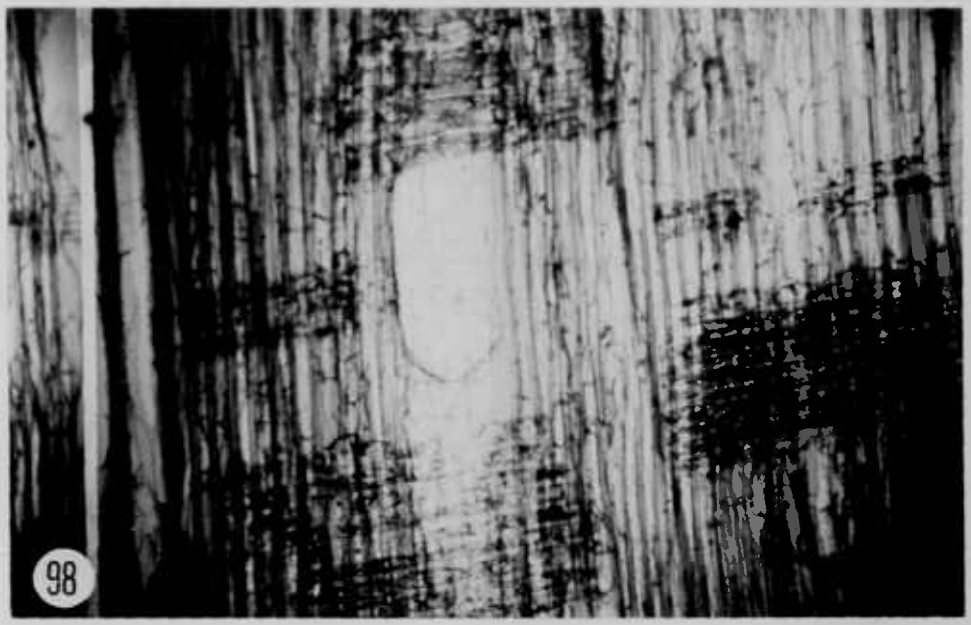
At later periods of exposure the number of patches of decay increased (Fig.105) and the older patches slowly enlarged to give regions of varying stages of colonization and decay. At 21 weeks there was still a large volume of the block that was not decayed or showed only slight colonization.

Cavity formation in the degraded zones indicated that the organisms responsible for such decay were soft-rot fungi. The purpose of extending the exposure period to 21 weeks was the slow rate of colonization and degradation throughout the blocks for even after this period of time the blocks were not or only slightly degraded in many regions.

Figure 98. R.L.S. untreated birch buried in soil showing colonisation of all tissues by fungal hyphae after 1 week exposure (x 160)

Figure 99. T.S. untreated birch buried in soil showing degradation of all tissues after 3 weeks exposure (x 610)

Figure 100. R.L.S. untreated birch buried in soil showing colonisation but no degradation in certain regions and both colonisation and degradation in other regions after 3 weeks exposure. Note the soft-rot type of decay by cavity formation (x 610). Polarized light.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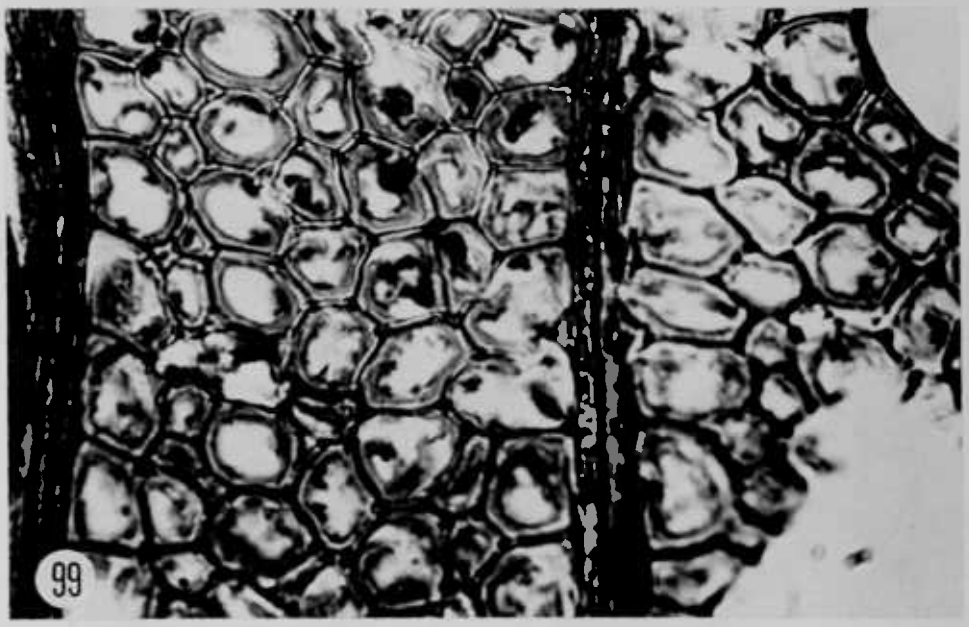
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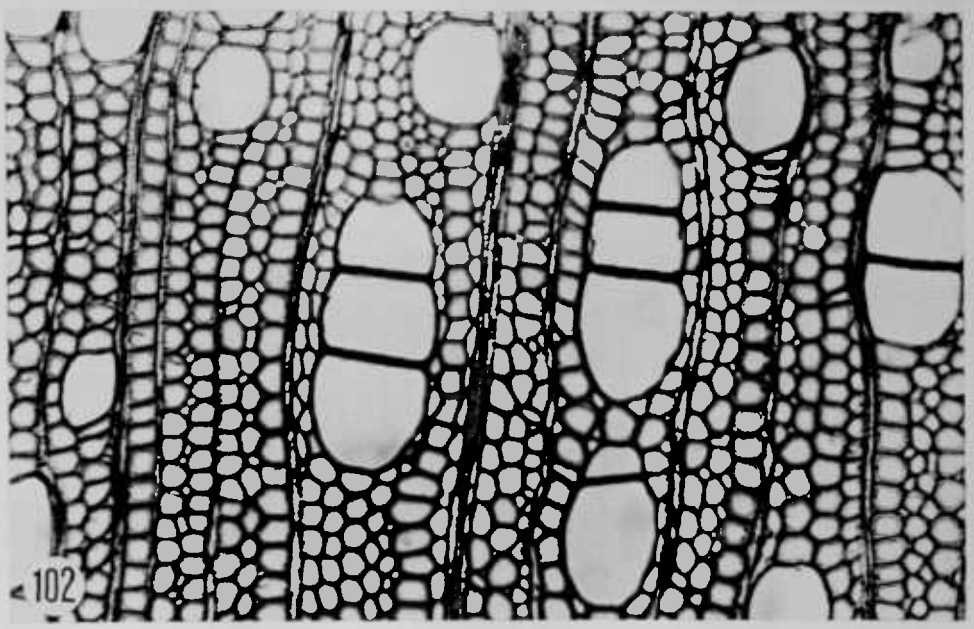
100

Figure 101. Untreated birch buried in soil showing heavily degraded wood after 12 weeks exposure (x 610)

Figure 102. T.S. treated birch buried in soil showing no colonisation or degradation after 6 weeks exposure (x 160)

Figure 103. R.L.S. treated birch buried in soil showing regions of soft-rot decay after 9 weeks exposure. Note that the rays are less degraded than the fibres. Polarised light (x 610)


101

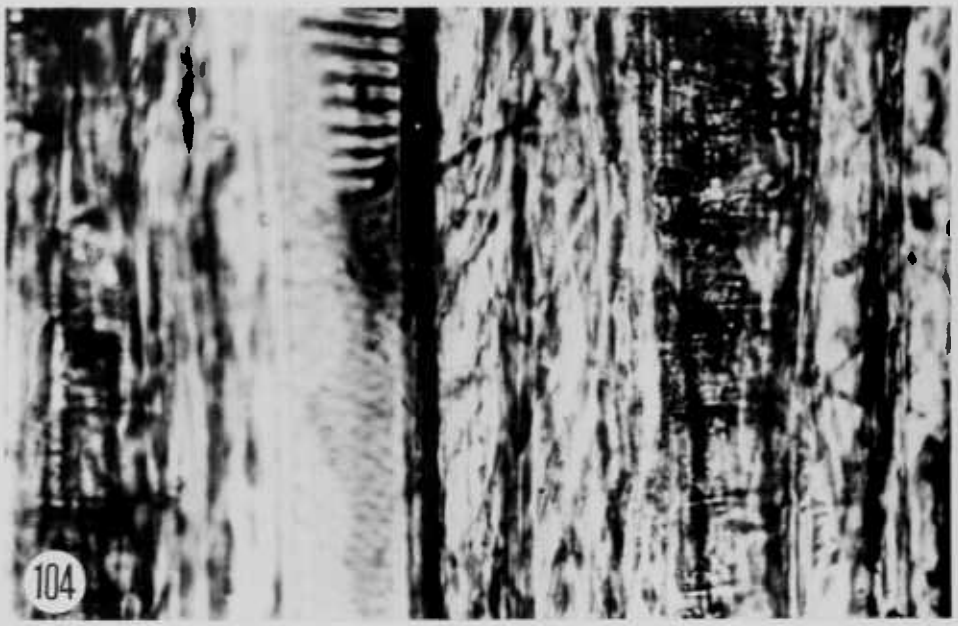

102


103

Figure 104. R.L.S. treated birch buried in soil showing heavily degraded regions after 9 weeks exposure (x 610)

Figure 105. R.L.S. treated birch buried in soil showing heavily degraded patches beside less degraded ones after 15 weeks exposure. Note that many ray cell walls are not decayed. Polarised light (x 610)

Figure 106. R.L.S. untreated Scots pine buried in soil showing degraded rays after 6 weeks exposure (x 610)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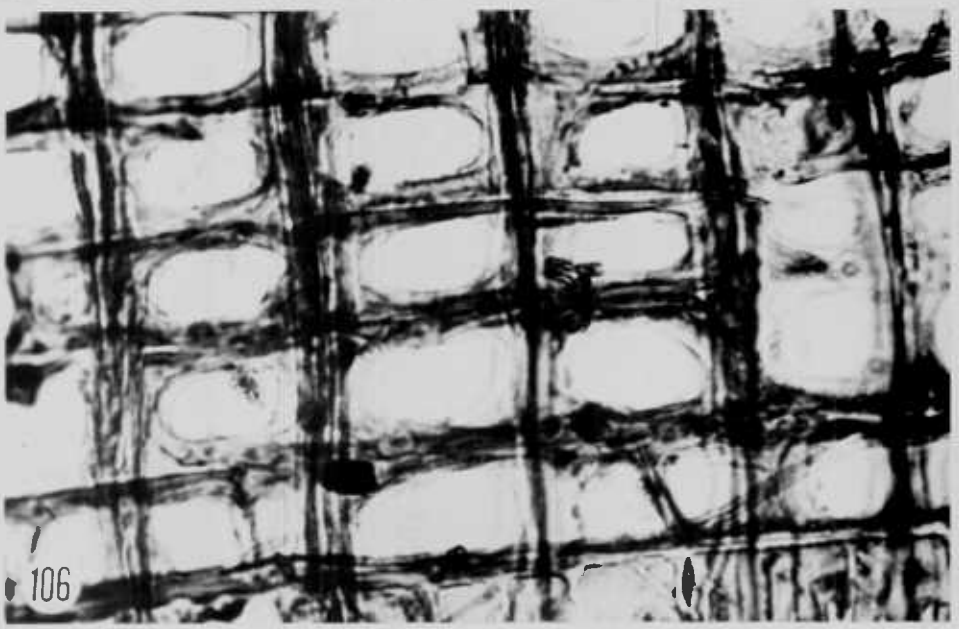

106

Untreated Scots pine: The whole block was heavily encrusted with soil at a later period of exposure than that of untreated birch. This was after 6 weeks exposure and after 9 to 12 weeks exposure it was very difficult to separate the blocks from the surrounding soil without damaging certain heavily degraded parts of the blocks. The blocks also gradually became slightly darker in colour towards the later periods of exposure.

After one week's exposure colonization was sparse and scattered in a few regions of the block but no sign of decay was observed. After 3 weeks exposure colonization was patchy, being heavy or sparse throughout the block but with still a few zones that contained no fungal hyphae. Rays and latewood zones in certain parts of the block were slightly degraded after this period. After 6 weeks exposure most of the block was heavily colonized and a few outer parts were found to be broken into fragments due to severe attack. Degradation was mostly in the rays (Fig.106) and latewood tracheids. The degradation pattern observed was that of a soft-rot type where cavities were formed in the S2 layer of the tracheid walls (Fig.107). The types of cavities formed resemble those described by Courtois (1963a) as being singly-occurring cylindrical cavities, conical at both ends changing into more narrow cylinders and then becoming spindle-shaped (Figs.108 and 109) or as narrow tube-shaped cavities (Fig.110). Courtois (1963a) goes on to suggest that the former type of cavity was orientated parallel to the longitudinal axis of the cell while the later were orientated as Z-helices to the longitudinal axis of the cell. From Figs. 108,109 and 110 it is clear that this differentiation is largely a matter of 3-dimensional geometry, in as much as the cavities parallel to the vertical axis of the cell are always at the edge of the cell so that

any deviation from the vertical viewed at right angles to the deviation will appear to be vertical. Serial sectioning would no doubt confirm this. With the progress of decay these cavities joined together causing the destruction of the whole S2 layer of the wall (Fig.111). Towards the later periods of exposure the whole block was heavily colonized and most parts were also heavily degraded. It was usually the case throughout the exposure periods that tracheids in the earlywood zones were degraded at a later stage and less than the rays or latewood tracheids (Figs.112 and 113). Degradation of the earlywood tracheids was of the same type observed in the latewood tracheids (Fig.114). At the later periods of exposure only one type of cavity was observed; the narrow tube-shaped cavities orientated asZ-helices to the longitudinal axis of the cell (Fig.115).

Treated Scots pine: Up to 21 weeks of exposure the blocks were sound and showed no change in colour or texture. They were also easily separated from the few surrounding particles of soil without causing the slightest damage to the blocks.

In the early periods of exposure colonization was very sparse and scattered in the outer regions of the block. Most of the hyphae present were unhealthy. After 9 weeks exposure more unhealthy hyphae were present at some depth in the block. This continued to be the case until the last week of exposure. There were more hyphae present in the latewood zones than the earlywood zones. No degradation was observed throughout the block even after 21 weeks of exposure.

In general the colonization and degradation of both species of untreated wood were slower than that observed in the monocultures. Treated birch was also colonized and gradually degraded at a late stage but even after 21 weeks of exposure many parts of the block were resistant to decay. Treated Scots pine as usual showed a high resistance to decay after a long period of exposure.

Figure 107. T.S. untreated Scots pine buried in soil showing soft-rot cavities in the S2 layer of the latewood tracheids after 6 weeks exposure (x 610)

Figure 108. R.L.S. untreated Scots pine buried in soil showing spindle-shaped soft-rot cavities (x 610)

Figure 109. As figure 108 but under polarised light (x 610)

107

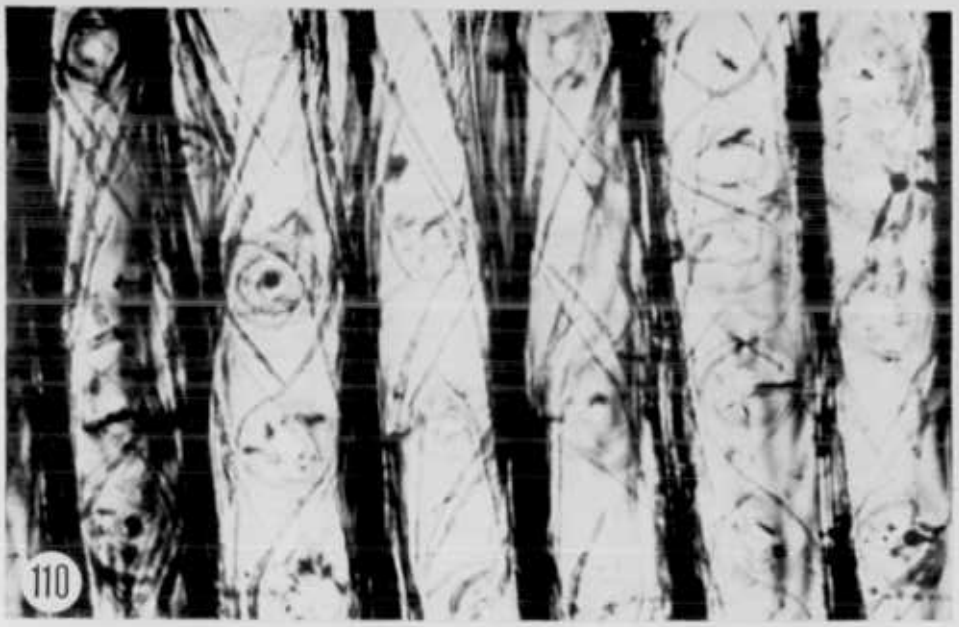
108

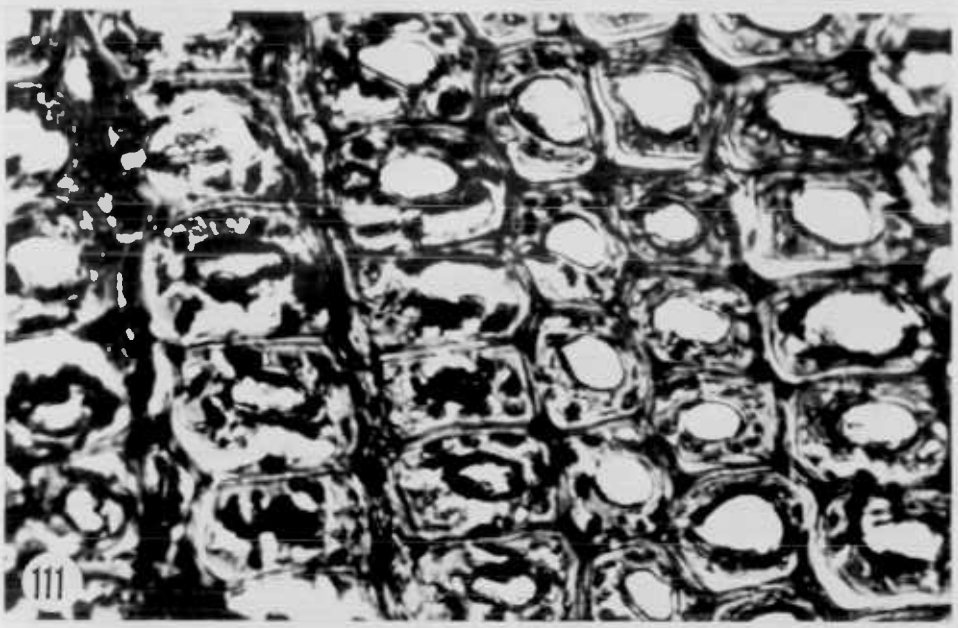
109

Figure 110. As figure 108 (x 610)

Figure 111. T.S. untreated Scots pine buried in soil showing heavily degraded S_2 layer in latewood tracheids by soft-rot attack after 9 weeks exposure. Note the highly resistant S_3 layer against decay (x 610)

Figure 112. T.S. untreated Scots pine buried in soil showing more degraded latewood tracheids than the earlywood tracheids after 9 weeks exposure (x 610)


110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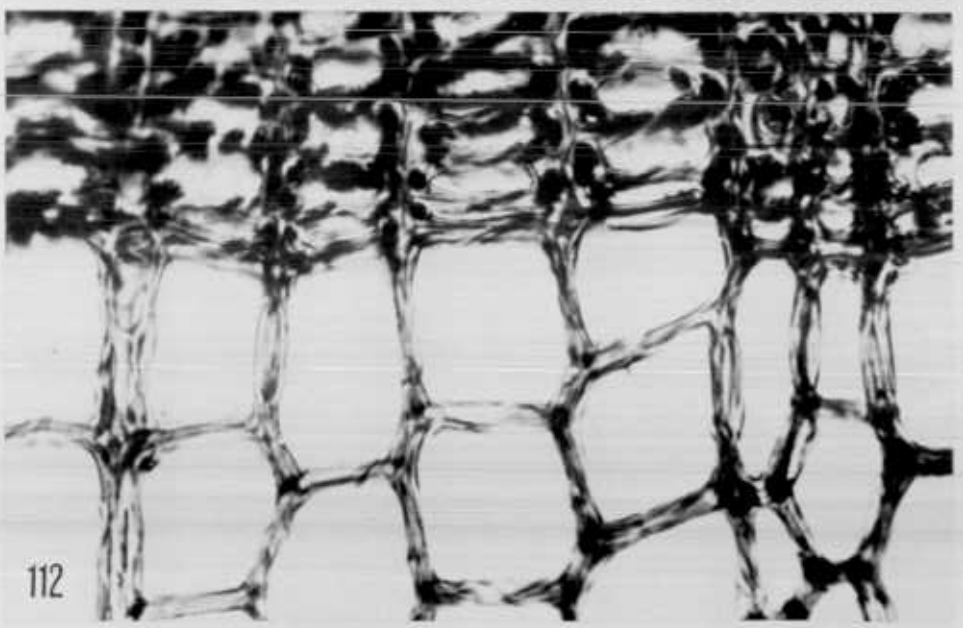

112

Figure 113. T.L.S. untreated Scots pine buried in soil showing heavier decay of latewood tracheids and rays than the earlywood tracheids after 9 weeks exposure (x 160)

Figure 114. T.S. untreated Scots pine buried in soil showing soft-rot cavities in earlywood tracheids after 9 weeks exposure (x 450)

Figure 115. R.L.S. untreated Scots pine buried in soil showing heavily degraded tracheids by soft-rot attack after 12 weeks exposure (x 610)

113

114

115

Plate 1. Diagram to illustrate colonisation and degradation by Phialophora fastigiata

Plate 2. Diagram to illustrate colonisation and degradation by Chaetomium globosum

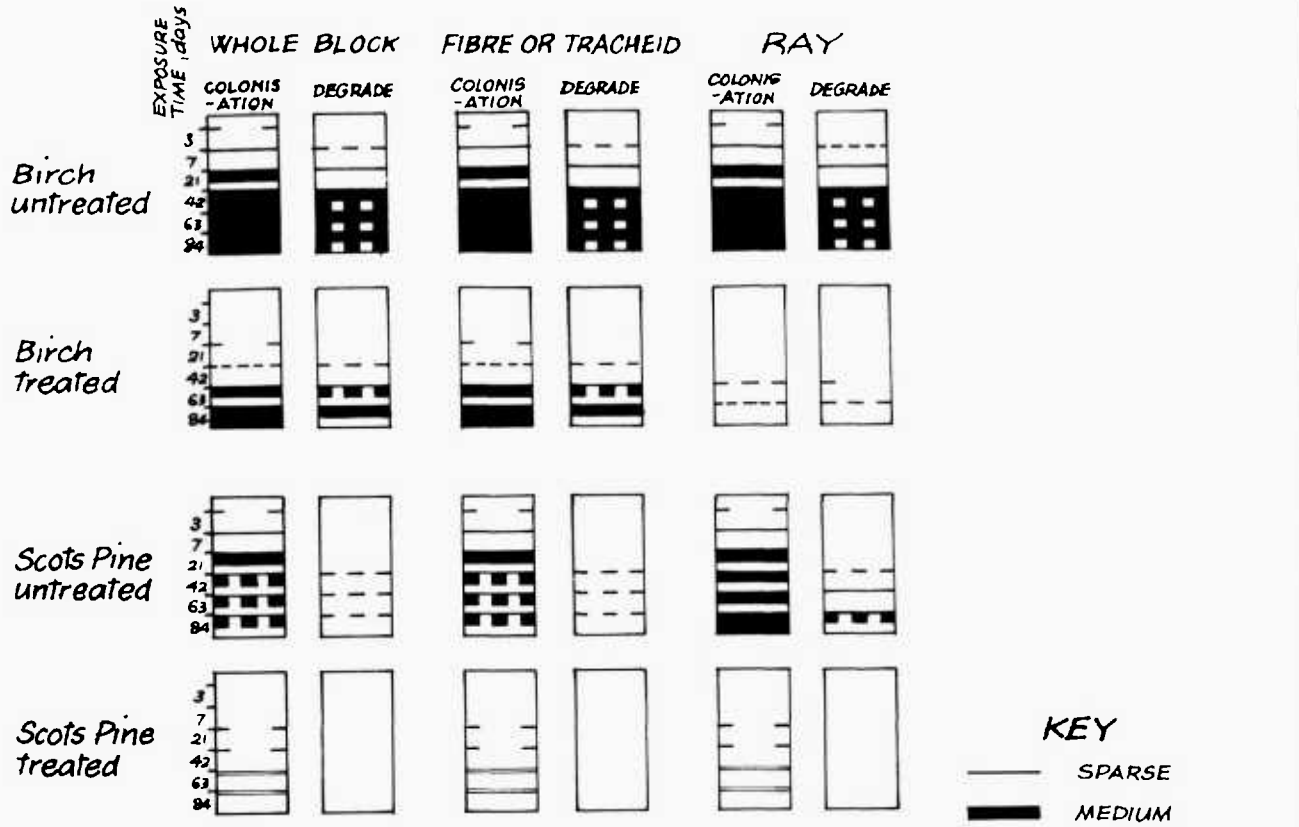


Plate 1 : *Phialophora fastigiata*

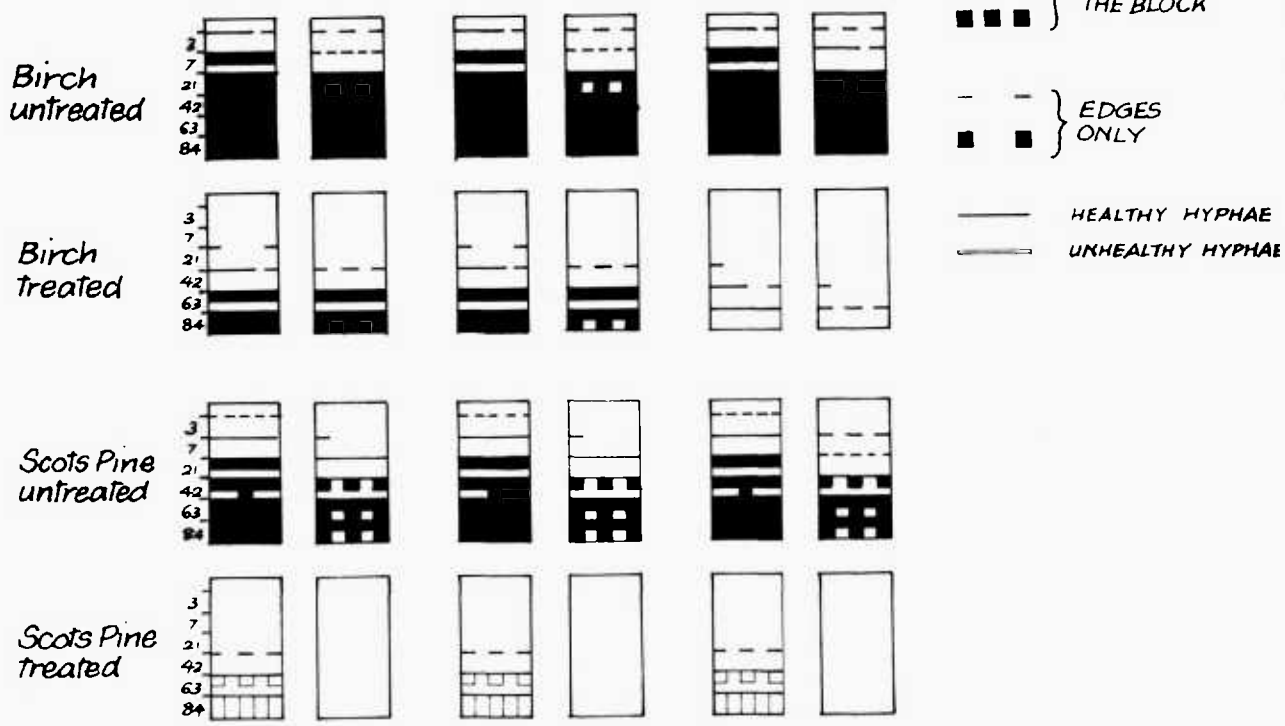


Plate 2 : *Chaetomium globosum*

Plate 3. Diagram to illustrate colonisation and degradation by *Coriolus versicolor*

Plate 4. Diagram to illustrate colonisation and degradation by *Coniophora puteana*

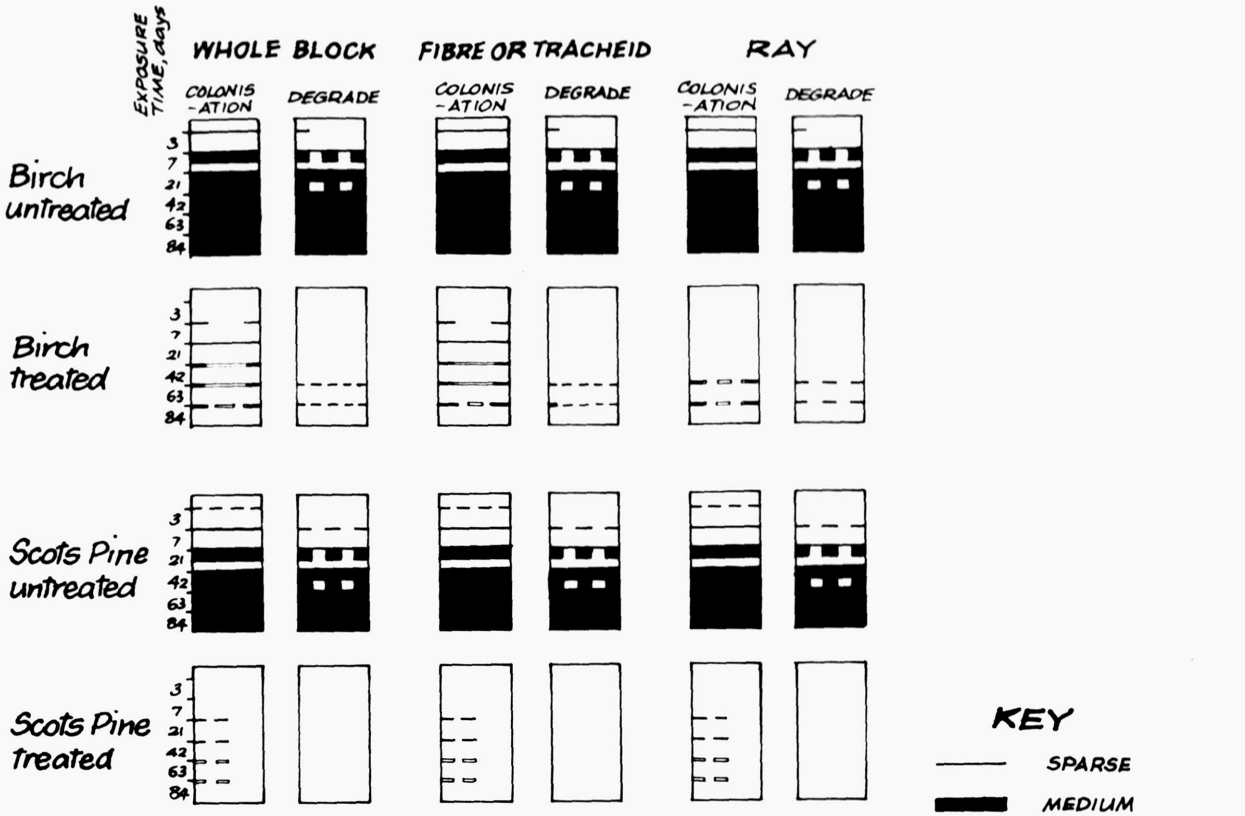


Plate 3 : Coriolus versicolor

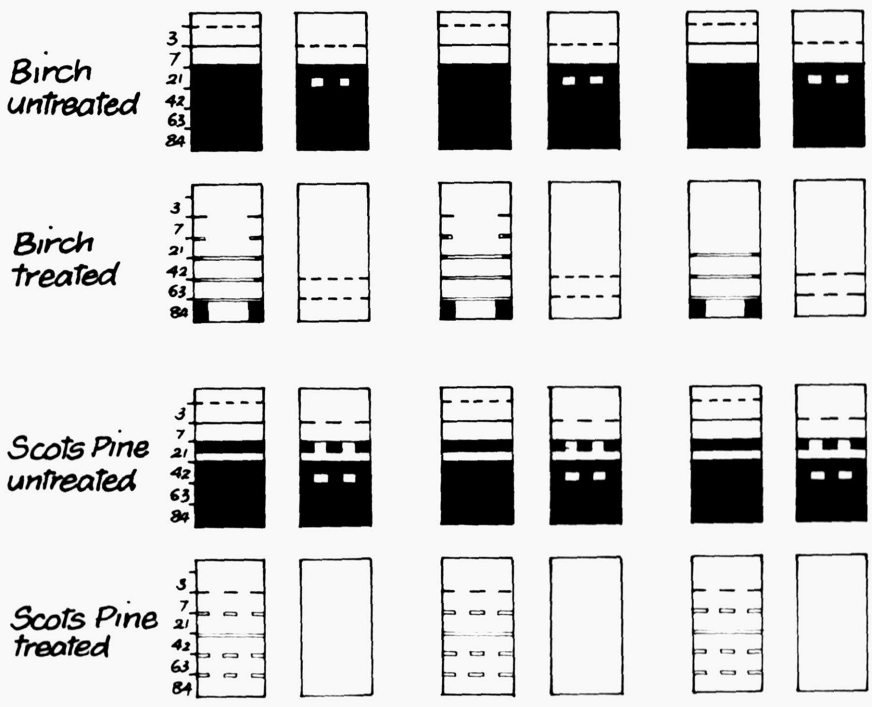


Plate 4 : Coniophora puteana

Plate 5. Diagram to illustrate colonisation and degradation by Botryodiplodia theobromae

Plate 6. Diagram to illustrate colonisation and degradation by mixed culture in unsterilised soil.

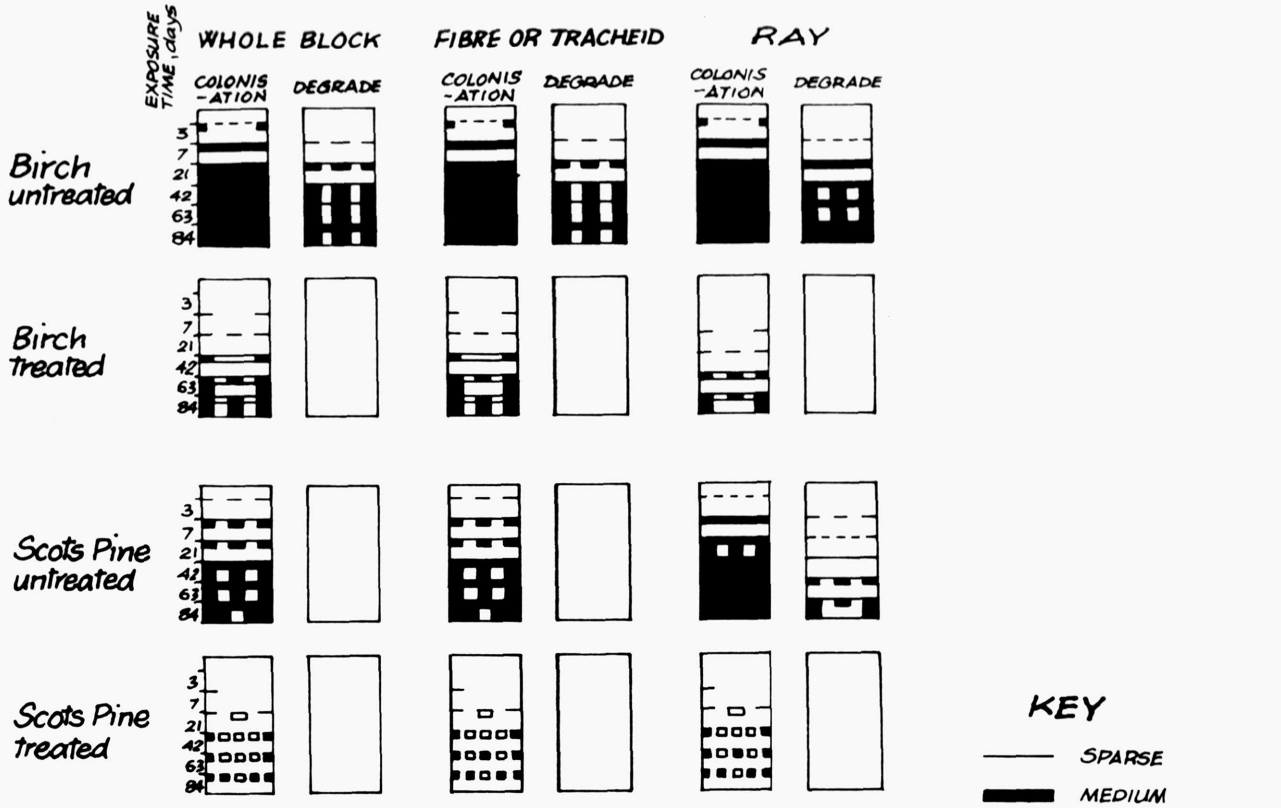


Plate 5 : Botryodiplodia theobromae

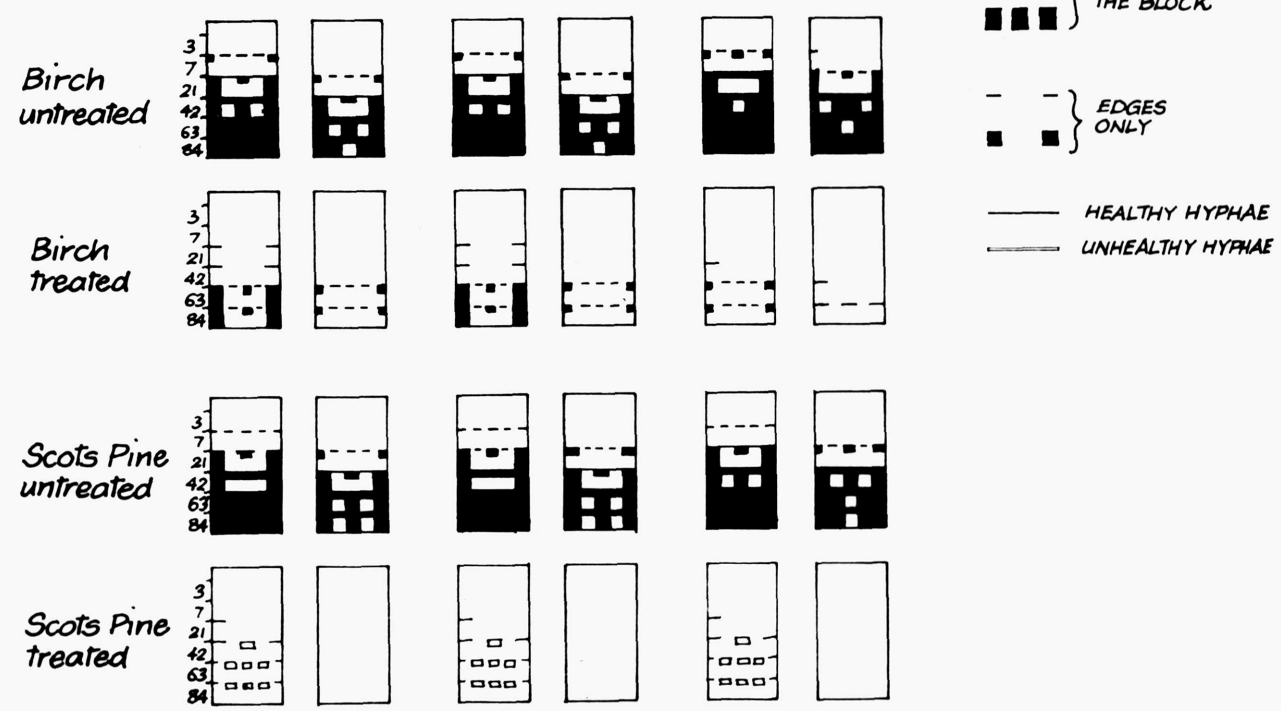


Plate 6 : Mixed culture (soil)

3.2. Moisture Content and Weight loss results

The mean percentage moisture content and mean percentage weight loss of 4 replicates of each species of wood was calculated in the way described in section 2.2.1.2. This was done for the 2.5%, 5% and untreated blocks after 3, 6,9 and 12 weeks exposure to each of the 5 species of fungi and unsterile soil. The same calculations were also made for the 4 replicates of the 2.5%, 5% and untreated control blocks of both species of wood (i.e., sterilized wood blocks on media without fungal mycelia or buried in sterile soil) in order to monitor any increase in weight due to the mineral uptake from the media or soil by the wood blocks.

This was carried out after 3, 6, 9 and 12 weeks exposure in the case of the sterile agar media and after 3 and 12 weeks exposure in the case of the sterile soil. A total of approximately 720 blocks of both species of wood were used for the weight loss and moisture content measurements.

The results showing erratic extremes were not considered in the calculations and the mean was taken for all the remaining blocks in such circumstances.

The results obtained from each fungal species or soil for each species of wood after the different exposure periods were tablulated in 12 separate tables. The results obtained from the control blocks were also included in each of the above-mentioned tables.

Key to abbreviations

E blocks exposed to the fungus

C control blocks

U untreated blocks

% M.C.% moisture content

% W.L.% weight loss

Exposure Period	% Preservative	E %M.C.	E %W.L.	C %M.C.	C %W.G.
3 weeks	2.5%	60.26	3.90	52.46	3.33
	5%	70.39	3.15	58.60	3.58
	U	159.39	7.90	76.97	1.90
6 weeks	2.5%	45.55	4.34	57.38	3.47
	5%	76.56	4.45	65.66	4.50
	U	107.37	11.87	49.15	2.58
9 weeks	2.5%	65.35	8.62	63.75	3.85
	5%	113.49	7.27	67.63	4.49
	U	98.08	17.31	63.87	3.34
12 weeks	2.5%	112.08	9.05	75.76	5.41
	5%	114.34	7.96	66.15	4.33
	U	122.78	19.77	66.00	3.73

Table (1) Phialophora fastigiata in birch

Exposure Period	% Preservative	E %M.C.	E %W.L.	C %M.C.	C %W.G.
3 weeks	2.5%	49.32	3.34	54.89	2.35
	5%	58.26	3.17	59.65	2.33
	U	162.70	3.83	141.99	2.93
6 weeks	2.5%	37.01	1.33	85.88	2.45
	5%	39.15	3.00	39.58	4.05
	U	152.33	5.48	133.08	2.81
9 weeks	2.5%	52.87	2.39	85.08	3.86
	5%	43.37	3.47	66.72	4.26
	U	136.42	7.12	126.33	3.32
12 weeks	2.5%	55.20	3.97	81.72	3.30
	5%	78.41	3.84	62.35	4.59
	U	149.37	8.65	124.78	2.75

Table (2) Phialophora fastigiata in Scots pine

Exposure Period	% Preservative	E %M.C.	E %W.L.	C %M.C.	C %W.G.
3 weeks	2.5%	73.18	6.21	52.46	3.33
	5%	92.60	5.12	58.60	3.58
	U	115.28	28.56	76.97	1.90
6 weeks	2.5%	63.61	11.67	57.38	3.47
	5%	81.49	9.51	65.66	4.50
	U	101.87	46.89	49.15	2.58
9 weeks	2.5%	105.74	19.95	63.75	3.85
	5%	130.41	19.69	67.63	4.49
	U	129.87	51.11	63.87	3.34
12 weeks	2.5%	96.88	28.94	75.76	5.41
	5%	79.80	21.46	66.15	4.33
	U	132.31	53.56	66.00	3.73

Table (3) Chaetomium globosum in birch

Exposure Period	% Preservative	E %M.C.	E %W.L.	C %M.C.	C %W.G.
3 weeks	2.5%	53.94	2.79	54.89	2.35
	5%	74.22	2.16	59.65	2.33
	U	167.61	8.39	141.99	2.93
6 weeks	2.5%	25.08	2.87	85.88	2.45
	5%	55.28	3.58	39.58	4.05
	U	85.88	10.25	133.08	2.81
9 weeks	2.5%	41.81	3.10	85.08	3.86
	5%	87.28	3.78	66.72	4.26
	U	132.73	12.23	126.33	3.32
12 weeks	2.5%	50.28	3.63	81.72	3.30
	5%	50.71	4.12	62.35	4.59
	U	118.62	17.34	124.78	2.75

Table (4) Chaetomium globosum in Scots pine

Exposure Period	% Preservative	E		C	
		%M.C.	%W.L.	%M.C.	%W.G.
3 weeks	2.5%	72.66	5.94	52.46	3.33
	5%	58.18	5.54	58.60	3.58
	U	231.44	51.39	76.97	1.90
6 weeks	2.5%	32.30	5.46	57 38	3.47
	5%	36.83	4.72	65.66	4.50
	U	295.74	70.58	49.15	2.58
9 weeks	2.5%	50.08	8.51	63.75	3.85
	5%	151.97	12.38	67.63	4.49
	U	734.17	87.07	63.87	3.34
12 weeks	2.5%	128.93	29.03	75.76	5.41
	5%	137.42	13.66	66.15	4.33
	U	555.48	91.77	66.00	3.73

Table (5) Coriolus versicolor in birch

Exposure Period	% Preservative	E		C	
		%M.C.	%W.L.	%M.C.	%W.G.
3 weeks	2.5%	75.29	3.49	54.89	2.35
	5%	74.25	2.70	59.65	2.33
	U	114.03	13.73	141.99	2.93
6 weeks	2.5%	36.23	3.96	85.88	2.45
	5%	59.42	3.75	39.58	4.05
	U	146.39	22.47	133.08	2.81
9 weeks	2.5%	74.94	3.29	85.08	3.86
	5%	58.39	2.74	66.72	4.26
	U	147.75	47.65	126.33	3.32
12 weeks	2.5%	81.51	4.38	81.72	3.30
	5%	29.38	3.64	62.35	4.59
	U	147.97	64.96	124.78	2.75

Table (6) Coriolus versicolor in Scots pine

Exposure Period	% Preservative	E %M.C.	E %W.L.	C %M.C.	C %W.G.
3 weeks	2.5%	77.52	2.34	52.46	3.33
	5%	72.61	2.97	58.60	3.58
	U	59.99	43. 6	76.97	1.90
6 weeks	2.5%	100.82	3.06	57.38	3.47
	5%	93.86	2.53	65.66	4.50
	U	148.09	53.00	49.15	2.58
9 weeks	2.5%	111.00	7.81	63.75	3.85
	5%	97.82	8.48	67.63	4.49
	U	140.01	65.01	63.87	3.34
12 weeks	2.5%	104.06	13.10	75.76	5.41
	5%	74.31	15.16	66.15	4.33
	U	525.72	81.50	66.00	3.73

Table (7) Coniophora puteana in birch

Exposure Period	% Preservative	E %M.C.	E %W.L.	C %M.C.	C %W.G.
3 weeks	2.5%	86.63	2.36	54.89	2.35
	5%	83.52	2.83	59.65	2.33
	U	148.98	12.79	141.99	2.93
6 weeks	2.5%	119.42	4.28	85.88	2.45
	5%	89.40	2.81	39.58	4.05
	U	202.10	17.79	133.08	2.81
9 weeks	2.5%	112.73	3.25	85.08	3.86
	5%	100.90	2.52	66.72	4.26
	U	421.73	54.26	126.33	3.32
12 weeks	2.5%	87.95	3.01	81.72	3.30
	5%	80.45	3.35	62.35	4.59
	U	234.71	72.50	124.78	2.75

Table (8) Coniophora puteana in Scots pine

Exposure Period	% Preservative	E		C	
		%M.C.	%W.L.	%M.C.	%W.G.
3 weeks	2.5%	42.85	3.45	52.46	3.33
	5%	90.32	3.76	58.60	3.58
	U	45.25	3.69	76.97	1.90
6 weeks	2.5%	40.91	4.94	57.38	3.47
	5%	45.78	3.96	65.66	4.50
	U	50.56	6.33	49.15	2.58
9 weeks	2.5%	36.50	4.09	63.75	3.85
	5%	59.52	3.98	67.63	4.49
	U	48.80	8.35	63.87	3.34
12 weeks	2.5%	23.37	4.74	75.76	5.41
	5%	25.10	4.20	66.15	4.33
	U	120.48	31.73	66.00	3.73

Table (9) Botryodiplodia theobromae in birch

Exposure Period	% Preservative	E		C	
		%M.C.	%W.L.	%M.C.	%W.G.
3 weeks	2.5%	138.92	3.96	54.89	2.35
	5%	57.52	3.50	59.65	2.33
	U	54.60	2.81	141.99	2.93
6 weeks	2.5%	30.25	3.84	85.88	2.45
	5%	45.51	3.98	39.58	4.05
	U	54.60	3.63	133.08	2.81
9 weeks	2.5%	72.47	4.03	85.08	3.86
	5%	55.51	3.91	66.72	4.26
	U	55.98	6.76	126.33	3.32
12 weeks	2.5%	46.75	4.05	81.72	3.30
	5%	25.66	4.08	62.35	4.59
	U	91.00	7.20	124.78	2.75

Table (10) Botryodiplodia theobromae in Scots pine

Exposure Period	% Preservative	E		C	
		%M.C.	%W.L.	%M.C.	%W.G.
3 weeks	2.5%	22.27	2.83	37.32	0.73
	5%	24.92	2.66	42.81	1.99
	U	110.74	29.61	29.68	1.77
6 weeks	2.5%	37.35	4.15		
	5%	30.90	2.81		
	U	73.06	30.66		
9 weeks	2.5%	61.65	12.45		
	5%	40.37	3.03		
	U	88.39	35.54		
12 weeks	2.5%	17.56	9.00	48.71	2.45
	5%	38.79	8.27	40.92	2.20
	U	102.42	54.83	34.04	3.30

Table (11) Birch buried in soil

Exposure Period	% Preservative	E		C	
		% M.C.	%W.L.	%M.C.	%W.G.
3 weeks	2.5%	18.83	3.51	32.62	3.45
	5%	30.07	2.80	26.15	1.31
	U	50.28	9.81	34.30	0.36
6 weeks	2.5%	28.65	5.80		
	5%	24.33	4.75		
	U	26.97	11.14		
9 weeks	2.5%	48.81	5.14		
	5%	38.76	4.56		
	U	45.21	13.90		
12 weeks	2.5%	15.37	3.92	35.18	5.01
	5%	24.86	4.49	55.03	3.36
	U	69.43	18.49	40.32	4.30

Table (12) Scots pine buried in soil

3.3. Preservative distribution results

3.3.1. X-ray micro-radiography results

By applying the X-ray micro-radiographic technique described in Section 2.2.3.2.1., a shadow image of the wood section was obtained on the H-R photographic plate. These images were examined under a light microscope and photographs of different magnifications were taken using a Reichert Kam ES camera. The contrast in these photographs was highly dependent upon that of the initial contact H-R plate.

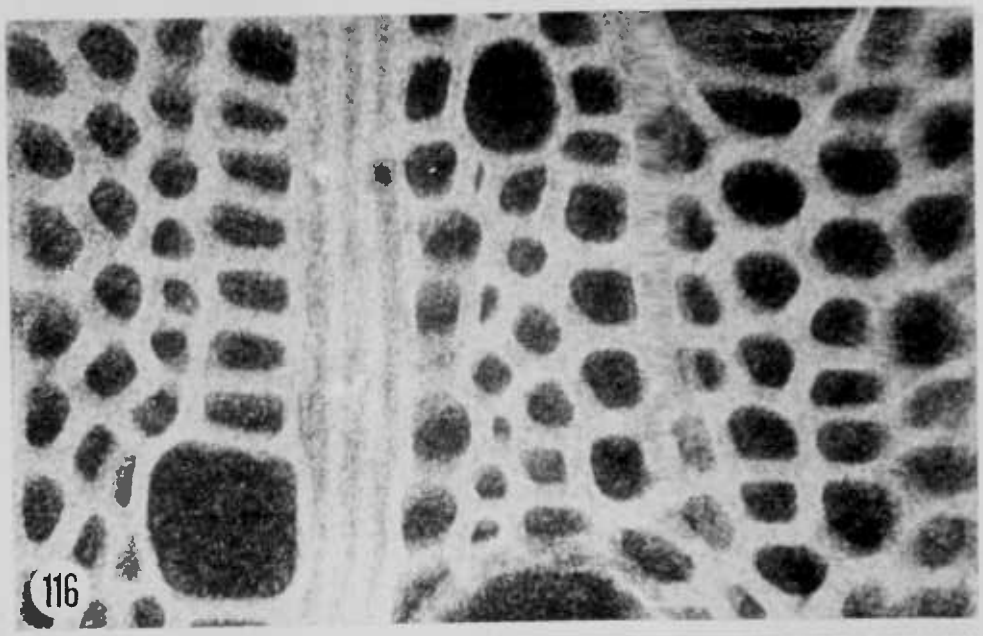
The shadow images were very sharp in the case of the sections obtained from the blocks treated with the higher preservative concentrations (i.e., 7.5% and 10%) compared to those of the lower preservative concentrations (i.e., 2.5% and 5%) or the even less distinct images of the sections obtained from the untreated control blocks. This was clearly distinguishable in the case of birch (Figs. 116, 117, 118 and 119) and to a lesser extent in the case of Scots pine (Figs. 120, 121, 122, 123 and 124). The different tones observed in the above-mentioned figures were the indication of the presence or absence of the preservative material in the wood sections. This was less informative in the final photographic plates than the direct microscopic observations of the original H-R plates.

The areas of the wood sections which appeared white in the photographic plates indicate a strong absorption of X-rays by these cell walls due to the presence of high concentrations of the preservative material, while the grey shades indicate the weak absorption of X-rays due to the absence or low concentration of the preservative in these areas. A non-absorbing material appeared as a black background in the photographic plates. The images obtained from the untreated control sections (Figs. 117 and 121) were rather confusing when compared to those obtained from the treated sections especially with the lower preservative concentrations,

Figure 116. An X-ray contact micro-radiograph of T.S. in birch treated with 7.5% copper-chrome-arsenic wood preservative (x 520)

Figure 117. An X-ray contact micro-radiograph of T.S. in untreated birch (x 520)

Figure 118. An X-ray contact micro-radiograph of T.S. in birch treated with 7.5% copper-chrome-arsenic wood preservative (x 160)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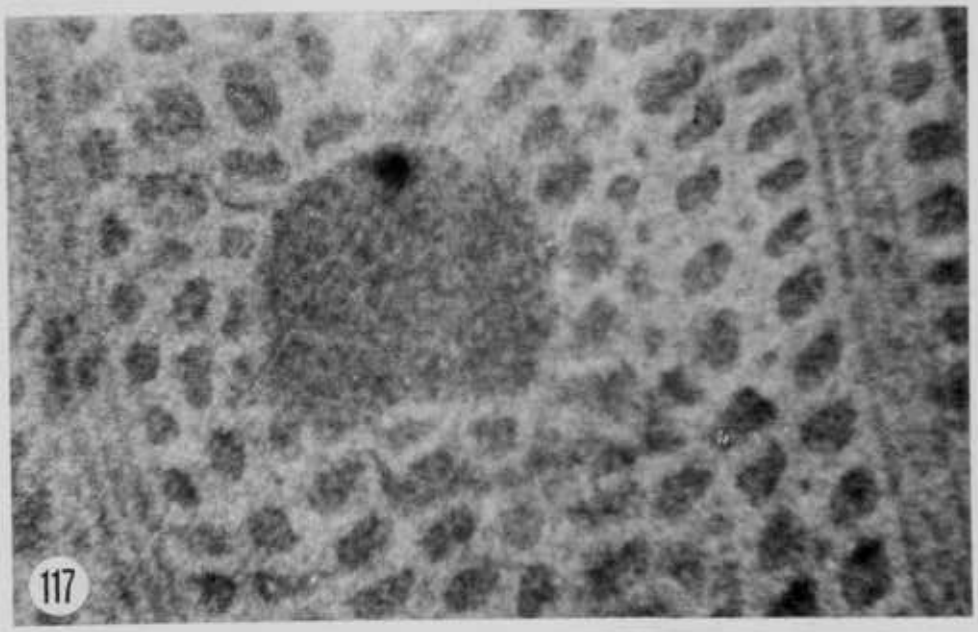

117

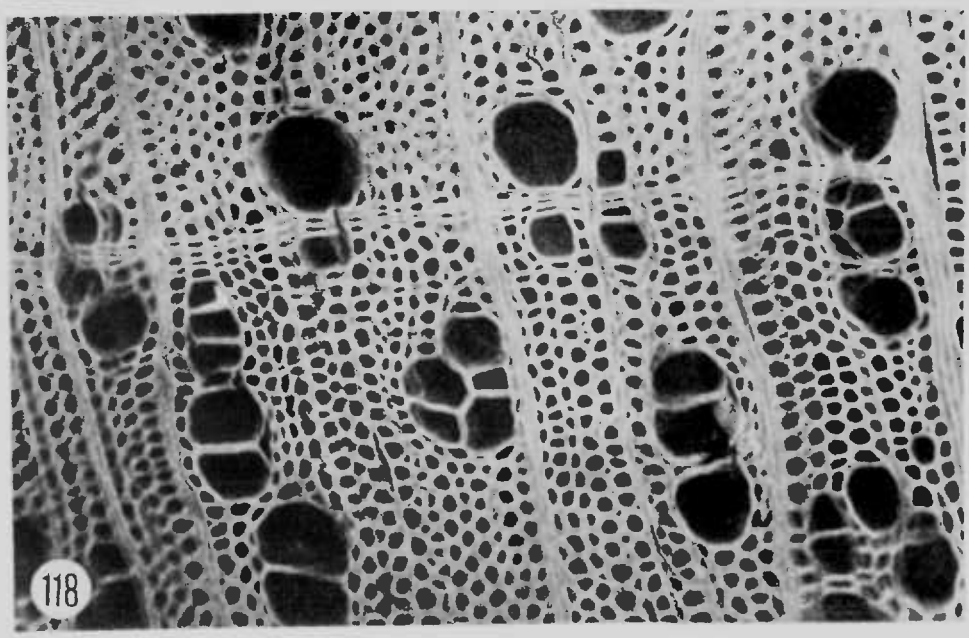
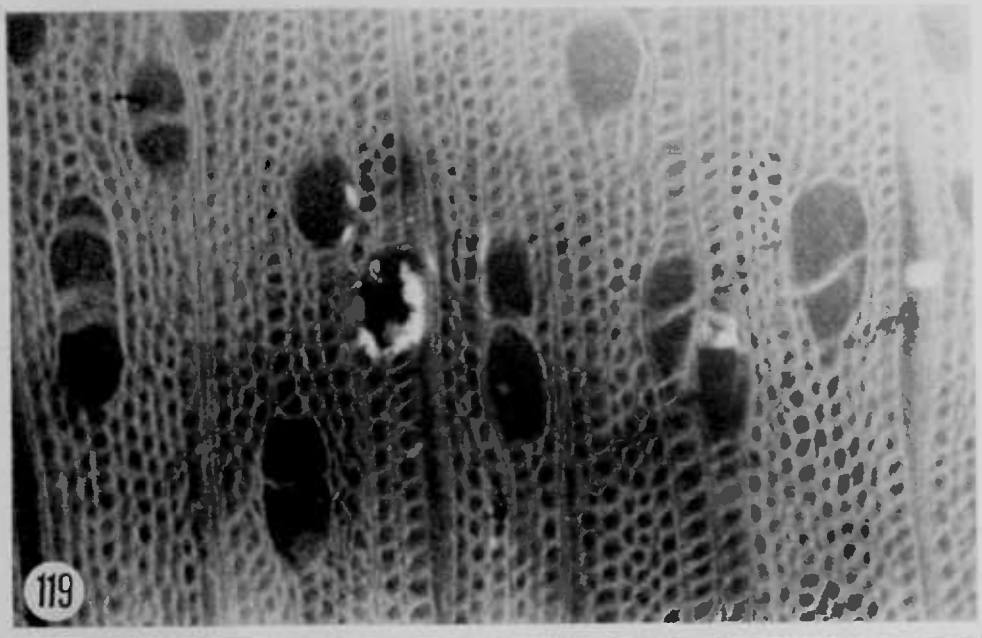

118

Figure 119. An X-ray contact micro-radiograph of T.S. in birch treated with 2.5% copper-chrome-arsenic wood preservative (x 160)

Figure 120. An X-ray contact micro-radiograph of T.S. in Scots pine treated with 7.5% copper-chrome-arsenic wood preservative (x 520)

Figure 121. An X-ray contact micro-radiograph of T.S. in untreated Scots pine (x 520)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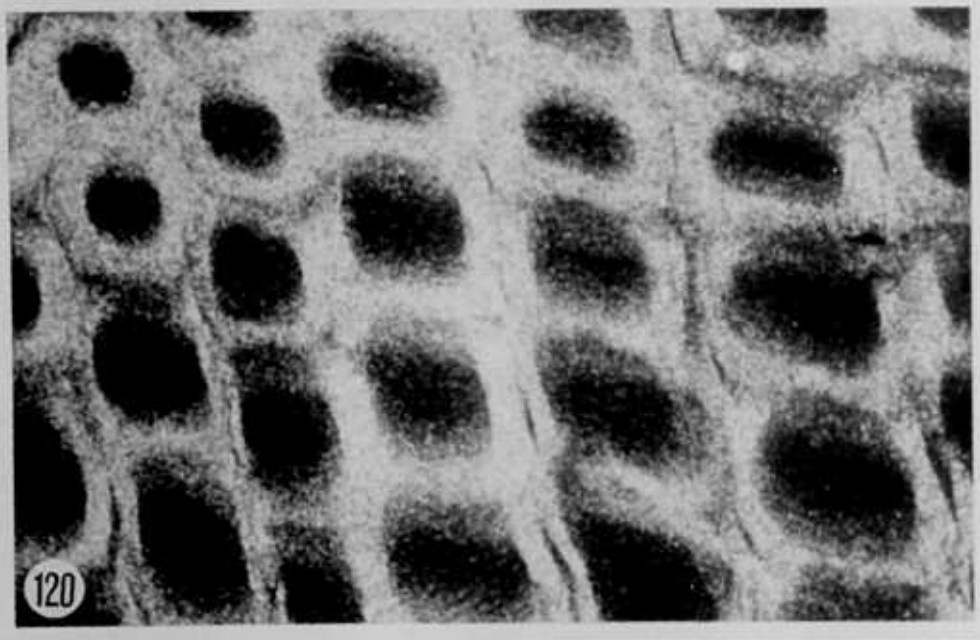
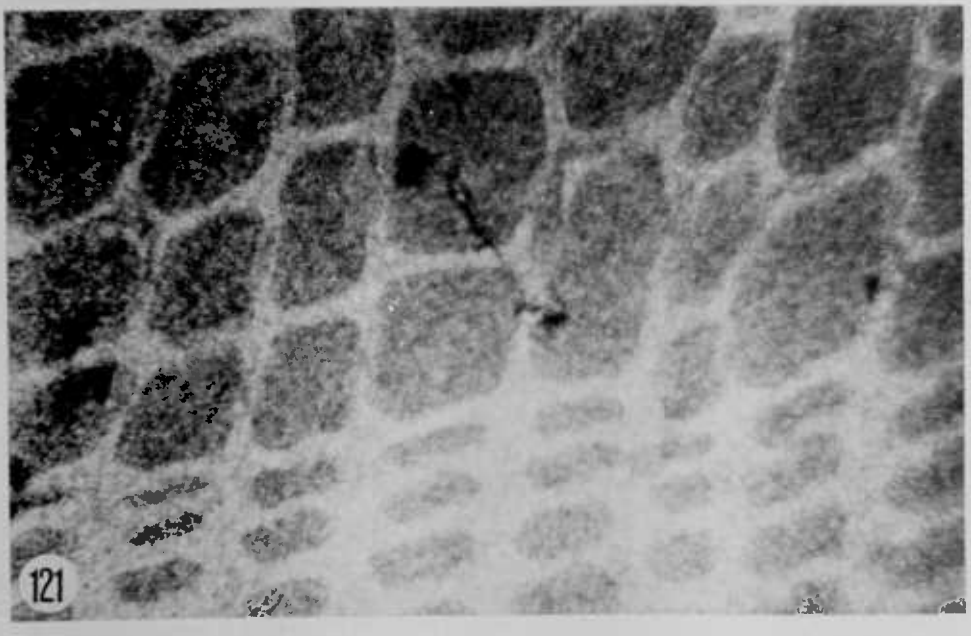
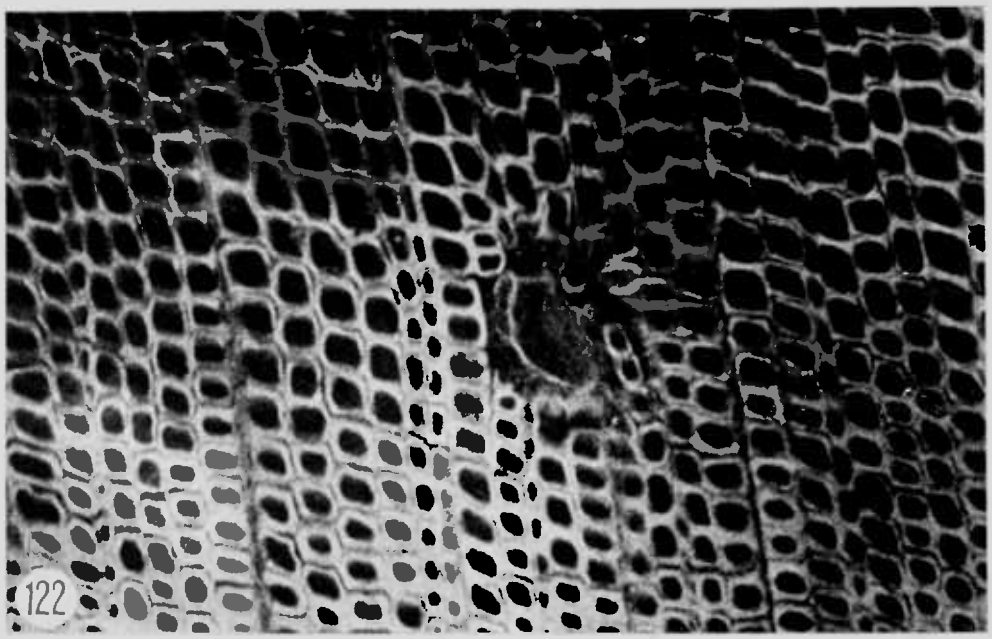
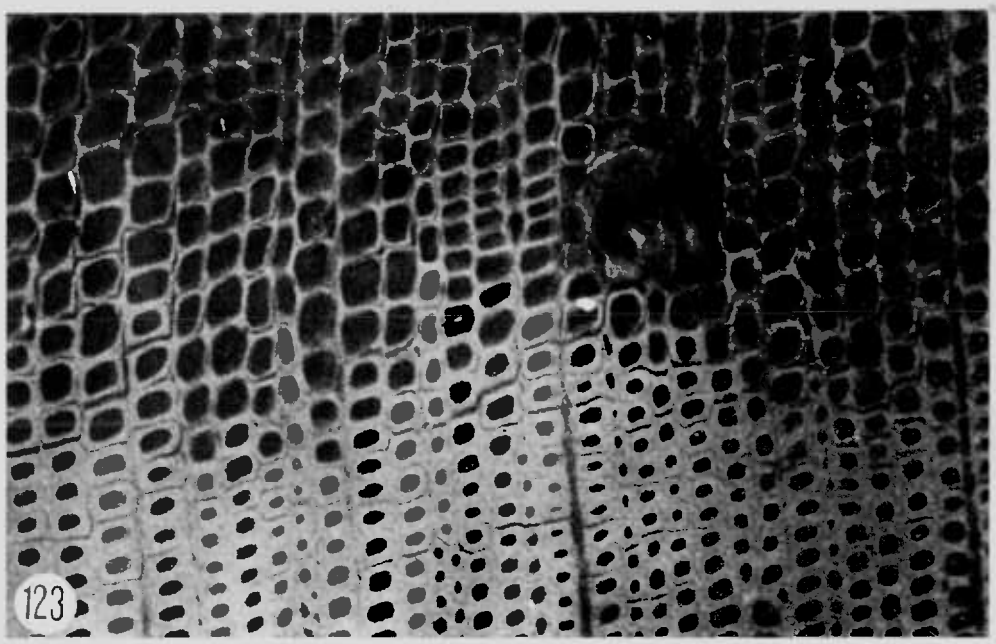

120

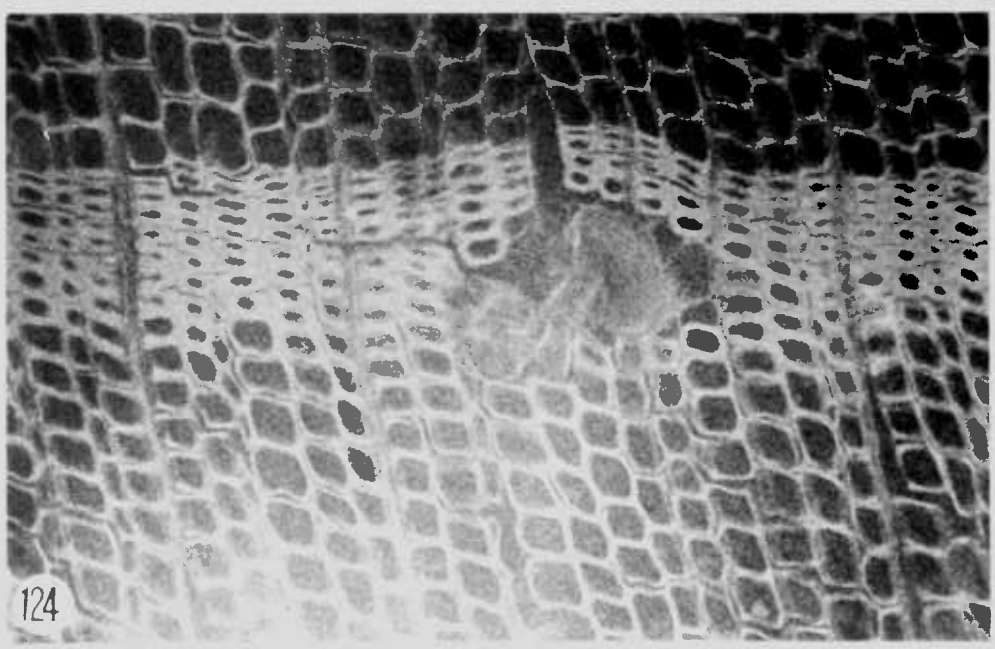

121

Figures 122, 123 and 124.

X-ray contact micro-radiographs of T.S. in Scots pine treated with 7.5%, 5% and 2.5% copper-chromo-arsenic wood preservative respectively (x 160)


122


123


124

particularly in the case of Scots pine because they were not easily distinguishable from each other in certain parts of the sections.

In the case of birch the preservative seems to be present in the wall layers of the vessels, fibres and rays (Fig. 116) and lumps of the preservative were also found deposited in certain vessels (Fig. 119). In the case of Scots pine the preservative also seems to be present in all cells with more preservative in the latewood zones than the earlywood zones (Figs. 120, 122, 123 and 124).

From these figures the apparent clarity is deceptive, since no quantification or comparison between timber species is possible. This aspect has been listed in Section 2.2.3.2.1. The limited information obtained when using this technique and its disadvantages make it inadequate for the detailed preservative distribution studies required and hence other techniques were developed for such work.

3.3.2. EDAX Results

The energy dispersive system (EDAX) used in this investigation proved to be a useful tool for the study of the general distribution of the different elements of the preservative at the tissue level. The system provided a clear visual aid for the distribution of all elements present in the preservative as well as the individual elements because an ordinary electron image scan of the area analysed in the wood section was produced in addition to an elemental image scan of the X-ray signal for all elements and for each individual element present in the analysed area. These figures were produced by utilizing the system in the way described in Section 2.2.3.2.2. The elemental image scan of the X-ray signal resulted in the formation of "spotty" X-ray pictures which represented the general mapping of all elements

present in the preservative and also for each individual element present in the analysed area. The "spotty" pictures were closely correlated to the anatomy of the wood section in the ordinary electron image scan of the analysed area. This useful visual information enabled areas of concentrations of preservative to be easily identified and then relative counts for the individual elements of the preservative over a 40 second time period of selected areas of the section was carried out. The counting procedure in the 2 species of wood as well as the different areas analysed was carried out under identical conditions (i.e., counting time periods and acceleration voltages were always the same) in order to be able to compare the results obtained from a certain area of a section or one species of wood with another. The relative counts of the individual elements over a 40 second time period were displayed on the T.V. screen of the EDAX system where a spectrum was also formed, each peak in the spectrum at a certain energy level represents a specific element. All counts were corrected for background by linear interpolation of the base line either side of the peak of interest (c.f. Section 2.2.3.2.2. for background in the spectrum).

The X-ray line scan (c.f. Section 2.2.3.2.2.) of the individual elements of the preservative was not found to be necessary for the present investigation because of the adequate information obtained from the previously mentioned techniques concerning the distribution of the preservative at tissue level. An example of the X-ray line scan technique is shown in figures 125, 126 and 127 for copper, chromium and arsenic respectively.

As previously described (c.f. Section 2.2.2.6.1.), considerable difficulties were encountered with the analysis due to the angle of specimen tilt necessary to optimise X-ray detection. The use of the

wrong tilt angle made it impossible to correlate the "spotty" picture produced for a particular area with its own electron image due to the fact that X-rays were emitted from all the cut faces and surfaces of the lumina which tended to obscure the cell outlines. This was particularly true in the case of Scots pine where a very even preservative distribution was obtained. Figures 128 and 129 show the electron image scan and the "spotty" X-ray picture for a transverse section of Scots pine scanned at 45^{o}. This lack of definition was avoided by using oblique transverse sections cut at 45^{o} to the main axis of the wood cells where the lumina of the cells were orientated in direct line with the electron beam, so that exposure and shadowing of the lumina faces was thus avoided and the optimum specimen tilt angle of 45^{o} was maintained. The correlation of the "spotty" X-ray picture (Fig. 170) with its own electron image (Fig. 172) was good. In the case of birch this technique was not necessary due to the uneven distribution of the preservative where certain elements were found to contain more preservative than other elements. This was clearly indicated in the "spotty" X-ray pictures for birch (Figs. 139, 140, 141 and 142).

The same problem (i.e. image correlation) was also experinced to some extent when analysis was carried out on longitudinal sections especially in the case of Scots pine. This was obviously due to the reasons described in the case of the transverse sections but no way was found to overcome the problem in this case, because the surface view of the cell wall provided X-ray emissions which made no distinction between lumen and cell wall.

Several areas from different wood sections of both species of wood were analysed. The relative counts over a 40 second time period obtained for the different elements of the preservative for each area analysed were tabulated in separate tables.

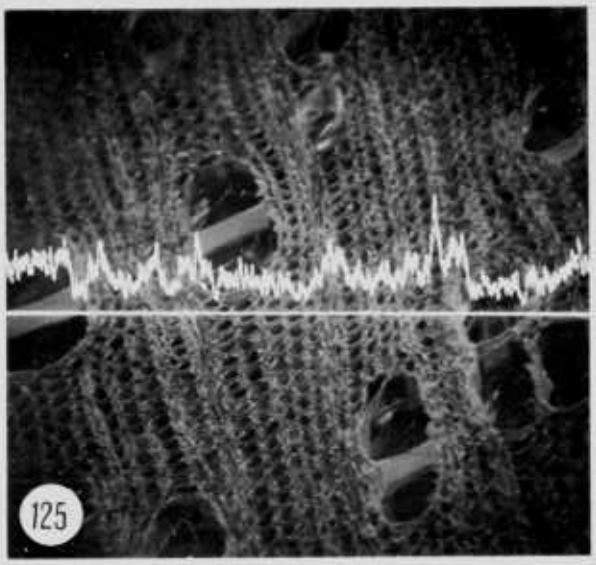
Figure 125. A line scan for copper in birch (x 140)

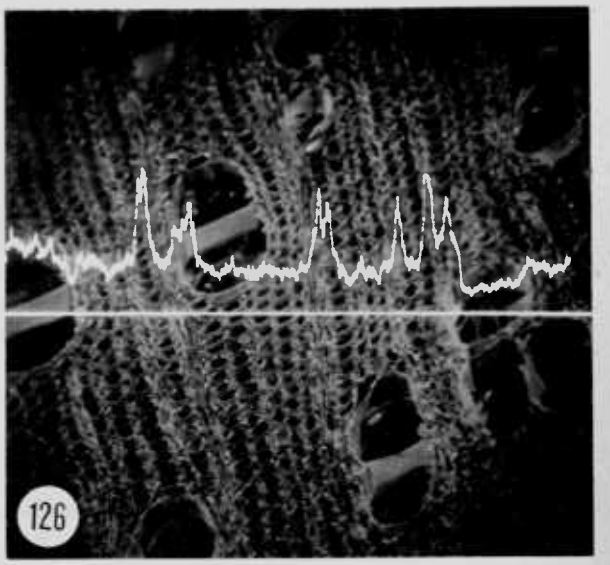
Figure 126. A line scan for chromium in birch (x 140)

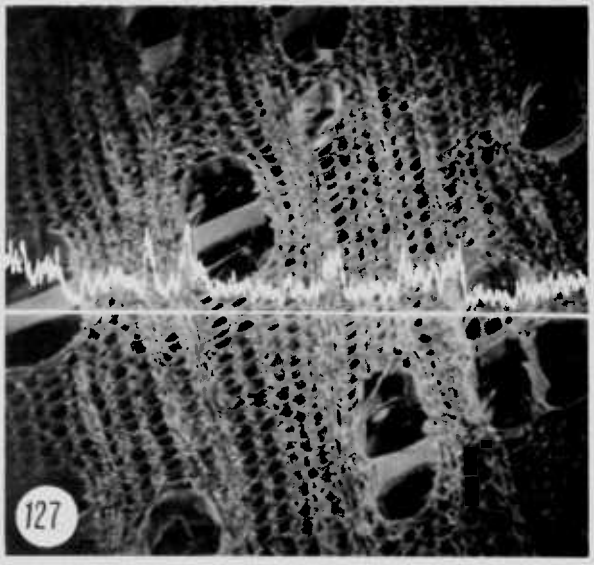
Figure 127. A line scan for arsenic in birch (x 140)

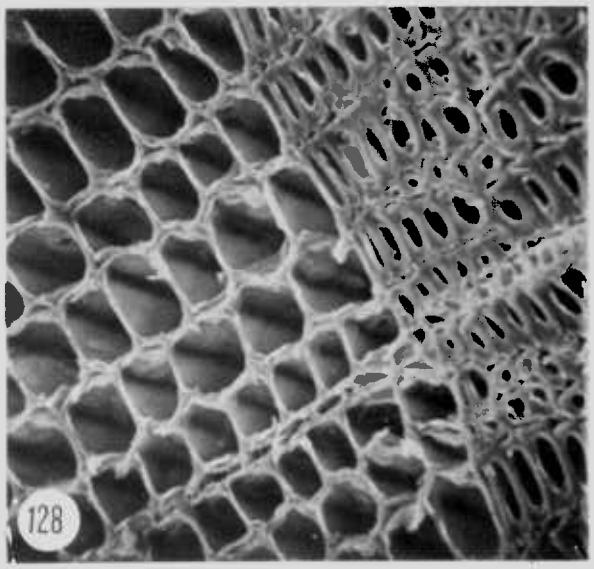
Figure 128. Electron image scan of normal T.S. of treated Scots pine (x 300)

Figure 129. X-ray image scan for copper, chromium and arsenic of field shown in figure 128 (x 300). Note that it is impossible to differentiate the cells.


125


126


127


128

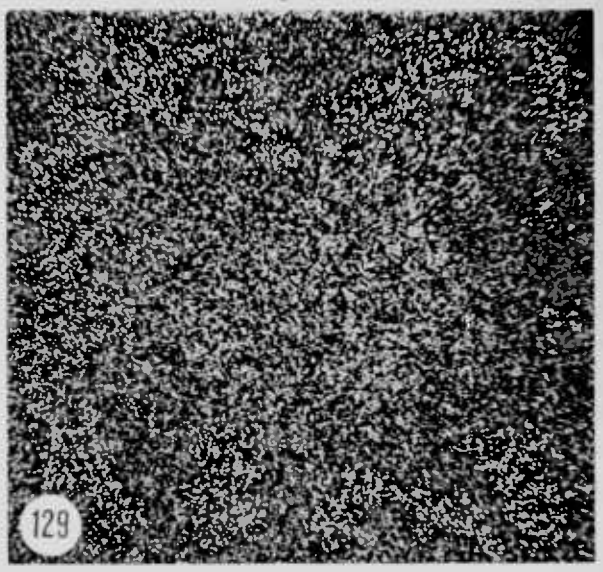

129

Figure 130. Edax spectrum for area (a) in figure 132.

Figure 131. Edax spectrum for area (c) in figure 132.

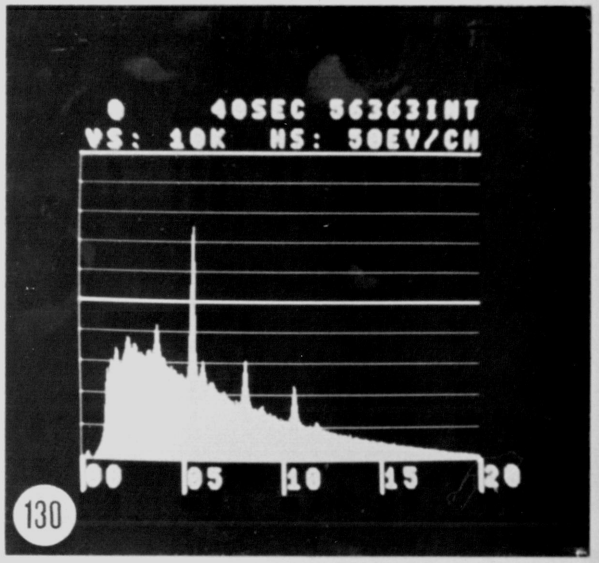
Figure 132. Electron image scan of T.S. in birch (x 300).

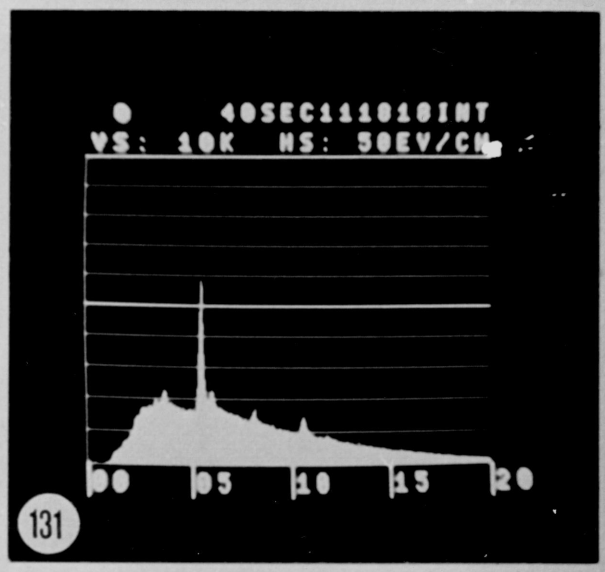
Figure 133. Edax spectrum for area (f) in figure 132.

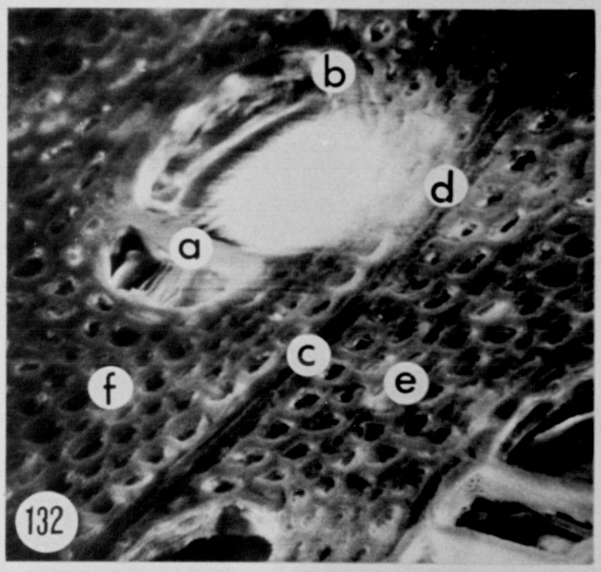
Figure 134. Edax spectrum for area (e) in figure 132.

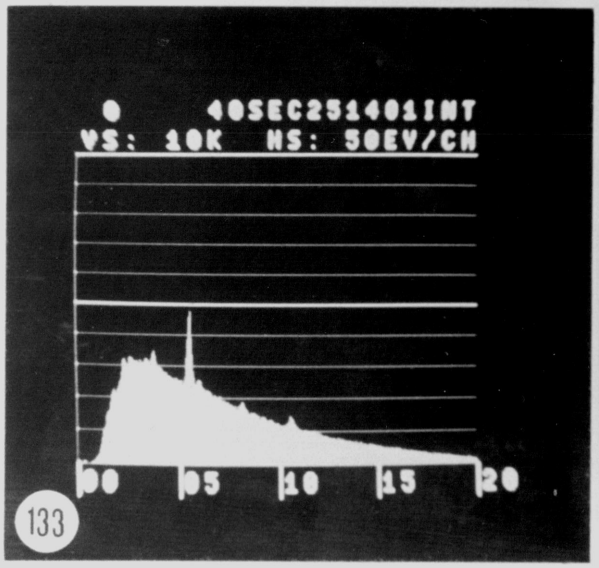
N.B.

The relative counts for Cu, Cr and Ae at points indicated in figure 132 are shown in Table (13).


40SEC 56263INT
VS: 10K HS: 50EV/CH
00
05
10
15
20
130


40SEC111818INT
VS: 10K HS: 50EV/CH
00
05
10
15
20
131


b
d
a
c
e
f
132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VS: 10K HS: 50EV/CH
00
05
10
15
20
133

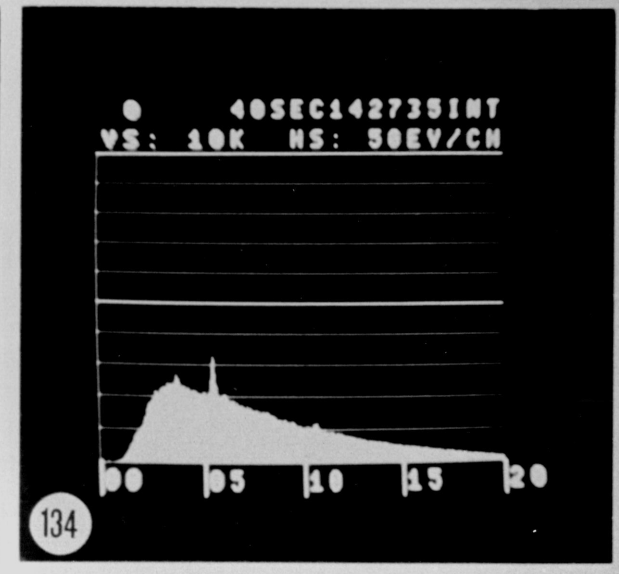

40SEC142735INT
VS: 10K HS: 50EV/CH
00
05
10
15
20
134

Figure 135. X-ray image scan for copper of field shown in figure 132 (x 300)

Figure 136. X-ray image scan for chromium of field shown in figure 132 (x 300)

Figure 132. Electron image scan of T.S. in birch (x 300)

Figure 137. X-ray image scan for arsenic of field shown in figure 132 (x 300)

Figure 138. Electron image scan of T.S. in birch (x 3080). A high magnification of area (f) indicated in figure 132.

Table (13). The distribution of the preservative elements in birch at points indicated in figure 132. (Relative counts per 40 seconds, background corrected).

Area	Copper	Chromium	Arsenic
a	1345	5116	1754
b	494	4266	662
c	340	4049	484
d	331	5310	560
e	277	2379	239
f	38	1114	142

a and b = vessel

c and d = rays

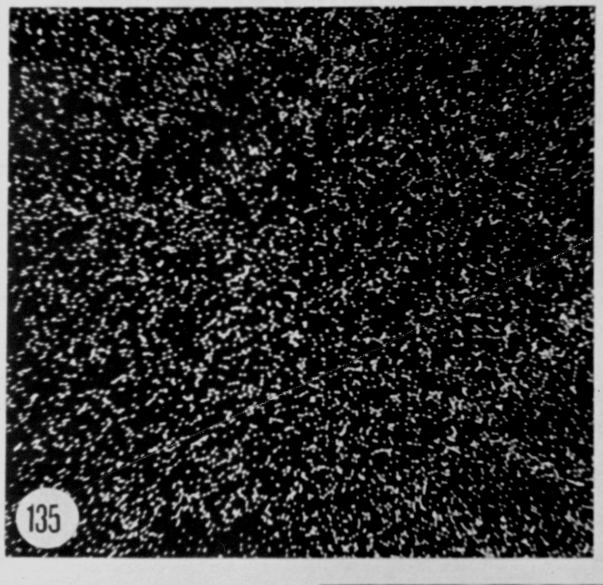
e and f = fibres

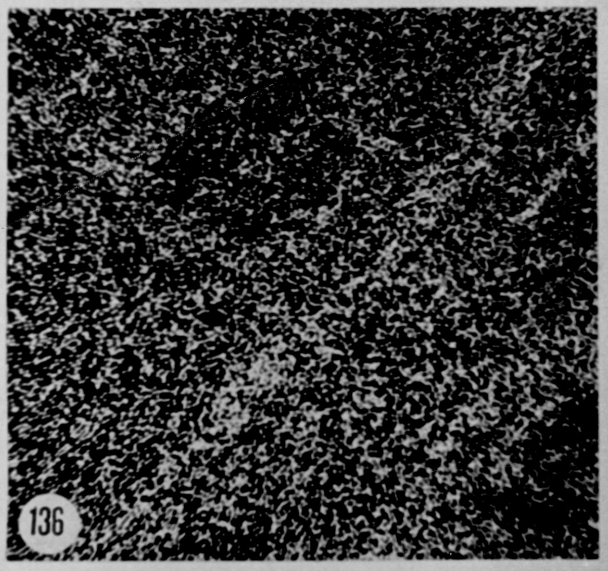
Table (14) The distribution of the preservative elements in birch at points indicated in Figure 138. (Relative counts per 40 seconds, background corrected).

Area	Copper	Chromium	Arsenic
A	15	859	67
B	96	828	*
C	36	648	139

A, B and C = fibre wall

* = not counted


135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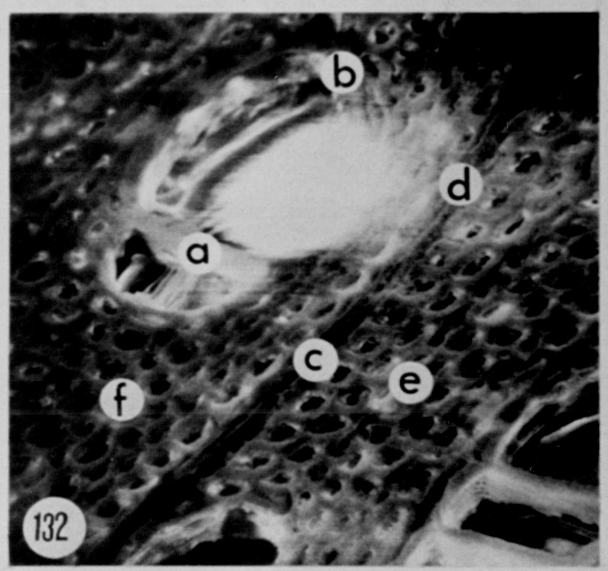
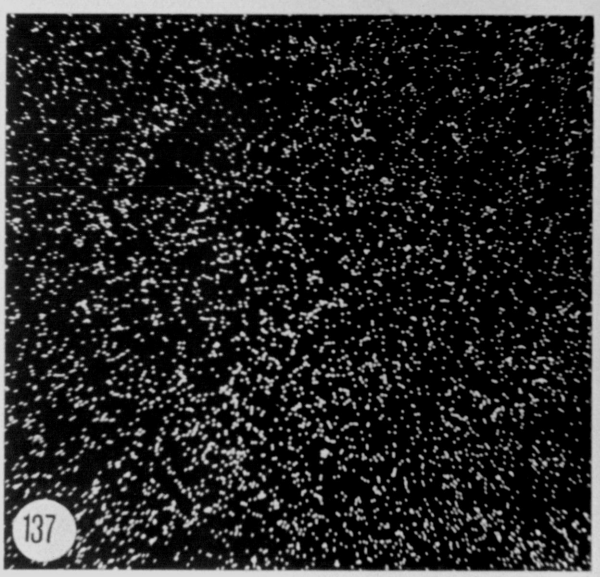

b
d
a
c
e
f
132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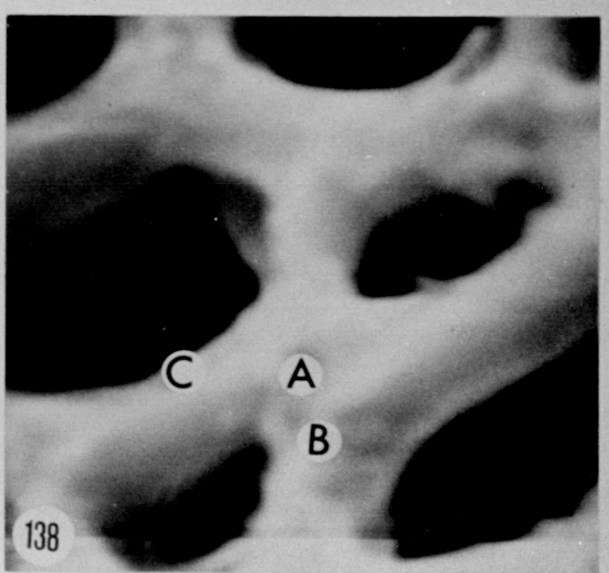

C
A
B
138

Figure 139. Electron image scan of T.S. in birch (x 300)

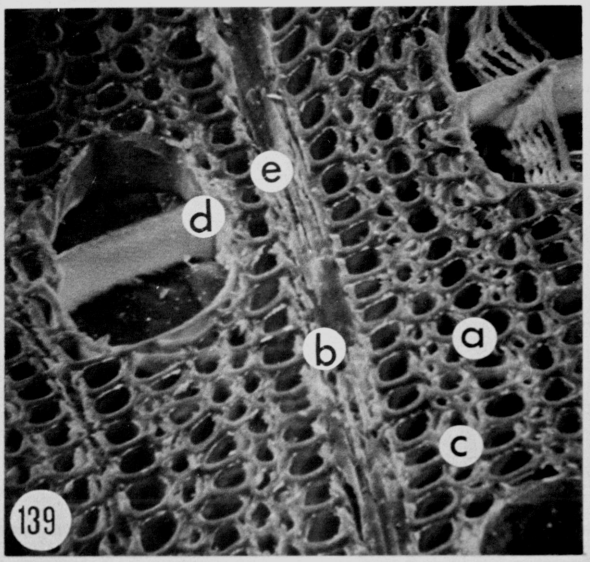
Figure 140. X-ray image scan for copper of field shown in figure 139. (x 300)

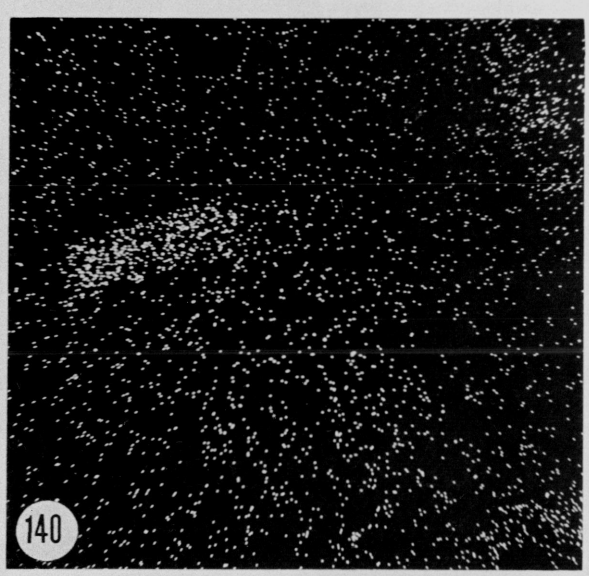
Figure 141. X-ray image scan for chromium of field shown in figure 139. (x 300)

Figure 142. X-ray image scan for arsenic of field shown in figure 139. (x 300)

N.B.

The relative counts for Cu, Cr and As at points indicated in figure 139 are shown in Table (15). The analysis was carried out in the whole area indicated in figure 139 but at a higher magnification.


e
d
b
a
c
139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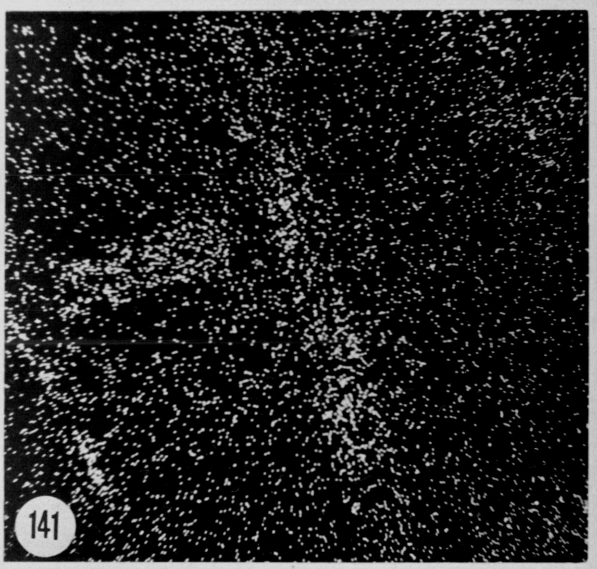
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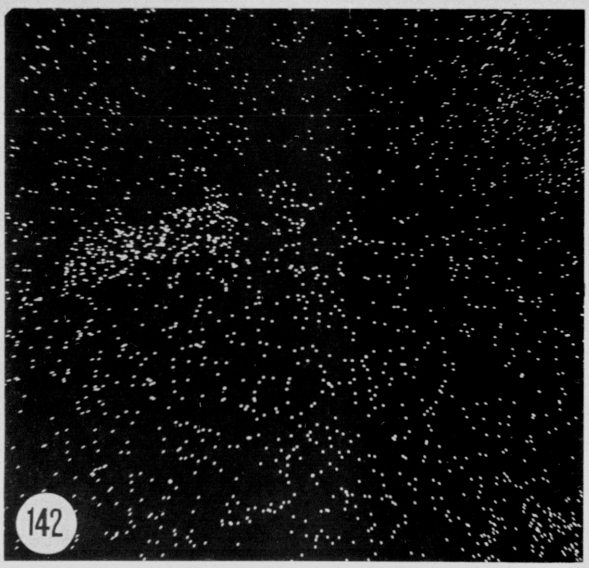

142

Figure 139. Electron image scan of T.S. in birch (x 300)

Figure 143. Electron image scan of area (a) in figure 139 (x 1640)

Figure 144. Electron image scan of area (b) in figure 139 (x 1640)

Figure 145. Electron image scan of area (c) in figure 139 (x 1640)

Figure 146. Electron image scan of area (d) in figure 139 (x 1640)

Figure 147. Electron image scan of area (e) in figure 139 (x 1640)

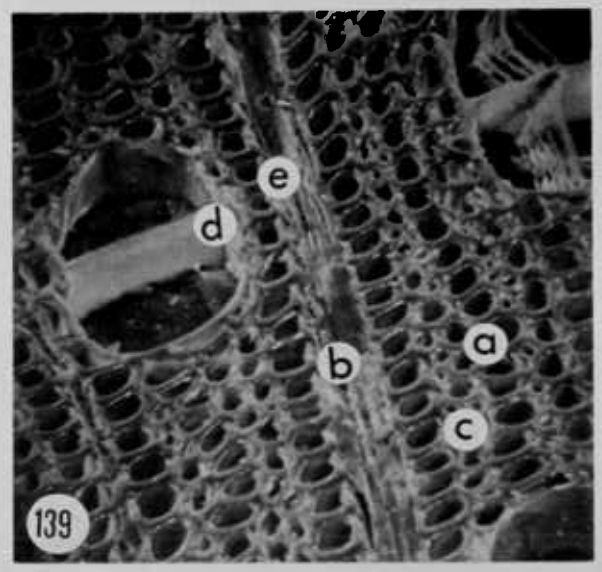
Table (15) The distribution of the preservative elements in birch at points indicated in figure 139. (Relative counts per 40 seconds, background corrected).

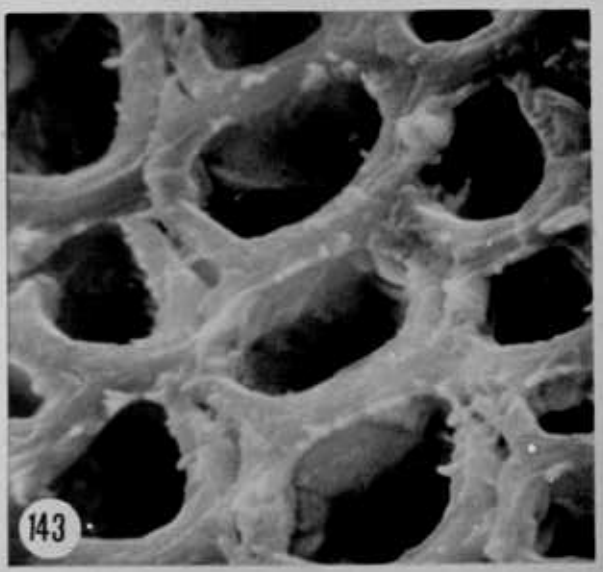
Area	Copper	Chromium	Arsenic
a	56	175	17
b	105	944	42
c	58	342	40
d	685	917	387
e	89	805	82

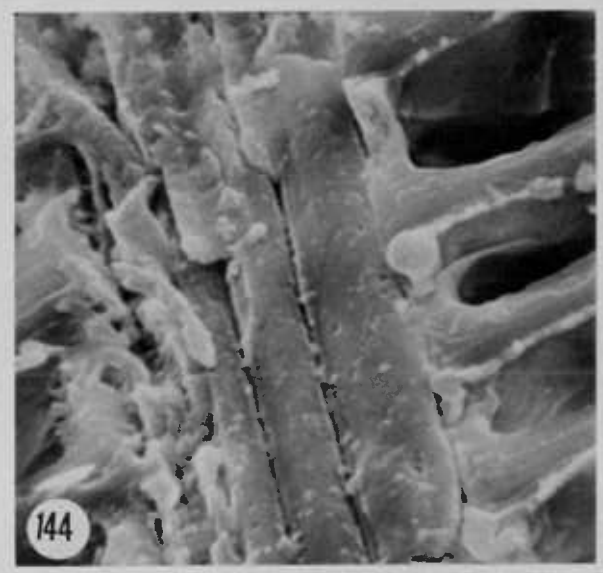
a and c = fibres

b and e = rays and fibres

d = vessel and fibres


e
d
b
a
c
139


143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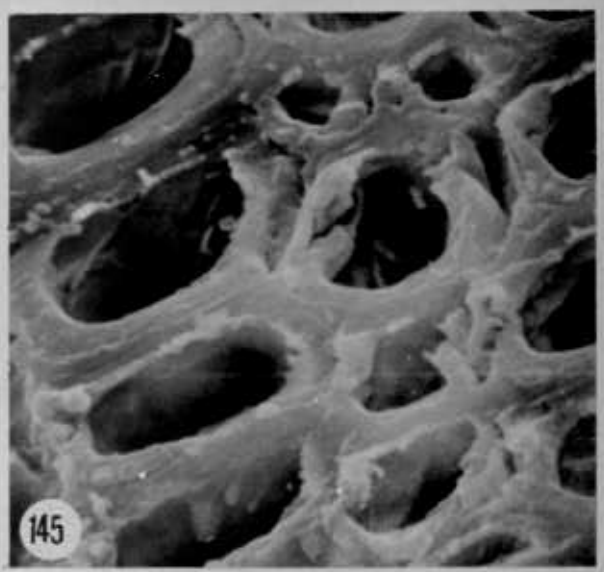
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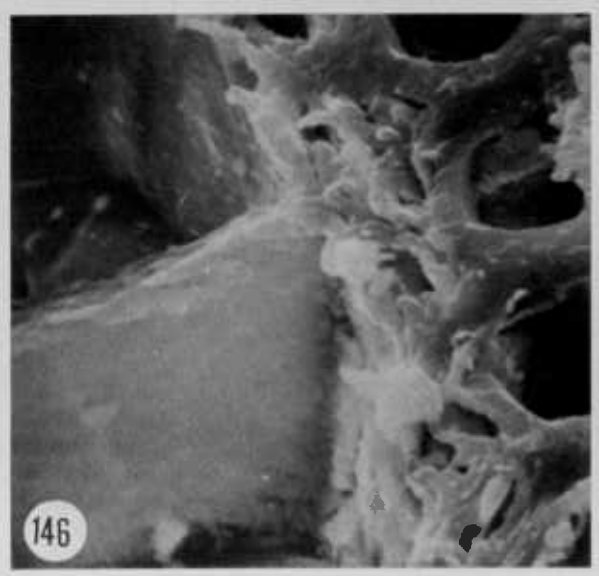
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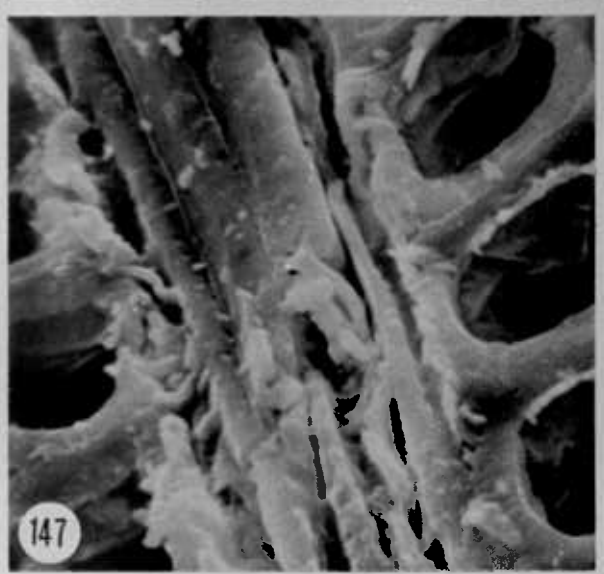

147

Figure 148. Electron image scan of T.S. in birch (x 140)

Figure 149. X-ray image scan for copper of field shown in figure 148 (x 140)

Figure 150. X-ray image scan for chromium of field shown in figure 148 (x 140)

Figure 151. X-ray image scan for arsenic of field shown in figure 148 (x 140)

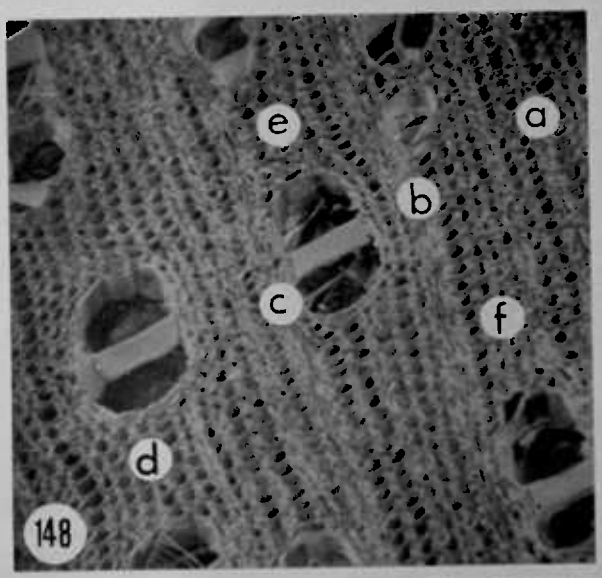
Table (16) The distribution of the preservative elements in birch at points indicated in figure 148, (Relative counts per 40 seconds, background corrected).

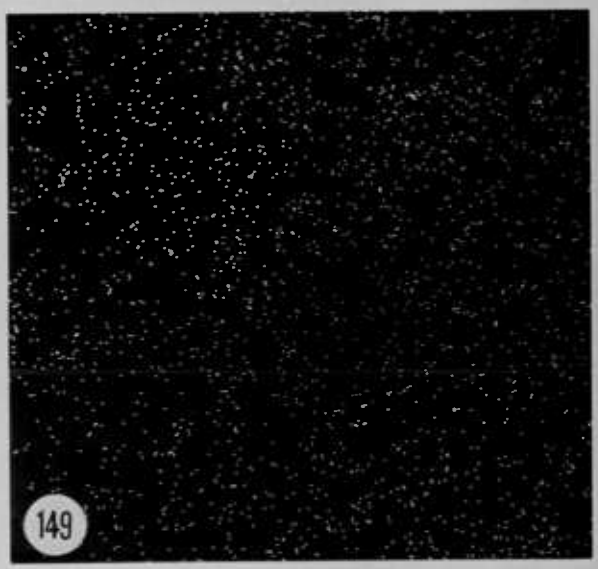
Area	Copper	Chromium	Arsenic
a	194	3905	357
b	239	2447	184
c	294	6249	561
d	140	1400	239
e	159	1121	131
f	208	1273	155

a and b = rays

c = vessel

d, e and f = fibres


e
a
b
c
f
d
148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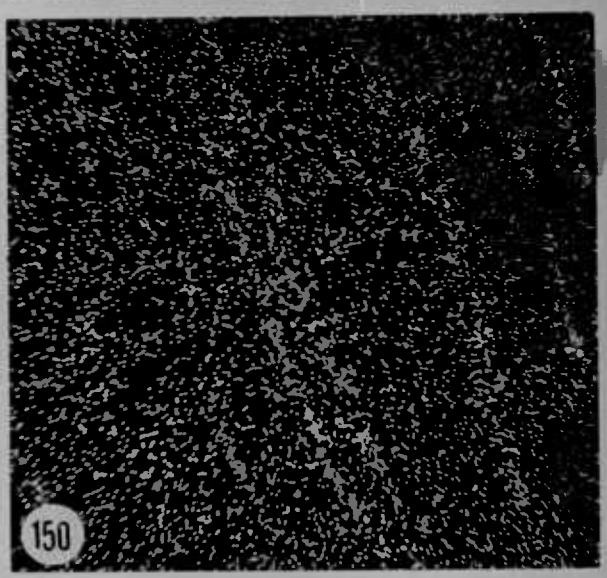
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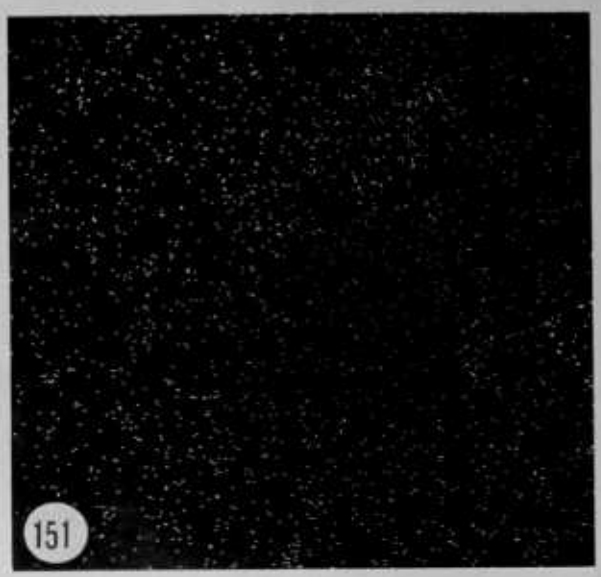

151

Figure 152. Electron image scan of T.L.S. in birch (x 420)

Figure 153. X-ray image scan for copper of field shown in figure 152 (x 420)

Figure 154. X-ray image scan for chromium of field shown in figure 152 (x 420)

Figure 155. X-ray image scan for arsenic of field shown in figure 152 (x 420)

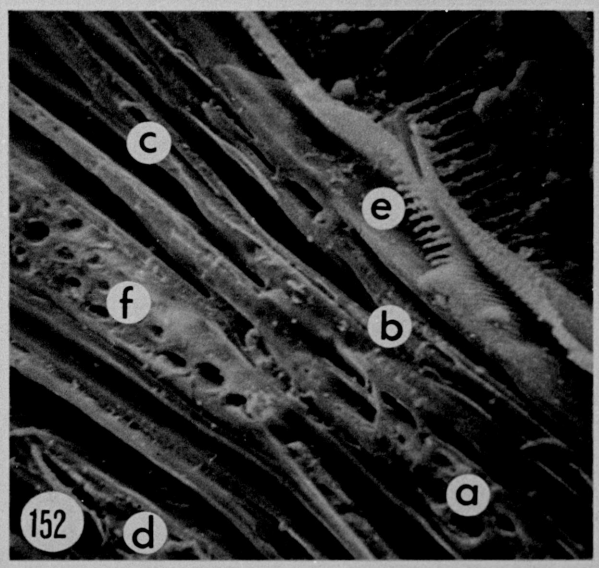
Table (17) The distribution of the preservative elements in birch at points indicated in figure 152. (Relative counts per 40 seconds, background corrected).

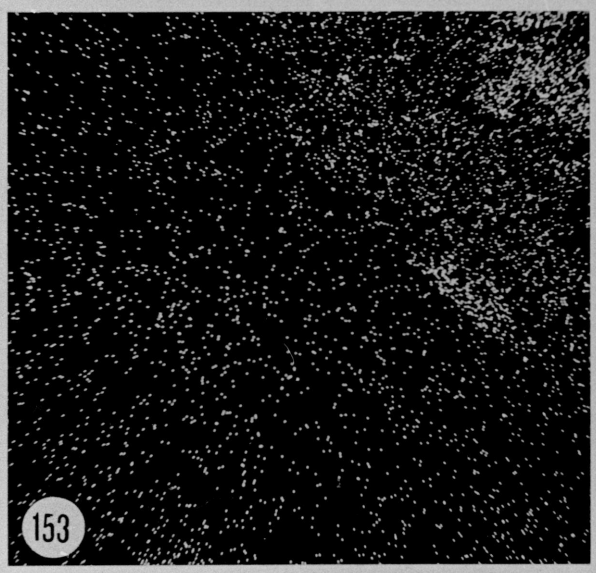
Area	Copper	Chromium	Arsenic
a	313	3592	388
b	681	1572	455
c	146	1281	207
d	2146	8700	1637
e	1366	4850	855
f	284	8046	524

a, d and f = rays

b and c = fibres

e = vessel


c
e
f
b
a
152
d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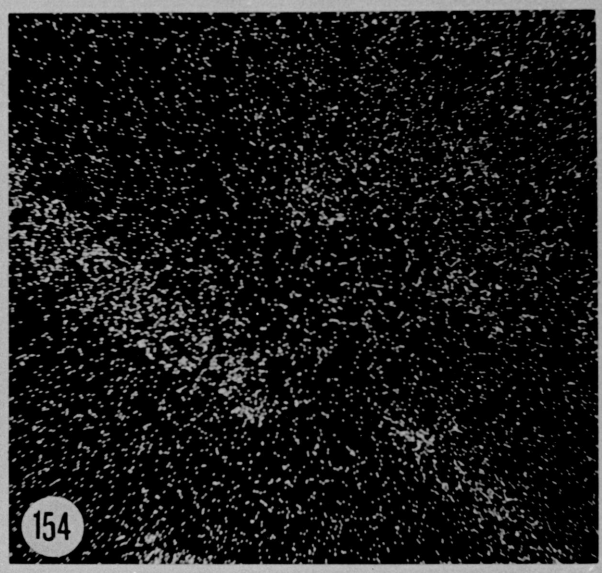
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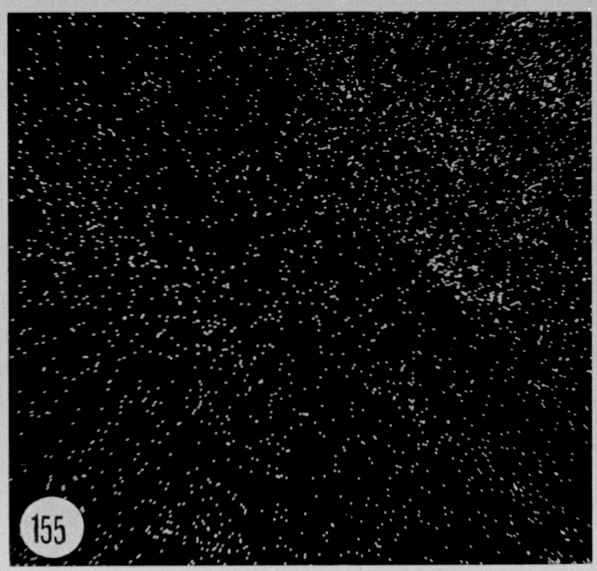
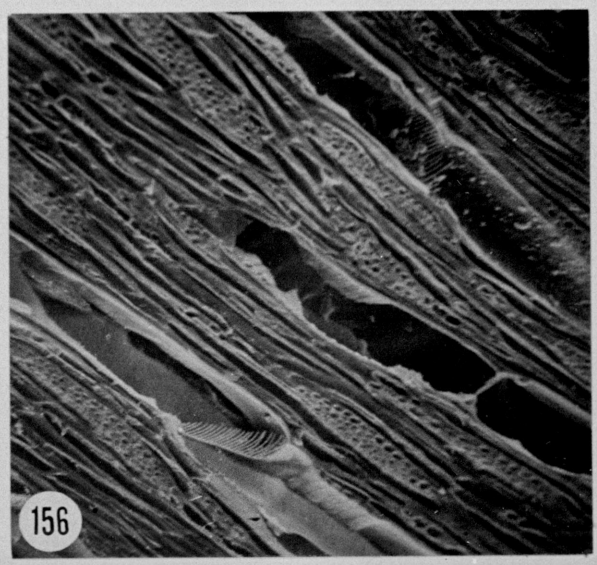

155

Figure 156. Electron image scan of T.L.S. in birch (x 120)

Figure 157. X-ray image scan for copper of field shown in figure 156 (x 120)

Figure 158. X-ray image scan for chromium of field shown in figure 156 (x 120)

Figure 159. X-ray image scan for arsenic of field shown in figure 156 (x 120)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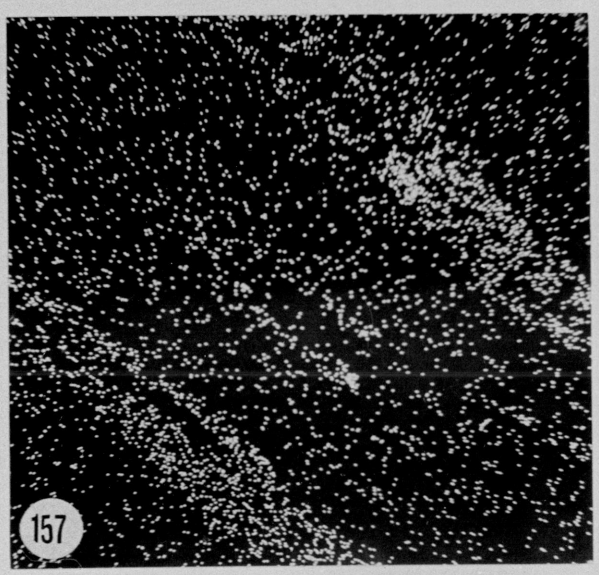
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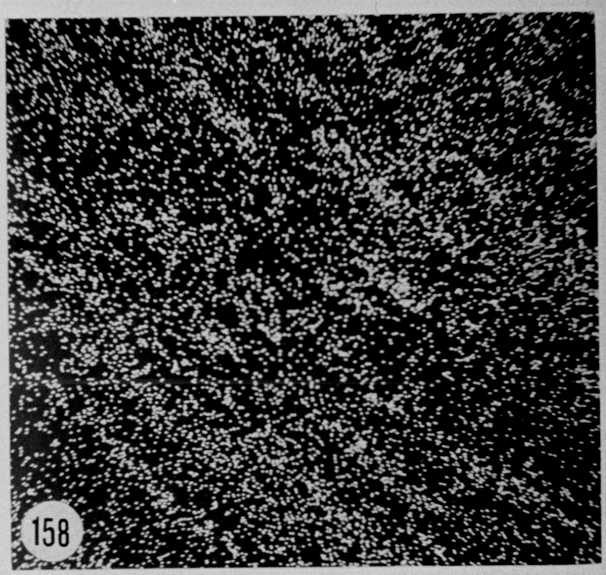
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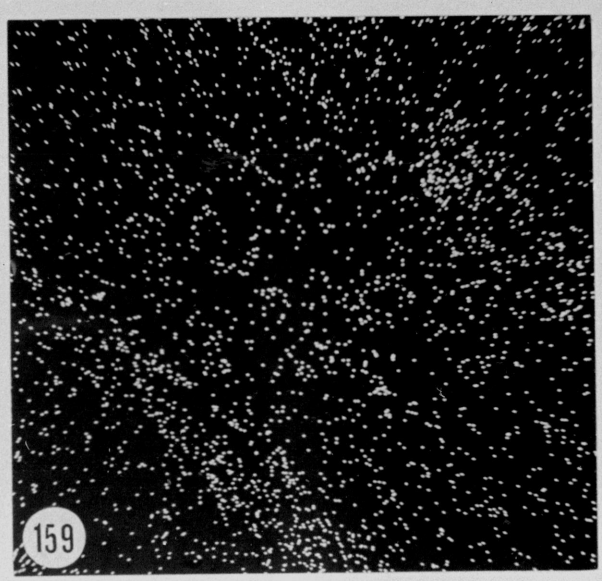

159

Figure 160. Electron image scan of R.L.S. in birch (x 160)

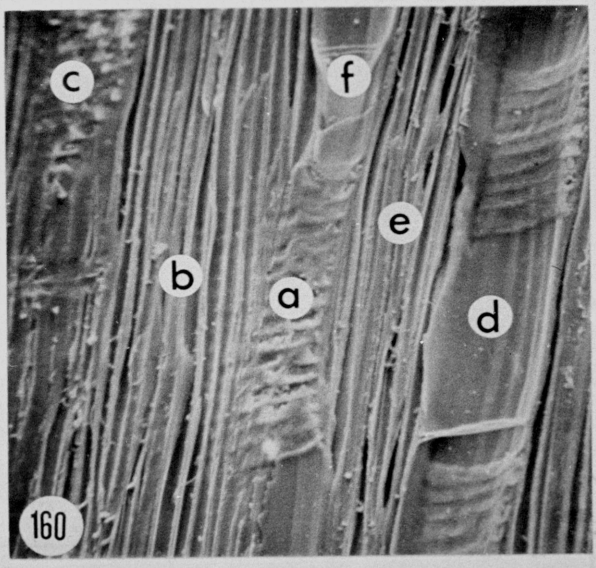
Figure 161. X-ray image scan for copper of field shown in figure 160 (x 160)

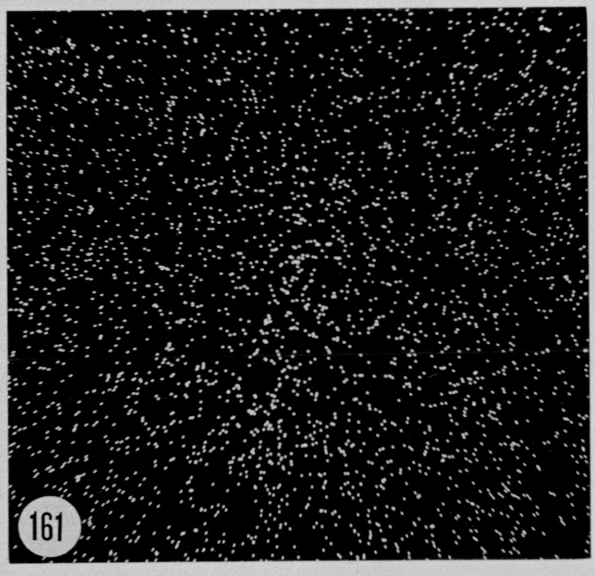
Figure 162. X-ray image scan for chromium of field shown in figure 160 (x 160)

Figure 163. X-ray image scan for arsenic of field shown in figure 160 (x 160)

N.B.

The relative counts for Cu, Cr and As at points indicated in figure 160 are shown in Table (18). The analysis was carried out in the whole area of the points indicated in figure 160 but at a higher magnification.


c
f
e
b
a
d
160


161


162

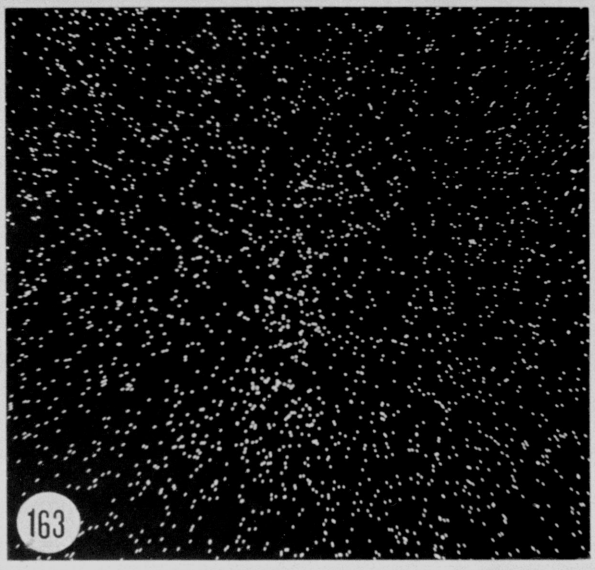

163

Figure 164. Electron image scan for area (a) in figure 160 (x 1640)

Figure 165. Electron image scan for area (b) in figure 160 (x 1640)

Figure 166. Electron image scan for area (c) in figure 160 (x 1640)

Figure 167. Electron image scan for area (d) in figure 160 (x 1640)

Figure 168. Electron image scan for area (e) in figure 160 (x 1640)

Figure 169. Electron image scan for area (f) in figure 160 (x 1640)

Table (18)

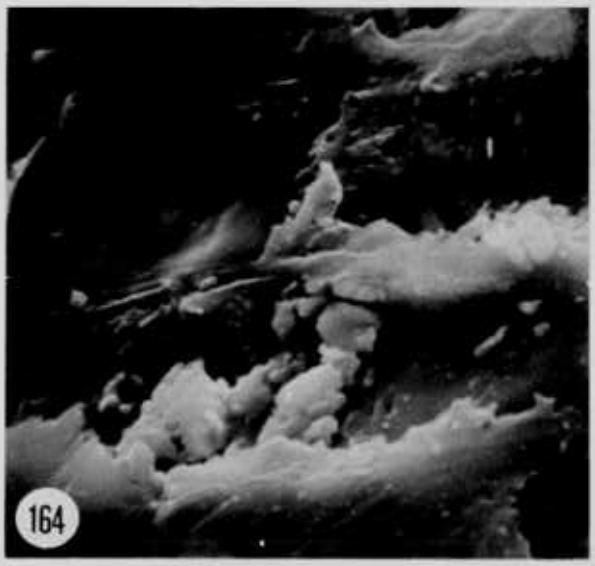
The distribution of the preservative elements in birch at points indicated in figure 160. (Relative counts per 40 seconds, background corrected).

Area	Copper	Chromium	Arsenic
a	270	2378	410
b	48	1046	145
c	192	2386	378
d	124	1281	127
e	91	919	77
f	113	1049	120

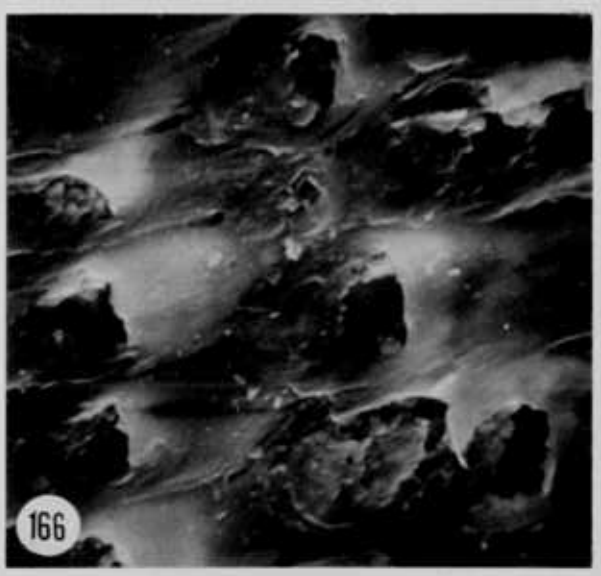
a and c = rays

b and e = fibres

d and f = vessel wall


164


165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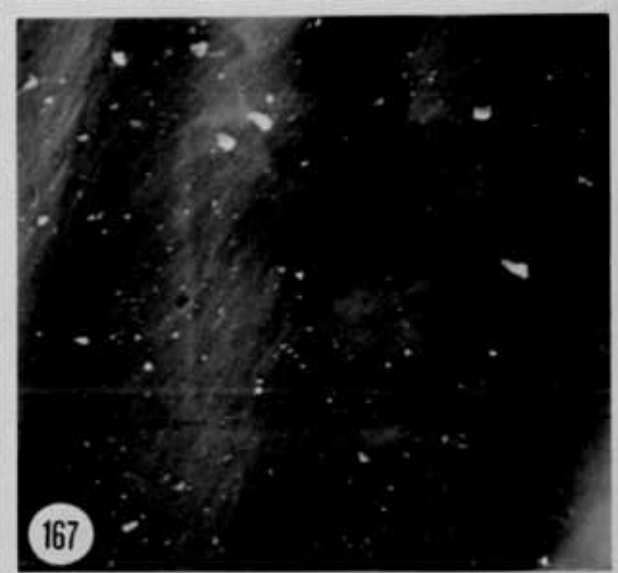
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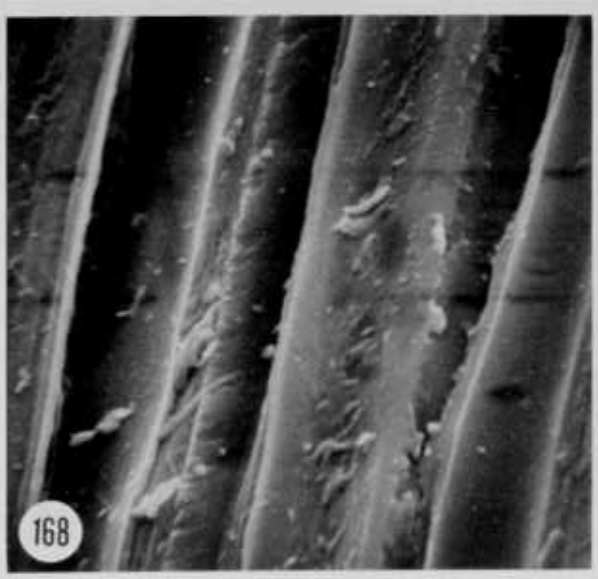
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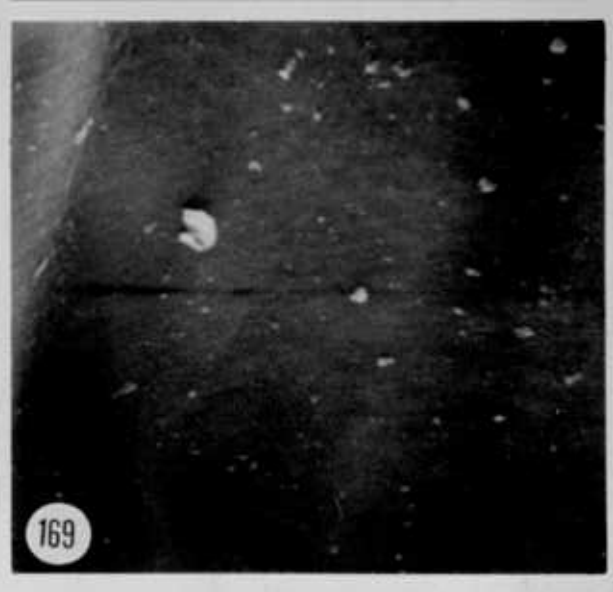

169

Figure 170. X-ray image scan for copper, chromium and arsenic of field shown in figure 172. (x 165)

Figure 171. X-ray image scan for copper of field shown in figure 172 (x 165)

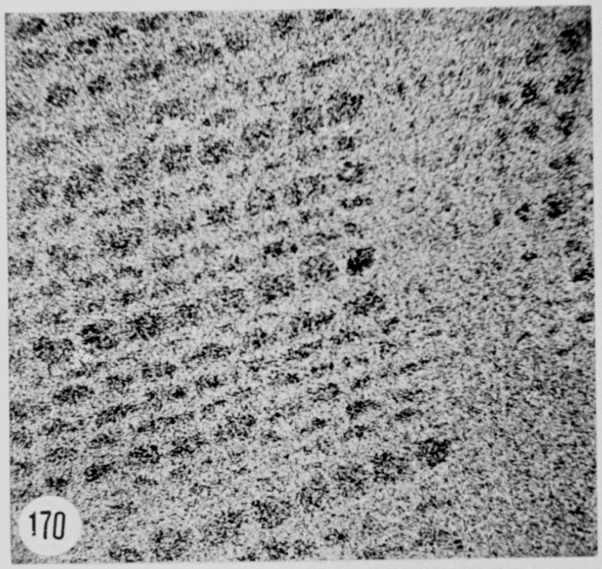
Figure 172. Electron image scan of T.S. in Scots pine (x 165)

Figure 173. X-ray image scan for chromium of field shown in figure 172 (x 165)

Figure 174. X-ray image scan for arsenic of field shown in figure 172 (x 165)

N.B.

The relative counts for Cu, Cr and As at points indicated in figure 172 are shown in Table (19). The analysis was carried out in the whole area of the points indicated in figure 172 but at a higher magnification.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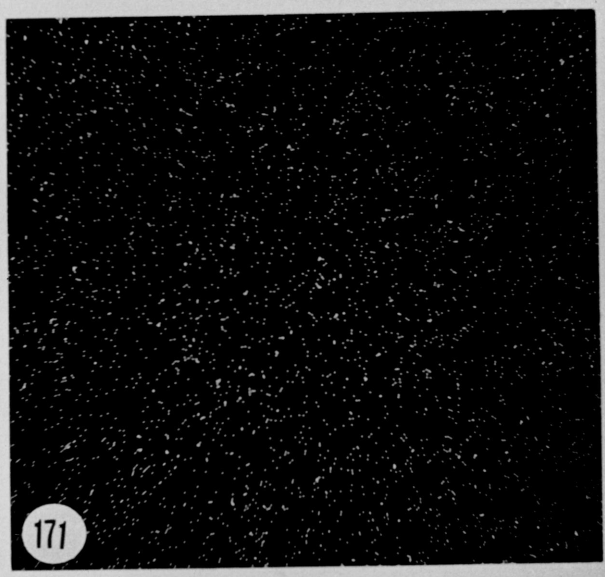
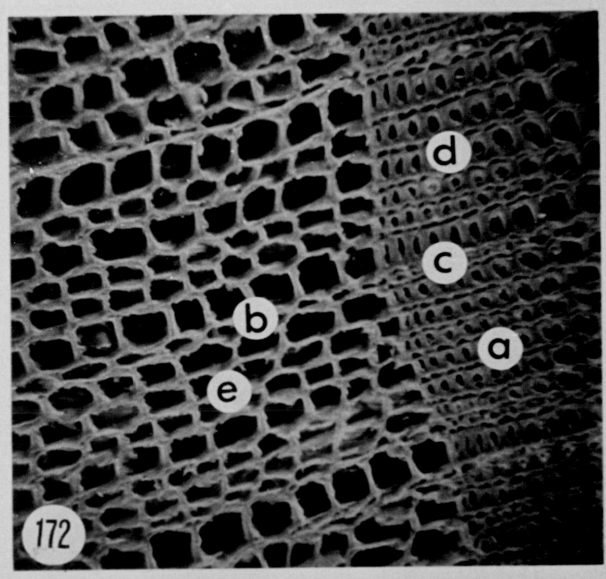
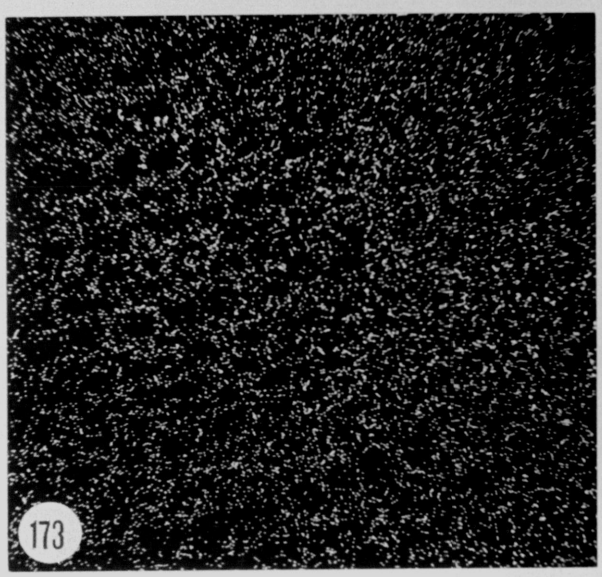

171


d
c
b
a
e
172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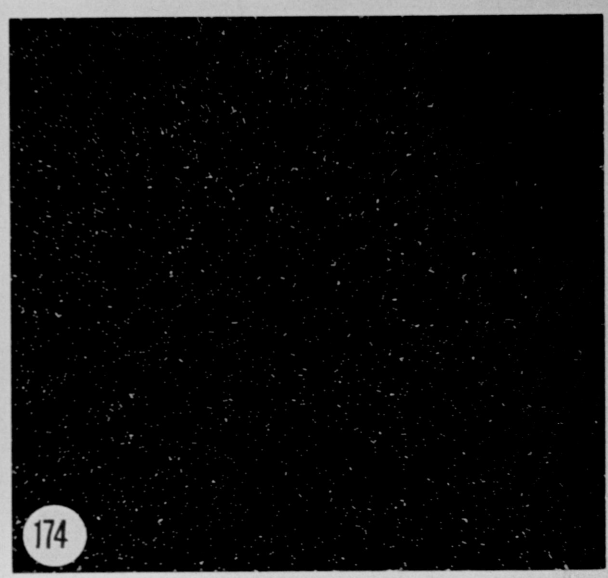

174

Figure 172. Electron image scan of T.S. in Scots pine (x 165)

Figure 175. Electron image scan for area (a) in figure 172 (x 820)

Figure 176. Electron image scan for area (b) in figure 172 (x 1640)

Figure 177. Electron image scan for area (c) in figure 172 (x 1640)

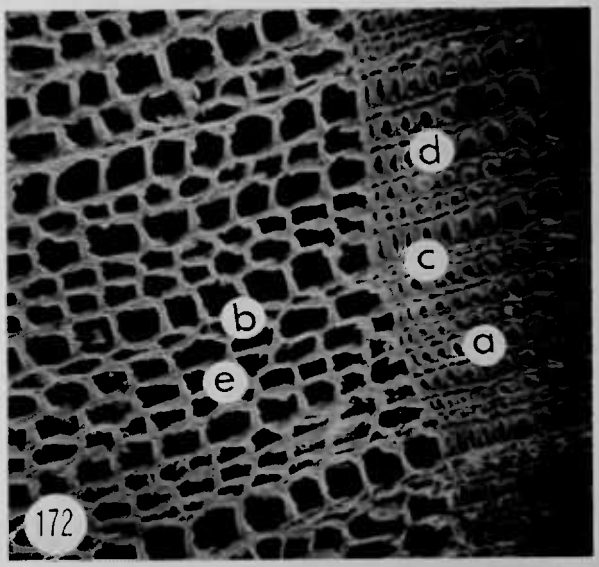
Figure 178. Electron image scan for area (d) in figure 172 (x 1640)

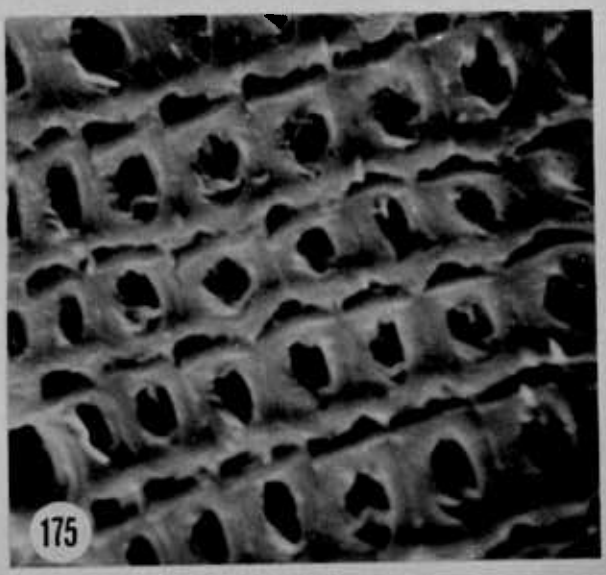
Figure 179. Electron image scan for area (e) in figure 172 (x 1640)

Table (19) The distribution of the preservative elements in Scots pine at points indicated in figure 172.
(Relative counts per 40 seconds, background corrected)

Area	Copper	Chromium	Arsenic
a	196	1391	262
b	261	850	176
c	321	1777	315
d	273	1728	261
e	227	1095	234

a and d = latewood tracheids
b = earlywood ray
c = ray cell and tracheids in latewood zone
e = earlywood tracheid


d
c
b
a
e
172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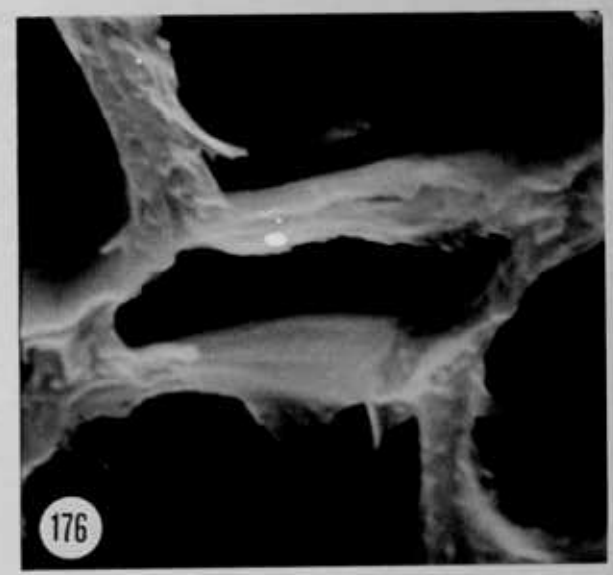
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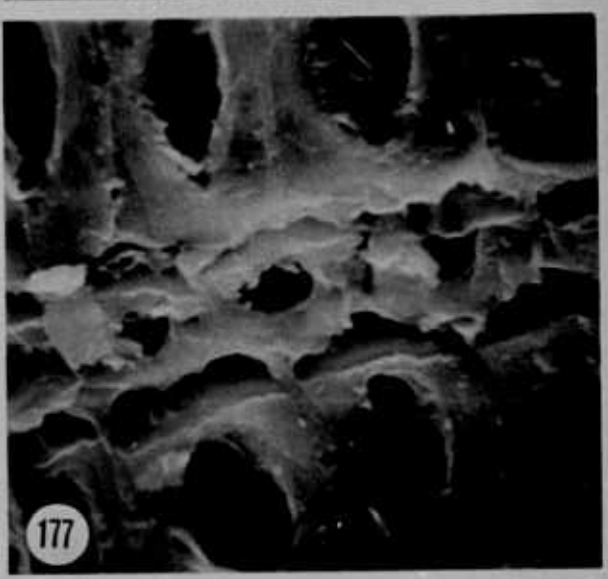
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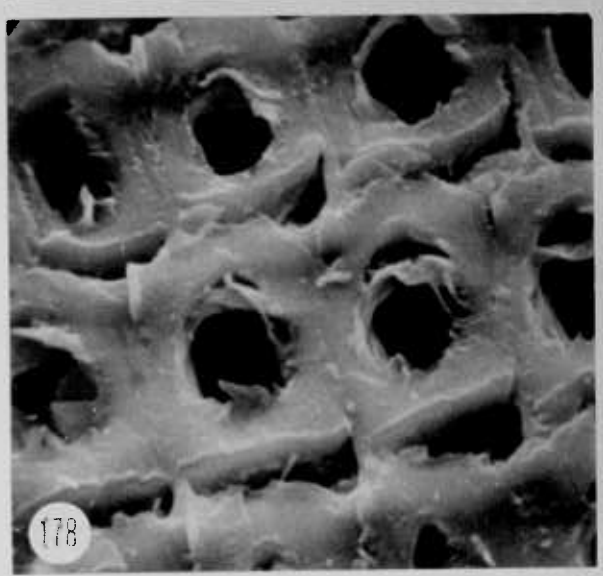
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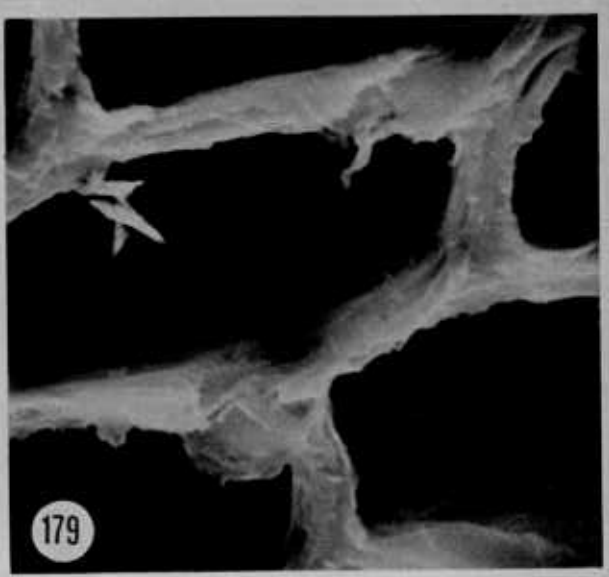

179

Figure 180. Electron image scan of T.S. in Scots pine (x 230)

Figure 181. X-ray image scan for copper of field shown in figure 180 (x 230)

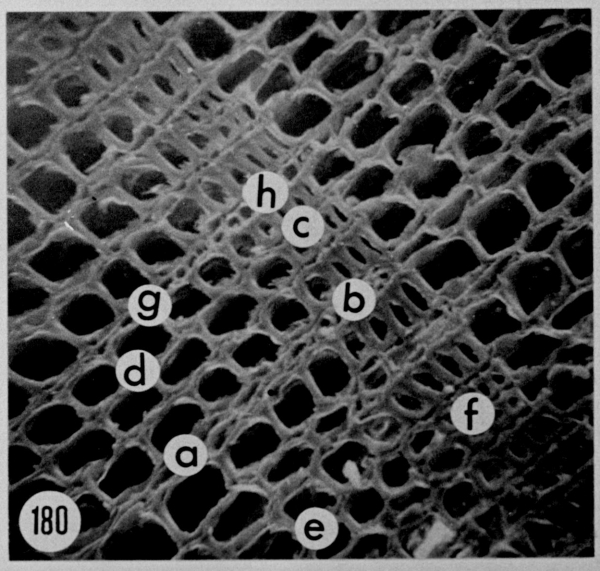
Figure 182. X-ray image scan for chromium of field shown in figure 180 (x 230)

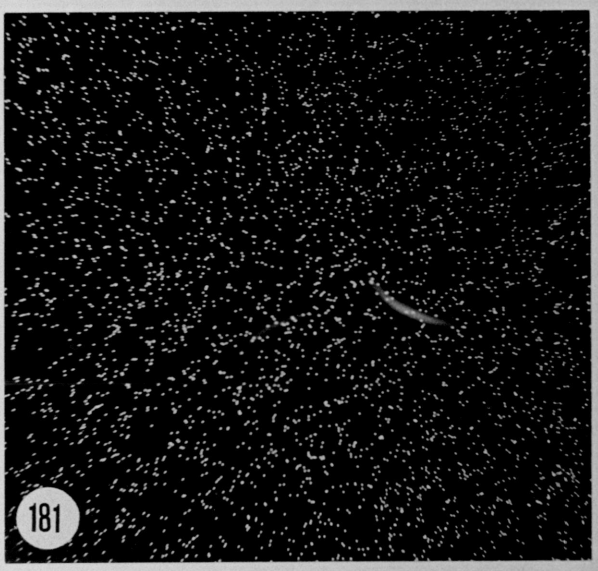
Figure 183. X-ray image scan for arsenic of field shown in figure 180 (x 230)

Table (20) The distribution of the preservative elements in Scots pine at points indicated in figure 180. (Relative counts per 40 seconds, background corrected).

Area	Copper	Chromium	Arsenic
a	684	4895	752
b	757	5282	868
c	738	4929	694
d	939	5848	935
e	779	4800	983
f	895	6026	1160
g	1031	5926	1105
h	806	5605	812

a and g = earlywood ray
b and h = latewood ray
c and f = tracheid in latewood
d and e = tracheid in earlywood


h
c
g
b
d
f
a
180
e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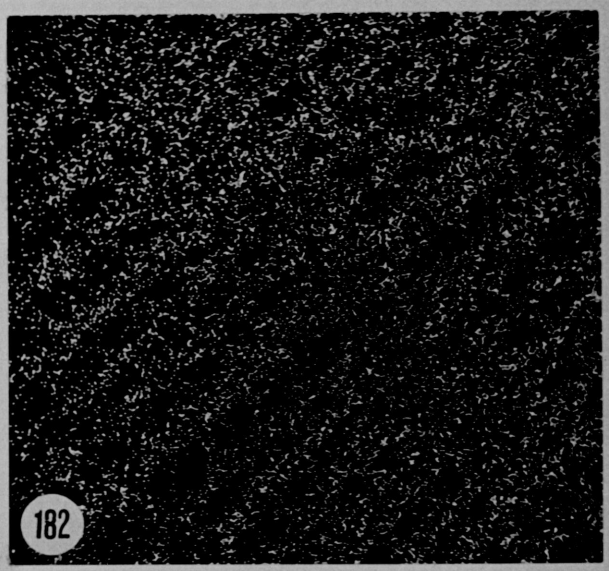
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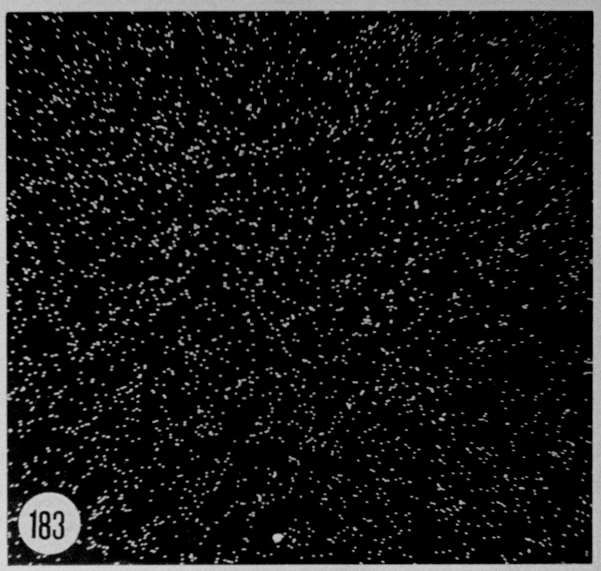

183

Figure 184. Electron image scan of R.L.S, in Scots pine (x 150)

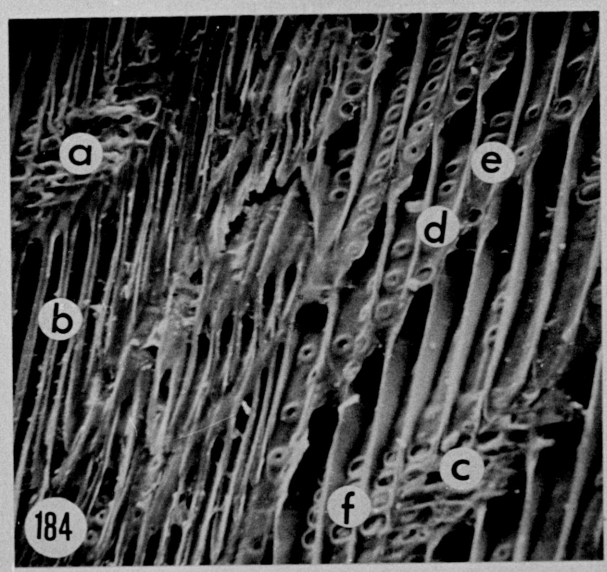
Figure 185. X-ray image scan for copper of field shown in figure 184 (x 150)

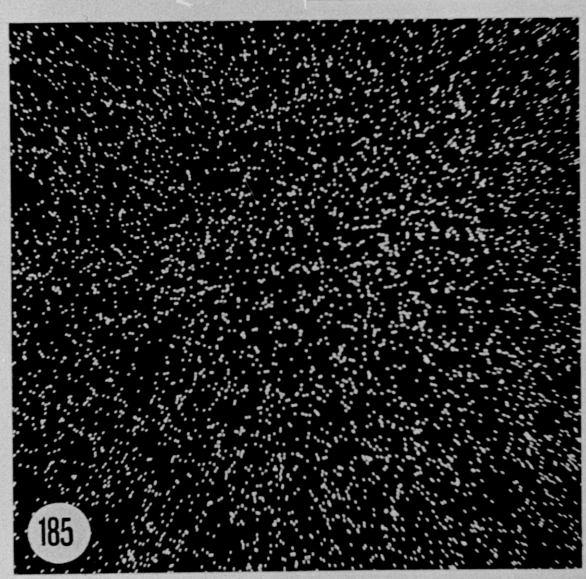
Figure 186. X-ray image scan for chromium of field shown in figure 184 (x 150)

Figure 187. X-ray image scan for arsenic of field shown in figure 184 (x 150)

N.B.

The relative counts for Cu, Cr and As at points indicated in figure 184 are shown in Table (21). The analysis was carried out in the whole area of the points indicated in figure 184 but at a higher magnification.


a
e
d
b
c
f
184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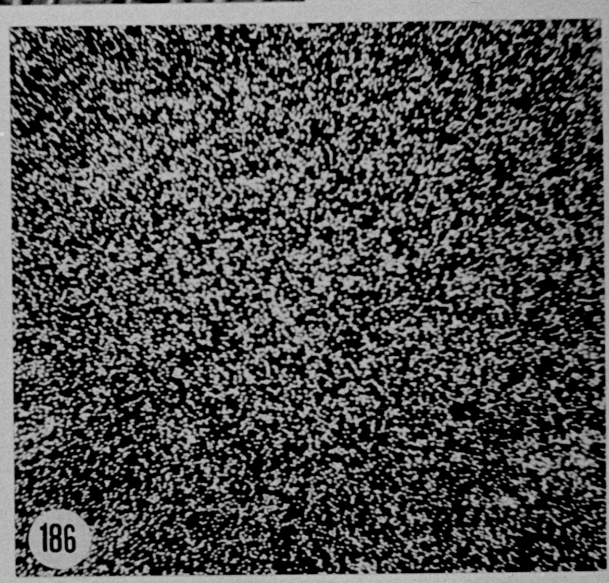

186

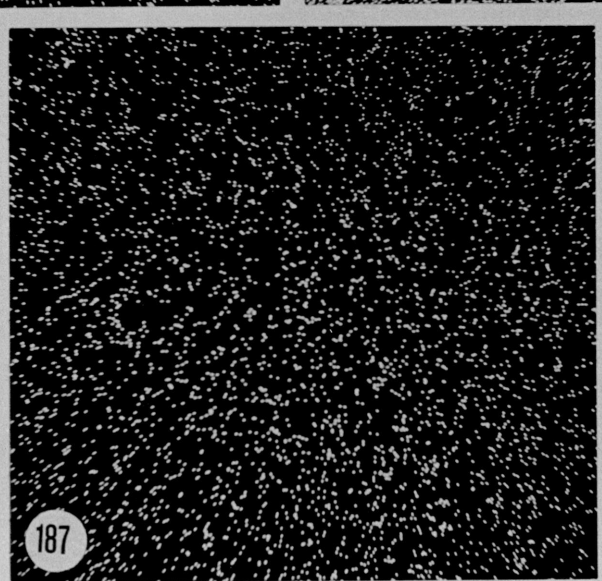

187

Figure 188. Electron image scan for area (a) in figure 184 (x 1640)

Figure 189. Electron image scan for area (b) in figure 184 (x 1640)

Figure 190. Electron image scan for area (c) in figure 184 (x 1640)

Figure 191. Electron image scan for area (d) in figure 184 (x 1640)

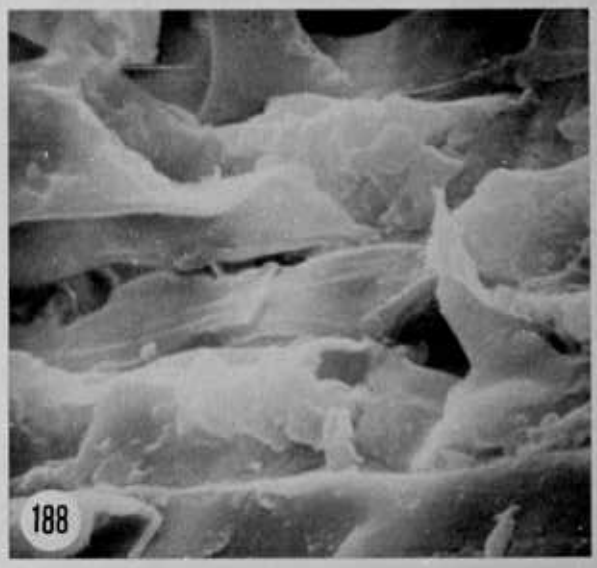
Figure 192. Electron image scan for area (e) in figure 184 (x 4060)

Figure 193. Electron image scan for area (f) in figure 184 (x 4060)

Table (21) The distribution of the preservative elements in Scots pine at points indicated in figure 184. (Relative counts per 40 seconds, background corrected).

Area	Copper	Chromium	Arsenic
a	622	3071	518
b	365	2459	433
c	823	4119	695
d	377	2459	405
e	553	2938	600
f	778	3924	643

a = latewood ray
b = latewood tracheid
c = earlywood ray
d = earlywood tracheid
e = bordered pit in earlywood
f = window pit in earlywood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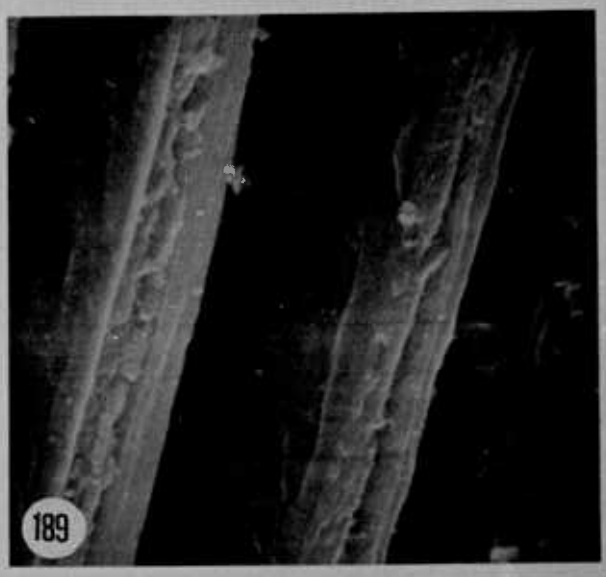
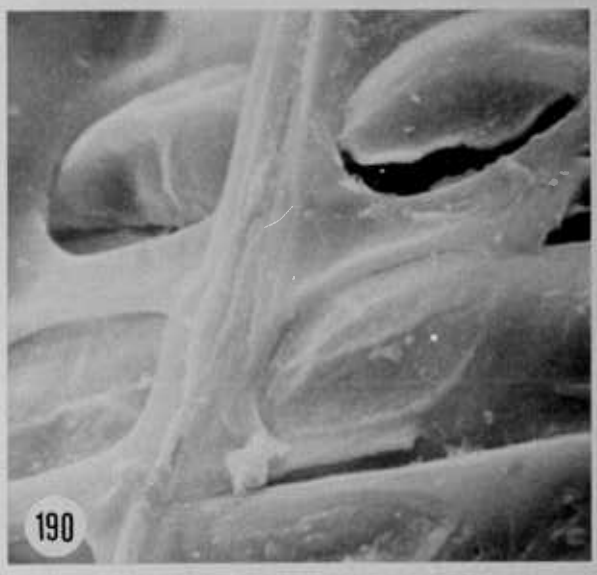

189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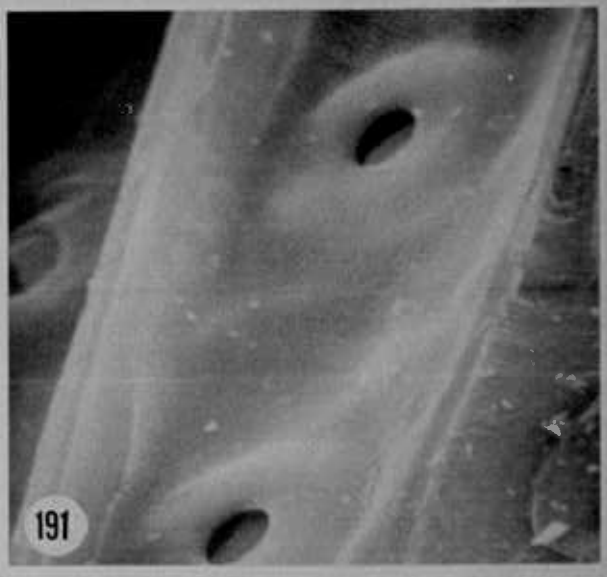

191


192

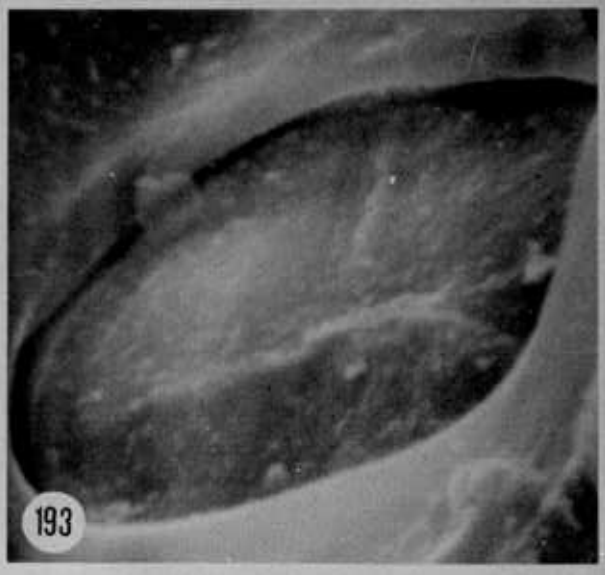

193

Figure 194. Electron image scan of R.L.S. in Scots pine (x 270)

Figure 195. X-ray image scan for copper of field shown in figure 194. (x 270)

Figure 196. X-ray image scan for chromium of field shown in figure 194. (x 270)

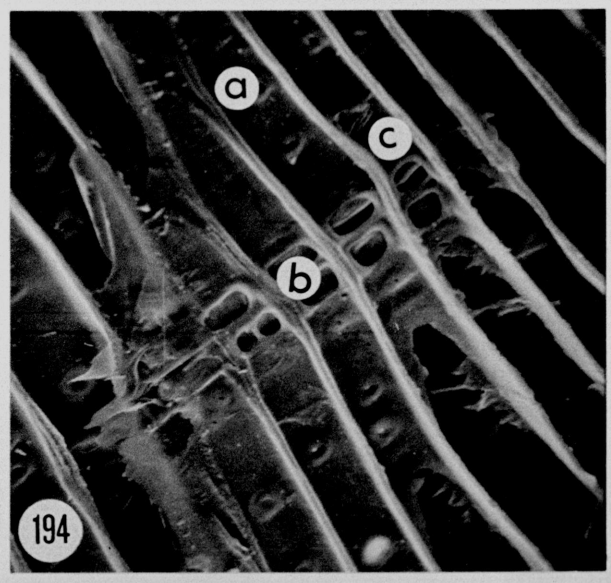
Figure 197. X-ray image scan for arsenic of field shown in figure 194. (x 270)

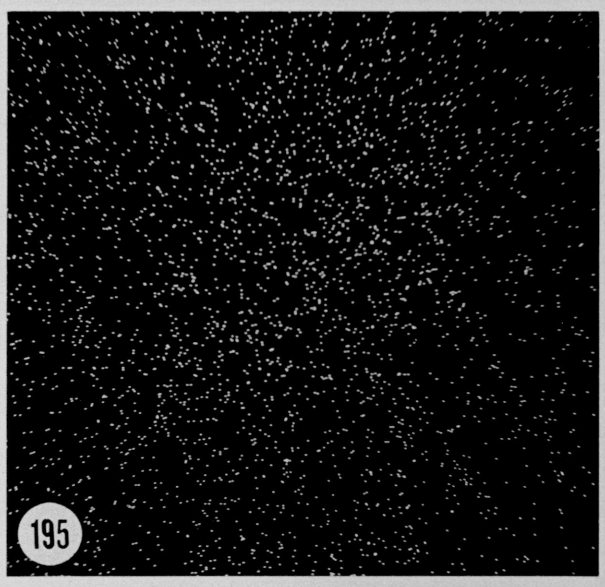
Table (22) The distribution of the preservative elements in Scots pine at points indicated in figure 194. (Relative counts per 40 seconds, background corrected).

Area	Copper	Chromium	Arsenic
a	459	1893	476
b	1352	7018	885
c	786	4140	752

a and c = earlywood tracheid

b = earlywood ray


a
c
b
194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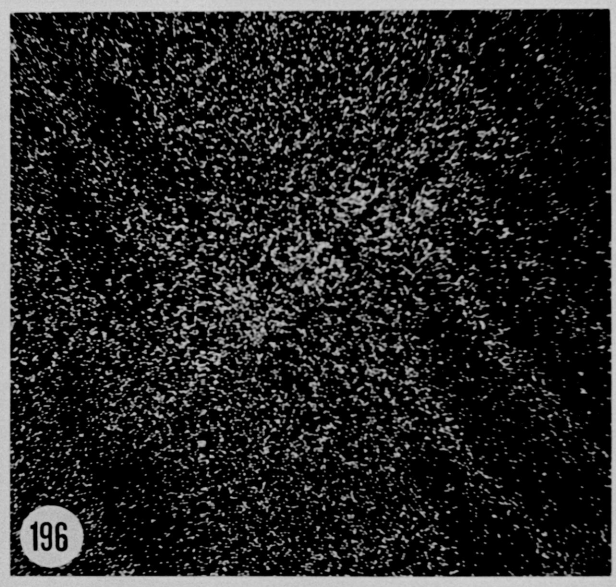
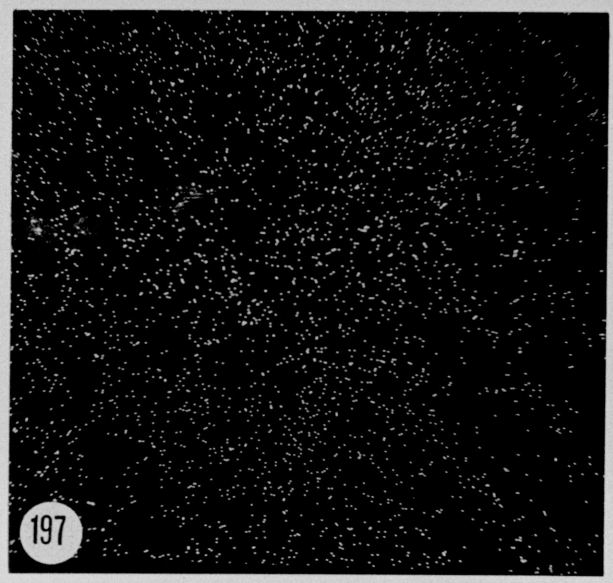

196


197

From the results obtained, certain general conclusions were made possible. These were as follows:

Birch

The results obtained clearly indicated an uneven distribution of the preservative elements in the birch blocks. The X-ray images obtained showed considerable differences in the distribution of the individual preservative elements in the different tissues of birch. The rays and vessels retained a high level of chromium which distinguished them from the surrounding fibres. A similar accumulation of copper and arsenic was also observed in the vessels. There was an overall lower distribution of the 3 elements across the fibres.

The relative counts for the individual elements confirmed these observations. High levels of chromium were present in the vessels and rays together with high levels of copper and arsenic in the vessels. The fibres gave relatively low counts for all 3 elements. The highest count in all tissues was always that for chromium in comparison to copper and arsenic(which were nearly the same)but usually much lower than chromium.

Scots pine

The X-ray images obtained clearly showed a very even distribution of copper, chromium and arsenic in the Scots pine block. The X-ray image for all elements clearly corresponds with the anatomy of the section (see figures 170 and 172).

The values obtained for the peak counts confirmed these observations where the area counts were nearly the same for each individual element in tracheids and rays of both earlywood and latewood zones. As in the case of birch, chromium always gave the highest count in all tissues, copper and arsenic were also nearly the same in each of the different tissues.

At a tissue level, it was clearly evident that an uneven micro-distribution had been achieved in the case of birch in comparison to the even distribution achieved in the case of Scots pine.

3.3.3. EMMA4 Results

"Epon" embedded and unembedded sections of both species of wood were examined in the college transmission electron microscope in order to select the best sections for analysis in the EMMA4 instrument. No heavy metals apart from the Preservative elements were present in these sections. The sections examined (Figs. 198-208) appeared to be clear and to a certain extent similar to those of the untreated stained sections. An electron-dense deposit in the lumen interface (i.e., the surface of the S3 layer of the cell wall that forms the boundary of the cell lumen) was observed especially in the case of Scots pine. These appeared as granules coating the lumen interface (Figs. 198, 199, 200 and 201). These granules were also found round the pit border in Scots pine and accumulated on the pit-tori (Figs. 202, 203 and 204). At high magnification the microfibrils in the S2 layer were clearly seen as dense lines running more or less parallel to each other (Fig. 205).

Several selected spots across the tracheid walls of Scots pine as well as the walls of fibres, rays and vessels of birch were analysed in the EMMA4 for copper, chromium and arsenic in both embedded and unembedded specimens. Relative counts per second for each element at each spot were obtained by counting over a 10 second time period. Although both spectrometers were used for the same elements, counts were only used from the unobscured spectrometer. Counts were used from one spectrometer in order to eliminate possible problems with shadowing due to grid bars of the holder on one side or the other. The NDD counts (c.f. section 2.2.3.2.3.)

were taken as an indication of section thickness. All the figures were corrected to a standard NDD in order to give relative comparative counts.

The results obtained from the analysis across each cell wall were tabulated in separate tables and the analysed spots were indicated in the figures obtained for the appropriate area of each wood section. Finally the data collected from various parts of the cell wall layers were averaged namely; S3/lumen interface, S2 and S1/middle lamella regions of a Scots pine tracheid wall and a birch ray parenchyma wall and a fibre wall. Histograms for these results were also drawn (Figs. 218, 219 and 220). This allowed a simple direct comparison to be made between the two species of wood, particularly in the S2 layer which was the region where attack of the hardwood fibres by soft-rot fungi occurred. Comparison between the treatment of the different distinguishable layers within each timber was also possible.

The results obtained from such analysis allowed certain generalizations to be made, these were as follows:

Birch

The results obtained indicated clearly the low levels of the preservative components in the fibre walls with higher levels in the ray parenchyma. The counts for arsenic are thought to be erroneous as arsenic detection was very unreliable in comparison to the copper and chromium.

Scots pine

The distribution of the preservative components was throughout the tracheid walls with relatively high levels of preservative. The low counts for arsenic were also considered to be unreliable due to difficulties in detecting this element (c.f. section 2.2.3.2.3.).

Figure 198. Transmission electron micrograph of 2.5% CCA treated Scots pine. Section obtained from "Epon" embedded material (x 4000)

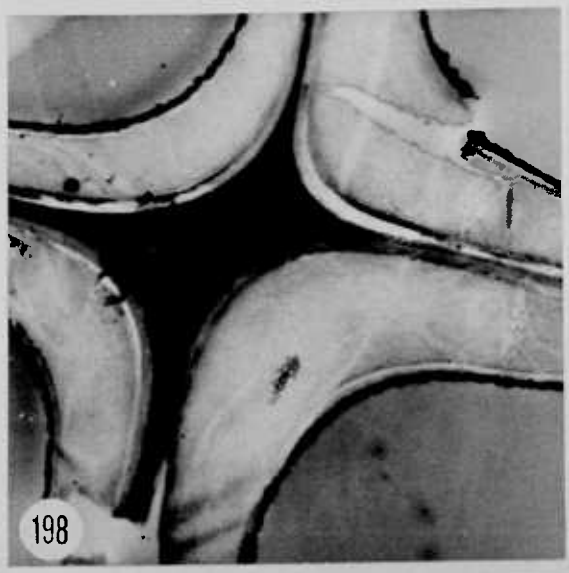
Figure 199. Transmission electron micrograph of 2.5% CCA treated Scots pine. Section obtained from unembedded material (x 10000)

Figure 200. Transmission electron micrograph of 2.5% CCA treated birch. Section obtained from "Epon" embedded material (x 10000)

Figure 201. Transmission electron micrograph of 5% CCA treated Scots pine. Section obtained from "Epon" embedded material (x 10000)

Figure 202. Transmission electron micrograph of 5% CCA treated Scots pine in a pit-region. Section obtained from "Epon" embedded material (x 6000)

Figure 203. Transmission electron micrograph of 5% CCA treated Scots pine in a pit-region. Section obtained from "Epon" embedded material (x 5000)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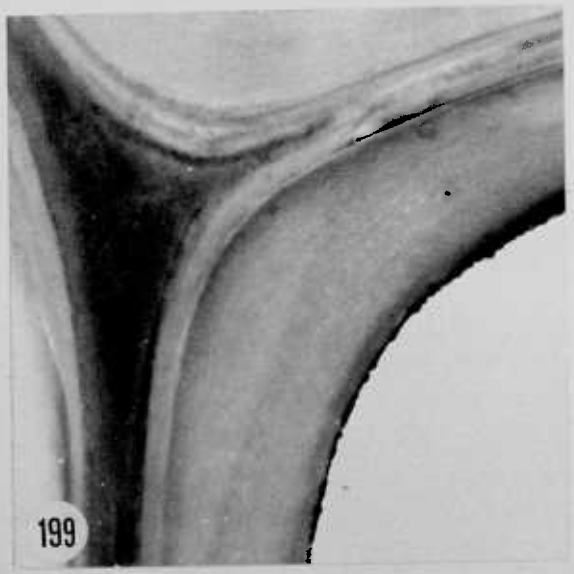
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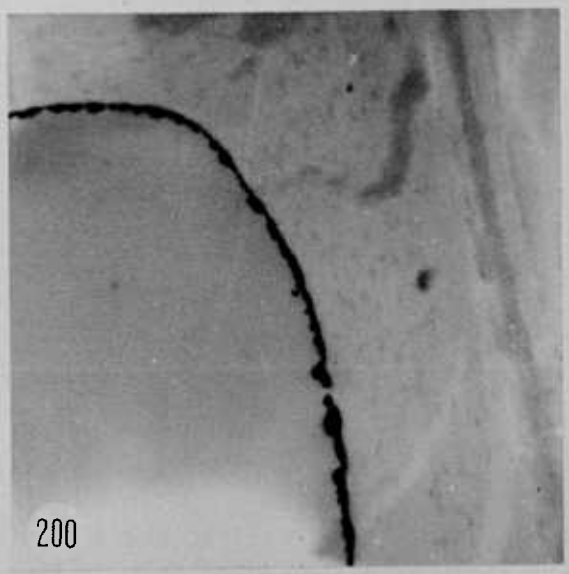
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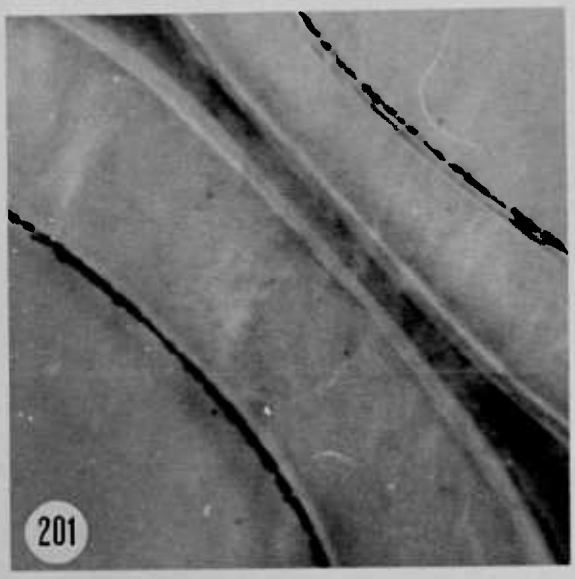
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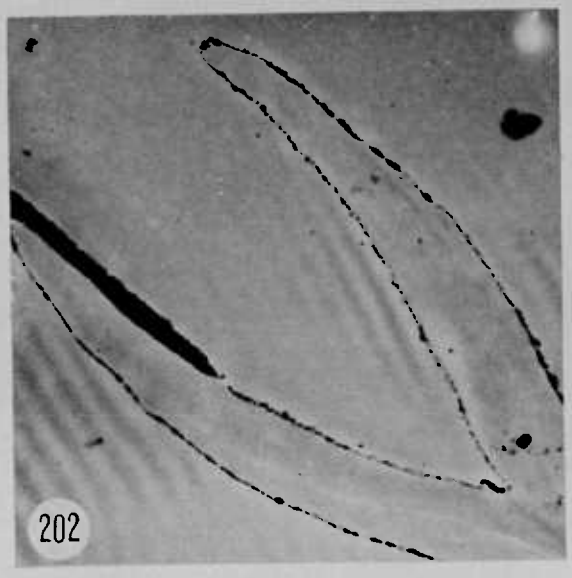
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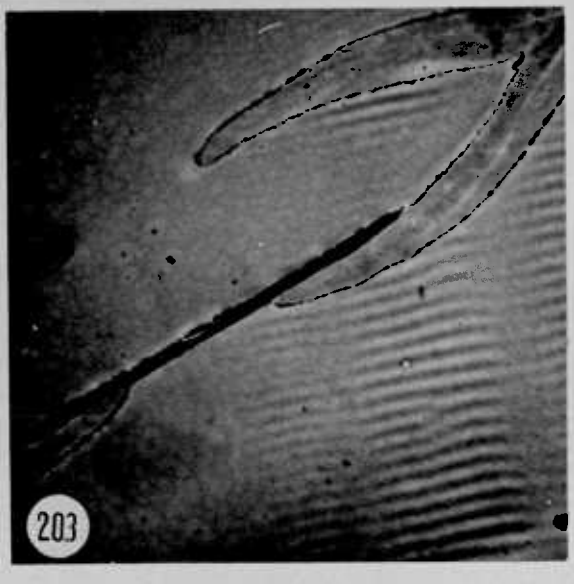

203

Figure 204. Transmission electron micrograph of 2.5% CCA treated Scots pine in a pit-region. Section obtained from "Epon" embedded material (x 4000)

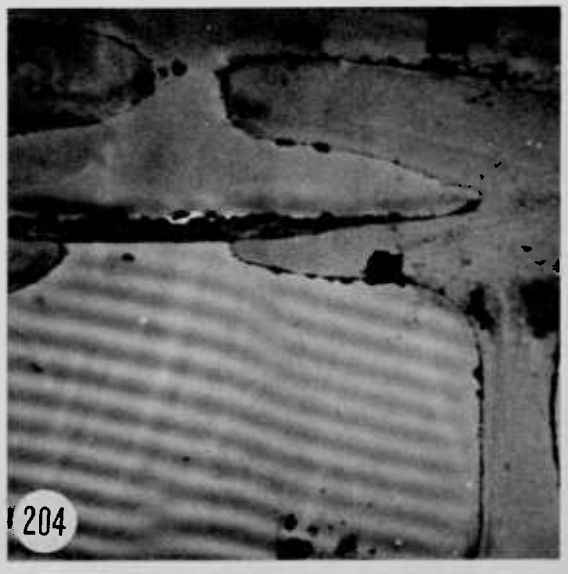
Figure 205. Transmission electron micrograph of 5% CCA treated Scots pine. Note the orientation of the microfibrils. Section obtained from "Epon" embedded material (x 20000)

Figure 206. Transmission electron micrograph of 5% CCA treated birch. Section obtained from unembedded material (x 2500)

Figure 207. Transmission electron micrograph of 2.5% CCA treated birch. Section obtained from "Epon" embedded material (x 2500)

Figure 208. Transmission electron micrograph of 5% CCA treated birch in a ray parenchyma region. Section obtained from "Epon" embedded material (x 5000)

N.B. Note the deposition of preservative as accumulated granules on the S_3/ cell lumen interface, round the pit border and pit-tori in the previous figures.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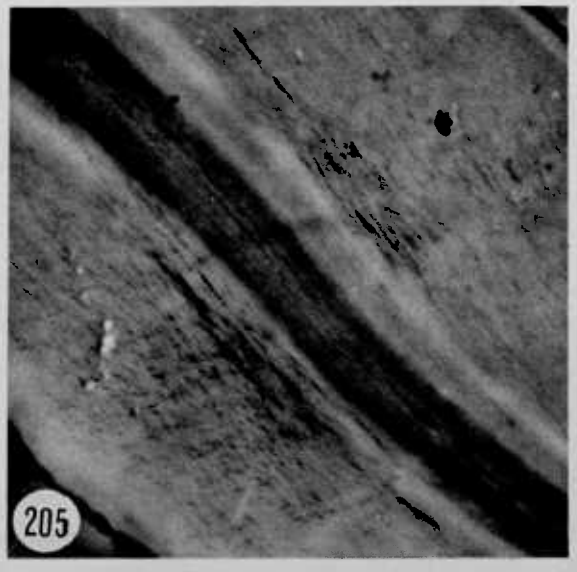
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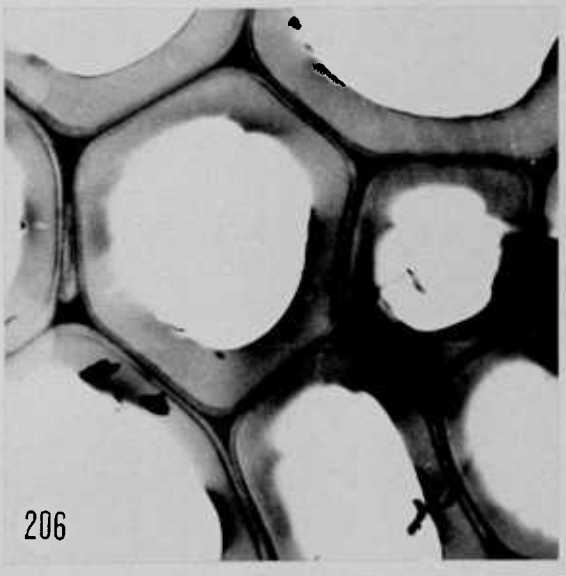
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207

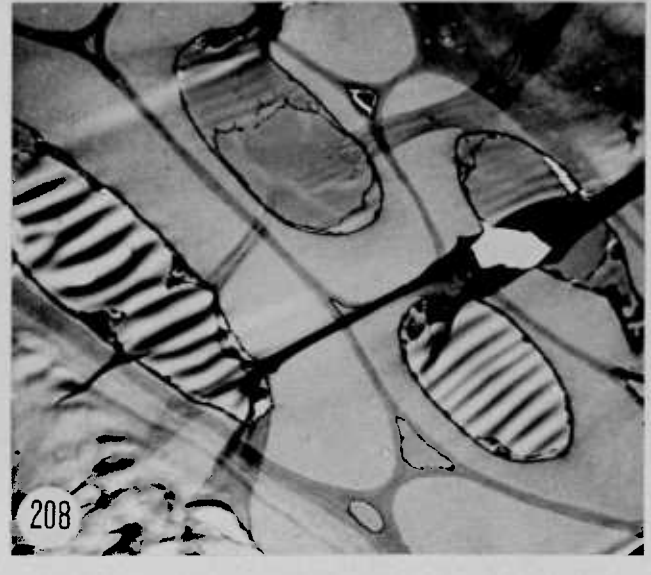

208

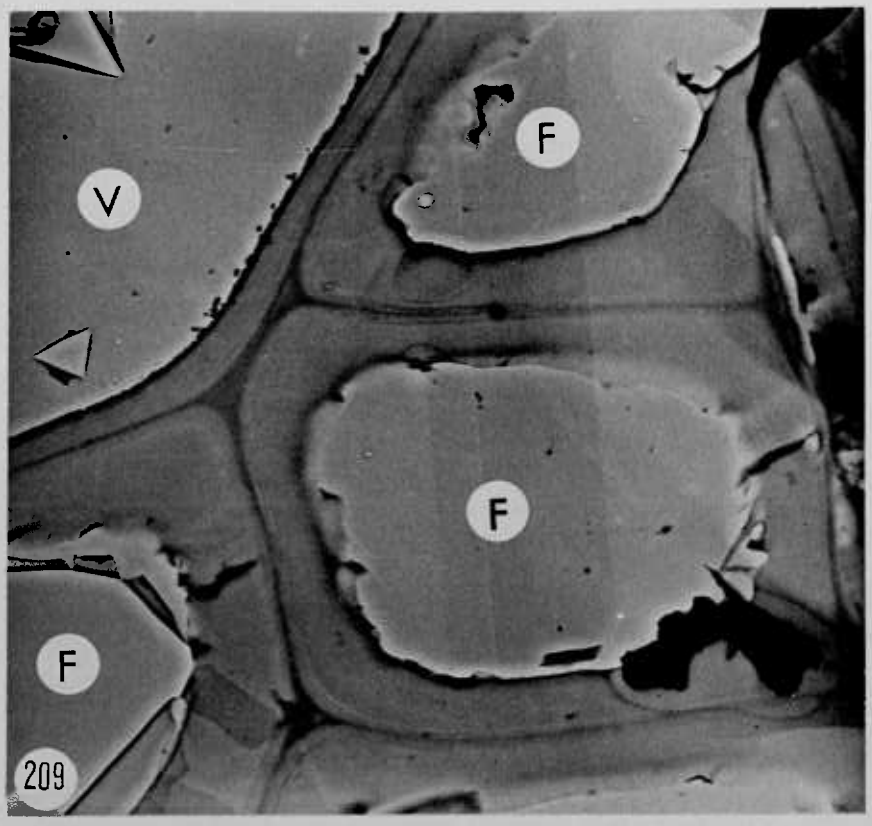
Figure 209. T.S. of unembedded and unstained birch showing a general view of the analysed area (x 3125)
V = vessel F = fibre

Figure 210. A higher magnification of figure 209 showing the analysed points across the cell walls of a vessel and a fibre (x 7875)
T = contamination spot

Table (23) Relative counts per second for copper, chromium and arsenic in points indicated in figure 210.

	Wall layer	Copper	Chromium	Arsenic
Vessel	a	0.0	3.8	0.0
	b	63.1	10.4	8.4
	c	12.8	2.7	1.5
	d	9.1	3.0	1.9
	e	25.7	9.9	1.4
Fibre	f	10.9	5.8	0.0
	g	20.7	6.4	0.0
	h	3.1	0.8	22.9
	i	13.2	5.7	5.1
	j	1.6	1.0	2.2

a and j = lumen
b, h and i = S_3 layer
c and g = S_2 layer
d and f = S_1 layer
e = middle lamella


V
F
F
F
209


a
b
c
d
e
f
g
h
i
j
T
210

Figure 211. T.S. of unembedded and unstained birch fibres showing a general view of the analysed area (x 3125)

Figure 212. A higher magnification of figure 211 showing the analysed points across fibre walls (x 12500)

L = cell lumen

T = contamination spot

Table (24) Relative counts per second for copper, chromium and arsenic in points indicated in figure 212.

Wall layer	Copper	Chromium	Arsenic
a	4.9	3.4	0.0
b	19.9	4.8	3.8
c	17.1	4.7	2.9
d	19.6	4.5	0.0
e	18.2	9.5	0.4

a and e = S_3 layer

b and d = S_2 layer

c = middle lamella


211

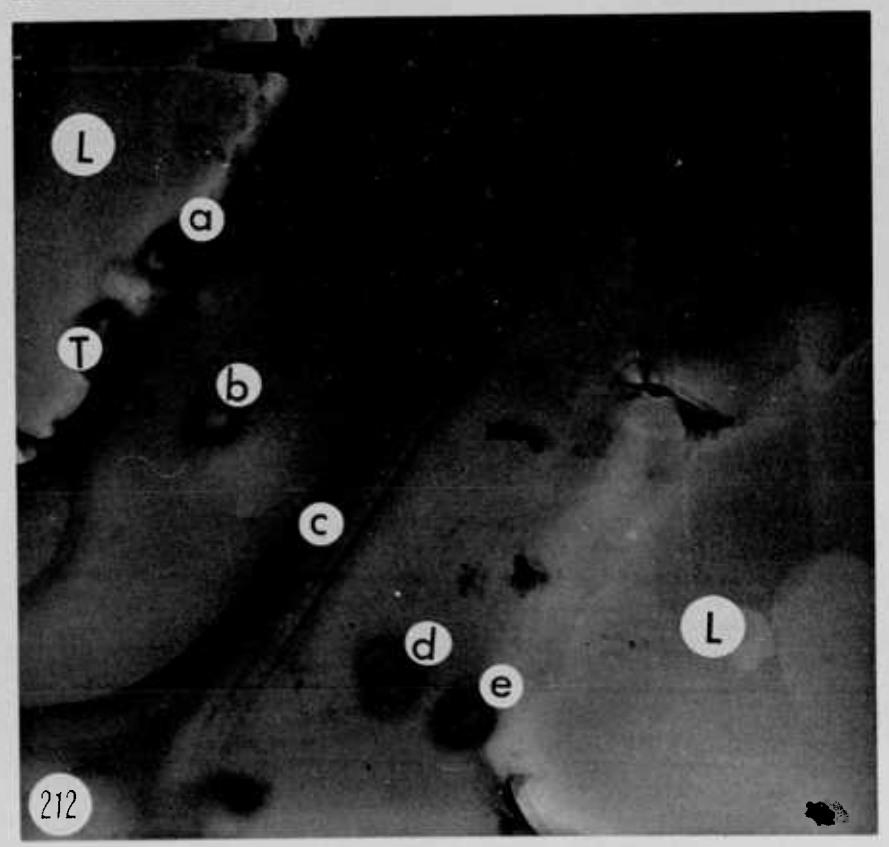

L
a
T
b
c
d
e
L
212

Figure 213. Section of embedded and unstained walls of birch fibres showing the points analysed across the cell walls (x 12500)

L = cell lumen

Table (25) Relative counts per second for copper, chromium and arsenic in points indicated in figure 213.

Wall layer	Copper	Chromium	Arsenic
a	7.4	7.5	0.0
b	5.4	3.8	0.0
c	6.0	4.6	0.3
d	7.4	4.0	1.4
e	5.6	2.0	8.1
f	5.2	6.9	0.0
g	4.8	2.0	0.0
h	3.3	1.7	0.0
i	4.8	3.5	0.0
j	6.2	11.0	9.3

a and j = S_3 layer

b, c h and i = S_2 layer

d and g = S_1 layer

e and f = middle lamella

Figure 214. Section of unembedded and unstained walls of Scots pine tracheids showing the points analysed across the cell walls (x 20000)

L = cell lumen

Table (26) Relative counts per second for copper, chromium and arsenic in points indicated in figure 214.

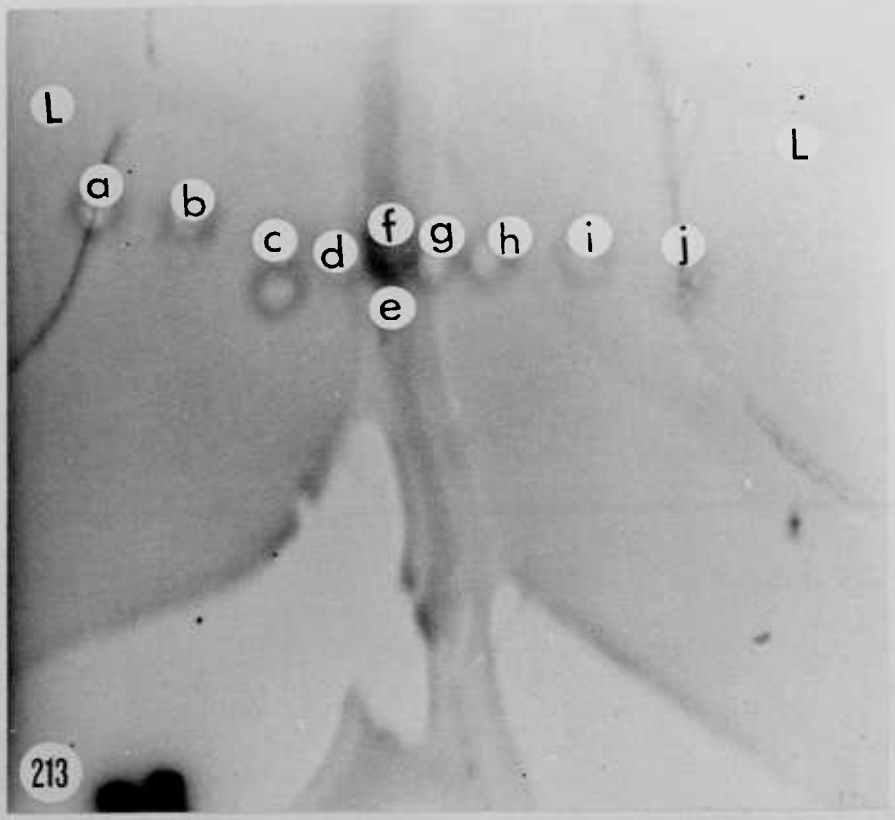
Wall layer	Copper	Chromium	Arsenic
a	334.1	215.9	0.0
b	204.5	323.6	46.4
c	323.5	355.6	0.0
d	147.9	198.6	95.7
e	217.8	303.9	1.3
f	162.0	207.6	0.0
g	143.5	141.3	0.0
h	224.2	195.6	0.0
i	198.4	251.6	39.7

a and g = S_3 layer

b, c, f and i = S_2 layer

h = S_1 layer

d and e = middle lamella


L
L
a
b
c
d
f
g
h
i
j
e
213

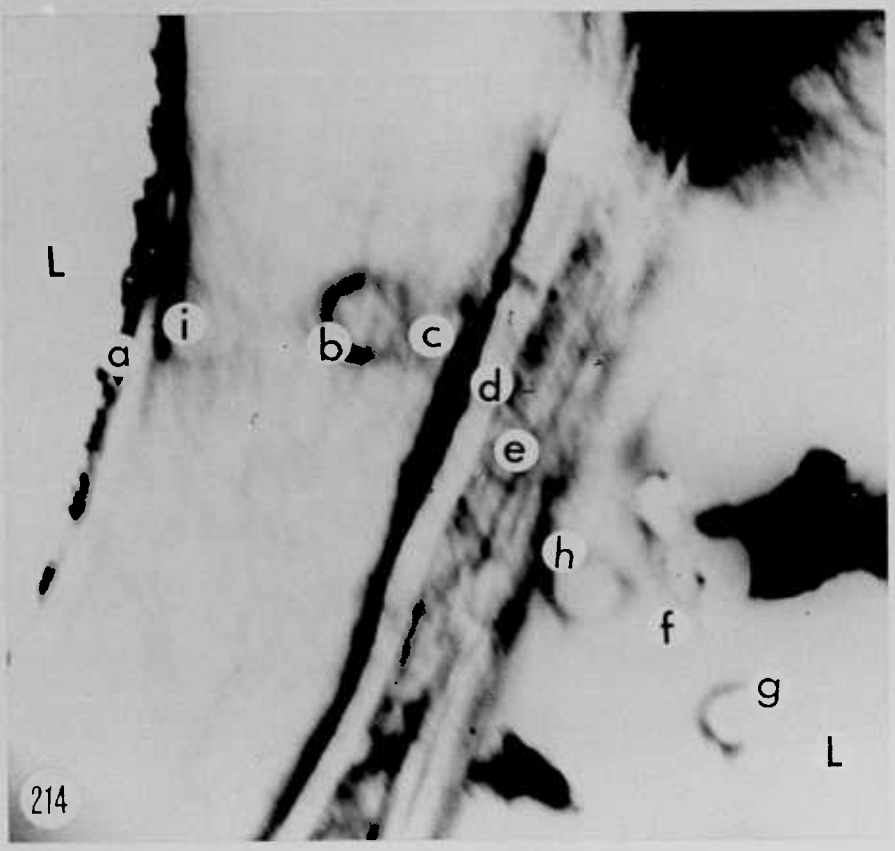

L
i
a
b
c
d
e
h
f
g
L
214

Figure 215. Section of embedded and unstained birch ray parenchyma cells showing a general view of the analysed area (x 12500)

Figure 216. A higher magnification of figure 215 showing the points analysed across the cell walls (x 20000)

Table (27) Relative counts per second for copper, chromium and arsenic in points indicated in figure 216.

Wall layer	Copper	Chromium	Arsenic
a	5.4	0,0	0.0
b	20.1	120,3	32.1
c	15.8	34,7	5.9
d	10.6	9,2	6.0
e	12.1	8,7	0.0
f	8.3	18,3	3.8
g	7.2	11.4	0.1
h	9.8	15.8	1.0
i	24.3	71.3	11.0
j	5.2	0.6	0.0

a and j = cell lumen

b, c and i = S_3 layer

d, e, g and h = S_2 layer

f = middle lamella


215

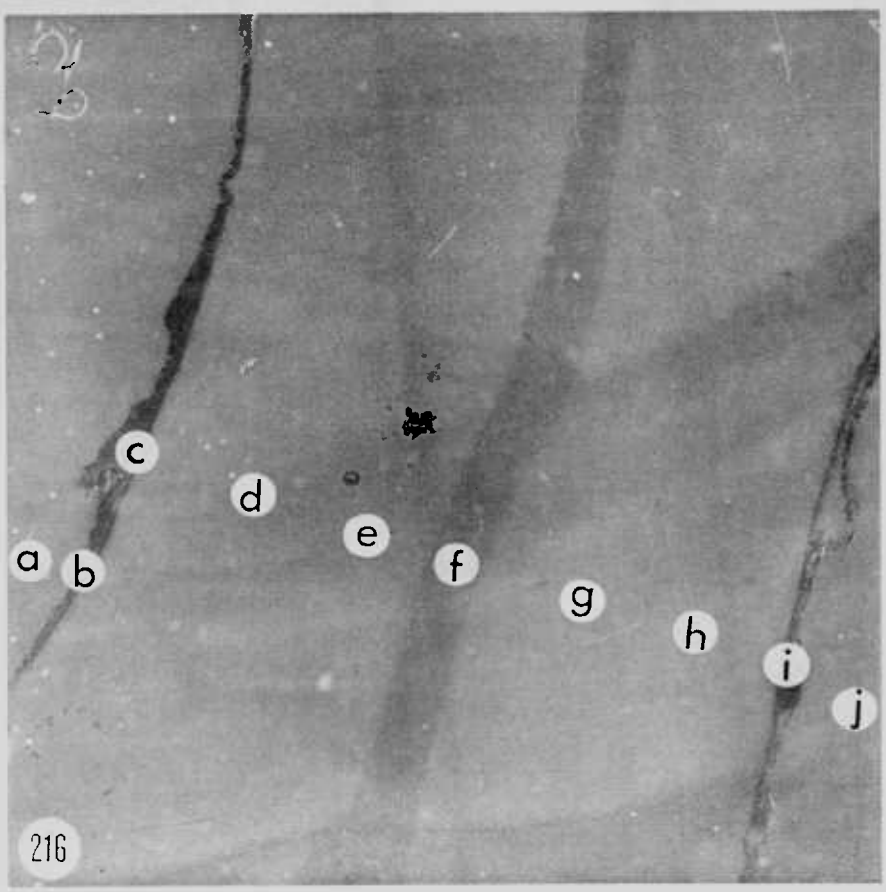

a
b
c
d
e
f
g
h
i
j
216

Figure 217. Section of embedded and unstained birch ray parenchyma cells showing the points analysed across the cell walls (x 12500)

Table (28) Relative counts per second for copper, chromium and arsenic in points indicated in figure 217.

Wall layer	Copper	Chromium	Arsenic
a	78.2	90.7	86.6
b	11.7	23.7	16.9
c	20.3	33.2	12.0
d	35.2	30.6	0.0
e	22.7	90.7	8.2

a and e = S_3 layer

b and d = S_2 layer

e = middle lamella

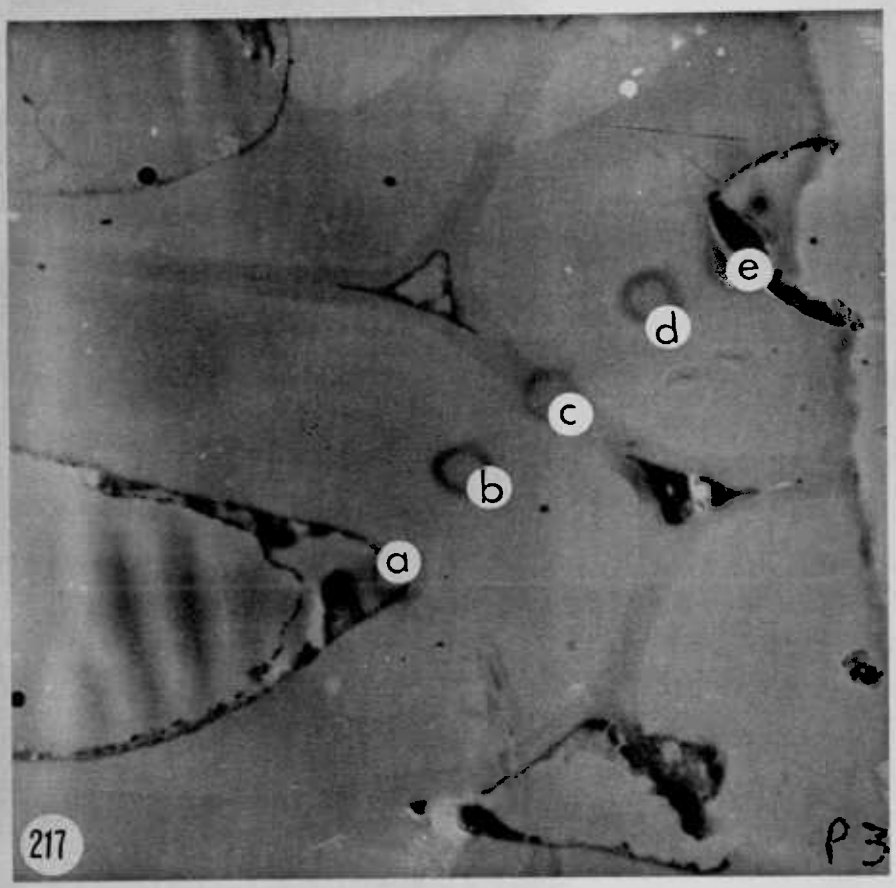

e
d
c
b
a
217
P3

Figure 218. Schematic representation of table (29).

Table (29) Average relative counts per second for copper, chromium and arsenic in wall layers of Scots pine tracheids.

Wall layers	Copper	Chromium	Arsenic
S_3	238.8	178.6	0.0
S_2	222.1	284.6	21.5
S_1	224.2	195.6	0.0
Middle lamella	182.9	251.3	48.5

Figure 219. Schematic representation of table (30)

Table (30) Average relative counts per second for copper, chromium and arsenic in wall layers of birch ray parenchyma cells.

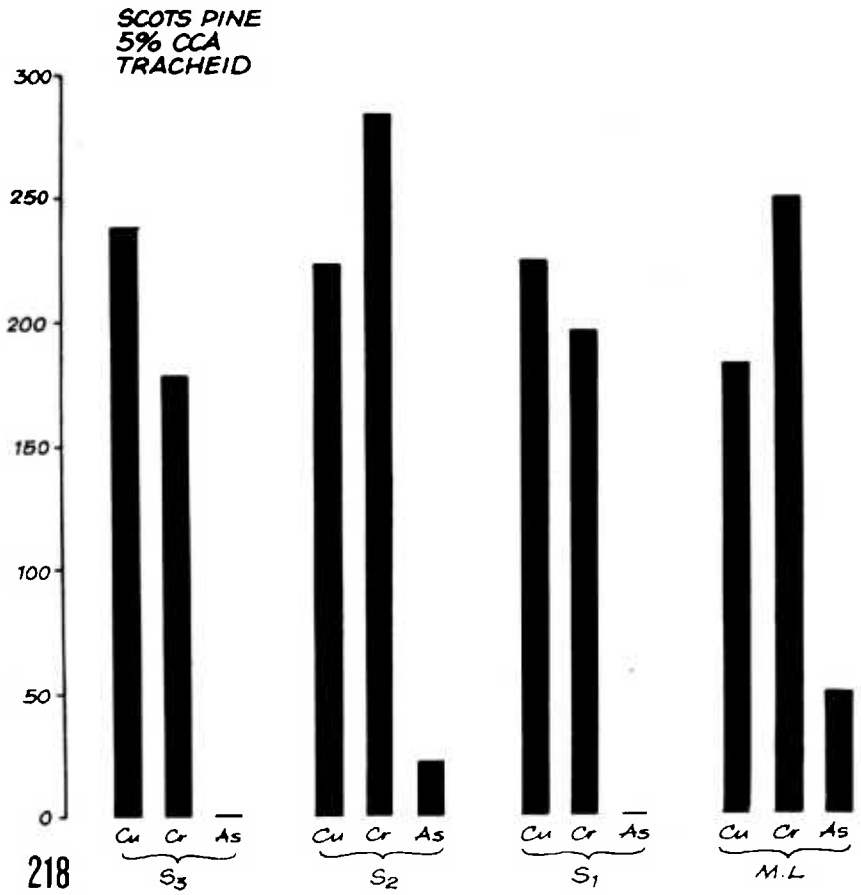
Wall layers	Copper	Chromium	Arsenic
S_3	50.5	90.7	47.4
S_2	23.5	27.2	8.5
S_1/ middle lamella	20.3	33.2	12.0

Figure 220. Schematic representation of table (31)

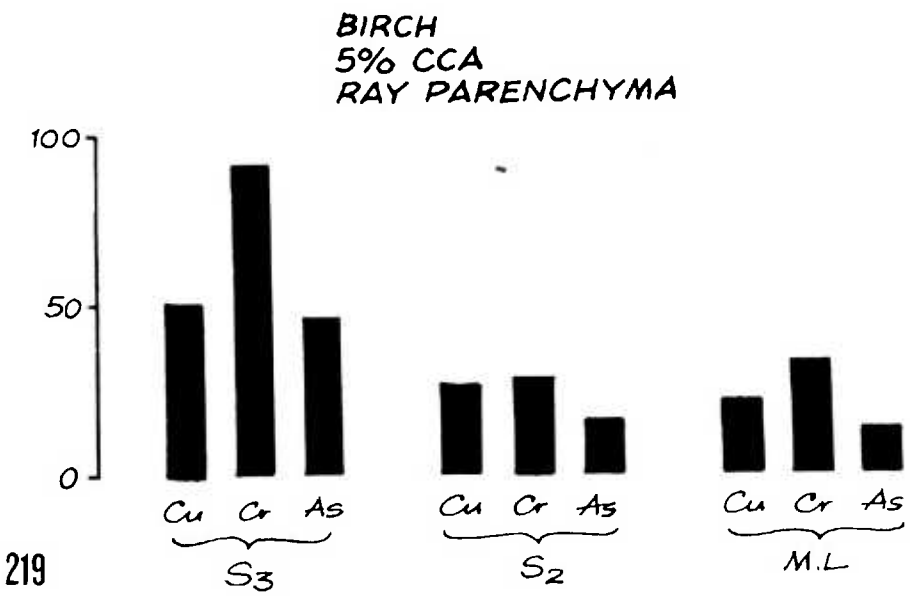
Table (31) Average relative counts per second for copper, chromium and arsenic in wall layers of birch fibres.

Wall layers	Copper	Chromium	Arsenic
S_3	6.8	9.3	4.7
S_2	4.9	3.4	0.1
S_1	6.1	2.0	0.7
Middle lamella	5.4	4.5	4.1

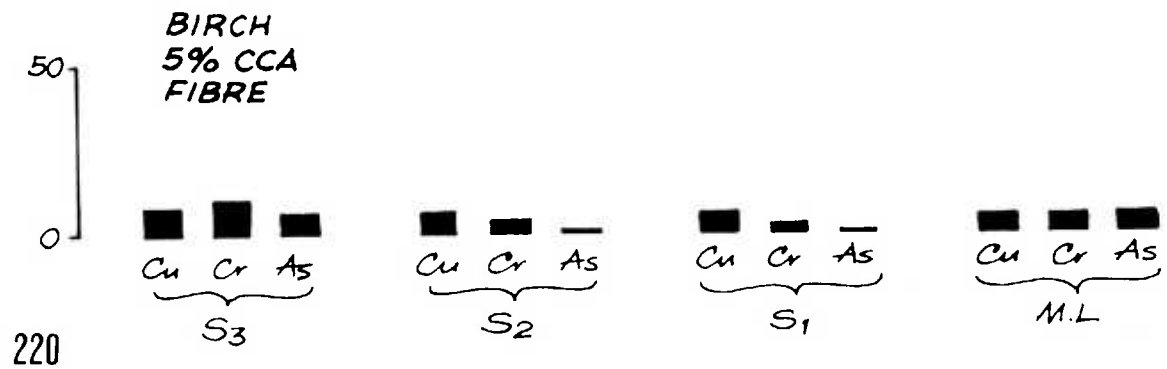
N.B. The vertical scale of figures 219 and 220 is approximately x 1.5 that of figure 218.


SCOTS PINE
5% CCA
TRACHEID
300
250
200
150
100
50
0
Cu Cr As
S3
S2
S1
M.L

218


BIRCH
5% CCA
RAY PARENCHYMA
100
50
0
Cu Cr As
S3
S2
M.L

219


BIRCH
5% CCA
FIBRE
50
0
Cu Cr As
S3
S2
S1
M.L

220

PART IV

GENERAL DISCUSSION AND CONCLUSIONS

4.1. GENERAL DISCUSSION

4.1.1. Microbiological studies

The original object of this work was to set up a series of experiments to establish the usual pattern of penetration, colonization and decay of a softwood and a hardwood by five different representative types of fungus, a stainer, a stainer/soft rot, a soft rot, a brown rot and a white rot. Once these had been recognised and recorded, the behaviour of the same organisms in the same timber species which had been treated with a wood preservative would be examined to see whether the preservative or method of treatment (or both) caused any changes in the patterns previously observed. Such changes, if they occured might suggest on the one hand, the mechanism of action of the wood preservative on the fungus and on the other, the ability of the fungus to mobilise or remove the preservative chemicals in the immediate vicinity of the fungus. The major part of the observations would be made using light microscopy, but higher magnifications would be necessary from time to time and some means had to be used to determine the amount of wood preservative chemical distributed between cells and within the individual layers of the cell walls. These would clearly involve some forms of electron microscopy.

The different methods of preservative treatment and the two concentrations of preservative used in the investigation showed no visual differences in the degree of protection of either species of wood.

The results obtained from the observations made on the behaviour of certain species of the test fungi on both species of wood were rather interesting and are of such importance as to be discussed

individually. Therefore the related aspects of the observations made will be generally discussed together and the differences observed in the behaviour of individual fungal species will be pointed out and discussed separately.

The periodical examination of the wood blocks exposed to each fungal species and soil after each exposure period was a very useful method to study the patterns of penetration, colonization and degradation caused by each species of fungi, since, it is often very difficult and sometimes impossible to distinguish between the different types of organism at the final stage of decay where the wood is almost completely broken down. This is especially true when more than one type of fungal decay occurs, as in the case of wood buried in unsterile soil. It is also an advantage to know the entire history of a degraded sample of wood where comparisons at a particular stage of attack can be easily made between preservative treated and untreated timber, between fungal species and between timber species under investigation.

As observed by the earlier workers (Corbett, 1963, Greaves, 1966 and Bravery, 1972) colonization of the untreated blocks of birch seemed to be initiated mainly in the rays and vessels with all species of fungi. The rays in untreated Scots pine afford the initial penetration pathway for the fungus and the resin canals were also initially colonized but to a lesser extent. The initial colonization of the rays may indicate that the fungus is stimulated by these nutrient rich centres which afford an easy source of food to the invading organism. The comparatively early destruction of the ray cells may support this view. The colonization of the vessels in birch and resin canals in Scots pine may simply be due to the nature of these cells in having large voids where the fungus can easily penetrate through.

It was interesting to note that earlywood and latewood tracheids were not equally attacked. P. fastigiata and C.globosum mostly colonized and degraded the latewood tracheids earlier than the earlywood tracheids. While, the opposite occurred in the case of C.versicolor and C.puteana where the earlywood tracheids were colonized and degraded before the latewood tracheids. A possible explanation for this phenomenon is that the soft-rot fungi do not prefer the thin-walled tracheids in the earlywood zones which contain more lignin than that of the latewood zones (Jane, 1970). They may also have been stimulated by the wide cellulose-rich S_2 layer in the latewood tracheids which affords more space in which the fungus can grow as it is a well known fact that the soft-rot fungi act within the S_2 layer of the cell wall. The preference of the earlywood tracheids in the case of C.versicolor and C.puteana may be related to the larger voids in these cells which afford easy penetration pathways to the fungi and that the space within the cell wall is not needed because of the nature of their process of degradation which acts from the cell lumen by a diffusable enzymic system (Liese, 1970 a, b and Bravery, 1972). Bailey et al (1968) suggested that brown-rot fungi have a much more efficient pre-cellulolytic system than soft-rot fungi and that the acidic conditions maintained by brown-rot fungi may play an important part in their pre-cellulolytic system. Although brown-rot fungi, as in the case of soft-rot fungi, are known to attack cellulose, the differences observed in the behaviour of C.puteana to that of the soft-rot fungi in initially attacking the more lignified earlywood tracheids may be related to its more efficient biochemical action than that of the soft-rot fungi. These are obviously speculations and further work is needed to explain such phenomena.

At the early stages of exposure to the test fungi, the fungal species usually colonized the outer regions of the block particularly the parts closer to the culture media. This was followed by a scattered form of colonization which eventually dominated the whole block. This usually took place within the first week of exposure in the case of the untreated blocks of both species of wood with a varied growth rate from one fungus to the other. No degradation was observed after the 3 day period of exposure which suggests a period of fungal establishment within the wood blocks. After this period of time slight degradation was observed in certain parts of the block as colonization was becoming heavier throughout the block. This was true with most species of fungi in both species of wood. The period of colonization prior to degradation was also valid in the case of the other species of fungi but varied from one species to the other. This phenomenon was also observed by Bravery (1972) and discussed by him in detail. Towards the later periods of exposure fungal colonization was heavy throughout the block where fungal hyphae densely filled the different cell lumina. The untreated blocks of birch were found to be more rapidly and heavily colonized with all species of fungi than the untreated blocks of Scots pine.

The untreated blocks of birch were found to be more susceptible to decay by all species of fungi than the untreated blocks of Scots pine. All tissues of birch were rapidly degraded, but this was comparatively slower in the case of Scots pine and the tracheids of this timber showed resistance to decay against B.theobromae and were only slightly degraded at a late period of exposure by P.fastigiata. The rays in both species of wood were usually the initial and most heavily

degraded regions compared to the fibres and the more resistant vessels in birch or the tracheids in Scots pine. The differences in the rates of decay between the 2 wood species and between the tissues in each wood species may be related to the differences in the chemical nature of such timber species or tissues and also of the degradation process of the particular fungal species. This will be discussed in more detail at a later part of this section.

It is interesting to note that the microbiological observations made on the preservative treated blocks suggested possible preservative distribution patterns within the 2 species of wood. This was strongly supported by the results obtained from the preservative distribution studies in the present work and the findings of other workers.

In the case of the preservative treated blocks of both species of wood, colonization by the different species of fungi was considerably delayed in comparison to the untreated wood blocks. The patterns of colonization in the treated blocks were, more or less, similar to that observed in the untreated blocks with the exception of the rays which were found to be more resistant to colonization. Unlike the untreated blocks, where the rays were generally found to be the initial pathways to the fungi and considerably degraded at an early stage of attack, the ray cells in the treated blocks were found to be colonized at a later period of exposure. This was strikingly obvious in the case of treated blocks of birch. This is in agreement with the observations made by other workers (Cavalcante, 1975 and Nilsson, 1976) where it has been demonstrated that at the higher preservative concentrations, the rays were found to be more resistant to colonization and decay. This is obviously due to the better preservative treatment of these

tissues which were clearly observed in the preservative distribution pictures of the present investigation (c.f. Section 3.3.). In addition, the hyphae present at the early exposure periods were mostly unhealthy and collapsed into fragments or consisting of a fluffy-like material, especially in the case of the Basidiomycetes, particularly in the treated blocks of Scots pine, where more collapsed hyphae were usually present than the normal ones at each of the different exposure periods. These hyphae were never observed in the untreated blocks of wood. It was also found that the fungal hyphae were not able to surround the treated blocks as was the case with the untreated blocks of both species of wood. This was especially noteworthy of the Basidiomycetes which surrounded the untreated blocks with a sheath of fungal mycelium. This phenomenon also occurred with untreated blocks buried in soil where a heavy soil mycelium encrustation of the blocks was observed but was not found to be present with the treated blocks. These observations indicate the toxic action of the preservative on the fungal hyphae. The light microscope observations made showed beyond doubt that the preservative had a harmful effect on the fungal hyphae. This is highly supported by the transmission electron microscope observations made by Chou (1971) where he found severely disrupted fungal hyphae in the CCA treated blocks of Scots pine which were not observed in the untreated blocks. The interactions between the fungal hyphae and the toxic elements of the preservative were not considered further in the present work mainly because of shortage of time.

The rates of growth of the different species of fungi in the treated blocks were also very slow and most parts of the blocks were

found to be free from fungal hyphae. Colonization was mostly towards the outer and lower parts of the blocks or slightly scattered in certain parts, with the exception of the soft-rot species in birch which succeeded in penetrating and colonising the deeper parts of the blocks. This colonization of the lower and outer parts is related to the continuous hyphal invasion from the culture media. The presence of most normal hyphae in these zones of the blocks supports this view. The scattered form of colonization may be related to the absence of the preservative in certain areas of the block which may not have received the preservative in the original treatment (Bravery, 1972 and Greaves, 1972b).

The degradation of the preservative treated birch blocks was also considerably delayed. This only started at a time the untreated blocks were, more or less, completely degraded by all species of fungi. This degradation was mainly caused by the two soft-rot fungi and at a very late stage slight degradation was also observed in the blocks exposed to the 2 Basidiomycetes. The treated blocks of birch buried in soil were also slightly degraded at a late exposure period and the degraded zones were characteristic of soft-rot type. The treated blocks of Scots pine were completely protected against all species of fungi even after the last exposure period.

The detailed ultrastructural degradation patterns of the individual cell walls of birch caused by the soft-rot species and the Basidiomycetes were not possible to observe under magnifications possible in the light microscope and only the gross patterns of degradation were observed. The fibres of birch were the most degraded tissue. Unlike the untreated blocks, the ray parenchyma cells in treated birch showed more resistance to decay at the early stages of attack but they were

eventually slightly degraded at a late exposure period. Such degradation of the rays was observed only in the regions of the highly degraded fibres. The vessels also showed high tolerance to degradation although they served as important pathways to the invading fungal hyphae. The decay caused by the soft-rot fungi rapidly progressed in the fibres after the initiation of attack, while this was much slower in the case of the Basidiomycetes. The failure of the fibres in treated birch and the complete protection of the treated blocks of Scots pine is most likely to be mainly due to the availability of the preservative elements in the different wood tissues as well as through the deeper parts of the cell walls.

The microdistribution analysis carried out in the present study showed up differences which can offer very acceptable reasons for the biological performance. The tissues of the softwood and the individual cell wall layers of the tracheids were very evenly treated with a comparatively much higher level of treatment than that of birch fibres. This could account for the complete protection of the treated blocks of Scots pine. In the case of birch, the rays and vessels had the highest treatment with some penetration of the wood preservative into the wall in depth but the fibres were poorly treated with little CCA treatment of the wall in depth, particularly the S2 layer. This again could account for the greater protection of the ray parenchyma in the birch in comparison to the fibres. The observations made on the preservative distribution patterns also showed high accumulation of preservative at the S3/lumen interface of the different tissues. This coating of the preservative undoubtedly has a direct toxic effect on the invading fungal hyphae and protects the deeper parts of the cell

wall against decay, since the majority of fungal hyphae invading wood are located in the cell lumina and mostly lie in close contact with the S3/lumen interface (Chou, 1971). The eventual success of the soft-rot fungi in degrading the poorly treated S2 layer of the birch fibres may be related to the ability of these fungi to cross the highly treated S3 layer and penetrate into the poorly treated S2 layer and cause the degradation of this layer. They do not cause degrade from the cell lumen as in the case of the brown and white-rot fungi. The nature of this crossing of the S3 layer is generally not clear and needs further investigation but it is possible that it may in part be due to a mechanical penetration through the S3.

The late failure of treated birch caused by C.versicolor and C.puteana may be related to the nature of the degradation process of these fungi in acting from the cell lumen by a diffusable enzymatic system. The preservative coating on the S3/lumen interface may, therefore, act as a barrier against enzyme diffusion through the wall or it may inactivate the extracellular enzymes produced by these fungi. The interactions occurring between the preservative and the extracellular enzymes are not clear but the slight late attack of birch caused by the Basidiomycetes may indicate that the continuous supply of healthy normal hyphae from the agar media and their interactions with the toxic material lead to the formation of clear zones from which the extracellular enzymes can freely diffuse or this continuous invasion by the fungal hyphae may, in some way or another, cause the gradual detoxification of the preservative elements. The presence of many collapsed unhealthy hyphae beside the normal hyphae which succeeded in slightly degrading the wood may support this possibility. The slow rate of decay caused by the Basidiomycetes in treated birch

suggests that it may take a long period of time before the complete destruction of the blocks occurs provided that new active hyphae are available to invade the blocks. The faster rate of decay caused by the soft-rot fungi in comparison to the late slow rate of decay caused by the Basidiomycetes suggests that the latter may be of no importance in causing the degradation of treated wood in ground contact because of the early destruction caused by the soft-rot fungi which will have destroyed the wood before the Basidiomycetes can begin.

It must be emphasised that these interpretations concerning the interactions between the fungal species and the toxic elements of the preservative in wood remain highly speculative and work of mainly biochemical nature is required before adequate postulations can be made.

Certain species of the test fungi showed some interesting behaviour in the 2 species of wood which were of such importance to be discussed separately.

The spores observed in the case of P.fastigiata in both species of wood during the first few days of incubation indicate the way in which this fungus colonises the wood. More spores were found than fungal hyphae during the first 3 days of incubation and gradually decreased in number towards the end of the first week of exposure where the blocks of wood were dominated by fungal hyphae. Many of these spores were found to be germinating into new fungal hyphae. This phenomenon of sporulation and spore germination was observed in the wood blocks exposed to the fungal cultures grown in each of the 3 different agar media. This was first thought to be due to the change of media to the invading fungus where the fungus grown on a malt extract media and invaded the wood block which is a different carbon source, but the use of wood agar media which is, more

or less, a similar carbon source to the wood blocks showed a similar phenomenon. A possible explanation for this phenomenon is that the actual colonization of the wood blocks is due to the fungal hyphae produced from the germination of the initially observed spores. The germination of spores at the initial periods of colonization and the gradual decrease or the absence of these spores towards the later periods of colonization may account for this.

It was generally believed that P.fastigiata behaves as a stainer fungus in Scots pine (Findlay, 1970). From the observations made in the present work, this was found to be true in the first few weeks of exposure but it has been clearly shown at a later period of exposure that the fungus succeeded in degrading the softwood tracheids by the formation of typical soft-rot cavities. This again supports the observations made by Zainal (1975) who observed a similar decay pattern in Scots pine. She concluded that the highly lignified tracheids in Scots pine delayed the decay by the soft-rot fungus and not as previously postulated that the higher lignin content prohibited P.fastigiata from causing soft-rot in softwoods (Findlay, 1970).

In comparison to C.globosum, P.fastigiata was also a slower soft-rot fungus in causing the degradation of both untreated and treated blocks of birch.

It was interesting to note that B.theobromae, which is generally known to act as a stainer fungus, behaved as a white-rot fungus in untreated birch where the gradual degradation of the fibre and vessel walls from the cell lumen towards the middle lamella region occurred in addition to the normal stainer behaviour. This was not observed in Scots pine or treated birch where the fungus behaved as a stainer only. The decay pattern caused by B.theobromae needs further investigation which is beyond the scope of this thesis.

The soil burial tests generally showed a slower rate of colonization and degradation in both species of wood than that observed in the pure culture tests. It was observed that the untreated blocks were completely and firmly encrusted with soil. This occurred when the colonization by the fungal hyphae was well advanced. This encrustation was believed to be a function of the enclosure of soil particles in the mycelial mat surrounding the blocks.

The exposure period in soil was extended to 21 weeks in the case of the treated blocks because of the very slow rate of colonization and degradation. The treated blocks of Scots pine were completely protected against decay even after the last exposure period. In the case of treated birch, slow rate of decay was observed which seemed to be mainly of the soft-rot type. Many parts of the blocks remained sound or only slightly degraded even after 21 weeks of exposure. This might indicate that it may take a long period of time before the complete destruction of these blocks occurs.

Although the weight loss results obtained were, more or less, in agreement with the micromorphological observations, these measurements were not very reliable as a measure of the decay capacity of a fungus. This will be more elaborated when discussing the results obtained from such studies in the following paragraphs.

Higher weight losses were recorded for both treated and untreated blocks of birch than those recorded for Scots pine with all species of fungi. This is in agreement with the microscopical observations with the exception of the early exposure periods of treated birch and throughout the exposure periods of treated Scots pine where no signs of degradation was observed but nevertheless the results showed certain weight losses. Although these weight loss results are rather negligible when compared to the higher weight losses obtained for the

untreated blocks, they may be related either to the unreliability of these measurements or to the possibility that these measurements indicate the utilization of certain chemical ingredients of the wood blocks which did not result in the formation of any signs of visual decay. The microscopical observations showed that the decay of treated birch caused by P.fastigiata was far more advanced and heavier than that caused by the Basidiomycetes, while the weight loss results in the case of P.fastigiata were lower than those obtained with C.versicolor or C.puteana. This may be also related to the degraded wood residues left by each species of fungus. These degraded substances may be lighter in the case of the Basidiomycetes than those left by P.fastigiata.

In general the present methods applied for the weight loss measurements as a decay capacity criterion are not adequately reliable by themselves to assess the decay capacity and other criteria should be considered such as biochemical and micromorphological studies.

4.1.2. Preservative distribution studies

The differences observed, in the microbiological studies, between the tissues and wood species from the colonization and degradation point of view suggested that the preservative distribution within each timber species was playing the most important role in causing such differences. It was therefore very important to find ways and means to learn more about the preservative distribution in order to understand the reasons for such differences. The results obtained from applying the different techniques for the study of the preservative distribution clearly pointed out reasons for such biological differences obtained.

The initial investigations carried out for the study of the preservative distribution by using an X-ray radiographic technique revealed unsatisfactory results and the need for the development of more precise techniques was found to be necessary. The results obtained were also misleading and did not show the exact microdistribution of the preservative in the 2 wood species as well as in the different wood tissues. It was not possible by applying this technique to reliably analyse or detect the individual elements present in the preservative. Other workers claimed the suitability of such technique for the preservative distribution studies in wood (Belford, 1960; Rudman, 1966 and Sharp, 1974). The radiographs shown by those workers were only for the preservative treated sections and although they claimed that these radiographs were distinguishable from the ones obtained from the untreated sections no radiographs were shown for such untreated sections. The present work clearly demonstrated that clear shadow-images were obtained from the untreated sections. Certain radiographs obtained from the treated sections were very similar to the ones obtained from the untreated sections especially in the case of Scots pine. This made it difficult or even impossible to locate the preservative distribution at a particular area of the wood section. In addition, the limited resolution of the light microscope, under which the radiographs were examined, it was difficult to determine the exact positions of the preservative especially in the case of the narrow cells where it was not possible to know whether the accumulation of the preservative was in the lumen or in the cell walls. More sophisticated techniques were needed in order to obtain a clearer picture of the preservative element distribution in the test material.

The utilization of two distinct systems, namely, the energy dispersive analytical technique in conjunction with scanning electron microscopy and the electron-probe micro-analysis in conjunction with a conventional transmission electron microscope made it possible to show the general distribution of the preservative elements across the cellular tissues and to examine the distribution of the elements within the cell wall layers respectively.

In spite of the difficulties encountered in the study of the preservative distribution in wood, the results obtained from the utilization of the 2 distinct systems were beyond doubt in presenting a better defined picture of the preservative distribution in wood.

The Edax system used in the present investigation has clearly shown marked differences between birch and Scots pine concerning the evenness of preservative distribution at tissue level. The X-ray image scans (i.e. Spotty pictures) obtained from the utilization of the system were a very useful visual aid in showing the general mapping of both the individual elements and the total mixture of the preservative which enabled areas of concentrations of preservative to be easily identified. These have shown considerable differences in the distribution of the individual elements in the different tissues of birch. The rays and vessels received a high level of preservative in comparison to an overall lower distribution of the 3 elements across the fibres. In the case of Scots pine a very even distribution of all elements was observed. The relative counts obtained for the individual elements confirmed these observations and also indicated high counts of all elements in the case of Scots pine while in the case of birch the rays and vessels showed higher counts than the fibres but still much lower than those of Scots pine. These results may account for the complete protection

observed in the case of Scots pine and the failure of the birch fibres at an early stage in comparison to the slight failure of the rays at a later period of exposure.

Greaves (1972b) using the same system, found a similar picture for Eucalyptus regnans to that observed for birch but found a more patchy distribution for Pinus radiata in comparison to Scots pine. He did however attempt to analyse his material to a finer level, where the present work was confined to establishing the preservative distribution at a tissue level only. He also reported considerable difficulties due to shadowing and the angle tilt of the specimen. This was overcome in this study by the use of thin oblique transverse sections as previously described (c.f. section 2.2.2.6.1).

The EMMA4 analysis confirmed the results obtained from the Edax system. The preservative elements penetrated deeply through the tracheid wall. This compares well with the reported observations of Petty and Preston (1968) and Chou (1971). The level of treatment in birch fibres was generally very low. All the wall layers were very poorly treated which agrees with previous observations on Sycamore and Eucalyptus (Dickinson, 1974). The ray parenchyma and vessel cell walls were generally treated much better than the fibre walls but not so well as the softwood tracheid walls. Nevertheless, this level of treatment was apparently high enough to afford a better protection to the ray parenchyma from the soft-rot fungi. The most significant factor of these observations is the dramatic differences between the treated hardwood fibre and the softwood tracheid, particularly in the S2 region. These differences are almost certainly involved in the susceptibility of the S2 layer in the birch fibres to soft-rot as demonstrated by the biological assay. The good treatment of the S2 layer of the tracheid walls explains the lack

of attack by all test fungi in that region as recorded earlier.

A high level of preservative deposition was observed lining the S3/lumen interface of the different tissues of both species of wood. This deposition was also found round the pit border in Scots pine and accumulated on the pit-tori. Such a deposition of the CCA preservative was also observed by Chou (1971) in Scots pine and Dickinson (1974) in Sycamore and Eucalyptus. Bravery et al (1974) found a similar situation with tributyl tin oxide in treated beech blocks. Such deposition was not observed in certain unembedded sections especially in the fibre region. The use of dry wood sections may lead to the loss of material which was highly preserved in the "Epon" embedded sections. Apart from this, no significant differences were observed between the analysis of the embedded and unembedded wood material. This suggests that the embedding of the wood specimens does not mobilise the preservative elements as was thought might possibly occur, so that much greater confidence can be placed on observations made with embedded material.

The mechanisms involved in the entry of the preservative into the different wood tissues and through the cell walls seems to be numerous. It is clear that the substrate and its components have an effect in the distribution of the preservative. This may be evident from the differences observed between the distribution of the preservative in birch and Scots pine. Such mechanisms are beyond the scope of this thesis and projects for the study of these mechanisms are urgently needed.

The present work suggests that the failure of the treated hardwood was not delayed or prevented by doubling the loading of the preservative and the complete protection of the softwood achieved by the higher preservative concentration was also achieved by the lower

preservative concentration. This is not in agreement with what has been indicated by other authors who have suggested that an increase in the preservative retention delayed or prevented the decay of wood (Chou, 1971; Bravery 1972; Levi, 1973 and Strank and Hulme, 1975). These differences may be related to the high concentrations of the preservative used in this investigation and it is possible that if concentrations at and below the toxic limits had been used, a difference in the degree of protection might have been observed. The concentrations of the preservative used in this study were chosen to represent the usual commercial level (i.e. 2.5%) and twice that concentration (i.e. 5%). The present results may possibly indicate that the increase in the preservative concentration delays the attack of wood by fungi up to a certain concentration limit but beyond this point a further increase may have no effect. The differences between the results of the present work and those of others may also be related to the different preservatives used and the timber species examined.

4.2. CONCLUSIONS

1. The untreated blocks of both species of wood were rapidly colonised and degraded by all species of fungi. The rate of decay was slower in the case of Scots pine compared to that observed in birch and the rays were usually the tissue degraded initially in all cases.

2. Phialophora fastigiata mainly behaved as a stainer in the untreated blocks of Scots pine but at a later period of exposure it caused the degradation of the tracheids by the formation of typical soft-rot cavities.

3. During the first few days of exposure, Phialophora fastigiata mainly colonised the untreated blocks of both species of wood by its spores which eventually germinated into new hyphae;spores gradually disappeared towards the later periods of exposure and the blocks were heavily colonised by the fungal hyphae.

4. The S3 layer in the tracheid wall of untreated Scots pine was highly resistant to decay by the soft-rot fungi even when the S2 layer was completely degraded.

5. Earlywood and latewood tracheids in untreated Scots pine were not equally attacked by the different species of fungi. The soft-rot fungi colonised and degraded the latewood tracheids earlier than the earlywood tracheids but the opposite was true of the white-rot and the brown-rot fungi.

6. Botryodiplodia theobromae behaved as a stainer in both species of wood but at a later period of exposure it behaved as a white-rot fungus and degraded the fibres and vessels of the untreated blocks of birch.

7. Increasing the loading of the preservative had no significant effect on the prevention or delay of the fungal attack because no difference in the patterns of colonisation and degradation were observed between the 2.5% and the 5% CCA treated blocks of both species of wood.

8. The colonisation and degradation of the preservative treated blocks of both species of wood by the different species of fungi were considerably delayed in comparison to the rapid colonisation and degradation of the untreated blocks.

9. The treated blocks of Scots pine were completely protected against all types of decay by the different species of the test fungi even after 12 weeks of exposure and mostly unhealthy hyphae or spores were present in such treated blocks.

10. The treated blocks of birch were heavily degraded by the soft-rot fungi particularly in the fibres but the rays showed slight degradation after a longer period of exposure. The blocks were also very slightly degraded by the white-rot and brown-rot fungi at a late period of exposure with many unhealthy hyphae present but completely protected against the stainer fungus Botryodiplodia theobromae.

11. The colonisation and degradation of the treated blocks of both species of wood buried in unsterile soil were considerably delayed. Treated Scots pine showed no signs of decay even after 21 weeks of exposure while the degradation of the treated blocks of birch was very slow where even after 21 weeks of exposure most of the block was not decayed. The decay of the treated birch blocks was of the soft-rot type.

12. The weight loss results were unreliable by themselves as a measure of the decay capacity of a fungus.

13. The transmission electron micrographs of the preservative treated specimens showed a deposition of the preservative as accumulated granules on the S3/cell lumen interface of the different cells of both species of wood and also round the pit borders and the pit tori of Scots pine. This deposition was not observed on certain walls of sections obtained from unembedded material.

14. The tissues of Scots pine and the individual cell wall layers of the tracheid were evenly treated with a high level of preservative treatment. In the case of birch, an uneven distribution of all preservative elements was observed; the rays and vessels having the highest treatment with some penetration of the wood preservative into the wall in depth whilst the fibres were poorly treated with little CCA treatment of the wall in depth, particularly the S2 layer. Although the level of treatment in the rays and vessels of birch was higher than that of the fibres it was still much lower than that of the Scots pine tracheids, but appeared to be sufficient to retard the onset of decay very considerably.

15. Apart from the losses of preservative from the S3/cell lumen interface no measurable difference was observed between the wood sections obtained from the "Epon" embedded material and those obtained from the unembedded material as regards the preservative distribution. This suggests that embedding with "Epon" does not affect the micro-distribution of the preservative.

ACKNOWLEDGEMENTS

It is with great pleasure that I sincerely acknowledge the advice, encouragement and guidance given by my supervisor, Dr. J.F. Levy throughout this work.

I would like to express my gratitude to many members of the Botany Department of the Imperial College; in particular Dr. D.J. Dickinson for advice and stimulating discussion and my colleagues in the Timber Technology section for their help in many ways. My sincere thanks also go to Dr. J.L. Gay, Miss M. Martin and the staff of the Electon Microscope Unit and I am deeply indebted to Mr. R. Adams, Mr. R. Baker and the technical staff.

I would further like to acknowledge the advice and assistance given by Professor E. Cohen, Mrs. M.E. Lewis and Mr. V. Harris of the Mining and Mineral Technology Department at Imperial College, on the X-ray radiographic technique.

For advice and assistance in the operation of the analytical microscopes, I gratefully acknowledge Mr. B. Kent of the National Physical Laboratory, Teddington and Mr. K. Lawson of the Electrical Research Association, Leatherhead.

The typing of the thesis by Mrs. S. Douglas and the photographic assistance of Mr. R. Brooks are greatly appreciated.

To the University of Kuwait I am much in debt for the financial support given throughout the course of this study.

Finally I would like to thank my wife for her patience during the preparation of this thesis.

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