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Effects of Environmental Factors on Wood Decay Micromorphology of *Pinus radiata* and *Fagus sylvatica*, and its Significance for In-ground Wood Preservative Performance – Volume I

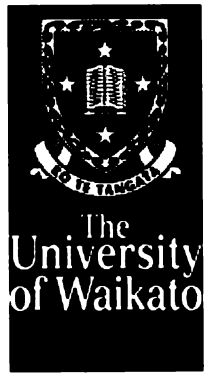
A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Biological Sciences

at

The University of Waikato

by

Robin Nicholas Wakeling



May 2003

This thesis is dedicated to my family
and
The New Zealand Forest Research Institute

ABSTRACT
Wood preservatives are essential to the continued use of wood in situations where fungal decay and damage from wood boring insects such as termites, would otherwise occur. Significant risks to health and the environment arising from their correct use are unproven but new legislation and consumer trends continue to cause a decline in the use of traditional and highly effective preservatives such as arsenical copper chromates (CCA). It is not certain that wood treated with alternative biocides will perform well in soil, an environment containing diverse decay flora and fauna and an aggressive preservative depletion hazard.

The objective of this work was to determine the effects of diverse test site conditions on in-ground performance of *Pinus radiata* D. Don. and *Fagus sylvatica* L. treated with new and traditional wood preservatives, determine wood decay type diversity, and test its significance on wood preservative performance and the ability of current decay classification nomenclature to describe it. *P. radiata* test stakes, 20 x 20 x 500 millimeters, were treated with ground contact retentions of CCA (0.72% mass/mass (m/m) active element), copper-azole (CuAz) (0.59% (m/m) active ingredient (a.i.): 0.57% m/m copper plus 0.02% m/m tebuconazole), an ammoniacal copper quaternary (ACQ) (1.56% m/m (a.i.): 0.87% m/m copper oxide plus 0.69% m/m didecyl dimethyl ammonium chloride (DDAC)), chlorothalonil plus chlorpyriphos in oil (CC) (1.07% (m/m) chlorothalonil) and a 60:40 mixture of creosote plus oil (37% m/m creosote). *F. sylvatica* stakes were treated with CCA (0.42% m/m a.i.), CuAz (0.54% m/m a.i. Cu) and CC (0.98% m/m chlorothalonil). For pine, 3 - 4 lower retentions of a 1.5 geometric series were also exposed to ensure advanced decay occurred within the 6.5 year exposure period. For beech, 1 - 2 lower retentions of a 2.25 geometric series were exposed for 5.5 years. Eleven New Zealand test sites of highly diverse soil type, vegetation and climate, plus a tropical and sub-tropical site in Australia were used. A Zeiss Axioplan II light microscope (LM) was used to determine decay micromorphology for each treatment (preservative + wood species) at each site and the effects of site, preservative and wood species on preservative performance, decay type and preservative depletion was determined. Suitability of epifluorescent confocal laser scanning microscopy (CLSM) for imaging decay micromorphology on the limits of LM resolution capability was determined.

Preservative performance

1. Across 13 sites, pine treated with CuAz (0.59% m/m a.i), ACQ (0.87% m/m CuO plus 0.69% m/m DDAC) and CCA (0.72% m/m a.i.) had mean soundness reduction (MSR) values of 15, 19 and 19% respectively. CuAz performed significantly better (5% level of probability (P)) than ACQ and CCA.
2. 6.5 years exposure is too short for prediction of long-term performance, however over widely varying conditions, including sites with extreme preservative depletion hazards, CuAz treated pine performed consistently. Performance of 0.39% m/m a.i., a retention 14% below the NZS3640 minimum requirement of 0.46% m/m a.i., gave significantly (5% P) poorer performance than the NZS3640 minimum requirement for CCA (0.72% m/m a.i.).
3. Pine treated with CuO plus DDAC (ACQ) at retentions 29 and 64% above the NZS3640 minimum requirements respectively, was susceptible to rapid failure from brown rot at some pastoral sites likely to be encountered in service. In contrast, CuAz treated pine gave superior performance against brown rot at the same sites compared to CCA treated pine.
4. Site had a significant (5% P) effect on preservative performance and a significant site-preservative interaction effect meant that overall site hazard index was not always a reliable indicator of performance, particularly at sites containing aggressive brown rot fungi (7 out of 13 sites).
5. Knowledge of the distribution of different decay types across sites, coupled with known decay hazards and preservative depletion hazard, suggested that 4 test sites of

clearly defined features would enable comprehensive field testing of preservative treated wood. Selection of sites is not straightforward and requires detailed knowledge of soil type, geology and climate, or comprehensive knowledge of the decay types present.

Preservative depletion

1. Site and wood species had a major effect on copper (Cu), chromium and arsenic depletion from CCA treated pine and beech and depletion of Cu and tebuconazole from CuAz treated pine and beech. Mean Cu depletion for radiata pine treated with 0.72% m/m a.i. CCA after 5.5 years, across 13 sites was less than 1% for above ground portions of stakes compared to 30% for below ground. However, below ground depletions at acidic sites located at a peat bog and a *Nothofagus* beech forest were 43 and 73% respectively. Mean below ground chromium and arsenic depletions were 9 and 21% respectively but were 22 and 41% at the most severe depletion site (*Nothofagus* forest).
2. Extremes of soil water availability and pH were the most important factors affecting depletion, where waterlogged sites with a high organic matter content (by inference high organic acid concentration) had the most severe depletion hazard.
3. Across all sites above ground depletion of copper and tebuconazole from radiata pine treated with 0.59% m/m a.i. CuAz was 19 and 42% compared to 47 and 55% for below ground. Substantially greater loss of copper from Cu-Az treated wood compared to CCA treated wood, especially for above ground exposure, across all sites, may become significant for wood in service situations where aquatic toxicity of copper is important.
4. Beech was more susceptible than pine to loss of copper for both CCA and CuAz. This may have been attributable to less efficient fixation reactions and preservative distribution (macro- and micro-) in beech.

Decay type diversity and classification nomenclature

1. Preservative treated wood was decayed by a complex mixture of decay types except at those sites where brown rot alone caused rapid failure. On the basis of unique mixtures of decay micromorphology, 18 decay types were identified of which 5 fitted within current decay classification nomenclature.
2. Based on mean soundness reduction caused by macroscopic decay types and their associated microscopic decay types, fifty percent of decay across all sites was caused by decay types not described (or inadequately described) by the currently accepted decay types: brown rot, soft rot (SR) (Corbett's type 1, 2 and diffuse cavitation), white rot (WR) (simultaneous and preferential) and bacterial decay (tunnelling, erosion and cavitation). This was in part attributed to presence of fungi which possessed diverse decay capabilities that produced mixtures of decay micromorphology preventing a neat fit within current classification, a finding that is concurrent with increasing evidence within the literature that basidiomycetes possess multiple decay capabilities. Current decay classification boundaries that place emphasis on taxonomic affinity and decay type expression under laboratory conditions, failed to accommodate the diversity of wood decay types found under field conditions for preservative treated pine and beech.
3. Close association of hyphae possessing clamp connections, with several unusual types of tunnelling and cavitation micromorphology in wood with a colour and texture typical of WR or intermediate between WR and SR, suggested that WR basidiomycetes were responsible for a significant proportion of the decay types observed.
4. Unidentified decay fungi that bypass the decay resistant S3 layer, causing complete concentric removal of the S2 layer by multi-branched hyphae under an overlying

lumen wall, were very important in preservative treated beech fibres and pine tracheids. Whilst some of the micromorphology associated with this type of decay has previously been reported as diffuse type 1 SR, its significance has been largely overlooked.

5. New insights into cavitation decay that forms near or at the S3—S2 interface, and where the overlying lumen wall disintegrates early in the decay sequence, has in the past been misdiagnosed as Corbett's type 2 (erosion) SR.
6. Simultaneous WR and type 2 SR were of little significance in preservative treated pine and beech, suggesting that decay fungi that produce it in other situations (e.g. untreated wood) adopt a tunnelling or cavitation mode in preservative treated woods. This was thought to be a stress response similar to the previously observed responses of basidiomycete decay fungi present in pathological reaction wood that contains allopathic chemicals and physical blockages of wood cell lumenae such as gums and resins. Therefore, latent genes encoding decay capabilities not expressed under laboratory conditions may be highly significant in preservative treated wood in ground contact.
7. Cellulose microfibril orientation in both the S2 and S1 layers determined orientation and positioning of the great majority of decay types, including those previously thought to be essentially random, such as tunnelling bacteria. Contrary to the literature, orientation parallel to, or affected by, the S1 cellulose microfibrils (CM) was of similar significance as alignment with the S2 CM. Position, orientation and limits of expansion of novel types of tunnelling and cavitation in both pine and beech suggested that concentric radial wood cell wall structure (cell lamellae and/or planes of resistance caused by differential lignin content) were important in determination of decay micromorphology.
8. A putative model was derived to account for the high frequency and morphology of concentrically aligned SR cavities, and cavities and tunnels of other decay types. The S1–middle lamella interface triggered formation of tangential hyphal branches followed by non-oscillatory or suppressed oscillatory growth and preferential expansion in the tangential plane. Whilst the S1 triggered tangential cavity orientation, it is too narrow to trigger normal SR oscillatory growth.
9. Protracted staining procedures, the need to embed samples and the time taken to manipulate images using computer assisted 3D modelling software, made epifluorescent CLSM unsuitable for examination of large numbers of samples, as required in ecological studies. Furthermore, the resolving capabilities and depth of field of the LM optics used in this study provided greater insight into the 3D nature of decay micromorphology.

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GLOSSARY OF ABBREVIATIONS

Test Sites

CC	Canal Creek
ESK	Esk Valley Vineyard
GB	Glenbervie
GBF	Glenbervie Forest
HH	Hari Hari
MM	Mount Mee
OAK	Oak forest
PT	Peat bog
TAP	Tapanui
TOM	Tombleson's mandarin orchard
TOT	Totatara flat *Nothofagus* forest
WKA	Whakarewarewa

Preservatives

CCA	Copper chrome arsenic (arsenical copper chromate)
CuAz	Copper Azole
ACQ	Ammoniacal copper quaternary
CC	Chlorothalonil plus chlorpyriphos in P9 Shell oil
C	Creosote plus oil (60:40)
DDAC	Didecyldimethyl ammonium chloride

Wood species / preservative treatments

P	Pine (*Pinus radiata* D. Don.)
B	Beech (*Fagus sylvatica* L.)
PC1	Pine treated with creosote, retention 1 (Table 2.1)
PC2	Pine treated with creosote, retention 2 (Table 2.1)
PC3	Pine treated with creosote, retention 3 (Table 2.1)
PC4	Pine treated with creosote, retention 4 (Table 2.1)
PACQ1	Pine treated with ACQ, retention 1 (Table 2.1)
PACQ2	Pine treated with ACQ, retention 2 (Table 2.1)
PACQ3	Pine treated with ACQ, retention 3 (Table 2.1)
PACQ4	Pine treated with ACQ, retention 4 (Table 2.1)
PCAZ1	Pine treated with CuAz, retention 1 (Table 2.1)
PCAZ2	Pine treated with CuAz, retention 2 (Table 2.1)
PCAZ3	Pine treated with CuAz, retention 3 (Table 2.1)
PCAZ4	Pine treated with CuAz, retention 4 (Table 2.1)
PCCA1	Pine treated with CCA, retention 1 (Table 2.1)
PCCA2	Pine treated with CCA, retention 2 (Table 2.1)
PCCA3	Pine treated with CCA, retention 3 (Table 2.1)
PCCA4	Pine treated with CCA, retention 4 (Table 2.1)
PCCA5	Pine treated with CCA, retention 5 (Table 2.1)
PCC1	Pine treated with CC, retention 1 (Table 2.1)
PCC2	Pine treated with CC, retention 2 (Table 2.1)
PCC3	Pine treated with CC, retention 3 (Table 2.1)
PCC4	Pine treated with CC, retention 4 (Table 2.1)
POIL1	Pine treated with P9 Shell oil, retention 1 (Table 2.1)
POIL2	Pine treated with P9 Shell oil, retention 2 (Table 2.1)
SOLV	Pine treated with Mobile dry cleaning solvent (Table 2.1)
BCCA1	Beech treated with CCA, retention 1 (Table 2.1)
BCCA2	Beech treated with CCA, retention 2 (Table 2.1)
BCCA3	Beech treated with CCA, retention 3 (Table 2.1)
BCAZ1	Beech treated with CuAz, retention 1 (Table 2.1)
BCAZ2	Beech treated with CuAz, retention 2 (Table 2.1)
BCC1	Beech treated with CuAz, retention 1 (Table 2.1)
BCC2	Beech treated with CuAz, retention 2 (Table 2.1)
BOIL	Beech treated with P9 Shell oil, (Table 2.1)

Decay Types

WR	White rot
BR	Brown rot
SR	Soft rot
WR/BR	Intermediate type: white rot / brown rot
TB	Tunnelling bacteria
EB	Erosion bacteria
T1	Tunnelling type 1
T2	Tunnelling type 2
T3	Tunnelling type 3
T4	Tunnelling type 4
C1	Cavitation type 1
C2	Cavitation type 2
C3	Cavitation type 3
C4	Cavitation type 4
C5	Cavitation type 5
C6	Cavitation type 6
C7	Cavitation type 7
E1	Erosion type 1
E2	Erosion type 2

Micrographs of decayed wood

M1-WR, M2-WR, M3-WR etc…	Micrographs 1, 2 and 3 for WR micromorphology
M1-BR, M2-BR, M3-BR etc…	Micrographs 1, 2 and 3 for BR micromorphology
M1-SR, M2-SR, M3-SR etc...	Micrographs 1, 2 and 3 for SR micromorphology
M1-WR/BR, M2-WR/BR, M3-WR/BR etc...	Micrographs 1, 2 and 3 for WR/BR micromorphology
M1-T1, M2-T1, M3-T1 etc…	Micrographs 1, 2 and 3 for T1 micromorphology
M1-T2, M2-T2, M3-T2 etc…	Micrographs 1, 2 and 3 for T2 micromorphology
M1-T3, M2-T3, M3-T3 etc…	Micrographs 1, 2 and 3 for T3 micromorphology
M1-T4, M2-T4, M3-T4 etc…	Micrographs 1, 2 and 3 for T4 micromorphology
M1-C1, M2-C1, M3-C1 etc…	Micrographs 1, 2 and 3 for C1 micromorphology
M1-C2, M2-C2, M3-C2 etc…	Micrographs 1, 2 and 3 for C2 micromorphology
M1-C3, M2-C3, M3-C3 etc…	Micrographs 1, 2 and 3 for C3 micromorphology
M1-C4, M2-C4, M3-C4 etc…	Micrographs 1, 2 and 3 for C4 micromorphology

M1-C5, M2-C5, M3-C5 etc...	Micrographs 1, 2 and 3 for C5 micromorphology
M1-C6, M2-C6, M3-C6 etc...	Micrographs 1, 2 and 3 for C6 micromorphology
M1-C7, M2-C7, M3-C7 etc...	Micrographs 1, 2 and 3 for C7 micromorphology
M1-E1, M2-E1, M3-E1 etc...	Micrographs 1, 2 and 3 for E1 micromorphology
M1-E2, M2-E2, M3-E2 etc...	Micrographs 1, 2 and 3 for E2 micromorphology
M1-TB, M2-TB, M3-TB etc...	Micrographs 1, 2 and 3 for TB micromorphology
M1-EB, M2-EB, M3-EB etc...	Micrographs 1, 2 and 3 for EB micromorphology

Wood Anatomy

ML	Middle lamella layer of wood cell wall (Figure 1.2)
S1	S1 layer of wood cell wall (Figure 1.2)
S2	S2 layer of wood cell wall (Figure 1.2)
S3	S3 layer of wood cell wall (Figure 1.2)
CM	Cellulose microfibrils (within wood cell wall)

Miscellaneous

BH	Bore hole
IUFRO	International Union of Forest Research Organisations
AWPA	American Wood Preservers' Association
MSR	Percentage mean soundness reduction
CLSM	Confocal laser scanning microscopy
CSA	Competitive sapropholic ability
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
LM	Light microscopy
WGA	Wheat germ agglutinin
NBD-PE	Nitrobenzoxadiazole glycerophosphoethanolamine
PBS	Phosphate buffered saline

Effects of environmental factors on wood decay micromorphology of *Pinus radiata* and *Fagus sylvatica*, and it's significance for in-ground wood preservative performance

CHAPTER 1
INTRODUCTION

Section 1.1
Role of wood in society

Wood is a highly versatile material of major importance in the world economy. It is widely used as a structural material, fuel and industrial raw material. As a structural material it has a high strength to weight ratio and is easily shaped and fastened. Its disadvantages as a structural material are largely related to its biodegradability, dimensional instability, property variability, combustibility and susceptibility to weathering. However these disadvantages are typically only a major issue when wood is used inappropriately. They can mostly be overcome by good design, product engineering and through use with speciality treatments such as coatings and preservatives.

There has been over-reliance on traditional sources of slow grown non-renewable or slow to renew forest, supplies of which are failing to meet world demand. Wood derived from increasingly important intensive forestry in countries like New Zealand and Chile does not always conform to traditional performance expectations. There has also been over-reliance on wood use as a replaceable low value commodity, rather than a value added product of enhanced performance and increased service life. This has created a legacy of reduced cost-effectiveness through wastage and remediation costs. These factors, coupled with increased competition from other materials such as plastic, steel, brick and concrete, have resulted in wood losing market share in some sectors of the construction industry. Wood has yet to realise its full potential as a renewable and versatile raw material for production of high performance engineered wood products.

The competitiveness of wood as a raw material could be increased by prevention of inappropriate use, through improved information transfer, by development of new technologies that will create new wood products of greater versatility than

raw wood and existing engineered wood products, and through increased recognition of the unique properties of wood. One of the great potential advantages of wood over other raw materials is its renewability and subsequent compatibility with socioeconomic and political trends such as sustainability and low environmental impact. Trees are efficient radiant energy transducers and as relative raw material costs and availability change, wood has the potential to replace petroleum as a raw material for production of a wide range of industrial chemicals and polymers. Provided it is derived from sustainable forestry, as a source of energy it has the merit of lower environmental impact compared to fossil fuels. This is because its combustion results in no net gain in CO_2 emission, thereby reducing global warming and its associated detrimental effects on the environment. Another advantage of wood is its natural image and aesthetically pleasing appearance, factors likely to become increasingly important in the built environment.

Whilst wood use is likely to continue to decline in some sectors of the construction industry, at least in the short term, it may increase in others in response to increased use of high performance engineered wood products, especially composites. The timing of increased demand that may result from wood use diversification, for example as a raw material replacement for petroleum, is difficult to forecast. However these factors coupled with declining wood availability from traditional sources, is likely to increase demand from intensively grown forests. This creates new opportunities and challenges for producer countries like New Zealand and highlights the importance of understanding the underlying mechanisms of wood performance. This study investigates the underlying wood decay mechanisms that determine the performance of preservative treated wood in ground contact.

Section 1.1.2

New issues for wood as a biodegradable material

Wood is a very durable organic material when properly used and maintained, lasting hundreds of years in buildings which have been appropriately designed to reduce the impact of biotic, chemical and physical factors that cause wood degradation. Once wood becomes wet its susceptibility to degradation increases greatly. Of the many factors that can affect wood soundness, presence of moisture

is the most important in many service situations. If wood cannot be kept dry in service, for example by use of building roof overhangs or through use of non-biocidal coatings such as acrylic paint, a wood preservative is required for the majority of wood species used. Whilst the primary function of a wood preservative is to prevent biotic degradation using a biocide, they may also contain substances that reduce contact with water (e.g. waxes and resins) and prevent or reduce chemical and physical degradation (e.g. additives that screen ultraviolet light, reducing lignin photodegradation). A major challenge facing the wood preservation industry is development of new preservative systems that meet both performance criteria and new expectations of low environmental impact and low human toxicity. Concerns over the acceptability of wood preservatives and a great many other substances typically go beyond actual risk and are driven by socioeconomic and consumer trends. In particular, the desire to minimise use of many substances and materials, especially those of artificial origin, on the basis of a perceived risk to health or the environment, is increasingly a driving force in selection of acceptable products.

Preservative development and registration for use is increasingly expensive and time consuming and this factor coupled with the shrinking availability of acceptable candidate wood preservatives, increases the importance of acquiring knowledge that makes the process more cost-effective. In particular, candidate wood preservatives that meet new health and safety requirements must be tested under a sufficiently diverse set of field conditions to ensure that strengths and weaknesses are identified before they become a problem in service. Part of this study was designed to determine the appropriate number and type of field test sites, and identify relative susceptibilities of different preservative-wood species combinations to different site hazards. This information can be used to identify critical parameters for the design of improved preservatives.

Section 1.2

Wood structure

To understand the basic properties of wood and its susceptibility to degradation, it is necessary to understand its basic structure and composition. The basic cellular structure of wood consists of many parallel, thick walled, elongated cells: tracheids in conifers, and fibres and vessels in hardwoods (Figure 1.1). This

accounts for approximately 90% of the mass of wood. These axial elements are interspersed with radially aligned ray tissue composed of thin walled parenchyma cells. Pits connect wood cells and, together with parenchyma tissues, are the primary routes of entry for wood decay microorganisms.

Wood cell walls consist largely of cellulose, hemicellulose and lignin and the way these are attacked by fungi and bacteria is a reflection of their chemistry and interrelationships. Cellulose is a linear homopolymer consisting of ß-D-glucose residues connected by (1-4) glycosidic linkages. Covalent bonding of glucose units accounts for the great tensile strength of cellulose. These cellulose chains are arranged in parallel to form microfibrils. Each microfibril consists of highly organised crystalline cellulose (60 – 70% in woody tissues) interspersed with non-crystalline cellulose. Microfibrils are laid down successively during tree growth to form laminae or layers. Microfibrils sit within a matrix of hemicellulose that probably serves a structural function by coating and binding them together. Hemicelluloses are much shorter polymers than cellulose, have side chains, and are comprised of various pentose and hexose sugars, the precise composition depending on tree species.

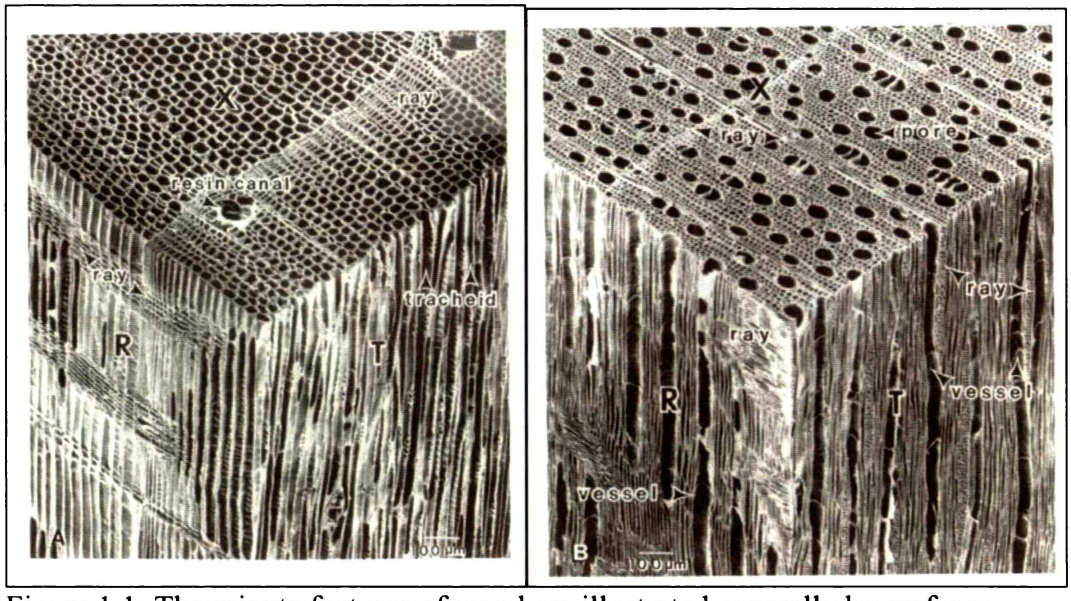


Figure 1.1: The minute features of wood are illustrated generally by surfaces through the cross or transverse (X) radial (R), and tangential (T) planes. Cubes of (A) eastern white pine (*Pinus strobus*) (a softwood) and (B) red maple (*Acer rubrum*) (a hardwood) indicate the planes and several important microscopic features typical of softwoods and hardwoods. Courtesy of Pofessor J.J. Morrell of Michigan State University, United States of America (originally by courtesy of Dr. W.A. Côté (1981), with permission of Springer-Verlag for (B)).

Lignin is believed to sit in spaces between the cellulose microfibrils and the hemicellulose, and amongst the hemicellulose matrix. Lignin forms covalent bonds with some hemicelluloses. Its primary function is probably provision of strength and cohesion to the wood cell wall as well as isolation of the cellulose and hemicellulose from the hydrolytic enzymes produced by wood decay fungi. Lignin is a large polyphenolic polymer formed by condensation of three types of propane unit. The type of propane units contained and the resultant polymer structure is essentially different for hardwoods and softwoods and affects susceptibility to decay, particularly soft rot, which preferentially attacks hardwood lignin.

Cellulose content is similar for most wood species (40 – 50%) whereas hemicelluloses are more abundant in hardwoods and lignin is more abundant in softwoods, with some exceptions occurring for tropical hardwoods which have high lignin content.

Wood cell walls of tracheids or fibres consist of a primary wall laid down during growth and maturation prior to successive deposition of the secondary cell wall comprised of 3 layers (S1, S2 and S3) (Figure 1.2). The central region of the primary cell wall that delineates adjoining cells is called the middle lamella. The S2 layer is sandwiched between the much thinner S1 that adjoins the middle lamella and the S3 that sits adjacent to the central cell lumen. The S2 layer accounts for the bulk of the cross-section and gives wood most of its strength, density and moisture properties. Cellulose concentration is highest in the secondary cell wall, hemicellulose concentration is highest in the S1 layer, and lignin concentration is highest in the middle lamella and primary wall (compound middle lamella).

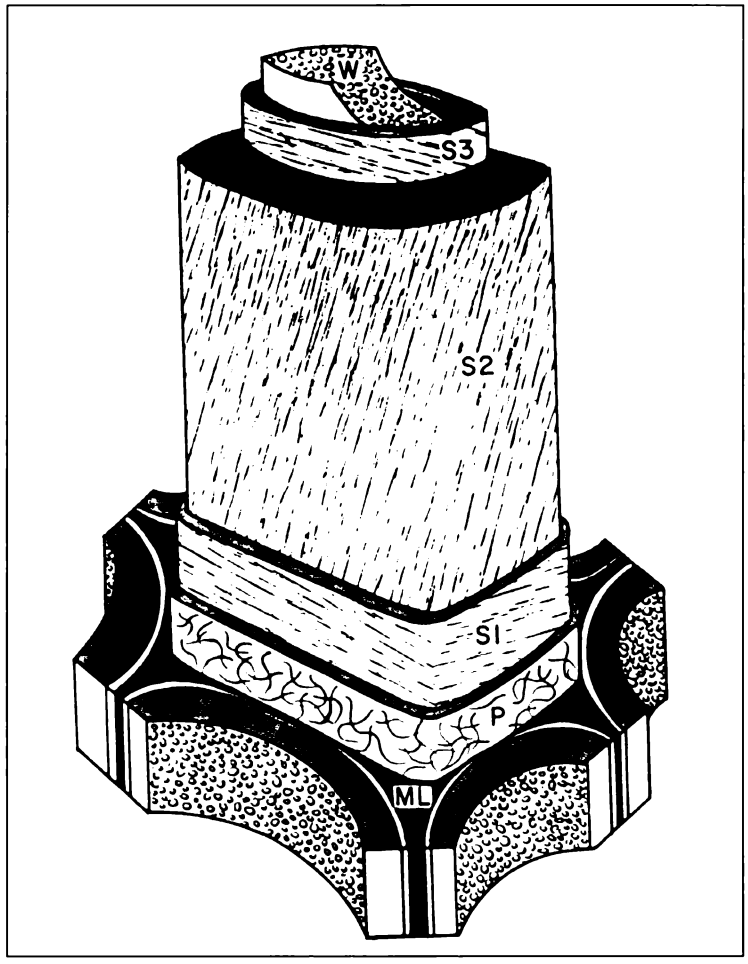


Figure 1.2: A model of several contiguous wood cells showing the organisation and microfibrillar orientation of the major cell-wall layers. The layers are identified from the middle lamella (ML) and outward as primary wall (P), the S1, S2 and S3 composing the secondary wall, and the warty (W) lining of the lumen surface. Courtesy of Professor J.J. Morrell (originally by courtesy of Dr. W.A. Côté (1981) with permission of the University of Washington Press).

Water is a prerequisite for wood decay and therefore an understanding of the relationship between wood and water is necessary. This is best understood by considering three organisational levels of the cell wall (Zabel and Morrell, 1992). The gross capillary zone consists of the lumenaee of cells and pit cavities and can contain both liquid water and water vapour. The transient capillary zone consisting of the non-crystalline cellulose zones of microfibrils contains bound water (hydrogen bonding). The transient capillary zone is that region which contracts as wood dries below the fibre saturation point when oven dried, and expands when remoistened above the fibre saturation point. The sorptive properties of wood are such that it can only become sufficiently wet for decay to occur if free liquid water is present.

Section 1.3

Wood decay

Man has been aware of the biodegradability of wood for thousands of years but Hartig (1874) was the first to describe the underlying cause. Hartig referred to wood biodegradation as wood decay and suggested a causal relationship between its occurrence in trees and the presence of fungi. Hartig (1878) went on to establish a link between particular fungal species and specific types of wood decay in different trees. A history of the various contributions made by different researchers to knowledge of wood decay has been dealt with elsewhere (Raynor and Boddy, 1988). Very few publications have attempted to bring together knowledge of the underlying microbiological and biochemical mechanisms of wood decay and the science of decay prevention (wood preservation) in a holistic manner (Zabel and Morrell, 1992; Eaton and Hale, 1993).

Wood decay involves digestion of wood by extracellular enzymes and oxidants produced by microorganisms, usually fungi that subsequently absorb the wood breakdown products for use as a food source. If the primary requirements for wood decay, water and oxygen are present, all wood species decay, although the rate of decay varies from a few months to hundreds of years (Raynor and Boddy, 1988). When wood decays it loses structural integrity making it unsuitable for the intended use, such as construction of buildings. Three approaches are used to prevent, or limit wood decay to acceptable levels: 1) keep wood dry (moisture below 18% of the oven dry weight), 2) select naturally durable wood species comprised largely of durable heartwood, 3) select wood species that are suitable for impregnation with wood preservatives.

Whilst the term wood decay is typically used to describe microbial destruction of wood, wood boring insects such as termites can also destroy wood in many of the situations where microbial degradation occurs and in other situations where it is prevented through inhibitory moisture conditions. Molluscs and crustacea (marine borers) also destroy wood submerged in marine situations. Whilst mastication is the primary step in wood destruction by insects, molluscs and crustaceans, microbial degradation of the masticated wood, for example by gut flora (bacteria and protozoa), typically follows. This is a reflection of the comparative rarity amongst living organisms of biodegradative mechanisms that breakdown the

lignin-cellulose complex that forms the basic structure of wood. Whilst wood destroying organisms are typically described within the context of "pests", they are very important recyclers in the carbon and nitrogen cycles of Earth's ecosystems. Apart from beneficial effects on the environment some wood degrading microorganims have biotechnological applications.

Section 1.3.1

Wood decay caused by fungi

White rot, brown rot and soft rot (Figure 1.3) are the 3 main types of wood decay currently recognised as being caused by fungi. The terms white rot and brown rot were first used by Hartig (1874) and were based on the colour of the decayed wood. It was assumed that the white colour was cellulose left behind by the white rot fungus and that the brown material left behind by brown rot fungi was lignin. Later research (Campbell, 1932; Scheffer, 1936) showed that white rot fungi breakdown all wood components (cellulose, hemicelluloses and lignin) whereas brown rot fungi only break down the cellulose and hemicelluloses, leaving behind a modified lignin. Hartig's grouping of decays into white and brown rot remains a very useful method of separation because it is based on two essentially different biochemical mechanisms (Section 1.4). The microscopic features of wood (micromorphology) attacked by white and brown rot, are also essentially different (Section 1.6).

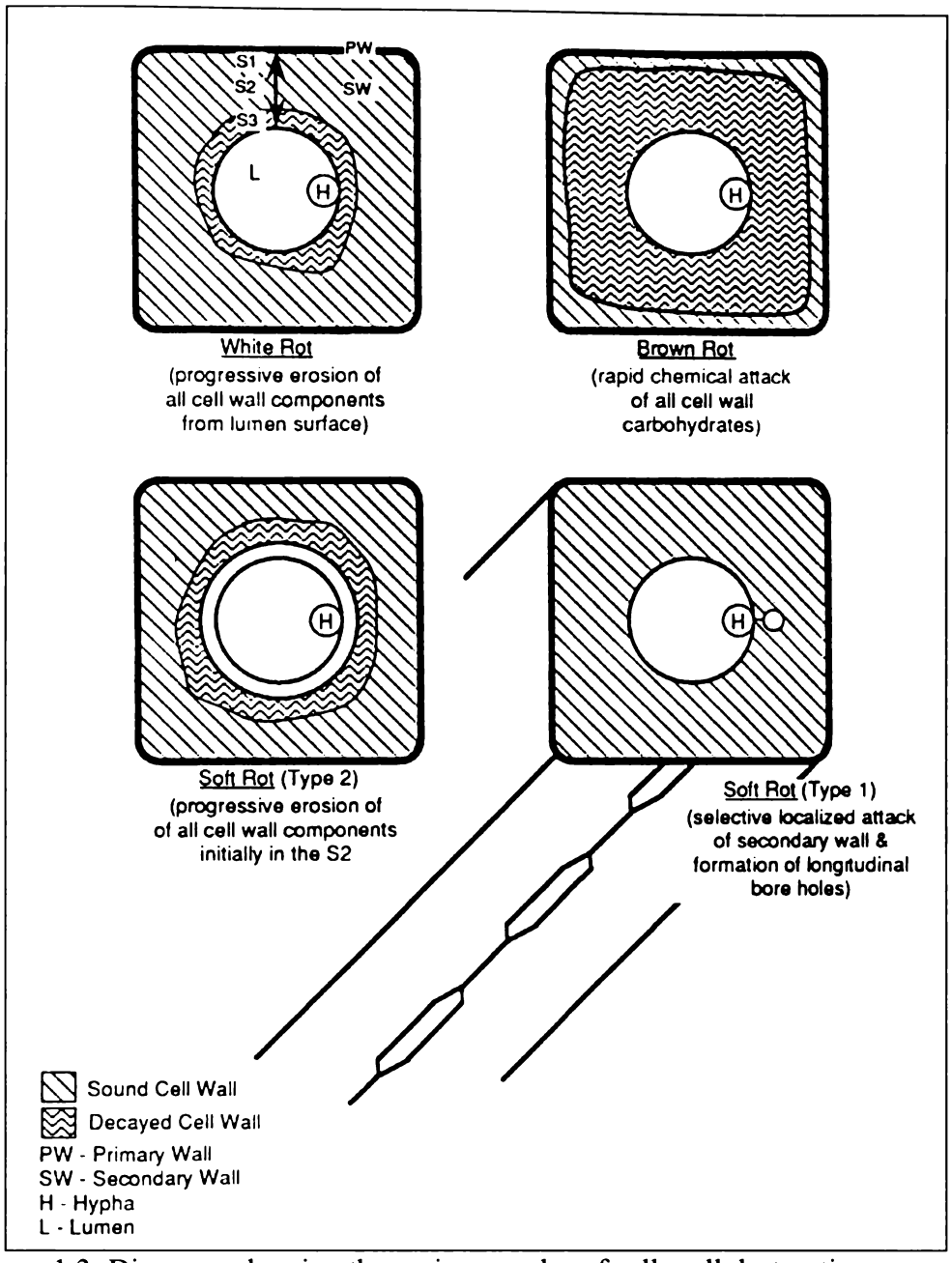


Figure 1.3: Diagrams showing the various modes of cell-wall destruction caused by white rot fungi, brown rot fungi, and two types of decay caused by soft rot fungi. Courtesy of Professor J.J. Morrell.

When Savory (1954) first used the term soft rot, it was described as unusual on the basis of possessing a soft-spongey texture, essentially different to the fibrous texture of white rot and the powdery texture of brown rot, and because of its unique micromorphology. The selective removal of the interior of the secondary cell wall, by formation of "bore holes" (soft rot cavities) parallel to the long axis of the wood cell wall was considered a unique feature of soft rot. Eaton and Hale (1993) refer to earlier reports describing decay symptoms now regarded as soft rot (Schacht, 1850 & 1863; Dippel, 1898, Bailey, 1913, Bailey and Vestal, 1937; Tamblyn, 1937, Barghoorn and Linder, 1944). Research has shown that many

ascomycetes and deuteromycetes can cause soft rot (Liese, 1955; Duncan, 1960; Nilsson, 1974; Seehan et al. 1974).

Wood breakdown products are metabolised rapidly by white rot so that the residual wood is largely unaltered (is not depolymerised) and remains insoluble in sodium hydroxide. In contrast, wood with brown rot undergoes extensive early depolymerisation of the structural carbohydrates before the brown rot fungus completes decay and metabolises the breakdown products, leaving behind a substantial residue of partially depolymerised carbohydrate of increased alkaline solubility. Since brown rot fungi only have a minor lignin degrading capability they are largely unaffected by the type of lignin in wood from different trees, unlike the white rot and soft rot fungi (Nilsson and Daniel, 1987).

Section 1.3.2

Wood decay caused by bacteria

The most compelling evidence for bacterial attack of wood has come from electron microscopy studies that have shown a causal relationship between unicellular bacteria and tunnels or troughs present in wood cell walls. Nilsson and Daniel (1988) have stated that only one wood degrading bacterium, tentatively identified as *Erwinia* sp. has been obtained in pure culture but subsequent studies do not appear to have proved beyond doubt that this isolate causes wood decay. The failure to link specific decay types with single species of bacteria is almost certainly due to the difficulty of reproducing environmental conditions known to favour bacterial wood decay, in a laboratory.

According to Daniel and Nilsson (1998) the potential role of actinomycetes to cause wood decay remains a controversial issue. Whilst reports of actinomycete isolations from wood are not uncommon (Calvante and Eaton, 1981; Safo-Saampah, 1985) evidence for their causing wood decay is lacking. Baeker *and King* (1981) assessed the potential of 20 *Streptomyces* species isolated from Baltic redwood (*P. sylvestris*) to decay lime wood (*Tilia vulgaris*) and Baltic redwood. Soft rot cavities were commonly observed in lime wood colonised by *S. xanthochromogenus*. Occassional soft rot cavities were observed in lime wood colonised by *S. thermovulgaris* and *S. tanashiensis*. No soft rot was observed in Baltic redwood. *S. xanthochromogenus* caused 12.5% weight loss in lime wood

after 35 weeks at 25^{0}C. Daniel and Nilsson (1998) reported that subsequent studies have not confirmed this work. Actinomycetes can degrade non-lignified tissues in wood such as parenchyma cells, phloem in hard- and softwoods and cellulosic G-layer in tension wood (Sutherland *at al.*, 1979; Eriksson *et al.*, 1990). Nilsson and Daniel (1990) described fine filaments within narrow branching channels in spruce driftwood (*Picea* sp.), Baltic redwood and *Alstonia scholaris* from unsterile soil, that were attributed to attack by actinomycetes. Isolates were tentatively identified as belonging to the genera *Streptomyces* and *Micromonospora*).

Eaton and Hale (1993) surmised that since actinomycetes are known to play an important role in decomposition and composting of lignin containing plant residues, then it might be expected that they also are capable of causing wood decay. However, as suggested by Cooke and Rayner (1984), the decomposition process typically associated with comminuted plant material, is characterised by high temperatures (40 – 60°C) and rapid rates of decomposition and is essentially different to the conditions that occur in wood in the great majority of exposure situations (Tuomela *et al.* 2000). Actinomycetes typically have rapid growth rates, short life cycles and many are thermotolerant, attributes that suit them to decomposition of comminuted plant residues but which probably do not confer a competitive advantage during wood decay.

Section 1.4

Wood decay biochemistry

This section deals with the salient differences between the biochemical processes of each of the major decay types and how these relate to known decay micromorphology. This is important because decay micromorphology, a subject of considerable importance to this study, is intimately linked to the biochemistry of decay.

Two approaches have dominated research into the biochemical basis of wood decay.

1. Determination of chemical changes in wood cell wall components during decay by known fungal species of different decay types.

2. Characterisation of hydrolytic and oxidative enzymes and other degradative products, produced by different decay types and elucidation of where they act at the fungal – wood interface.

Advances in understanding of wood decay chemistry since the early 1970's have been based on detailed studies of only a few fungi. *Phanaerochaete chrysosporium* and *Trametes versicolor* appear to be the most extensively studied white rot fungi and of the brown rot fungi *Postia placenta, Tyromyces pulustris* and *Gloephyllum trabeum* have been studied in the most detail. Of the soft rot fungi *Chaetomium globosum* features prominently in the literature (Eaton and Hale, 1993; Cameron and Aust, 2001).

Very little is known about the chemistry of wood decay caused by ascomycetes, litter decomposing basidiomycetes, soil fungi (mainly deuteromycetes), and bacteria (particularly unicellular bacteria). It is also important to realise that most decay-mechanism studies have been based on single enzyme tests on substrates (wood and its components: cellulose, hemicelluloses and lignin) in fermentation flasks, an artificial system unlikely to produce results that reflect wood decay chemistry that occurs in nature. Recent reviews suggest that wood decay biochemistry involves a complex series of interconnected reactions with numerous associated positive and negative feedback mechanisms (Leonowicz *et al.*, 1997; Goodell and Jellison, 1998; Highley *et al.*, 1998; Hofrichter *et al.*, 2002).

Enzymatic degradation of wood carbohydrate is caused primarily by hydrolytic enzymes whereas lignin is largely degraded by oxidative enzymes. However, cellulose oxidising enzymes have also been reported for soft rot (e.g. *C. globosum*) and white rot fungi (e.g. *P. chrysosporium*) (Zabel and Morrell, 1992; Ding-Zuolong *et al.*, 1997; Cameron and Aust, 2001). Non-enzymatic reactive oxygen species (ROS) and chelators are also important in the decay process. These enzymes and other degradative products act outside the fungal cell and must bind to or form a complex with the carbohydrate polymer or lignin macromolecule. The major cellulose degrading enzymes are endo-1,4-ß-glucanase, exo-1,4-ß-glucanase and, 1,4-ß-glucosidase. Hemicelluloses are structurally more complex than cellulose and require a correspondingly complex set of enzymes to debranch and remove side chain moieties as well as

depolymerise the polymer backbone. These enzymes include endo-1,4-ß-xylanase, 1,4-ß-xylosidase, their mannan equivalents, and several acetyl esterases. Lignin is degraded by a complex of enzymes including two hydrogen peroxide (H_2O_2) dependant peroxidases (lignin peroxidase (LIP) & manganese peroxidase (MnP)), H_2O_2 producing enzymes and other phenol oxidising enzymes including laccase (Kirk and Shimada, 1985; Kirk, 1988; Leonowicz *et al.*, 1997).

Section 1.4.1

Brown rot

The ability of brown rot fungi to cause decay large distances from their hyphae and without producing lysis zones is a reflection of the important role of diffusible degradative agents much smaller than known fungal enzymes. Evidence in general suggests that the dimensional constraints of crystalline cellulose prevent entry of enzymes by diffusion, at least in the early stages of decay when the wood cell wall pore size remains unaltered. Cobb (1982), showed that cellulose degradation occurred when *Postia placenta* was isolated by an ultrafiltration membrane that excluded molecules with a molecular weight greater than 1000 (fungal endoglucanases, some of the smaller cellulase enzymes have molecular weights ranging from 30,000 to 35,000).

Several degradative models that utilise low molecular weight agents have been proposed. One of the earliest, called the Fenton reagent, involves hydrogen peroxide (H_2O_2) and iron (II) ions (Fe^{2+}), and was reported to depolymerise cellulose microfibrils (Koenigs, 1974; in Eaton and Hale, 1993). Schmidt *et al.* (1981) proposed a model involving Fe^{2+}, H_2O_2, oxalic acid and production of hydroxyl radicals. Theories to account for the sources of these low molecular weight agents and associated cyclic redox reactions are still evolving as new research is conducted. Low molecular weight degradative agents are not restricted to brown rot fungi. Hydrogen peroxide producing enzymes and peroxide dependant enzymes (e.g. lignin-peroxidase) linked to oxidation, hydroxylation and cleavage reactions of lignin have also been isolated from the white rot fungus *Phanerochaete chrysosporium* (Kirk, 1988). Other reactive oxygen species (ROS) have also been implicated in brown rot decay and it is believed that chelators play an important role in their generation and concentration. For example Goodell and Jellison (1998) discussed the possible role of phenolate chelators.

Shimada (1991) questioned the importance of the Fe^{2+}/H_2O_2 oxidative system, pointing out that the low pH (1.3) from oxalic acid production by *Tyromyces palustris* would cause acid hydrolysis of cellulose and that such a pH would also inhibit the Fe^{2+}/H_2O_2 oxidative system. Clausen and Green (2003) and Green and Clausen (2003) have implicated apparent over-production of oxalic acid in degradation of pine treated with non-arsenical wood preservatives (e.g. copper citrate) by copper tolerant brown rot fungi. Oxalic acid has also been implicated as a chelator of reactive Mn^{3+} produced by manganese peroxidase in white rot fungi (Hofrichter *et al.*, 2002) suggesting oxalic acid is produced by both white and brown fungi but that its respective role is different for the two decay types. However, during studies with *Postia placenta*, Illman and Highley (1989) concluded that, due to the rapidity of Fe^{2+}/H_2O_2 oxidative degradation compared to acid hydrolysis of cellulose, it is likely that cellulose-metal complexes and oxidation reactions by oxygen radicals are important. Low molecular weight metal chelators, referred to as siderophores by Jellison *et al.* (1991), have been suggested to play an important role in cellulose oxidation, including systems that lack H_2O_2 and other oxidants. Cations of iron, manganese and calcium have been shown to accumulate during colonisation and decay of wood by brown rot fungi (Jellison *et al.*, 1997).

Hemicellulose is the first component of the wood cell wall removed by brown rot fungi. Cellulose protection from oxidative agents afforded by encrusting lignin is probably reduced by its demethoxylation (Zabel and Morrell, 1992). Similarly, removal of the hemicellulose matrix surrounding cellulose microfibrils may also increase access to cellulose by degradative agents. It is unclear if hydrolytic enzymes are able to penetrate brown rotted wood. Brown rot fungi possess endo-glucanases but lack exoglucanases. Only the Coniophoraceae have been shown to degrade cystalline cellulose under laboratory conditions but *Postia placenta* (Polyporaceae) has been shown to degrade cystalline cellulose in the presence of hemicellulose. Hemicellulose may be an important substrate for H_2O_2 production (Zabel and Morrell, 1992).

At completion of the decay process, brown rot fungi leave behind a demethoxylated lignin matrix. Since brown rot fungi only have a minor lignin degrading capability they are largely unaffected by the type of lignin in wood

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from different trees, unlike the white rot and soft rot fungi (Nilsson and Daniel, 1987). Unlike white rot fungi, brown rot fungi exhibit much less variation in the sequential attack of the wood cell wall, and the great majority of species studied adhere to the sequence of events described above.

Section 1.4.2

White rot

White rot fungi are amongst the very few microorganisms able to depolymerise and metabolise lignin. All cell wall components are ultimately removed if ideal conditions prevail except for a residue of minerals (95 – 97% wood weight-loss). Increasing evidence suggests that white rot fungi are a heterogenous group with diverse degradative mechanisms. Liese (1970) made the distinction between *simultaneous* white rot fungi (e.g. *Trametes versicolor)* that utilise all cell wall components essentially uniformly, and white rot fungi (now usually termed *preferential* or *sequential* white rot fungi) which initially utilise lignin more rapidly than cellulose (e.g. *Phellinus pini*). *Ganoderma applanatum* is an example of one of the few white rot fungi known to remove carbohydrate more rapidly than lignin (Zabel and Morrell, 1992). The terms are misleading because some species can cause both types together under laboratory culture conditions and culture conditions such as nitrogen availability can influence the type of decay caused (Eaton and Hale, 1993).

Hemicellulose, cellulose and lignin remains essentially unaltered in undecayed portions of wood, which indicates that degradative agents of white rot fungi act primarily on exposed cell-wall surfaces. Enzymes slowly erode cell wall surfaces forming pits, holes and grooves, in contrast to brown rot fungi that do not form such lysis zones. However recent ultrastructural studies of white rotted wood have shown that wood cell wall modification can occur at greater distances from hyphae than the limits of lysis zones (section 1.6.1.1).

Detailed studies of carbohydrate degrading enzymes have been conducted for only a few white rot fungi. *P. chrysosporium* is the most extensively studied and is know to possess all three types of hydrolytic enzyme for degradation of cellulose and cellulosic residues (endo-1,4-ß-glucanase, exo-1,4-ß-glucanase and, 1,4-ß-glucosidase).

A variety of enzymes are believed to be important for lignin breakdown to CO_2 (Kirk and Shimada, 1985; Kirk and Farrell, 1987; Shimada and Higuchi, 1991; in Eaton and Hale, 1993; Highley *et al.*, 1998; Leonowicz *et al.*, 1997; Hofrichter *et al.*, 2002). These include lignin-peroxidase, laccase, manganese-dependant peroxidases, demethoxylases, H_2O_2-generating enzymes and the monomer degrading enzymes, cellobiose dehydrogenase, vanillic acid hydroxylase and trihydroxybenzene dioxygenase. Unlike brown rot fungi, white rot fungi and some soft rot fungi produce phenol oxidase enzymes that are necessary for lignin breakdown beyond demethoxylation. The glcosylated heme protein manganese peroxidase (MnP) is the most common lignin-modifiying peroxidase produced by the majority of white rot basidiomycetes. Manganese (II) ions (Mn^{2+}) which are usually present in wood or associated with soil clay micelles and humic molecules, is oxidised to the more reactive Mn^{3+} which is then chelated, for example by oxalic acid (Hofrichter *et al.*, 2002). Chelated Mn^{3+} acts as low-molecular weight diffusible redox-mediator that attacks phenolic lignin structures resulting in formation of instable free radicals that tend to disintegrate spontaneously (Leonowicz *et al.*, 1997; Hofrichter *et al.*, 2002).

Unlike cellulose and hemicellulose breakdown that occurs during the primary growth phase, biochemical studies of white rot fungi have suggested that lignin breakdown is initiated when growth slows down and enters a stationary phase. When the fungal mycelium enters a secondary metabolic phase the lignin-degrading system is released. Unlike polysaccharide breakdown, lignin breakdown is not induced by the presence of the substrate. Lignin-breakdown only occurs in the presence of carbohydrate that provides an energy source which lignin is unable to provide. However these observations may be partly related to the laboratory culture conditions employed since they do not entirely fit within the concept of a gradual, local erosion of the wood cell wall which must involve iterative degradation of all cell wall components.

Section 1.4.3

Soft rot

Soft rot fungi break down all wood carbohydrates but are considered to have inferior lignin degrading capabilities compared to white rot fungi. Soft rot and white rot fungi degrade cellulose and hemicellulose at similar rates. Currently, 3

major classes of cellulase enzymes (endo- and exocellulases and ß-glucosidase) are accepted, although a variety of isozymes and cellulases that act in different stereospecific ways are known to be important in many soft rot fungi (Eaton and Hale, 1993). In common with white and brown rot fungi, the soft rot fungus *C. globossum* produces cellobiose dehydrogenase (Cameron and Aust, 2001). The soft rot fungus *T. reesei* has been shown to produce an oxidative cellulose-degrading enzyme system (Vaheri, 1982). Some of the earliest detailed studies of fungal cellulase systems were conducted on *Trichoderma viride,* one of a relatively small group of fungi tested that is able to degrade cellulose in the native highly crystalline form (cellulolytic fungi). *T. viride* was shown to possess a cellulase complex of endo- and exocellulases thought to act in a multi-step synergistic process to effect degradation of crystalline cellulose (Reese *et al.* 1950, in Eaton and Hale, 1993). However in laboratory studies *T. viride* has variable soft rot capabilities that are believed to reflect both the strain used and the experimental conditions employed. Greeves and Savory (1965) showed no cavities (type 1 soft rot) in birch and Nilsson (1973), in Eaton and Hale (1993), only found erosion decay (type 2 soft rot). T. viride has produced soft rot cavities in birch in other studies (Liese and Pechman, 1959; Levy, 1969) but Levy found that only 1 out of 8 strains produced cavities. Clearly, the possession of a cellulase complex capable of degrading crystalline cellulose is not a reliable indication of soft rot capability under laboratory conditions.

It is generally accepted that cellulase enzymes produced by soft rot fungi are too large to diffuse into wood cell walls and thus both erosion and cavity formation are restricted to surfaces (Bailey *et al.*, 1972).

Savory and Pinion (1958), in Eaton and Hale, reported that *Chaetomium globosum* caused 92% weight loss of *Fagus sylvatica*, and since this tree species contains 23% lignin this suggests that substantial lignin breakdown occurred. However the literature in general shows that whilst several soft rot fungi can modify lignin (demethoxylation) and/or attack the phenol ring and its side chain moieties, soft rot fungi in general have a greatly inferior lignin degrading capability compared to white rots.

Levi and Preston (1965) showed that the amount of lignin present in wood affects the overall wood decay rate and Butcher and Nilsson (1982) showed that the type of lignin was important. Wood cell walls containing a high concentration of lignin with the propane unit syringyl (e.g. *F. sylvatica*) is more susceptible to soft rot than those containing a high concentration of guaiacyl lignin (e.g. *Pinus radiata*). Ryan and Drysdale (1988) postulated that improved resistance to soft rot decay of hardwood containing a high lignin concentration such as *Alstonia scholaris* compared to *Betula alba*, a hardwood containing less lignin, was of particular significance when the wood was treated with a CCA preservative. Earlier observations had shown that many hardwoods had poor microdistribution of copper in the S2 layer, and a concentration of CCA on the lumen face of the S3 layer. It was thought that the greater protection of cellulose microfibrils from encrusting lignin that occurs in the S2 layer of high lignin containing hardwoods compensated for the low copper concentration. Singh *et al.* (1992) referred to the high resistance to soft rot of hardwoods that contained of high proportion of guaiacyl lignin (*Alstonia* spp.), a feature normally associated with softwoods. Presumably the combined effects of cellulose protection from high lignin, in combination with a high concentration of guaiacyl lignin resulted in a particularly high soft rot decay resistance.

Section 1.5

Microscopy for investigation of wood decay

Some features of decayed wood and the causitive microorganisms are discernible to the unaided human eye and are termed macroscopic features. These include changes of wood colour and texture and presence of associated fungal growths (e.g. fruiting bodies, mycelial strands and pigmentation produced by pseudosclerotial plates and other fungal tissues). However, these changes are not reliable diagnostic features if used in isolation and provide very limited information concerning the nature of the decay process. This is because most evidence of wood decay is invisible to the naked eye, partly because wood decay microorganisms and the surrounding wood cells are microscopic but also because wood is an opaque solid material that hides internal decay.

The three major wood cell wall constituents (cellulose, hemicelluose and lignin) can be identified using various stains specific to one of them (Zabel and Morrell,

1992). For example potassium permanganate and bromine fix preferentially to lignin and Periodic acid-thiocarbohydrazide silver proteinate (PAT Ag) fixes preferentially to hemicellulose. The position of cellulose in the wood cell wall relative to hemicellulose and lignin can be determined by a process of elimination. Therefore specific staining techniques can be used to study where the three components are located in the wood cell wall and in what order they are removed after exposure to specific decay fungi or their enzymes.

Specific stains are also available for fungi and bacteria. For example the lectin wheat germ agglutinin (WGA) is specific for fungal chitin, a cell wall constituent of most higher fungi (Morrell *et al.*, 1985). WGA can be visualised in wood by coupling to a fluorescent or metal compound and observing it under an appropriately equipped microscope. However the massive improvement in resolution provided by the development of electron microscopy (EM) in the late 1940's and 50's tended to steer most researchers of wood decay processes away from LM towards EM.

Since the 1950's electron microscopy has provided useful information about changes in wood structure during the decay process and the location of fungi and bacteria in wood. However, staining techniques that permitted determination of the biochemical nature of changes at specific sites in the wood were not available until the development of immunocytological techniques. Development of antibodies specific to fungal enzymes coupled with specific staining techniques, or colloidal gold-coupled antibody techniques, have enabled detection of enzyme activity within specific areas of the fungus and the wood cell wall (Blanchette and Abad, 1988; Daniel *et al.*, 1989; Srebotnik and Messner, 1991). For example these techniques have identified and located lignin peroxidase in the fungal cell and have indicated that this enzyme is capable of moving into decayed wood, but not sound wood.

Section 1.5.1

Light microscopy

The maximum resolving power of a light microscope, defined as the minimum distance by which, two points must be separated in order that they appear as two distinct points within the image, is 0.2 micrometers (μm) ($1x10^{-6}$m) (Roberts,

1982). This is achieved by placing a series of lenses of appropriate curvature and positioning between the eye and an illumenated thin specimen. The resolution limit of a light microscope is defined by the wavelength of light, which prevents resolution above a magnification of X1500. In addition to the magnification step, production of magnified images often requires several specimen pretreatment steps, the objective of which is to improve contrast between the decay microorganism, their degradative agents, and the various components of the decayed and sound wood cell wall.

Light microscopy (LM) allows rapid viewing of many cells with minimal specimen preparation. To a limited degree LM can give a three-dimensional perspective by viewing features a short distance either side of the focal plane. Whilst these features are out of focus and therefore not sharp enough for photography, useful information is acquired by the operator concerning the spatial relationship between the decay microorganism and the wood cell wall. LM with polarised light is one of the few techniques that can measure loss of wood cell wall birefringence, a diagnostic feature of wood with brown rot (Wilcox, 1993, in Anagnost, 1998).

Disadvantages of LM are primarily associated with its magnification limits. Some hyphal features and their effects on wood cell walls are at the resolving limit of the light microscope. In order to obtain resolution at the maximum magnification of LM (approximately X1500) time consuming specimen preparation techniques are typically required (fixation, staining, embedding and microtome sectioning). Superior resolution is achievable using transmission electron microscopy (TEM) (Singh and Butcher, 1991) and confocal laser scanning microscopy (CLSM) (Singh *et al.* 1997) and therefore the value of time consuming specimen preparation for LM is questionable. LM is especially suited to research that involves examination of many sections cut using a hand-held blade (1000's). The thickness of such sections (20 –30 μm) prevents maximum resolution but is sufficient for distinguishing many diagnostic wood decay features (Anagnost, 1998). An integration of microscopic techniques where a large number of specimens are screened using LM and a small sub-sample are then used for TEM and CLSM, is perhaps the best approach for exploitation of the advantages of each (Singh *et al.*, 1994a).

Section 1.5.2

Transmission electron microscopy

Whereas light microscopy can resolve features up to a few micrometers with ease and has a maximum resolution of 0.2 μm, resolution with TEM is measured in Ångstroms ($1x10^{-10}$m) and can achieve magnifications of up to X1,000,000. Unlike light microscopy in which light absorption by the specimen decreases image brightness, thereby limiting resolving power, deflection of the electron beam employed in TEM plays an important role in image contrast. An electron beam produced by an incandescent tungsten filament under vacuum, is accelerated by high voltage and focused using electromagetic "lenses" onto a small area of the specimen (3 to 5 μm). After passing through the specimen, electrons are focussed using various intermediate and projective lenses, onto a fluorescent screen or photographic plate. Heavier materials are more electron-dense and can be used as stains (e.g. potassium permananate ($KMnO_4$)), uranyl acetate and lead citrate) to improve contrast (Zabel and Morrell, 1993). For example, $KMnO_4$ fixes preferentially to wood cell wall lignin which then appears as dark areas on the image thereby increasing contrast with surrounding lighter areas rich in carbohydrate (Singh *et al.*, 1994). The major disadvantages of TEM are the time consuming specimen preparation involving dehydration, fixation and embedding, and the necessity to examine specimens under a vacuum.

Section 1.5.3

Scanning electron microscopy (SEM)

In SEM two condenser lenses are used to focus an electron beam onto an area of small diameter (10-20 nanometers (nm)). Two pairs of scanning coils in the second condenser lens deflect the focussed electron beam so it can scan a square area of the specimen surface. Wood specimens are typically coated with either carbon or a heavy metal (e.g. gold-palladium). As the beam penetrates the specimen, secondary electrons are emitted from the surface, collected and counted by an electron detector and converted to light. Light is conducted through a photomultiplier tube and visualised with a cathode-ray tube. Magnifications between X50 and X20,000 can be obtained. One of the most useful capabilities of SEM is examination of surfaces at great magnifications with superior depth of field giving excellent topographical imaging. An extension of SEM is energy dispersive X-ray analysis (EDXA). The electron beam generates X-rays

characteristic of the various elements present in the specimen that can be collected and identified. It is therefore a powerful technique for determination of the relative concentrations of elements present, included some wood preservative active ingredients such as copper.

Section 1.5.4

Confocal laser scanning microscopy (CLSM)

The confocal microscope was patented in 1961 (Lichtman, 1994) but has only seen widespread application since the early 1980's. CLSM has increasingly seen application in medical science (Boon *et al.*, 1994; Mills *et al.*, 1994), biology (Shotton, 1989), fungal cytology (Knight *et al.*, 1993; Kwon *et al.*, 1993), and wood structure (Knebel and Schepf, 1991; Donaldson and Lausberg, 1998). However, at the commencement of this study CLSM had not yet been applied to wood biodegradation studies. To apply CLSM to examination of wood requires use of epifluorescent techniques. One of likely challenges of this procedure is selection of fluorescent stains that enable differentiation of decay microorganisms from wood cell walls which autofluoresce over a wide wavelength band when illumenaeted by a laser light source. Fluorescent lectins specific to chitin in fungal cell walls have been employed for examining fungi in wood using LM (Morrell *et al.*, 1985; Krahmer *et al.*, 1986) but little information is available for fluorescent staining of bacteria in wood. In this study the feasibility of combining fluorescent stains specific to fungi and bacteria with CLSM was investigated.

A potential advantage of CLSM over other microscopy techniques can be appreciated by considering a comment of Zabel and Morrell 1992) that referred to the difficulty of examining small multidirectional decay features inside an opaque material. Many decay features move through wood in a random fashion and it is therefore difficult to gain a three dimensional perspective of their orientation when thin sections are examined using TEM. Whilst SEM is excellent for examining surface topography of substrates with complex surface relief like wood, it cannot produce images of internal decay features. Images of multiple sequential sections through the depth of relatively thick sections (5 – 8 μm) produced by CLSM, can be built up into an image that has 3D qualities using mathematical interpolation software. Whilst the epifluorescent techniques employed by CLSM are more complicated and time consuming than simple LM

staining techniques such as use of aniline blue, this inconvenience may be offset by it's multiple non-destructive sectioning capability. Unlike LM that requires examination of many sections within different planes to gain an appreciation of the 3D interconnectedness of decay features like tunnels, the penetrative nature of laser light produced by CLSM coupled with the unique optical arrangement of the pin-hole aperture (Donaldson and Lausberg, 1998), enables production of a sequential "stack" of images without cutting the sample.

Section 1.6

Wood decay micromorphology

Entry into wood by saprophytic microorganisms is primarily via cuts in cell walls of wood rays and axial cells (e.g. trachieds, fibres and vessels). Contact with wood can be effected by windborne spores or mycelial fragments and via contact with substrates that already harbour microorganisms such as soil, water and woody detritus. Microfauna and insects may act as vectors for some wood decay microorganisms (Zabel and Morrell, 1992). Initial colonisation is sustained by food reserves of the inoculum and available carbon sources present in the lumenae of wood cells. Reports of fungi in the monokaryon condition suggest single basidiospores (haploid) are able to initiate decay (Raynor and Boddy, 1988).

Section 1.6.1

White rot micromorphology

In the early stages of decay, hyphae of white rot fungi are more abundant in the wood cell lumenae than brown rot fungi. Later in decay many hyphae autolyse and are generally less abundant. In softwoods, early colonisation occurs in the ray parenchyma and resin canals and in hardwoods, ray parenchyma and vessels are first colonised. Ray parenchyma cells are typically decayed early on in the decay process in hardwoods but this is not always the case (Blanchette, 1994). Initially, passage of hyphae between cells occurs via the pits that can become enlarged due to erosion, but later hyphae pass between cells via bore holes.

Section 1.6.1.1

Simultaneous white rot micromorphology

Simultaneous or non-selective white rot, studied in most detail for *Trametes versicolor* (Eaton and Hale, 1993), is characterised by erosion of the cell wall

adjacent to hyphae growing in the lumen. Typically, erosion corresponds closely with the location of hyphae that sit on or close to the cell wall surface, more or less in the centre of a U-shaped trough (Blanchette, 1995a). A more expansive cell wall thinning is also widely reported. Liese (1970a), in Eaton and Hale (1993), suggested this was the result of coalescence of neighbouring lysis zones caused by individual hyphae. However TEM micrographs showing cell wall thinning of tracheid walls of *Liquidambar styraciflua* (Cowling, 1961) and *Tsuga heterophylla* (Highley and Murmanis, 1984) by *T. versicolor* and erosion of pine cell walls by *T. hirsuta* (Messner and Stachelberger, 1984) showed that wood cell wall thinning occurred evenly over the entire surface area. In some cases, where this even thinning occurred, the hyphae appeared to be less closely associated with the cell wall than was the case for U-shaped erosion troughs. Erosion of fibres of *Phoenix canariensis* by *Wolfiporia cocos* (Adaskaveg *et al.* 1991) appeared to be intermediate between the U-shaped erosion troughs described by Blanchette (1995a) and the even thinning described by Cowling (1961) and Highley (1987). Here the epicenter of deepest erosion was clearly linked to the position of individual hyphae in the approximate centre of the fibre lumen but the erosion trough was much wider, covering approximately half the lumen width.

The middle lamella is usually the last part of wood cell wall to be degraded, and although the corner middle lamellae often persist, ultimately the whole cell wall is eroded away. During the decay process hyphae traversing pits often cause significant erosion of the surrounding cell wall, typically causing 2 tapering angular cavities either side of the pit. Bore holes widen during decay from an initial diameter of 0.5 to 2 – 3 μm (Eaton and Hale, 1993). Anagnost (1998) reported round and oval bore holes of diameters between 4 and 8 μm depending on species of both fungus and wood (hardwood and softwood). Anagnost also reported large openings up to 22 μm diameter that resulted from erosion of intertracheid pit borders. Bore holes, cell wall thinning and enlargement of pits are readily observed with a light microscope (Eaton and Hale, 1993). Erosion troughs can be seen with the aid of polarised light using LM but are best observed using SEM (Morrell and Zobel, 1992).

According to Eaton and Hale (1993) and Ruel *et al.* (1981) and Ruel and Barnoud (1984), with simultaneous white rot, activity of fungal enzymes is restricted to

regions close to the hyphae. This is supported by several TEM micrographs showing the close contact between hyphae and the wood cell wall (Highley and Murmanis, 1984; Foisner *et al.*, 1985). However TEM micrographs of erosion of fibres of *Phoenix canariensis* by *Wolfiporia cocos* (Adaskaveg *et al.* 1991) indicated that hyphae are not always positioned close to the eroded cell wall but appear in the central region of the lumen.

It is possible that enzymes arrive at the wood cell surface via extracellular membranous structures (EMS) or extracellular slime (Foisner *et al.*, 1984; Foisner *et al.*, 1985) or fungal sheath (Nicole *et al.*, 1995). These extracellular materials are believed to act as a matrix for channelling and concentrating both extracellular fungal enzymes and wood cell wall breakdown products. Immunolocalisation studies of wood degrading enzymes have provided evidence that fungal sheaths are directly involved in wood degradation (Green *et al.*, 1992; Nicole *et al.*, 1995).

Whilst there are a number of TEM micrographs available that illustrate that hyphae and associated extracellular slime and sheath are closely adpressed to the wood cell wall, this may to some extent be misleading. This is because it is likely that during the dehydration step of specimen preparation these structures become more closely adpressed to the wood cell wall during shrinkage. During the decay process the hyphae and associated extracellular material may be further away from the cell wall.

Section 1.6.1.2

Preferential white rot micromorphology (and combined preferential and simultaneous WR)

Ultrastructural changes during wood decay have been more extensively studied for the preferential type of white rot that cause selective delignification because of their potential value for lignin modification and removal in biopulping and bleaching processes. Delignification of the wood cell wall begins at the lumen cell wall inner surface and progresses through the S3, S2 and S1 layers (Eaton and Hale, 1993). Eventually the compound middle lamellae is delignified and because of its high lignin content it often disintegrates resulting in defibrillation (separation) of the wood cells. The extent of defibrillation depends on the fungal species (Otjen and Blanchette, 1987). Advanced delignification is apparent from

ultrastructural changes such as defibrillation and by determining the relative electron density of the cell wall during the decay process using $KMnO_4$ stain (Yoshizawa *et al*, 1990). Early delignification has been studied using uranyl acetate stain. Whilst the mechanism is not fully understood uranyl acetate appears to react with modified polysacharide during lignin removal. As decay progresses the zone stained by electron dense uranyl acetate progresses deeper into the cell wall and becomes more intense (Blanchette, 1995a).

The extent of delignification varies greatly between species of white rot fungus such that it is possible to find white rotted wood with almost any ratio of lignin:cellulose:hemicellulose, and this is a function of both fungal species and wood species (Blanchette, 1995a). The most obvious consequence of this for LM studies is the extent of defibrillation seen and for TEM the rate and extent of lignin removal can be determined using stains such as $KMnO_4$ and uranyl acetate. *Phellinus pini*, *Phellinus nigrolimitus* and *Inonotus dryophyllus* are examples of fungi (white pocket rots) which remove lignin without appreciable removal of cellulose (Blanchette, 1980; Otjen and Blanchette, 1986).

Ganoderma applanatum, *G. tsugae* and *Heterobasidion annosum* are examples of fungi that can cause both simultaneous and preferential white rot in the same substrate (Blanchette 1995). TEM studies of Schmidt *et al.* (1997) showed very small erosion troughs (0.3 – 0.5 μm accross) produced by the white pocket rot *Physisporinus vitreus* in *Pinus sylvestris* tracheids. Hyphae adjacent to these erosion troughs were 1.5 – 2 μm diameter. Schmidt referred to similar erosion troughs cited by Eriksson *et al.* (1980) caused by *Phellinus pini* and *Phlebia tremellosa*. This pattern of erosion appears essentially different to both U-shaped erosion and wall thinning and it is difficult to account for in terms of the position of the trough relative to the hyphae. Presumably this type of erosion would be seen as a stripy pattern in longitudinal section using LM. In heavily degraded wood the S3 and S2 layers were completely removed as occurs for typical simultaneous white rot, but the S1 and middle lamella was delignified. This suggests that expression of either simultaneous or preferential white rot by *P. vitreus* was dependant on the wood cell wall layer.

One of the most spectacular examples of wood species affecting ultrastructure of preferentially white rotted wood occurs when species with high syringyl lignin containing parenchyma and fibres are completely removed and only vessels high in resistant guaiacyl lignin remains (Blanchette *et al.*, 1988). Whilst syringyl lignin is preferentially degraded by white rot fungi, many species (*Heterobasidion annosum*, *Armillaria ostoyae*, *Phellinus pini*, *Phellinus weirii* etc.) are associated with decay of soft woods that do not contain syringyl lignin (Blanchette, 1995a). One of the most decay resistant types of wood is reaction wood that contains abnormally thick wood cell walls high in lignin. However, Blanchette *et al.* (1994) reported that both a simultaneous white rot species (*T. versicolor*) and preferential types (*Phellinus pini*, *Phlebia tremellosa*) caused appreciable lignin loss.

Section 1.6.1.3

White rot fungi causing tunnelling and cavitation micromorphology

Several white rot fungi produce tunnelling and cavitation micromorphology. Tunnel and cavity size and form and associated hyphal branching patterns vary considerably and whilst some are similar to SR micromorphology others appear unique. This micromorphology is of special significance to those decay types that do not fit neatly within the decay types described by current decay classification nomenclature and is therefore introduced in Section 1.7 and discussed in detail in chapter 4.

Section 1.6.2

Brown rot micromorphology

Hypha are relatively sparse in cell lumenae during early decay but become more prevalent as decay progresses and splits and voids that develop due to shrinkage become filled with mycelium (Blanchette, 1995a). Wilcox (1973) stated that single hyphae are present in nearly every cell during early colonisation. Using TM and SEM Wilcox (1978) reported that the earliest diagnostic feature to appear in brown rotted wood was hyphae in the earlywood lumenae. Hyphae gained access to the axial cells (fibres and tracheids) via the radial wood pathways such as parenchyma. Pit tori were traversed and degraded by hyphae (Zabel and Morrell, 1992; Eaton and Hale, 1993).

In wood with early brown rot, typically the only significant wood cell wall lysis occurred close to the bore hole hyphae. TEM studies have suggested that bore holes are formed primarily by release of degradative agents from the hyphal tip (unpublished data cited by Zabel and Morrell, 1992). Bore holes are typically larger than the penetrating hyphae (Blanchette, 1995a). Wilcox (1978) reported increases in hyphal and bore hole diameter as decay progressed for brown rot in Douglas-fir (*Pseudotsuga menziessi*) and white fir (*Abies concolor*) caused by *Gloeophyllum trabeum* and *Postia placenta*. Wilcox (1978) concluded that the size of bore holes and hyphae could be used as a diagnostic feature of the extent of brown rot attack in wood. Messner and Stachelberger (1984) produced TEM micrographs of brown rot in pine caused by *Fomitopsis pinicola* and observed one to two hyphae in earlywood tracheids at a wood weight loss of 2.5% but bore holes were rare even at 15% weight loss.

Kuo *et al.* (1988) examined the progression of the early stages of brown rot in cottonwood (*Populus deltoides*) caused by *G. trabeum* and found that presence of hyphae was the only feature which distinguished sound wood from wood with a weight loss of 2.7%. At a weight loss of 4.3% loss of wood cell wall birefringence had occurred, and small bore holes were present. Wilcox (1993) reported that loss of birefringence was the first diagnostic feature of attack in Douglas-fir and white fir decayed by *G. trabeum* and *Postia placenta*. *G. trabeum* produced a significant amount of weight loss prior to loss of birefringence which was first seen in Douglas-fir at 7% weight loss and 15% for white fir. *P. placenta* produced loss of birefringence in the earliest weight losses recorded of 3% for Douglas-fir and 2% in white fir. These differences in onset of birefringence loss suggested different degradative mechanisms for the two fungi. The loss of birefringence at only 4.3% weight loss, reported for *G. trabeum* in cottonwood (Kuo *et al.*, 1988) was considered not to discount this suggestion since the result was for a hardwood. Khramer (1982) used a fluorescent staining technique based on acridine orange to detect incipient brown rot in southern yellow pine (*Pinus* spp.) with weight losses in excess of 3%.

Attack of the cell wall is predominantly initiated by hyphae growing in the lumenae in contact with or close to the lumen wall (Wilcox, 1978). Hyphae are often attached by an extracellular complex multi-layered hyphal sheath, although

this is often less prominent in advanced decay (Wilcox, 1978). Typically the S3 remains undegraded until late in the decay process compared to the S2 which is heavily degraded much earlier. Whilst erosion troughs are not a prominent feature, a shallow indentation in the S3 has been observed directly underneath hyphae for *T. palustris* in spruce tracheids (Kim *et al.* 1992). Kim *et al.* (1992) also cited similar depressions caused by *Postia placenta.* Intense degradation of localised areas of the middle lamella, particularly the corner middle lamella have been reported (Highley and Murmanis, 1985). TEM micrographs of brown rotted wood fixed with $KMnO_4$ shows that the lamellar structure of lignin is lost in all cell wall layers indicating degradation or modification of lignin (Highley and Murmanis, 1985). TEM studies have shown that the S2 sometimes has a layered appearance, indicating preferential dissolution in definite zones which might be related to it's lamellate structure (Wilcox *et al.* 1974). This same study also reported that considerable shrinkage of the cell wall takes place during decay and before subsequent shrinkage resulting from dehydration. In particular, delamination at the S1/S2 boundary was observed and it was suggested that this was a zone of weakness during brown rot decay. Alternatively a differential susceptibility to brown rot of the S1 and S2 that resulted in preferential decay of one, would be expected to cause shrinkage stress at the interface.

Palmer *et al.* (1983) used TEM to observe hyphal sheaths and found that all 12 brown rot fungi (9 genera from several orders of basidiomycotina) studied produced abundant hyphal sheath. TEM studies have provided insights into the possible structure and functioning of multi-structured hyphal sheath (Palmer *et al.*, 1983; Green *et al.* 1992).

In most studies penetration of the cell wall by brown rot fungi is infrequent and is considered not to be an important degradative feature, bore hole forming hyphae causing only localised wood cell wall lysis (Highley and Murmanis, 1985). TEM micrographs suggest that bore hole forming hyphae degrade all cell wall components but only in the immediate vicinity of the hypha. This is in contrast to hyphae in the cell lumenae that cause the widespread degradation of the S2 layer that is typical of brown rot (Wilcox, 1970; Liese, 1970a). It might be expected that initial attack by brown rot fungi would open up the cell wall structure, giving access to enzymes (Flourney *et al.* 1991). Flourney *et al.* (1991) employed

molecular probes to investigate changes in wood pore size during decay and concluded that the cellulose-depolymerising agent of *P. placenta* must be between 12Å and 38Å. Studies of penetrability of enzymes into the cell wall by use of TEM and immunogold labeling found that lignin peroxidase (M_{r}- 42,000, ovalbumin (M_{r}- 45,000) and myoglobulin (M_{r}- 16,700) were incapable of penetrating brown rotted wood (Srebotnik and Messner, 1991; Flourney *et al.*, 1991). One of the smallest known proteins is ribonuclease (M_{r}- 13,000) with a diameter of approximately 30Å. A few small cellulolytic proteins, M_{r}- 11,000 to 13,000, have been reported (Flourney *et al.*, 1991).

Several publications have reported observations of radial configurations in wood decayed by brown rot fungi (Green *et al.* 1889; Highley and Murmanis, 1985; Larsen *et al.* 1995). Larsen *et al.* 1995 suggested that these configurations in decayed wood were a reflection of the pre-existence of planes of weakness in sound wood. It was further suggested that this radial utrastructure coexists as a sub-level of the concentric lamellate structure reported elsewhere (Sulaiman and Murphy, 1995; Nilsson and Daniel, 1983). These radial planes of symmetry were not continuous in the radial plane but were staggered across the S2 layer, being described as linear/curvilinear. Singh *et al.* (2002) also reported that lignin had a heterogenous distribution within the S2 layer of radiata pine. These continuous radially orientated hemicellulose configurations were suggested to account for the radial penetration of hyphal sheath structures subsequent to the rapid penetration of the hydronium ion (Green *et al.* 1989; Green *et al.* 1991; Larsen and Green, 1992). In addition it was suggested that radial planes of hemicellulose could be exploited by brown rot fungi that produce large amounts of oxalic acid, shown to hydrolyse hemicellulose in early stages of decay (Larsen *et al.* 1995). Once radial pathways were thus opened up by oxidative degradative processes penetretration of enzymes in association with hyphal sheath was facilated. It was hypothesised that this radial configuration was a primary factor contributing to the rapid degradation of wood cell wall by brown rot fungi.

Section 1.6.3

Soft rot micromorphology

Predominant wood colonisation occurs via the rays and also the vessels in hardwoods. Once hyphae move into the axial wood cells further colonisation is

typically via penetration of pits. However some fungi produce specialised penetration hyphae that facilitate movement between cells (Eaton and Hale, 1993).

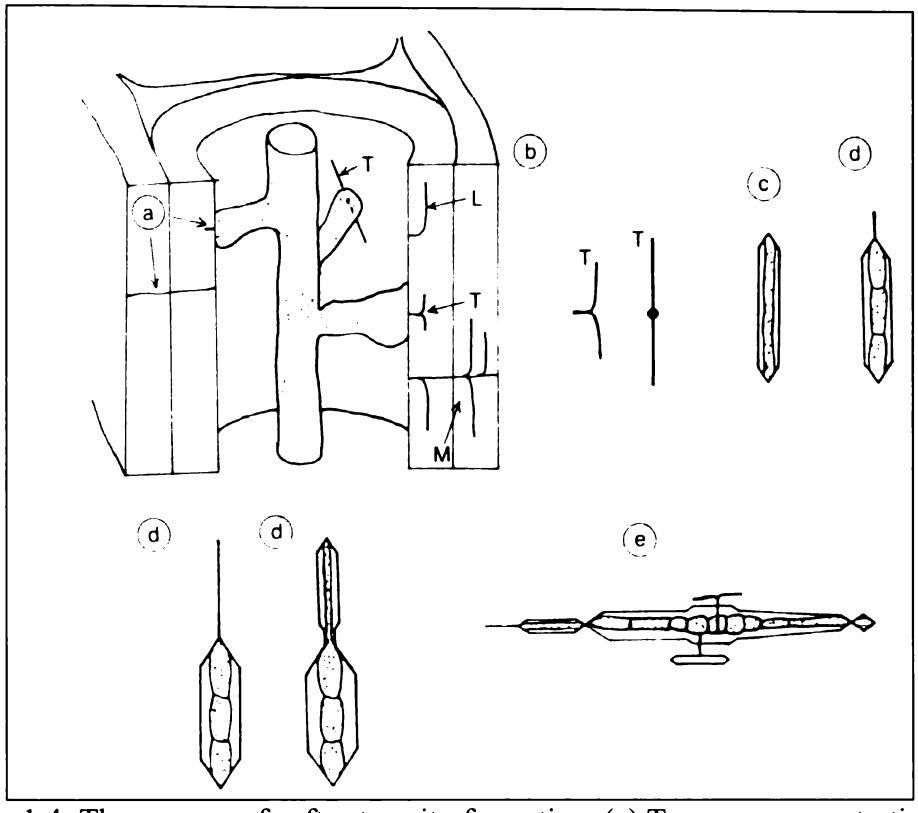


Figure 1.4: The process of soft rot cavity formation. (a) Transverse penetration into and through the cell wall; (b) T-branching (T), L-bending (L) and continued penetration through the wall with multiple branching (M); (c) cell wall dissolution around the T-branch hypha to produce the cavity; (d) proboscis hyphal growth and formation of a second cavity; (e) repeated proboscis hyphal growth results in a chain of cavities. Courtesy of Dr. R.A. Eaton, Portsmouth University, United Kingdom (UK) and Dr. M.D.C. Hale, University of Wales, Bangor, UK.

Section 1.6.3.1

Cavitation soft rot

Corbett (1965) distinguished two different types of soft rot called type 1 (T1) cavity attack and type 2 (T2) cell wall erosion attack. The most comprehensive studies of the mechanics of cavity formation were conducted by Hale and Eaton (1985). The first step in cavity initiation is transverse penetration of the S3 and S2 wood cell wall layers by a fine penetration hypha (0.2-0.4 μm diameter) produced by a hypha in the cell lumen (Figure 1.4). Penetration hyphae that originate from hyphae growing in intercellular spaces have also been observed (Courtois, 1963). At some point in the cell wall, usually the S2 but sometimes the S1, either an L-branch or T-branch occurs, typically parallel to the cellulose microfibrils. Multiple

branching is also sometimes observed (Daniel and Nilsson, 1997). Branching does not always occur and the penetration hyphae can form a fine bore hole by continuing across the paired cell walls of two adjacent wood cells, until it reaches the cell lumen of the second wood cell where it then enlarges to its original size. Alternatively, branching and continued transverse apical extension can occur simultaneously (Eaton and Hale, 1993). Typically, the fine bore hole formed, does not enlarge significantly beyond the diameter of the penetration hyphae. However bore hole widening has been reported by Liese (1970a) for wood attacked by *Chaetomium globossum*, a fungus generally considered to be a typical soft rot fungus.

Once T-branching has occurred the longitudinally aligned hypha extends a short distance and then apical growth stops. Hyphal diameter increases and lateral dissolution of the surrounding wood cell wall occurs forming a cavity with conical ends. Apical extension in the longitudinal plane then continues beyond the first hypha, stops, and then a second cavity is formed. This alternating sequence of apical growth followed by lateral expansion of hypha and cavity has been referred to as an oscillatory growth pattern and results in formation of a chain of discrete cavities. Liese (1970a) suggested that the size and form of cavities are greatly influenced by the chemistry and morphological structure of the wall layers, by the species of wood and fungus and ecological conditions. Soft rot cavities are formed in most types of wood cell including fibres, tracheids, fibre-tracheids, axial and radial parenchyma cells, ray tracheids and vessels.

Observations from TEM micrographs suggest that transverse hyphal penetration is accomplished primarily by a chemical degradation process, in contrast to the apparently mechanical penetration by sapstain. On the basis of two types of halo around the tip of penetration hyphae, Hale and Eaton (1985) suggested that two types of chemical degradative system may operate at the tip of penetration hyphae of *Phialophora hofmannii.* It was suggested that a pre-cellulolytic system similar to the Fe: peroxide pre-cellulolytic system of brown rot fungi would allow fine hyphae to penetrate structurally disorganised wood cell wall. It was further suggested that localisation of this putative diffusible degradative mechanism was contained by hyphal release under pressure at the tip region only. Presumably

degradation was limited by the rapid deployment and rapid expenditure of the active degradative agent immediately ahead of the penetration hyphae.

Daniel and Nilsson (1998) reported that an ordered microfibrillar arrangement within a wall layer of sufficient thickness appears to be a prerequisite for cavity formation and suggested that it might act as a trigger for enzyme release by cavity forming hyphae. Khalili *et al.* (2000) suggested that the minimum width of cell wall of kapok fibre necessary for proboscis hypha formation was >0.3 μm on the basis that proboscis hyphae are 0.2 – 0.4 μm diameter (Hale and Eaton, 1985). The process of formation of T- and L-branching by soft rot fungi is not fully understood but it has been linked to changes in wood cell wall structure and/or, chemistry encountered by the penetration hypa. Soft rot cavities are typically aligned with the long axis of the helical orientation of the cellulose microfibrils in wood and only occur in substrates like wood that have an ordered microfibrillar arrangement. As a result of this link it has been suggested that this ultrastructural feature is a requirement for soft rot cavity formation and that it induces production of cell wall-degrading enzymes (Daniel and Nilsson, 1997).

If an ultrastructural feature of the wood cell wall triggers T- and L-branch formation it is reasonable to suggest that some significant non-homogeneity must exist in either the radial or tangential plane. Levi and Preston (1965), cited by Sulaiman and Murphy (1995) suggested that the presence of physical barriers and localised regions of weakness within the cell wall might trigger formation of penetration hyphae. The barriers might consist of regions of high lignin and regions of weakness might consist of areas where carbohydrates are more readily accessible. Nilsson and Daniel (1983a) produced LM and TEM micrographs that showed cavity formation was linked to the position of concentric layers in wood cell walls. TEM micrographs (Nilsson and Daniel, 1983a) showed concentrically arranged half-moon shaped cavities in the fibre cell walls of *H. foetidium*. The argument that the lateral expansion of cavities was restricted by the high lignin content of concentric narrow lamellae, resulting in a half-moon shaped cavities, was very convincing.

A slightly different phenomena was observed in tracheid walls of Baltic redwood (*P. sylvestris*) and fibre walls of *Dillenia philippensis* and *Laurelia novae-*

zelandiae in which cavity formation occurred within concentric narrow layers high in lignin (Nilsson and Daniel, 1983a). It was suggested that these thin concentric layers were essentially different to surrounding wood and therefore triggered T-branching and formation of longitudinal cavities. TEM micrographs of cavity margins showed preferential cell wall degradaton along the narrow lamellae suggesting that this was a zone of cell wall weakness in these wood species. This was different to the situation in *H. foetidium* where the thick lamellae between narrow lamellae was less resistant and the high lignin containing narrow lamellae restricted lateral cavity extension due to its inherent resistance to degradation. In these studies wood blocks were buried in sterile soil and cavity-forming fungi were not identified.

Formation of similar crescent-shaped cavities by a soft rot fungus, *C. globossum*, in bamboo (Sulaiman and Murphy, 1995) was attributed to initiation of tangential T-branch hyphae at narrow lamella of the parenchyma cell wall. Bamboo parenchyma cell walls have alternating narrow (0.04 μm) and broad (0.2 μm) lamellae, thought to possess different physical and chemical composition (e.g. lignin type and content or cellulose microfibril orientation). TEM micrographs showed that initiation of T-branch formation occurred at the thin lamellae. Subsequent extension and expansion of hyphae within the broad lamella at the boundary of the narrow lamellae resulted in formation of a crescent-shaped cavity. The T-branch that results in a tangential cavity is approximately perpendicular to the long axis of the cellulose microfibrils. In this example it is logical that the penetration hyphae would turn through 90^0 into the tangential plane upon encountering the narrow lamella. Sulaiman and Murphy (1995) also observed typical longitudinal T-branches that resulted in typical soft rot cavities aligned with the long axis of the cellulose microfibrils. However no TEM micrographs were available that showed a close correlation between location of longitudinal T-branching and position of a thin lamella, as was the case for the tangential T-branch. A complete explanation to account for these two different T-branch orientations is currently not available. Possibly, tangential T-branching is only a significant phenomenon in wood cell walls with a pronounced multi-lamellate structure, were stimuli for tangential branching override stimuli for longitudinal T-branching. However the studies of Nilsson and Daniel (1983) suggested that initiation of longitudinal T-branching was triggered by concentric wood cell wall

layers high in lignin for several hardwood species and one soft wood which suggested that both types of T-branch are triggered by similar phenomena in some wood species. Singh *et al.* (2002) reported that lignin had a heterogenous distribution within the S2 layer of radiata pine and such a configuration might account for the more random distribution of T-branches seen in some wood species.

It is not known why soft rot cavities often remain as discrete chains and do not form a continuous tunnel by longitudinal coalescence of individual cavities. This feature is probably the consequence of a combination of the oscillatory growth pattern plus an unknown feature of the chemistry of wood cell wall degradation. Preston (1979) developed a mathematical model to account for the shape of soft rot cavities. It was proposed that enzymes released by cavity hyphae diffuse at different rates across and along the cellulose microfibrils, and that these rates are so regulated that a diamond or conical shaped cavity is produced. Daniel and Nilsson (1998) suggested that absence of enzyme secretion from hyphal segments involved, or incorrect stereochemistry of cell wall polymer groups hindering enzyme activity, could also be involved. Lignocellulosic substrates in which soft rot fungi form cavities appear to have in common an ordered (parallel) cellulose microfibrillar arrangement within plant cell walls. Several authors cited by Daniel and Nilsson (1998), have reported cavity formation by soft rot fungi in a range of substrates including: cotton, seed hairs, bast fibres (Nilsson, 1974), bamboo (Sulaiman and Murphy, 1995), reed (Bosman, 1985; Kirby and Rayner, 1989), pampass grass (Fukuda, 1991) and pine needles (Gourbiere *et al.* 1989).

Section 1.6.3.2

Erosion soft rot

Corbett (1965) referred to erosion attack as type 2 attack and it is generally considered to be the result of enzyme secretion from hyphae in the wood cell lumen. Corbett (1965), as cited in Eaton and Hale (1993) observed V-shaped notches in the wood cell wall that occurred contiguous to the hyphae growing along the lumen surface. Fine hyphae were also seen penetrating the cell wall, surrounded by a notch. Another form of erosion attack consisted of hyphae lying in a smooth erosion trough (Leightley and Eaton, 1977; as cited in Eaton and Hale, 1993). According to Daniel and Nilsson (1998), in hardwoods a gradual

erosion from the lumen towards the middle lamella occurs, causing cell wall thinning and/or formation of erosion troughs of various shapes. In softwoods, where the S3 layer is usually highly lignified, this layer appears to remain intact whilst the underlying S2 erodes. It is not known if the S3 becomes partially degraded in the process. However this seems likely based on observations that suggest a non-diffusible mechanism of action for enzymes, produced along the length of cavity forming hyphae, which presumably would only be able to traverse the S3 layer if its pore size had increased due to fungal degradation. It is possible that lumen hyphae that cause type 2 erosion and cavity hyphae that cause type 1 attack, possess different degradative mechanisms. Furthermore, evidence suggests that in the case of brown rot and white rot, cell wall porosity would not allow passage of enzymes, even during decay (Flournoy *et al.*, 1991; Srebotnik and Messner 1991: as cited in Anagnost *et al.*, 1994). Resistance of the S3 layer of softwoods is thought to be due to the amount or type of lignin present, and/or the presence of the hemicellulose glucuroarabinoxylan (Liese, 1970; as cited in Anagnost *et al.*, 1994). In later stages of type 2 erosion attack hyphae can be seen associated with lysis zones in the S2 layer (Daniel and Nilsson, 1998)although their mechanism of entry appears unclear. In softwoods the S1 layer and middle lamella remain largely intact in wood with advanced decay. In hardwoods the erosion pattern is difficult to distinguish from that produced by white rot fungi although Daniel and Nilsson (1998) suggest that extensive middle lamella breakdown is diagnostic for white rot. Zabel and Morrell 1992) and Eaton and Hale (1993) point out the much greater susceptibility of hardwoods to soft rot type 2 erosion compared to softwoods but do not refer to the essential difference in type of erosion between hardwoods and softwoods as explained by Daniel and Nilsson (1998).

Most species of fungi that cause extensive erosion in hardwoods cause less severe attack in softwoods which is believed to be related to lignin type and content. This is supported by the observation that hardwoods with unusually high lignin and a low syringyl:guaiacyl (S/G) ratio have a similar resistance to soft rot erosion to that shown by soft woods which also have high lignin and a low S:G ratio. Nilsson (1973) stated that most cellulolytic fungi that have been tested are able to cause at least some erosion of the fibre walls of hardwoods. This includes mould fungi of the genera *Aspergillus*, *Gliocladium*, *Penicillium* and *Trichoderma* that

cause slight erosion of birch fibres. Birch does not have an S3 layer but it is believed to have a thin layer adjacent to the lumen that is more resistant to attack than the underlying S2 layer (Zabel and Morrell, 1992). Cavity formation by *Aspergillus*, *Penicillium* and *Trichoderma* species has been reported (Liese and Pechman, 1959: Courtois, 1963; Fukuda and Haraguchi, 1975: as cited in Daniel and Nilsson, 1997) but Daniel and Nilsson (1998) pointed out that this has not been substantiated by later studies.

Chemical delignification, particularly for softwoods, greatly increases susceptibility to soft rot (Daniel and Nilsson, 1998). Erosion type soft rot appears to be the primary form of decay in delignified wood and Nilsson *et al.* (1989) reported that cavity formation was impeded by delignification. Encinas and Daniel (1997) reported erosion of non-lignified cellulose in wood such as the gelatinous layers in tension wood fibres of hardwood and Nilsson (1974) reported cavity formation in cotton which lacks lignin. These observations suggest that lignin content and/or possibly a low S:G ratio impedes erosion attack by soft rot, typically in softwood species, and is a trigger for cavity formation in many but not all wood species.

Several authors have suggested a possible need for recognition of a third type of soft rot referred to as diffuse cavity attack (Eaton and Hale, 1993; Anagnost, 1994; Daniel and Nilsson, 1998). Daniel and Nilsson (1998) describe a typical type 1 cavity having well defined side-walls compromising what appears to be sound wood cell wall. Their description of a diffuse cavity attack is as follows: "diffuse degradation sometimes occurred, which extended beyond the cavity edge". They went on to say, "observations using polarised light suggest that the cellulose is degraded in a way reminiscent of brown rot attack". Anagnost *et al.* (1994) described a diffuse expansion and coalescence of cavities that caused complete destruction of the S2 layer. No reference was made to similarities with brown rot attack although the extensive loss of wood cell wall brightness across several wood cells seen using interference contrast optics was essentially the same as would occur had polarised light been employed. This was attributed to complete loss of the S2 layer due to cavity coalescence. Anagnost *et al.* (1994) reported diffuse cavity attack caused by *Arthrographis cuboidea*, *Scytalidium lignicola, C. funicola* and *C. globosum* in southern yellow pine (*Pinus taeda*).

Section 1.6.4

Tunnelling bacteria (TB)

Tunnelling bacteria have been described as Gram negative, motile but non-flagellate, pleomorphic (variable shape capable of conforming to surrounding surfaces), slime producers, cellulolytic and lignolytic (Eaton and Hale, 1993). These features suggest tunnelling bacteria are gliding bacteria, possibly belonging to the Myxobacteriales or Cytophagales (Eaton and Hale, 1993). Several authors have reported pure cultures of tunnelling bacteria isolated from wood with tunnelling bacteria attack but it appears that Koch's postulates were not applied (Drysdale *et al.*, 1986; Schmidt *et al.*, 1995). Daniel and Nilsson (1998) reported that the taxonomic classification of wood degrading bacteria may lie with the cytophages or sporocytophages or more likely within the poorly known genus *Nevskia* (Famintzin, 1892; as cited in Daniel and Nilsson, 1997). This genus shares many morphological features seen for tunnelling bacteria, for example the production of slime behind the bacterium.

In early stages of attack or around the margins of more advanced attack, tunnels are easily distinguishable using LM but in later stages wood appears granulated. Individual bacteria within tunnels are also distinguishable using LM when a suitable stain such as aniline blue in lactic acid was used (Nilsson and Daniel, 1983b). In SEM micrographs, tunnels are often observed as elongated depressions that result from collapse of the S3 layer of the wood cell wall. TEM studies have shown that a single bacterium occupies each tunnel apex. One of the most distinctive features of tunnelling bacteria (TB) attack is the production of regular cross walls that are concave relative to the tunnel apex. Mouzouras *et al.* (1986), as cited in Singh and Butcher (1991), reported two types of bacterial tunnelling, one with cross walls and one without. The type that lacked cross walls had a larger diameter.

Mouzouras *et al.* (1986) suggested that cross walls reflect a start-stop growth mechanism of tunnelling bacteria. Singh and Butcher (1991) suggested that slime (mucopolysacharide) is produced continually and therefore when the bacterium is imobile a localised buildup occurs, which is then exposed once the bacterium recommences tunnel formation. An interesting feature of heavily attacked wood is the extent of close association between tunnels without their coalescence,

suggesting bacteria possess an avoidance mechanism (Eaton and Hale, 1993). Eaton and Hale (1993) reported that only in wood with very advanced attack does breakdown of partitioning wood cell wall layers occur. In high magnification TEM micrographs it is not uncommon to observe adjacent tunnels with no discernible partitioning wood cell wall.

Production of cross walls also gives clues as to the mechanism of entry into the cell wall from the wood cell lumen. TEM micrographs presented by Singh and Butcher (1991) show that cross walls are produced within a glycocalyx layer that surrounds bacteria during penetration of the S3 wood cell wall layer. This suggested that the mechanism of movement in the glycocalyx and the within the cell wall was essentially the same. These micrographs also showed that the narrow end of a pear-shaped bacterium penetrates the S3 layer of the wood cell wall. Micrographs showing later stages of bacterium cell wall penetration suggest that the bacterium changed its form to essentially fill an elongated oval-shaped cavity that presumably was the beginning of a tunnel. In the micrographs shown, the long axis of the bacterium appears to be aligned with the tangential plane, possibly at the interface of the S2 and S3 layers. Such a sequence is supportive of the pleomorphic nature of TB. Singh and Butcher (1991) suggested that wood cell wall entry into fibres of the high lignin containing tropical hardwood *Eusideroxylon zwageri* was via the margins of pit apertures, on the basis that cell lumenae were full of impassable extractives. However, TEM micrographs of Singh and Butcher (1991) also showed tunnelling could occur within the extractives and it is possible that access to the cell wall could be gained via tunnelling within the lumen extractives.

Section 1.6.5

Erosion bacteria

Erosion bacteria are Gram negative, exhibit cell wall plasticity and are believed to share taxonomic affinity with tunnelling bacteria (Eaton and Hale, 1993). Wood cell walls attacked by erosion bacteria seen using LM lose their smooth appearance and become pitted and irregular. Grooves may be seen in transverse section but are more easily observed in longitudinal section. Under SEM, rod-shaped bacteria with pointed ends (1 – 2 μm long) can be observed sitting within grooves (Eaton and Hale, 1993). Cell division causes extension of grooves into

long channels. Under LM this pattern is sometimes referred to as stripy erosion. The long axis of erosion bacteria and the underlying channels associated with stripy erosion are typically aligned with the cellulose microfibrils (Eaton and Hale, 1993).

Singh and Butcher (1991) separated erosion bacteria attack into stripy erosion and V-shaped erosion. With V-shaped erosion a V-shaped trough forms in the wood cell wall with the apex towards the middle lamella. Remnants of S3 layer overlying heavily degraded S2 is typical for this pattern. This is similar to some type 2 soft rot erosion. TEM micrographs of stripy erosion suggest that the S3 layer is removed. One of the less convincing aspects of reports of V-shaped erosion bacteria attack is the apparent evanescent nature of the bacteria within the trough. Micrographs that clearly illustrate the relationship between stripy erosion bacteria and the wood cell wall surface were presented by Singh and Butcher (1991) but equally convincing micrographs are lacking for V-shaped erosion. TEM micrographs of V-shaped erosion attack presented in the review by Singh and Butcher (1991) showed no clearly defined bacteria at the wood surface. There is potential for confusion between type 2 soft rot erosion and V-shaped bacterial erosion. A later review of Daniel and Nilsson (1998) did not refer to V-shaped erosion although the occurrence of several different erosion patterns was mentioned but not defined. LM micrographs of Greaves (1968) showed continuous, concentric V-shaped erosion troughs perpendicular to the long axis of radiata pine tracheids that were attributed to attack by erosion bacteria, although no bacteria were visible.

Daniel and Nilsson (1998) listed 3 features of decay micromorphology shared by erosion bacteria decay and soft rot decay.

1. Alignment with cellulose microfibrils.
2. Inability to degrade middle lamella and difficulty degrading the S3 layer in softwoods.
3. Frequent production of a discrete decay pattern with angular cavities.

Whilst this comparison appears essentially sound for stripy erosion it is possibly not completely valid for V-shaped erosion patterns. This is because V-shaped erosion troughs are often perpendicular to the long axis of the cellulose

microfibrils and this pattern is more likely to reflect radial concentric wood cell wall symmetry rather than the longitudinal orientation of cellulose microfibrils. As reported by Daniel and Nilsson (1998) cell division of bacteria aligned with the CM of the S2 layer expands the colony in the longitudinal plane and therefore it would be expected that the depressions formed would be longer in the longitudinal plane, unlike concentric V-shaped erosion of the type reported by Singh and Butcher (1991) and Greaves (1968).

Nilsson and Singh (1984) were the first to describe another bacterial decay pattern called cavitation bacteria attack but subsequent publications have questioned the validity of separating this bacterial decay type from erosion bacteria decay (Blanchette 1995; Daniel and Nilsson, 1998). This appears to be because the "cavities" are continuous with erosion troughs caused by erosion bacteria (Daniel and Nilsson, 1998). Such a configuration is particularly likely with erosion bacteria decay of softwoods because of the increased resistance of the S3 layer conferred by a high lignin content compared to hardwoods. Once bacteria have gained access to the S2 layer of the wood cell wall, ongoing expansion within this layer occurs in preference to degradation of the overlying S3 layer, resulting in cavity formation. Daniel and Nilsson (1998) reported that cavitation bacteria appear to be short-lived on the basis that bacteria are typically absent from cavities.

Section 1.7

Wood decay classification

Forest pathologists produced some of the earliest decay type classifications based on macroscopic appearance (colour and texture), fungal fruiting body anatomy and the pathological symptoms of the host tree (Hubert, 1931; Baxter, 1952; as in Zabel and Morrell, 1992). However this system was primarily intended for heart rot decays and could not accommodate the diversity of decay types found in nature and in wood products. One of the earliest detailed descriptions of wood decay was published by Cartwright and Findlay (1958) in which five groups were identified on the basis of wood use category and the type of wood affected.

1. Decays of standing timber (softwoods and hardwoods).
2. Decays of felled timber and timber in service exposed to the open.

3. Decays of timber in buildings and structures.
4. Decays in timber during storage, conversion, and shipment.
5. Decays of timber in various uses.

Other similar classifications have been developed based on where the decay occurs and have been summarised in a review by Zabel and Morrell 1992) (commonly used terms are shown in italics).

1. Stem decays in standing timber were grouped into *heart rots* and *sapwood decays* of the living trees; *slash rots* are those that develop in wind-throw timber, branches, and logging slash.
2. *Storage decays* occur during the storage and processing of pulpwood, pulp chips, logs, and piling.
3. Special commodity and use categories are the *building-rot decays*, decays of, *utility poles* (power poles), *mine timbers*, and piling.
4. *Conifer* (softwood) decays were often contrasted with *hardwood* decays.

Knowledge in four areas contributes to current decay type classification.

1. Microorganism taxonomy.
2. Nature of enzymes and other degradative agents produced by a decay fungus and the order in which wood cell wall components are removed.
3. Macroscopic features of decay (texture and colour).
4. Decay micromorphology (ultrastructure of damaged wood cell wall and its spatial relationship with the causative microorganism).

The current contribution made by microorganism taxonomy, to decay classification, is relatively straightforward. Some types of decay are primarily caused by a few large subdivisions of fungi. For example fungi belonging to the fungal division Basidiomycotina cause the great majority of brown rot and white rot decays. (The most important basidiomycete decay fungi, in terms of damage to wood products, belong to the order Aphyllophorales.) The majority of fungi known to cause soft rot are lignolytic members of the Deuteromycotina and Ascomycotina. Nilsson (1988) proposed that decay produced by all

deuteromycetes and ascomycetes should be considered soft rot. Inevitably, there are deviations from these phylogenetic groupings including the following.

- Some Xylariaceous ascomycetes (*Xylaria polymorpha*, *Daldinia concentrica*, *Hypoxylon* sp. and others) cause wood decay similar to simultaneous white rot in hardwoods but also produce typical soft rot cavities in softwoods and hardwoods (Kistler and Merrill, 1968; Nilsson *et al.*, 1989; in Zabel and Morrell, 1992; Eaton and Hale, 1993; Worrall *et al.*, 1997).
- A significant number of white rot basidiomycetes produce cavities and hyphal T-branch patterns in the cell wall of hardwoods and softwoods that share some of the features of soft rot cavities (Ruel *et al.*, 1981; Kim *et al.*, 1988; Daniel *et al.*, 1992; Nicole *et al.*, 1995; Schwarze *et al.*, 1995a; Worrall *et al.* 1997; Anagnost, 1998; Schwarze and Engels, 1998; Schwarze and Fink, 1998; Schwarze and Baum, 2000).
- The basidiomycete *Fistulina hepatica* has been reported to cause brown rot in fibre tracheids of *Quercus robur* heartwood and soft rot cavities in xylem parenchyma and fibres (Schwarze *et al.* 2000). The Ascomycete *Hypoxylon mediterraneum* was reported to cause brown rot and soft rot decay in wood of *Pinus* sp. (Francis and Leightley, 1984).
- Some soft rot fungi appear able to produce a type of diffuse soft rot that shares some of the features of brown rot decay (Anagnost, 1994; Daniel and Nilsson, 1998).

Liese (1970) published a classification of decay type based on the ultrastructure of damaged wood cell walls and their spatial relationship with the causative microorganism (micromorphology) that underpins much of the decay type terminology that has been used since. Primary emphasis was placed on the order that wood cell wall components were removed and secondary emphasis was placed on the mechanism of hyphal penetration into wood fibres and tracheids. A classification system from Zabel and Morrell (1992) based partly on the system of Liese included details of decay biochemistry and broad taxonomic affiliation (Table 1.1). It includes a section on non-decay fungi, defined as wood colonisers that utilise non-structural wood components, or tissues that do not contribute significantly to wood strength. Their associated wood cell wall micromorphology

is limited to production of hyphal bore-holes typically no bigger than the diameter of the penetrating hypha, and damage of the thin-walled parenchyma cells.

Table 1.1: A Summary of the anatomical and chemical features of the major types of wood-inhabiting microorganisms (Zabel and Morrell, 1992)

Wood-inhabiting microorganisms	Cell-wall constituents used	Anatomical features	Causal agents
Decayers (Cell-wall erosion and/or large bore holes formed > 2 μm)			
Simultaneous white rot	All	Cell walls attacked progressively from lumen surface	Basidiomycotina Some Ascomycotina
Sequential white rot	All, but hemicelluloses and lignin used selectively initially	Cell walls attacked progressively from lumen surface	Basidiomycotina Some Ascomycotina
Brown rots	Carbohydrates, but lignin-modified	Entire wall zone attacked rapidly	Basidiomycotina
Type 1 soft rot	Carbohydrates	Longitudinal bore holes develop in secondary wall	Deuteromycotina Some bacteria
Type 2 soft rot	Carbohydrates	Secondary wall erosion from lumen surface (in conifers, mainly the S2)	Ascomycotina Deuteromycotina Some Bacteria
Nondecayers: (No cell-wall erosion and occasional bore holes are minute < 1 μm)			
Sapstainers	Wood extractives	Invade parenchyma cells in sapwood primarily	Ascomycotina Deuteromycotina
Moulds	Wood extractives	Surface growth on wet wood	Zycomycotina Ascomycotina Deuteromycotina
Scavengers	Wood extractives and decay residues	Penetrate wood cells primarily through pits	Bacteria Zygomycotina Ascomycotina Basidiomycotina Deuteromycotina

Section 1.7.1

Evolutionary considerations for decay classification based on micromorphology

Zabel and Morrell 1992) suggested that similarities in decay patterns produced by divergent microorganims probably reflect a convergence in attack strategies determined by wood structure. However, Worrall *et al.* (1997) pointed out that although decay capability may have evolved independently in several groups of

fungi, it is also possible that the capability was maintained and enhanced as some groups arose from earlier ones. He went on to point out that if this is the case then decay features may provide clues to phylogeny and developed some convincing arguments in support of this. According to Worrall *et al.* (1997) the ability to decay wood probably evolved slowly, beginning with soft rot fungi belonging to the Ascomycotina. White rot fungi belonging to the Basidiomycotina probably evolved from fungi causing a soft rot type of decay, and may have arisen independently along several lines in the Basidiomycotina. As fungi became better able to degrade lignin (white rot), perhaps cavities were no longer a necessary strategy. Brown rot fungi then arose from white rot fungi in many groups, particularly the polypores. This latter hypothesis was first suggested by Gilbertson (1980), as cited in Worrall *et al.* (1997), who argued that the unifactorial mating system, common in brown rot fungi, is considered to have been derived by simplification of the bifactorial system that is common in white rots. He also pointed out that brown rot fungi are generally more efficient at obtaining energy from wood, particularly when soft-woods are attacked.

Nobles (1958), in Worrall *et al.* (1997), considered lack of the phenoloxidases by brown rot, required for complete lignin breakdown as occurs during white rot decay, a primitive feature. However, Worrall *et al.* (1997) argued that development of a brown rot fungus from a white rot fungus was a simplification process involving conversion of the bifactorial to a unifactorial mating system and loss of extracellular phenoloxidase and exogluconase. Worrall *et al.* (1997) went on to point out that members of the coniophoraceae 'still' have an exo-1,4-ß-gluconase. In common with brown rot fungi the white rot fungus *P. Chrysosporium* possesses hydrogen peroxide producing enzymes and peroxide dependant enzymes (e.g. lignin-peroxidase) linked to oxidation, hydroxylation and cleavage reactions of lignin (Kirk, 1988; in Eaton and Hale, 1993). Possibly this is further evidence of an evolutionary link between white and brown rot fungi. According to Worrall *et al.* (1997), once the non-enzymatic low molecular weight degradation system of brown rot had evolved, extensive delignification and fully enzymatic depolymerisation of wood cell wall carbohydrate would no longer be necessary and would eventually be lost. If this hypothesis is correct then as Worrall *et al.* (1997) points out, it is ironic to contemplate that only after white rot

fungi had conquered lignin, the great barrier to wood decomposition, did brown rot fungi find a way to largely circumvent it via brown rot.

Fistulina hepatica caused brown rot in fibre tracheids of *Quercus robur* heartwood and soft rot cavities in xylem parenchyma and fibres (Schwarze *et al.* 2000) and how this fits with the hypothesis of white rots being an evolutionary intermediate between soft rot and brown rot fungi is not clear. Similarly, the significance of the production of diffuse cavities by soft rot fungi that may involve degradative mechanisms similar to those of early brown rot decay (Anagnost, 1994; Daniel and Nilsson, 1998) remains unclear. The TEM observations of Eaton and Hale (1985) also suggested that soft rot penetration hyphae produced a local decay pattern suggestive of a pre-enzymatic oxidative mechanism similar to that of early brown rot. Furthermore, the significance of production of soft rot-like cavities by several basidiomycete white rot fungi is unclear. Whatever the evolutionary significance of these observations, they suggest that mechanisms of decay form a continuum across fungal decay types and across taxonomic groupings of fungi. This feature is likely to undermine attempts to separate decay types on the basis of taxonomic affinities such as Nilsson's (1988) suggestion that all decay caused by Deuteromycotina and Ascomycotina be termed soft rot.

Section 1.7.2

Challenges facing decay classification nomenclature

One of the challenges facing decay classification relates to the limited number of decay fungi that have been studied and the artificial laboratory conditions that have typically been used to demonstrate decay capability. Zabel and Morrell 1992) pointed out that many forest floor decay fungi associated with slash (fallen or pruned branches) have yet to be extensively studied, including members of the Tremallales, Dacrymycetales, Auriculariales and Gasteromycetes. Many of these fungi may play an important role in carbon recycling within forest ecosystems and probably represent a significant part of the gene pool that determines wood decay diversity. They may therefore hold many of the keys required, to unlock a complete understanding of the biochemistry of wood decay.

As pointed out by Rayner and Boddy (1988) "there has been a tendency to regard wood decay as though the resource and the organism were two simple reagents off

a laboratory shelf whose interaction results in a consistent and predictable outcome." They went on to say "this is, of course, far from the truth: not only is wood itself a variable and heterogenous resource but as with all other eukaryotes the decay fungi cannot be regarded as fixed entities. Rather they vary, and this variation occurs not only between different individuals, but also because of the indeterminate nature if the mycelial body form, within an individual. Causes of variation include genotypic differences, direct effects of environment on metabolic functioning, changes to differences in gene regulation and random effects. The implications of variation, both between individuals and during the development of single individuals, are considerable, not only with respect to taxonomy and diagnosis but also in terms of the ways in which populations of decay fungi establish in and interact with woody resources." The complexity of the determinate factors described by Rayner and Boddy is such that attempts to link a single phenomenon such as taxonomic affinity or any single determinate factor with the final outcome i.e. decay type, is likely to be overly simplistic.

Worrall *et al.* (1997) carried out decay tests on 98 isolates (78 species) of lignicolous fungi, including several orders of the Basidiomycotina (Agaricales, Aphyllophorales, Auricales, Dacrymycetales and Exidiaceae), several xylariaceous ascomycetes (Xylariales), and soft rot fungi belonging to the Ascomycotina and Deuteromycotina. Chemical and anatomical wood analysis showed that the validity of the generally accepted major decay types (white, brown and soft rot) was confirmed, and no new major types were proposed. However, one of challenges facing wood decay classification is that information concerning decay biochemistry and micromorphology derived from laboratory studies, fails to take account of the inevitable complexity of environmental influences that occur in the field. Wood decay is a result of a combination of factors which can be expressed as microorganism + wood + environment. Whilst some decay fungi may cause a single decay type irrespective of environmental conditions there is evidence that some fungi have the ability to cause a mixture of decay micromorphological features that are considered to be representative of more than one decay type or which do not fit well within any of the existing types (see Table 1.3). Possibly, many decay fungi produce diverse decay micromorphology but this has yet to be fully appreciated due to a lack of

investigations carried out that have taken account of the significance of fungus-wood species-field interactions.

It is generally accepted that some basidiomycetes produce both simultaneous and preferential white rot and whilst in some instances this has been linked to wood species effects, both types are produced simultaneously by single fungal species under controlled laboratory conditions (Blanchette *et al.*, 1990). Some soft rot fungi produce both cavitation and erosion decay. Soft rot erosion, especially in hardwoods is sometimes difficult to distinguish from erosion produced by basidiomycete white rots, especially early decay. It has also been shown that basidiomycetes produce "cavitation" micromorphology very similar to soft rot caused by ascomycetes and deutermycetes. It has also been suggested that some soft rot fungi that produce diffuse cavities may possess degradative mechanisms similar to early brown rot decay. Ascomycetes have been shown to produce micromorphology very similar to both classical soft rot and white rot. All these observations taken as a whole suggest that separation of decay types on the basis of taxonomic affinity is unlikely to constitute a robust system. Furthermore, most of these observations have come from laboratory studies and therefore have not taken account of the possible effects of environmental factors on expression of decay type, an influence likely to increase expression of decay type diversity.

Table 1.2 shows the definitions of decay micromorphology of decay types within current decay classification nomenclature. Table 1.3 gives examples of micromorphological features, and mixtures of features produced by single fungal species, that do not fit neatly within any of the single categories listed in Table 1.2. The wood and fungal species known to be responsible for intermediate decay are given with the reference in Table 1.4.

It may be significant that many of these examples (1, 2, 7, 8, 13, 14, and 19) are of micromorphology produced by wood decay fungi associated with heart rots or pathogenic reaction wood of standing trees and that most of the observations where made within the last 10 years. Most of the decay micromorphology observations that have influenced decay classification in the last 50 years have come from workers in the wood products field. It is perhaps not surprising to find that observations from a different but nevertheless closely related field have

produced a high proportion of the examples of decay micromorphology that is poorly defined by current decay classification nomenclature. Since taxonomic affinities of the fungi involved are similar to many of the decay fungi studied in the wood products and wood fibre modification fields, it seems unlikely that the micromorphology reported was unique to the fungal species reported. Infact a number of the micromorphological features reported have also been reported by

Table 1.2: Current decay classification nomenclature and definitions of wood decay micromorphology

1	**Brown rot**
	Loss of wood cellulose birefringence
	Small bore holes
2	**Simultaneous white rot**
	U-shaped troughs closely associated with lumen hyphae
	Cell wall thinning caused by lumen hyphae
	Large bore holes
3	**Sequential white rot**
	Uptake of specific stains by the cell wall in response early lignin removal and defibrillation
4	**Combined simultaneous & sequential WR caused by single fungal species**
5	**Classical soft rot** (Corbett's type 1: cavitation)
	Cavities - biconical or cylindrical with conical ends (smooth, irregular or serrated cavity walls). Typically aligned with S2 layer cellulose microfibrils (CM). Cavities initiated by proboscis hyphae and associated T- and L-branching/bending
6	**Soft rot erosion** (Corbett's type 2: erosion)
	U- and V-shaped troughs in lumen wall or cavities in S2 layer below thin overlying layer of lumen wall, caused by lumen hyphae.
7	**Combined cavitation and erosion SR caused by single fungal species**
8	**Tunnelling bacteria micromorphology**
	Tunnels with "cross-walls" containing pleomorphic bacteria at apex
9	**Erosion bacteria micromorphology** (includes cavitation bacteria micromorphology)
	Pits and troughs of various shapes containing rod shaped bacteria typically aligned with S2 layer cellulose microfibrils

Table 1.3: Examples of intermediate decay types with mixtures of micromorphology that are difficult to classify using current decay nomenclature

Micromorphology		Wood and fungal species reference (Table1.4)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Initiatory micromorphology															
1	Bore holes	×	×	×	✓	–	–	–	–	–	–	✓	–	✓	–	–
2	Proboscis hyphae[1]	–	–	–	–	–	–	–	–	–	–	–	✓	✓	–	–
3	Classical T-, L-, Y-branches (longitudinal) / aligned with cellulose microfibrils	×	×	×	✓	–	–	×	–	–	✓	✓	✓	✓	–	–
4	T-, L-, Y-branches (tangential-concentric) / inter-lamellate	–	–	–	–	–	–	–	?	–	✓	–	✓	–	–	–
	Erosion micromorphology															
5	U-shaped troughs plus hyphae (WR-type)	–	–	–	✓	–	–	–	–	–	–	–	–	–	–	–
6	Cell wall thinning	–	–	–	–	–	–	–	–	–	–	–	–	✓	✓	✓
7	U-, V-shaped troughs (notches in R.S.). SR erosion (Corbetts Type 1) or notched WR erosion:	–	–	–	–	–	–	–	–	–	–	–	–	✓	–	–
	Other WR micromorphology															
8	Small erosion troughs (0.3 - 0.5µm)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	Microhyphae[1] within wood cell walls	✓	✓	✓		–	–	–	–	–	–	–	–	–	–	–
10	Preferential delignification	–	–	✓	–	–	–	✓	✓		–	–	–	✓		✓
	Cavitation micromorphology															
11	SR-type cavities - biconical or cylindrical with conical ends, typically parallel with CM and with oscillatory growth pattern	–	–	–	–	–	–	–	–	–	✓	✓	–	✓	✓	✓
12	Cavities at S2-lumen wall interface caused by lumen hyphae	–	–	–	–	–	–	✓	✓	–	–	–	–	–	–	–
13	Crescent-shaped / concentric cavities (seen in T.S.)	–	–	–	–	–	–		✓	–	–	✓	–	–	–	–
14	SR-like cavities caused by bacteria (actinomycetes).	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	Diffuse SR cavitation: loss of wall birefringence beyond cavity wall or diffuse cavity walls	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	**Tunneling by fungal hyphae:** non-oscillatory extension of tunnels with rounded ends	–	–	–	✓	✓	✓		–	–	✓	–	✓	–	–	–
	Other															
17	Multiple T- and L-branching	–	–	–	✓	–	–	–	–	–	–	✓	–	✓	–	–
18	Reticulum of tunnels formed by multiple T-, and L- branching	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	Reticulum formed by cavity hyphae side branching	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

See "Table continued" on next page for key.

Table 1.3 continued

	Micromorphology	Wood and fungal species reference (Table 1.4)									
		16	17	18	19	20	21	22	23	24	25
	Initiatory micromorphology										
1	Bore holes	–	✓	–	–	–	–	–	–	–	–
2	Proboscis hyphae[1]	–	–	–	–	–	–	–	–	–	–
3	T-, L-, Y-branches (longitudinal)	–	–	–	–	–	–	–	–	–	–
4	T-, L-, Y-branches (tangential-concentric)	–	–	–	–	–	–	–	–	–	–
	Erosion micromorphology										–
5	U-shaped troughs plus hyphae (WR-type)	–	✓	–	–	–	✓	–	–	–	–
6	Cell wall thinning	–	–	✓	–	–	–	–	–	–	–
7	U-, V-shaped troughs (notches in R.S.). SR erosion (Corbetts Type 1) or notched WR erosion:	–	–	–	–	–	–	–	✓	–	–
	Other WR micromorphology										–
8	Small erosion troughs (0.3 - 0.5μm)	–	–	–	–	–	–	–	–	✓	–
9	Microhyphae[1] within wood cell walls	–	–	–	–	–	–	–	–	–	–
10	Preferential delignification	✓	–	–	–	–	–	–	–	–	–
	Cavitation micromorphology										–
11	SR-type cavities - biconical or cylindrical with conical ends, typically parallel with CM and with oscillatory growth pattern	–	✓	✓	✓	✓	✓	–	–	–	–
12	Cavities at S2-lumen wall interface caused by lumen hyphae	–	–	–	–	–	–	–	–	–	–
13	Crescent-shaped (seen in T.S.) / concentric cavities (L.S.)	–	–	–	–	–	–	✓	–	–	–
14	SR-like cavities caused by bacteria (actinomycetes).	–	–	–	–	–	–	–	–	–	✓
15	Expansive or diffuse SR cavitation	–	–	–	–	–	–	–	✓	–	–
16	**Tunnelling by fungal hyphae:** non-oscillatory extension of tunnels with rounded ends	✓	–	–	–	–	–	–	–	–	–
	Other										–
17	Multiple T- and L-branching	–	–	–	–	–	–	–	–	–	–
18	Reticulum of tunnels formed by multiple T-, and L- branching	–	–	–	–	–	–	–	–	–	–
19	Reticulum formed by coalescence of cavities at 90^0	–	–	–	–	–	–	✓	–	–	–
20	**Loss of birefringence (brown rot)**	–	–	–	✓	✓	–	–	–	–	–

✓ = feature present. – = feature absent. × = absence of feature is of particular significance. [1] = microhyphae of basidiomycete WR fungi and proboscis hyphae of SR fungi may be very similar in terms of ultrastructure and penetrative function.? = not reported but other features indicated their presence.

Table 1.4: Wood and fungal species associated with intermediate decay types shown in Table 1.3

	Fungus	Wood	Reference
1	*Phellinus noxious*[B]	*Betula papyrifera*	Nicole *et al.* 1995
2	*Fomes annosus*[B]	*?*	Peek *et al.* 1972
3	*Ganoderma applanatum*[B]	*Tsuga heterophylla*	Murmanis *et al.* 1984
4	*Auricularia auricula-judea*[B]	*B. alleghaniensis*	Anagnost *et al.* 1998
5	(natural infection)	*Eusideroxylon zwageri*	Singh & Wong, 1996
6	(natural infection)	*Pinus radiata*	Xiao *et al.* 1997
7	*Armillaria ostyae*[B]	*Picea abies*	Schwarze & Engels, 1998
	A. tabescens	*Acer patenoides*	
	Stereum sanguinolentum[B]	*P. abies*	
	G. applanatum	*Fagus sylvatica*	
	Grifolia frondosa[B]	*Quercus robur*	
	S. sanguinolentum	*P. abies**	
	G. frondosa	*Q. robur**	
8	*Meripilus giganteus*[B]	*F. sylvatica*	Swharze & Fink, 1998
	M. giganteus	*Tillia platyphyllos*	
9	*M. giganteus*	*F. sylvatica**	
10	*Chaetomium globossum*[D]	*Phyllostachys virideglaucascens*	Sulaiman & Murphy, 1995
11	*Oudemansiella mucida*[B]	*Pinus sylvestris*	Daniel *et al.* 1992
12	(ponded wood)	*Picea glauca*	Unligil & Chafe, 1974
13	*Inonotus hispidus*[B]	*Platinus x hispanica*	Scwharze *et al.* 1995a
	I. hispidus	*Platinus x hispanica**	
	I. hispidus	*Fraxinus excelsior*	
14	*I. hispidus*	*F. sylvatica*	Schwarze & Baum, 2000
15	*G. adspersum*	*F. sylvatica*	
16	*Phanerochaete chrysosporium*[B]	*P. abies*	Ruel *et al.* 1981
17	*Lentinus edodes*[B]	*Q. serrata***	Kim *et al.* 1988
18	*Ustilina deusta*[XA] (syn. *Hypoxylon deusta*)	*F. sylvatica* & *T. platyphyllos*	Swharze *et al.* 1995b
19	*Fistulina hepatica*[B]	*Q. robur*	Schwarze *et al.* 2000
20	*Hypoxylan mediterraneum*[XA]	*P. elliottii, P. radiata*	Francis and Leightley, 1984
21	*Hypoxylan mediterraneum*[XA]	*Eucalyptus maculata*	Francis and Leightley, 1984
22	*Neottiosporella radicata*	*P. elliottii*	Leightley, 1980
23	*C. globossum, Arthrographis cuboidea, Scytalidium lignicola, C. funicola*	*P. taeda*	Anagnost *et al.* 1994
24	*Wolfiporia cocos*[B]	*Phoenix canariensis*	Adaskaveg *et al.* 1991
25	*Streptomyces. thermovulgaris* and *S. tanashiensis*	*Tilia vulgaris*	Baeker and King, 1981

B = basidiomycete; XA = xylariaceous ascomycete; D = deuteromycete; * = naturally infected wood from living host tree; ** = naturally infected wood from log; ? = unknown.

workers in the wood products field (Anagnost *et al.*, 1998; Daniel *et al.* 1992). Possibly the key to understanding the expression of a given decay micromorphology lies in understanding the nature of the interaction of fungal species, wood species, other wood factors (e.g. extractive content of healthy wood and allopathic chemical content in stressed pathological reaction wood) and preservative content. Most of the citations in Table 3.4 involve laboratory studies and therefore the most important influences for these particular examples are probably related to the nature of interactions between fungal species and wood factors. Wood is a highly heterogenous material and considerable opportunity exists for great diversity of wood properties between wood samples of a single wood species, both within and between studies. Therefore different wood decay micromorphology may occur for a single fungal species-wood species combination. The possible significance of these factors and their interactions on decay type, particularly those possessing unusual mixtures of micromorphology are discussed in chapters 4 and 5.

Section 1.8

Ecology of wood decay

Ecology is essentially a holistic science that attempts to take account of all the contributing components of a system whilst maintaining a primary focus on the system as a single interconnected entity. Ecological studies of wood decay are usually designed to quantify the relative effects of all factors involved in determination of the severity and type of wood decay that developes and the sequence of arrival of different wood decay micoroorganisms on or in wood. These include all environmental effects, decay microorganism species effects (genotypic effects), inherent wood species effects, wood product effects such as addition of a wood preservative, and all their interaction effects. Occurrence and type of wood decay has been studied for standing trees, forest floor wood and wood products exposed to a variety of environmental conditions and publications have been reviewed in Raynor and Boddy (1988), Zabel and Morrell 1992) and Eaton and Hale (1993).

Ecology of microbial wood decay is poorly understood and studies in this field have lagged far behind ecological studies of higher plants and animals (Morrel and Zabel, 1992). According to Zabel and Morrell 1992) this is a reflection of the

difficulty of measuring decay development non-destructively within an opaque material. It is also extremely difficult to determine the relative role and importance of individual species in the decay process. This is partly a reflection of the complexity of interactions that affect the decay process but is also because isolation of the causative species is extremely difficult. Furthermore, application of Koch's postulates is very difficult in ecological studies because it is almost impossible to reproduce the complexity of natural field conditions in a laboratory. Most studies of wood decay ecology have focussed on succession of microorganims and many have been reviewed in Raynor and Boddy (1985). The term succession was originally coined to describe a predictable series of changes within a community of higher plants or animals, ending with a steady-state condition (Zabel and Morrell, 1992). Zabel and Morrell 1992) suggested that the use of this term is not entirely appropriate because almost without exception claims of succession in the wood decay process start with sound wood and end with onset of decay. This describes early colonisation only since the end of decay is marked by mineralisation of the wood. Lack of information for later stages of wood decay ecology is a reflection of the length of time wood takes to decay under field conditions, usually measured in years rather than months.

Several studies have reported the sequence of fungal species that arrive on untreated wood in ground contact. Typically, moulds such as *Trichoderma* spp., *Fusarium* spp. and *Penicillium* spp. arrive before wood decay fungi, usually within a few weeks (Corbett and Levy, 1963; Merrill and French, 1965; käärick, 1968; Butcher, 1968). Arrival of soft rot decay micromorphology typically occurs before brown rot. Concurrently, the frequency of isolation of species with known soft rot potential is greater than for brown rot species early on in wood (Merrill and French, 1965; käärick, 1968). Merrill and French (1965), as cited in Zabel and Morrell 1992), reported that soft rot arrived after 6 weeks and pockets of brown rot arrived after 12 weeks. Kaarick (1968) conducted a 4-year study of decay development in pine and spruce poles in Sweden and found that the vertical positioning relative to the ground line and soil type affected fungal isolations. A concept sometimes suggested by successional investigations is that early colonisers prepare wood for later colonisers. Alternatively the stage of arrival on wood may primarily be a reflection of inherent attributes which enable adoption of the most effective colonisation strategy for the prevailing conditions at a

particular time. Raynor and Boddy (1988) separated fungi into opportunists, stress resistors and combatants in an attempt to describe their relative attributes and suitability to the various stages of wood colonisation. Opportunists are usually bacteria and fungi adapted to rapid exploitation of wood and typically utilise non-structural wood components such as sugars, starch, resin-acids and other wood extractives and ray parenchyma cell wall carbohydrates. They tend to have rapid growth rates and rapid, prolific reproductive cycles. Many moulds and sapstain fungi have been implicated as opportunists, although some moulds arrive later and utilise breakdown products produced by decay fungi (secondary moulds) and some moulds and sapstain fungi can cause soft rot decay. Some decay fungi are considered opportunistic colonisers of stems, slash, stumps and wood products including *Schizophyllum commune*, *Phlebiopsis gigantea*, *Stereum sanguinolentum* and *Irpex lacteus* (Zabel and Morrell, 1992).

Stress resistors are adapted to adverse growth conditions such as moisture stress (high or low), low aeration and presence of toxic substances such as allopathic chemicals produced by host trees or wood preservatives added to wood products. For example wood degrading bacteria and soft rot fungi are often found in situations of high water stress (Kim and Singh, 2000; Blanchette, 1995b) and erosion bacteria appear to be adapted to low aeration (Singh and Butcher, 1991). Tolerance of copper containing preservatives has been reported for some soft rot fungi (e.g. *Lecythophora hofmannii* in Nilsson and Henningsson, 1978), some brown rots (e.g. *Antrodia vaillantii* in Eaton and Hale, 1993) and for wood degrading bacteria (Singh and Butcher, 1991). Some brown rot fungi produce copious amounts of oxalic acid, believed to be an important degradative product (Larsen *et al.* 1995) and it has been suggested that oxalic acid reacts with copper to form insoluble copper oxalate, thereby detoxifying copper containing preservatives (Eaton and Hale, 1993). A number of studies have shown that presence of wood preservatives in wood alters microbial colonisation, favouring those fungi possessing wood preservative tolerance (Greaves and Savory, 1965; Henningsson and Nilsson, 1976; Nilsson and Henningsson, 1978).

It has also been reported, that colonisation strategy is affected by wood preservative distribution across the wood cell wall. Eaton and Hale (1993) suggested that preferential uptake of CCA by the S3 wood cell wall layer enabled

soft rot fungi to decay the S2 layer. The white rot *M. giganteus* was capable of producing several types of erosion of fibre walls of dead *F. sylvatica* but only produced a soft rot like cavity attack in naturally infected (pathogenic) wood of *F. sylvatica* (Schwarze and Fink, 1998). It was hypothesised that this soft-rot mode of attack was better suited to conditions of stress that prevailed in the living host, a 90 – 100 year old beech tree. It was further suggested that this was partly a response to production of fungitoxic phenols by the living beech wood that concentrated in the cell lumen, thereby forcing cavitation attack rather than erosion of the lumen wall.

Combatants are adapted to defense of wood and can repel other fungi, either by antibiosis or pathogenesis (Zabel and Morrell, 1992). *Lenzites betulina* parasitises and replaces some *Coriolus* sp. (Rayner *et al.*, 1987; as cited in Zabel and Morrell, 1992). Many fungi possess attributes that favour adaption to more than one colonisation strategy (Raynor and Boddy, 1988).

Section 1.9

Wood preservation

The approach taken in decay prevention continues to be largely a reflection of the use situation, geography and economics i.e. selection of the cheapest form of adequately durable wood. (The increasing importance of health and environmental issues associated with use of wood preservative is covered in section 1.9.1). There are four principle hazard situations: 1) protected from the weather, 2) exposed to weather but not the ground, 3) exposed to the ground, 4) marine exposure. Naturally durable wood species have been used extensively in all these hazard situations without preservative treatment with varying degrees of success. However based on forecasts of world availablity of naturally durable wood species, it is highly unlikely that they will play a major role in ongoing traditional and new wood fibre uses. For example in New Zealand radiata pine power poles treated with preservatives containing salts of copper, chromium and arsenic (CCA) have replaced naturally durable Australian eucalypt poles.

The choice and concentration of wood preservative used to treat wood is closely related to the type and severity of hazard. Hazard level one is essentially not a microbial decay situation because it is used to describe those situations were wood

is expected to remain dry and is therefore not prone to microbial decay, but is prone to attack by some species of wood boring insects. Therefore an insectide-based wood preservative is used to protect wood in this situation in those countries where a hazard exists. Nethertheless buildings are prone to leaks, especially some modern designs, and therefore a wood preservative effective against wood decay fungi is necessary for some building designs in some geographical locations. In marine exposure situations the highest hazard is typically not fungi but marine borers such as crustacea belonging to the genus *Limnoria* and molluscs of the genus *Teredo*. However the above-water portions of marine constructions (e.g pilings and wharfs) are also subject to attack by wood decay fungi and therefore a dual wood preservative affective against both hazards is essential. Furthermore, marine fungi are known to cause soft rot and to a lesser exent, white rot, in submerged situations (Mouzouras, 1989). The requirement for a wood preservative to protect against wood decay fungi and insects at the same time is common in some countries or usage situations. For example in Australia, termites are a major problem and if sub-floor soil poisoning with insecticides is not an option, and physical barriers are inadequate, wood exposed to the weather requires treatment with a dual-acting wood preservative.

A considerable diversity of wood preservatives have been tested or used for a limited duration within the last 100 years (Richardson, 1993) but within the context of pesticide chemistry very few biocides have seen sustained commercial use as wood preservatives. Historically this has been a reflection of the ready availablity of relatively cheap raw materials however Preston (1999) suggested that this situation is likely to persist for the foreseeable future because of the cost of developing and registering new biocides. Development of broad-spectrum preservatives for use in high hazard situations was as much about availability of cheap raw materials, as specific design of mixtures of complementary biocides. For example the coal tar distillate creosote is a natural cocktail of biocides that poisons most living organisms and is likely to remain relatively cheap all the while an international coal industry flourishes. In addition to its broad-spectrum activity, the second key to the effectiveness of creosote as a wood preservative is its insolubility in water and subsequent resistance to depletion by leaching in the wet conditions typical of high hazard situations. A significant disadvantage of

creosote is its propensity to ooze from treated wood thereby exposing people, livestock and the environment to a poisonous substance.

The most significant water based wood preservative development was CCA. It was, and remains based on a combination of salts or oxides of a proven fungicide (copper), an insecticide (arsenic) and a chromium salt that mixed together in the correct ratio and incorporated into wood, react chemically to form a preservative system inherently resistant to leaching in wet situations. CCA was a landmark in wood preservative development because its combined broad-spectrum activity with longevity of efficacy was significantly beyond that provided by previous water based treatments. Acid copper chromate (ACC) (a $CuSO_4 + Na_2Cr_2O_7$ mixture) predated CCA but treated wood was susceptible to attack by copper tolerant brown rot fungi (e.g. *Poria* sp.) and termites. At about the time that wood treated with ACC was experiencing problems Kamesan patented the first CCA preservative in 1933 (Richardson, 1993). With only a few exceptions, CCA has given outstanding performance in diverse exposure situations with a service life of 30 years or more (Eaton and Hale, 1993). CCA is toxic to non-target organisms if ingested but once incorporated into wood it is relatively unavailable compared to creosote, and has not been proven to cause a significant health hazard (www.woodpreservation.com). The formulation invented by Kamesan was prepared as a dry powder and contained:

1 part $As_2O_5.2H_20$ – arsenic pentoxide,
3 parts $CuSO_4.5H_20$ – copper sulphate,
5 parts $K_2Cr_2O_7$ – potassium dichromate.

This formulation was used successfully during the 1930's and 1940's, and by the 1950's national specifications had been written to define the chemical composition of each CCA. In the United Kingdom (UK), salt-type CCAs are categorised into two types, I and II (Table 1.5) (Eaton and Hale, 1993) and a third type based on an oxide based, salt-free CAA is referred to as type III (BS 4072:1987 as cited in Eaton and Hale, 1993). The specifications for salt-type CCAs are based on the percentage composition of each compound in the preservative.

In the USA CCAs are classed as types A, B and C which specify the relative amounts of active oxides (CuO, CrO_3, As_2O_5) present in the formulation (Table 1.6) (Eaton and Hale, 1993).

Table 1.5: The percentage composition of type I (Celcure A) and type II (Tanalith C) CCA preservatives in the UK

Compound	Type I	Type II
$CuSO_4.5H_20$	33	35
$Na_2Cr_2O_7.2H_20$	41	45
$As_2O_5.2H_20$	26	20

Table 1.6: The percentage composition of type A, B and C CCA preservatives in the USA (pH limits between 2.5-3.2)

Oxide	Type A	Type B	Type C
CuO	18.1	19.6	18.5
CrO_3	65.5	35.3	47.5
As_2O_5	16.4	45.1	34.0

Complex reactions occur between the constituent salts of CCA in solution and wood and whilst not completely understood a comprehensive account of many of the reactions involved was provided in a review by Plackett (1983). It is generally accepted that immediately after CCA treatment there is an increase in pH caused by ion-exchange and adsorption reactions with the wood. A maximum pH of 5 is reached at the point when all chromium is consumed, the culmination of a series of precipitations of the active ingredients. After a complex series of oxidation and reduction reactions, copper fixes to the cell wall by ion exchange and other reaction products include Cr_2O_4, $Cu(OH)CuAs_2O_4$ and $Cr(OH)_3$. Treated wood must remain wet during fixation and the time to completion of fixation reactions depends largely on temperature and whilst 2 weeks has traditionally been recommended greatly accelerated fixation at high temperatures is possible (Nasheri, *et al.*, 1998).

Other preservatives are used in high hazard situations (Richardson, 1993) and this is typically either a legacy of a local established approach or because of local restrictions related to what constitutes acceptability in terms of potential health and environmental hazards. For example in the United States of America (USA) a traditional oil-based mixture of the fungicide pentachlorophenol and the insecticide dichlorvos is used to treat utility (power) poles. In a few European countries CCA has been banned because of health and/or environmental concerns

and in the USA the wood preservation industry in association with the Environmental Protection Agency (EPA) has decided to phase out its use for many applications by early 2003.

Broad-spectrum preservatives like creosote and CCA were developed for high hazard situations such as exposure to the ground, marine exposure and aquatic industrial situations (e.g. cooling towers). The decay hazard encountered by preservative treated wood exposed to the weather but not ground contact, such as decks and building claddings, is less than occurs for wood in ground contact. This is primarily because preservative depletion mechanisms are less severe (biodeterioration, weathering and leaching), decay microorganims typically arrive in the form of spores that are less invasive than actively growing mycelium present in soil, and wood moisture levels suitable for fungal establishment and subsequent decay are less sustained. The lower decay hazard typical for wood kept out of ground contact enables use of lower preservative concentration or use of preservatives with a narrower spectrum of biocidal activity and/or those of adequate persistence under a lower depletion hazard. Another factor that affects the selection of wood preservative is the need to avoid inducing wood dimensional instability by using water-based preservatives such as CCA, particularly for wood used in construction. This led to the development of solvent-based preservatives such as those containing copper and tin naphthenate used to prevent fungal decay and those containing organophosphate compounds and synthetic pyrethroid insectides for control of insect borers, or mixtures thereof.

A vital part of decay prevention is homogenous wood treatment or use of adequate envelope treatments that encapsulate untreated wood. Typically, this is achieved by selection of a treatable wood species and pressure impregnation of the preservative. Typically wood is placed inside a cylinder, a partial vaccuum created, then the cylinder is flooded with the preservative. For water-borne and heavy oil preservatives a pressure is usually then applied for a defined period of time to force more liquid into the wood but pressures are not used for solvent based preservatives due to their inherent flammability. Once the cylinder has been drained of preservative solution a final vacuum is often applied for a short period to drain some preservative solution from the surface wood layers to prevent dripping after wood is removed from the cylinder. Pressures are used for oil-based

systems that have a high flash point temperature such as creosote or pentachlorophenol in oil mixtures but for lighter solvents that have a lower flash point, pressures are not used. Typically, pressures are not required for light organic solvent based preservatives (LOSP) because they are usually used for treating wood in lower hazard situations that do not require complete impregnation, or because adequate impregnation is easily achieved for the size of commodity typically treated.

A vaccuum-flood-pressure treatment cycle like that developed by Bethel (Richardson, 1993) is sometimes referred to as a full-cell process because the wood cells are essentially filled with the preservative solution after treatment. This has the advantage that high preservative concentration can be achieved with relatively low solution concentration. However in some situations it is desirable to use less preservative solution. This can be achieved by removing the vacuum step (Lowry process) or by applying a small initial pressure (<4 bar) before the flood step (Rüping process) (Eaton and Hale, 1993). These processes entrap air in the wood cells and therefore less preservative solution is absorbed and in the case of the Rüping process the initial pressure step results in excess preservative being forced out of the wood after the final pressure is removed. Between 50 – 60% of initial absorption can be recovered with a Rüping type process and this coupled with lower initial uptake, compared to a Bethell process, results in much lower total uptake. Whilst not strictly accurate, these processes are often referred to as empty cell processes. The extent and duration of pressure used in commercial processes varies depending on the wood species, commodity type and preservative retention aimed for. For easy to treat softwoods 10 – 14 bar is typical but for difficult to treat (refractory) Australian eucalypts up to 69 bars have been used (Eaton and Hale, 1993). Temperature of the treatment solution is sometimes raised to improve uptakes of heavy oils or to assist fixation of water borne preservatives (Nasheri *et al.*, 1998).

Many wood species with desirable physical attributes that make them preferred species for construction are refractory. This is usually because they are comprised of a high percentage of heartwood with ultrastructural characteristics that make them difficult to impregnate with liquids, even under enormous pressures (Wilkinson, 1979). For example aspiration (closing-off) of pits, blocking of cell

lumenae by tyloses and presence of extractives in parenchyma and other woody tissues all contribute to the refractory nature of heartwood of many commercially important wood species. For example Douglas-fir (*Pseudotsuga menziessii*) is a preferred constructional wood species in many areas of North America but is notoriously difficult to treat with wood preservatives. In an attempt to combat this situation enormous effort has been invested in development of incising techniques. Incising involves introducing many small longitudinal cuts by passing wood through rollers with many strategically placed shallow knives (some techniques involve drilling). This is very effective at improving radial penetration of wood preservative but less effective at achieving tangential penetration. The effectiveness of incising is controversial. For wood exposed in lower and moderate hazard situations it is probably a largely satisfactory way of providing greatly improved decay resistance.

Section 1.9.1

Selection and testing new wood preservatives

Eaton and Hale (1993) described the types and properties of candidate new wood preservatives that were, and in many cases still are under investigation internationally. This short list has not expanded appreciably since and Preston (1999) discussed many of the reasons for the shortness of the list of potential biocides that might be used as wood preservatives. The rigorous toxicological, health and environmental safety testing regimes required by pesticide registration authorities are prohibitively expensive if derived biocides can only be applied as wood preservatives. Therefore new molecules have usually been sourced from other industrial sectors. For example agricultural fungicides tested as candidate wood preservatives typically have already undergone thorough screening against many plant pathogenic fungi, some of which are likely to be of the same genera and species as wood decay fungi. Preston (1999) suggested that the short list of available molecules is likely to get shorter and that well-established molecules are likely to continue to play leading roles in new preservative systems well into the future.

Two new wood preservative systems that are now approved for use in Europe, Japan, North America and Australasia are ammoniacal copper quaternary (ACQ) preservatives and copper-azole (CuAz) preservatives. ACQ is a slightly

misleading term now because modern versions have moved away from use of ammonia as a solvent for the copper salts used (e.g. copper chloride and copper carbonate), towards use of other alkaline solvents such as ethanolamine which have greater stability and fewer health concerns. Quaternary ammonium compounds, including alkyl ammonium compounds, are used widely as cleaning agents, disinfectants and sterilants in many industrial, municipal and domestic sectors. They have a broad spectrum of biocidal activity, are relatively cheap and are not readily leached from wood (Drysdale, 1983). The azole component of CuAz refers to use of a triazole fungicide such as tebuconazole (Exner, 1991).

Preservative test methodology typically involves the following sequence of tests.

1. Rapid laboratory screening of a relatively large number of chemicals.
2. Use of standard laboratory test procedures, normally on wood, according to national or international standards, to test performance of a short list of candidate treatments against fungi and insects.
3. Field testing.

The focus of phase one is on provision of comparative performance data of many candidate mixtures and concentration ranges of chemicals. Therefore the methodology must be straightforward and rapid providing data within a few weeks. Many agar plate and mini-wood block methods have been developed for this purpose (reviewed in Eaton and Hale, 1993; Zabel and Morrell, 1992). In agar plate tests candidate chemicals are mixed with agar over a range of concentrations and the linear growth rate of test fungi across the plate can then be measured. Alternatively, a 5 mm diameter filter paper disc pre-soaked in the test chemical is placed in the centre of a plate which is then either evenly or spot inoculated with the test decay fungus. The concentration of test chemical that just prevents fungal growth is termed the minimum toxic threshold concentration for control. The main disadvantage of agar plate tests is that the nature of the "carrier" agar is very different to wood and therefore important wood-preservative interactions are not tested. It is therefore difficult to extrapolate toxic threshold concentrations derived from agar tests to wood. In mini-block tests the chemical is impregnated into small (e.g. 7.5 x 7.5 x 40 mm) wood blocks and these are then placed onto an agar culture of the test fungus, usually astride a plastic mesh. Wood loss is used as a

measure of decay and toxic threshold concentrations that prevent decay can be derived as described for the national standard methods below.

National standard methods used in the second phase are more time consuming because great care is taken to follow precise and meticulous methodology and additional standard methods that stipulate prior wood conditioning and leaching regimes are also applied. In North America, the standard ASTM D1423-61 (cited in Eaton and Hale, 1993) uses soil as a medium for supporting fungal growth prior to and during exposure of test blocks. A loam soil at about 130% of its water holding capacity is placed in a glass jar so that it occupies about half its volume. Untreated wood "feeder-strips" are placed on the surface of soil and sealed jars are then sterilised by autoclaving. Following sterilisation, the feeder strips are inoculated with either *Lentinus* (*Neolentinus*) *lepideus, Gloeophyllum trabeum, Oligoporus* (*Postia) placenta* or *Trametes* (*Coriolus*) *versicolor* and incubated at optimal temperature for about three weeks to enable the fungus to become well established in the feeder strip and associated soil. Chemicals can be tested against these fungi in softwood or hardwood 19 mm sapwood cubes; the first three fungi against treated softwood cubes, all four against a treated hardwood species. Leaching and evaporative ageing tests are conducted on preservative treated cubes, followed by sterilisation by autoclaving, prior to placing two cubes on to the infected feeder strips in the test jars. Jars are then incubated for 12 weeks and the weight loss due to fungal decay is measured for each cube. By using a graded, usually geometric concentration range of treatment solutions for each set of replicate test blocks, a range of preservative retentions can be achieved. The retention is calculated by measuring the weight of blocks before and after treatment. The threshold retention is that retention of test chemical that prevents weight loss from decay by a particular fungus.

The advantage of using agar instead of soil is that it can be standardised anywhere and agar is also an inherently easier material to work with under laboratory condtions. The European standard EN 113 is based on a malt extract agar (Eaton and Hale, 1993). Two obligatory fungi are included, the brown rot fungus *Coniophora puteana* and the white rot fungus *Trametes* (*Coriolus*) *versicolor*. Two additional fungi must also be included according to the test product. The suggested fungi are *Lentinus* (*Neolentinus*) *lepideus* and *L. cyathiformis*

(*Neolentinus schaefferi*) for use with tar oil-based products and *Oligoporus* (*Postia*) *placenta*, *Gloeophyllum trabeum* or other local fungi of importance, or fungi of particular importance to special use situations or product types. Test blocks 50 x 25 x 15 mm are prepared from *Pinus sylvestris* or *Fagus sylvatica* sapwood and are treated with a range of concentrations of the test chemical. After sterilisation of blocks using either ionising radiation or gas sterilisation using ethylene or propylene oxide, two blocks per jar are placed on glass rod supports above the fungus where one block is an untreated control. Incubation is for 16 weeks and decay is measured as average % weight loss to determine the toxic values of the chemical against each of the test fungi. Two toxic values, expressed in kg of preservative per cubic meter of the wood are derived. Firstly, that concentration which protects the wood (mean mass loss is less than 3% of the initial dry mass of the blocks after exposure) and secondly, the next concentration down the series where decay does occur. Prior to exposure of blocks to test fungi leaching and evaporative ageing standard methods are used. Leaching involves impregnating blocks with water followed by soaking in water that is changed regularly over a two-week period. This is designed to remove unfixed chemical and simulate the leaching that would occur when wood is used in exposed service situations. Evaporative ageing involves placing the blocks in a temperature controlled wind tunnel and is designed to accelerate evaporative loss of solvents and active ingredients.

Testing new candidate wood preservatives against soft rot fungi is usually carried out in jars or bins of unsterile soil. The "fungus-cellar" test uses soil beds kept under optimal temperatures and high humidity. Stakelets 5 x 10 x 150 mm are placed vertically to one half their length in soil beds and are assessed approximately every 3 months for decay. The extent of decay is assessed using a rating scale similar to AWPA Standard M7-83 (AWPA, 1999):

Grade No.	Description of Condition
10	Sound
9	Trace of decay or termite attack
7	Moderate decay or termite attack
4	Heavy decay or termite attack
0	Failure to decay or termite attack

In some countries, toxic values derived from laboratory tests are required for registration of the preservative and are presumably assumed to be proof of efficacy (Hedley, 1993). Hedley and Butcher (1985) reviewed the rationale behind laboratory decay tests in New Zealand and it was concluded they achieve the following.

1. Provide a reference point of the basic toxicity of a given preservative when challenged by a selection of decay organisms.

2. Generate a base value (threshold value, toxic limits) which may then be used as a meaningful starting point for further evaluation using field tests.

In field stake tests the timber is selected for uniformity and treatment is designed to give even distribution of preservative throughout the test specimens as is the case for laboratory trials. However in field tests the conditions and performance vary according to the climate and soil type (Wakeling, 1991; Hedley *et al.*, 1996; Edlund, 1998). Hedley (1993) compared toxic limits derived from laboratory trials with performance data derived over 40 years of field testing using field test stakes, fence post tests and pole tests. For all three types of preservative examined (CCA, creosote and creosote plus oil, and alkyl ammonium compounds) there was no consistent correlation between threshold values from laboratory tests and field performance. Hedley (1993) went on to question the value of laboratory tests as a relevant component of an approval-seeking testing protocol.

Hedley (1993) further suggested that provisional approval for ground contact use should be given to the retention in stakes which were 100% sound after 5 years. At 10 years this retention would need to be not less than 95% sound to gain full approval. The basis for this suggestion appeared to be the field performance of recognised standards after 5 and 10 years exposure in ground contact, as given in Table 1.7.

Table 1.7: Field performance of 20 x 20 x 500 mm stakes after 5 and 10 years in ground contact at Whakarewarewa field test site (Rotorua, New Zealand).

			Mean % Soundness**	
	Retention Kg/m^3	Hazard Class*	5 Years	10 Years
CCA (AWPA Type A)	2.3 TAE	H3	100	85
	4.6 TAE	H4	100	98
Creosote	90	H3	98	85
	191	H4	100	98
5% PCP/oil	4.5 PCP	H3	100	84
	8.8 PCP	H4	100	94
Cu Naphthenate/oil	0.9 Cu	H3	100	92
	1.8 Cu	H4	100	99

*Hazard Class according to the New Zealand Timber Preservation Council (MP3640, 1992). **Mean soundness was calculated from the soundness values derived from 10 replicate stakes. Stakes were given a rating that best described their condition according to the following scale. TAE = total active element.

1 = zero decay,

2 = 90% sound (10% of cross-section removed where decay was greatest),

$^{+}$3 = 80% sound (20% of cross-section removed where decay was greatest),

3 = 70% sound (30% of cross-section removed where decay was greatest),

$^{-}$3 = 60% sound (40% of cross-section removed where decay was greatest),

4 = 40% sound (60% of cross-section removed where decay was greatest),

5 = zero soundness (stake failed – breaks upon light tap on side of boot).

However a later publication (Hedley *et al.*, 1996) showed that Whakarewarewa test site had a less severe decay hazard than was demonstrated at the two test sites located at Glenbervie and Hari Hari (Table 1.8). Whilst the data given was for replicate sets of stakes after 16 years it seems unlikely that the soundness values at ten years would have met the 95% soundness criteria outlined by Hedley (1993). The ground contact retention of Tanalith NCA (AWPA Type B) closest to the current New Zealand Standard 3640 minimum requirement (MP3640, 1992) for CCA in radiata pine for Hazard Class 4 (ground contact) (0.72 % m/m TAE, equivalent to approximately 3.24 kg/m^3, based on a wood density of 450 kg/m^3) was 3.3 kg/m^3 TAE. At 16 years the mean soundness of radiata pine stakes treated with 3.3 kg/m^3 TAE of Tanalith NCA was 68 and 57% at Hari Hari and

Glenbervie respectively. It is highly unlikely that stakes were 95% sound at 10 years exposure and then lost 32 and 43% of their mean soundness at Hari Hari and Glenbervie respectively, over the 6 year period between 10 and 16 years. On the other hand, if performance had decreased at this rate over this 6 year period then this would also cause concern regarding the validity of the guideline of 95% soundness at 10 years being considered a requirement for determining adequate performance of new candidate preservatives.

Table 1.8: Comparison of performance of 25 x 50 x 500 mm (IUFRO*) radiata pine stakes treated with tanalith NCA (Hedley *et al.*, 1996) after 16 years

Treatment / Retention TAE Kg/m^3	% Mean Soundness Reduction			
	Site			
	Glenbervie	Wakarewarewa	Hanmer	Hari Hari
6.2	80	96	99	80
4.5	71	86	98	73
3.3	57	74	92	68
2.4	43	66	70	66

*IUFRO stipulates the required size of field test stake.

These results suggested that the guidelines of Hedley (1993) require revision. They also show the inter-site variability of decay hazard and it is not clear from the literature how many and what type of sites should be used for field testing new candidate wood preservatives, a subject which is addressed in this study.

Section 1.9.2

Biochemical mode of action of wood preservatives

The mode of action of biocides is a complex and incompletely understood subject and only a few basic principals are covered here. Many biocides prevent normal cellular activity by denaturing proteins and disrupting plasma membranes and because thay exert an effect across many basic cellular functions they are typically referred to as non-specific (Eaton and Hale, 1993). For other biocidal molecules the molecular mode of action is known. For example triazole fungicides, including tebuconazole used in the copper-azole wood preservative Tanalith E tested in this study, act by blocking specific steps in the ergosterol biosynthesis pathway. The specific actions of many of the active ingredients in wood preservatives are not known. Eaton and Hale (1993) divided specific acting fungicides into, a) inhibitors of respiration that prevent normal functioning of the tricarboxylic acid cycle (TCA or Krebs cycle), b) inhibitors of biosynthetic reactions in cells, and c)

inhibitors of cell division. For a biocide to exert a toxic effect it must be mobile upon contact with the microbial cell wall so that it can enter the cytoplasm and disrupt normal cellular and metabolic functioning. The mechanism of transfer across the microbial cell wall and plasma membrane is poorly understood for many biocides and the chemistry of some biocides changes once inside microbial cells. Copper chelates such as a 1:2 copper:8-hydroxyquinolinolate are more biocidal than Cu^{2+}, and this is believed to be because the chelate is lipophilic and is therefore more mobile across the lipid membrane of fungal cells (Eaton and Hale, 1993). Copper in CCA preservatives is in the form of Cu^{2+}, and this must therefore pass into the cell by a different mechanism. Transfer of anions and cations across microbial cells involves active transport mechanisms, often referred to as "ionic pumps" (Alberts *et al.*, 1983) and it is likely that these are involved in movement of biocidal ions such as Cu^{2+}. Some brown rot fungi for example *Poria* sp. produce oxalic acid (Larsen *et al.*, 1995) which is believed to detoxify copper by forming insoluble copper oxalate (Green and Clausen, 2003). This suggests that some fungi have a limited ability to regulate uptake of Cu^{2+} by modifying active ionic uptake or passive diffusion. Possibly the transport mechanisms involved do not distinguish biocidal ions such as Cu^{2+} from essential bivalent cations ions like Ca^{2+} (calcium).

Within the cytoplasm Cu^{2+} has an affinity for thiol groups, resulting in denaturation of proteins and enzymes (Siegel and Sisler, 1977). Copper ions are also implicated as inhibitors of pyruvate dehydrogenase which converts pyruvate to acetyl CoA, a step between glycolysis and reactions within the TCA cyle. Arsenic, phenolic groups (e.g. those of pentachlorophenol) and tri-butyl tin interferes with oxidative phosphorylation. Quaternary ammonium compounds such as didecyl dimethyl ammonium chloride are believed to disrupt the lipid bilayer of semipermeable membranes, causing cell constituents to leak out of cells and cell organelles, and by allowing water to leak in thereby disrupting osmoregulation (Siegel and Sisler, 1977). Some of the more recently discovered fungicide reaction sites have been reviewed by Henry and Gustafson (2003).

Wood preservatives used in ground contact have tended to rely on use of broad spectrum biocides such as mixtures of biocidal salts (e.g. CCA) and natural cocktails of toxic substances like creosote (Richardson, 1993). As these "robust"

preservatives loose favour because of health and environmental concerns it is increasingly important to consider the mode of action of candidate molecules so that they can be combined to exert an appropriate spectrum of activity and longevity of effectiveness. For example copper is a broad spectrum fungicide in general terms but has known weakness against some copper tolerant fungi, such as some brown rot fungi in soft woods (Wakeling and Singh, 1993) and soft rot fungi in hardwoods (Johnston, 1986). It is therefore recognised that copper needs to be combined with another fungicide when protection in high biological hazard situations is required. Triazoles have been shown to control brown rot fungi in laboratory trials (Valcke and Goodwin, 1985; Valcke, 1989). Possibly this is the rational for combining triazole fungicides with copper. For example the copper-azole fungicide Tanalith E contains copper, boric acid and tebuconazole in a ratio of 25:10:1 (Creffield *et al.* 1996). The fungicidal value of boric acid is somewhat contentious but in above ground situations it is likely to contribute fungicidal and insectidal activity for several years (probably less than 5 years). As well as the complementary activity of a copper plus tebuconazole mixture against groups of decay fungi such as copper tolerant brown rots (softwoods) and soft rots (hardwoods), it has been suggested that copper exerts a protective effect over the tebuconazole, an organic molecule that might otherwise be susceptible to biodegradation.

Section 1.10

Objectives of this investigation

The influence of environmental factors on wood decay type has rarely been investigated systematically across diverse field test site conditions. Most observations of decay micromorphology in wood that was naturally infected in the field have been reported because they were related to in-service wood failure, were from wood preservative field test sites where the primary objective was wood preservative performance, or were from extreme exposure environments such as industrial cooling towers (Eaton, 1994). Rarely has wood been exposed for the purpose of determining the effects of field conditions on decay type diversity and elucidation of associated decay type effects on wood preservative performance. Preservative treated wood is known to have differential susceptibilities to different decay fungi and there are specific examples of preservative tolerance. For example soft rot fungi are tolerant of high retentions of

CCA in hardwoods (Eaton and Hale, 1993), CCA treated pine is susceptible to attack from tunnelling bacteria in horticultural soils (Singh and Butcher, 1991) and to brown rot in some situations were soil inoculum potential is elevated (Wakeling and Singh, 1993) and also to some soft rot fungi (e.g. *Phialophora* sp.) in certain situations (Henningsson and Nilsson, 1976).

However, in general, traditional wood preservatives such as creosote, pentachlorophenol and CCA have given reliable performance across diverse field conditions because of well-defined properties and it is not certain that candidate new preservative systems will be of a similarly robust nature. They tend to contain biocides of a narrower specificity in terms of toxicity towards the target pests and may also be more susceptible to depletion mechanisms such as biodegradation and leaching. It is therefore vital to test them over a wide range of field conditions and gain insight into their relative strengths and weaknesses. The objective of this thesis was to investigate these factors, improve understanding of the diversity of decay type in the field, assess its implications for decay classification nomenclature, and assess the merits of light microscopy and confocal laser scanning microscopy as tools for field investigations compared to other microscopy techniques. The following studies addressed these objectives.

1. The performance of radiata pine (*Pinus radiata* D. Don.) and European beech (*Fagus sylvatica* L.) 20 x 20 x 500 mm field test stakes treated with modern wood preservatives was compared to performance given by traditional preservatives in diverse environments after 5.5 – 6.5 years in ground contact (chapter 3).

2. Determination of wood decay-type diversity in radiata pine and European beech using light microscopy, and determination of the ability of current wood decay classification nomenclature to describe it (chapter 4).

3. Effect of environmental factors, preservative type, wood species and their interactions on wood decay type as defined by wood cell wall decay micromorphology (chapter 5).

4. Effect of field test site conditions on preservative depletion (CCA and CuAz) from pine and beech (chapter 6).

5. Suitability of confocal laser scanning microscopy for characterisation of wood decay in field test stakes (chapter 7).

CHAPTER TWO
MATERIALS AND METHODS

Section 2.1
Effect of test site location on in-ground preservative performance

Section 2.1.1
Preservative treatment of field test stakes

Radiata pine (*Pinus radiata* D. Don) defect-free, sapwood boards were selected at a local sawmill and kiln dried using dry and wet bulb temperatures of 70 and 60°C, respectively. Pre-dried (method unknown) European beech (*Fagus sylvatica* L.) from Yugoslavia and Denmark was obtained from a local timber merchant. Darkly stained heartwood was rejected. Boards were sawn and planed to a final size of 20 x 20 mm, prior to sawing into stakes, 500 mm long. Grain orientation in the stakes varied.

The following preservatives were used.

a. Tanalith C®, a class 1 (New Zealand Timber Preservation Council (MP3640, 1992)) preservative containing copper (Cu), chromium (Cr) and arsenic (As) (CCA), (8.9% mass/mass (m/m) Cu, 15.9% m/m Cr and 11.3% m/m As) (this is very close to the active element ratio of an American Wood Preservers Association (AWPA) Type C CCA) (Section 1.9).
b. A 60/40 mixture of high temperature Australian creosote and oil (C).
c. Ammoniacal copper (7.76 % m/m Cu as copper oxide) plus didecyl dimethyl ammonium chloride (DDAC) (1:0.8 ratio of CuO:DDAC) (ACQ). The intended ratio of CuO:DDAC was 2:1 however the concentration of DDAC added to the ammoniacal copper treatment solution was too high.
d. Tanalith E® (formerly known as Tanalith 3485), a formulation containing copper, boric acid and tebuconazole (α-[2-(4-chlorophenyl)ethyl]- α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) in a ratio of 25:10:1 (CuAz).
e. Chlorothalonil (4.55% m/m) plus chlorpyriphos (concentration unknown) in P9 Shell oil (CC).

Tanalith C® and Tanalith E® are registered trademarks of Arch Chemicals and were supplied by Koppers Arch Wood Protection (NZ) Ltd. ACQ was supplied by Chemical Specialities Inc (USA). Chlorothalonil plus chlorpyriphos in P9 Shell oil was supplied by ISK Biosciences (USA).

Sufficient stakes were treated to allow rejection of 30%, representing the extremes of preservative solution uptake, prior to installation in the test sites. Target preservative retentions are given in Table 2.1. For water borne preservatives, treatment solution concentrations used were based on a predicted uptake of 630 litres per cubic meter for radiata pine and 550 litres per cubic meter for beech using a conventional Bethel type treatment process. For CC, a double vacuum treatment cycle was used in an attempt to achieve an average uptake of 90 litres per cubic meter so that the target highest retention could be achieved without dilution of the CC preservative. For creosote, working solution concentrations were calculated based on an uptake of 200 litres per cubic meter using a vacuum only treatment cycle. For beech treated with water borne preservatives and for pine treated with solvent based preservatives a trial treatment cycle (highest retention only) using 20 stakes was carried out to determine solution uptakes, whereas for pine treated with water borne preservatives, predicted uptakes were based on previous *Forest Research* experience.

Working solutions of CCA, ACQ and CuAz were water based and were diluted with water to achieve the required treatment working solution. CC was an oil-based formulation and was used undiluted for the highest retention aimed for and was diluted with toluene to achieve the lower chlorothalonil solution concentrations required for lower retentions. The creosote plus oil mixture was used undiluted for the highest concentration and was diluted with Mobil dry cleaner hydrocarbon solvent for the lower solution concentrations required. All preservatives were used as supplied by the manufacturer except ACQ for which the ammoniacal copper oxide concentrate was supplied, but the DDAC was mixed into the working solution using Bardac 2280 (80% DDAC plus 20% ethanol) (Chemcolour Industries, (NZ) Ltd.).

All preservative solution concentrations were expressed as units of percentage mass/mass and all preservative concentrates were weighed out prior to mixing with

the diluent (water, toluene or Mobil dry cleaner solvent). The preservative concentrate was weighed into a glass vessel which was then washed out into a 100 litre drum placed on a digital scale using the appropriate diluent. Diluent was then added until the required weight of solution was present to achieve the desired solution concentration.

For all treatments, stakes were stacked in filleted layers using 20 x 20 x 500 mm untreated stakes within a stainless steel tank. For water borne preservatives and creosote the tank was placed in a vacuum pressure cylinder before submerging in the preservative which was subsequently fed into the tank via a plastic tube at the appropriate time during the treatment cycle. For CC the tank was flooded prior to installation in the vacuum cylinder.

Preservative Treatment Cycles

Water borne preservatives

1. Vacuum (-90 kilopascals (KPa)) maintained for 20 minutes.
2. Tank flooded during which vacuum dropped to close to -85KPa (8 - 10 mins).
3. Pressure of 660KPa maintained for 60 minutes.

Creosote

1. Vacuum (-90 KPa) maintained for 20 minutes.
2. Tank flooded and vacuum maintained for 10 minutes.
3. Soaked at atmospheric pressure for 30 minutes.

CC

1. Tank flooded.
2. Vacuum (-70KPa) reached after 2 minutes and maintained for a further 10 minutes.
3. Soaked at atmospheric pressure for 10 minutes
4. Tank drained (10 minutes).
5. Second vacuum (-80KPa) for 20 minutes.

At completion of the treatment cycle the preservative solution remaining in the treatment tank was pumped out into a holding tank and the stakes allowed to drain for 10 minutes prior to their removal. For water borne preservatives, the first treatment cycle for each preservative was carried out using the lowest solution treatment solution concentration (for the lowest target retention). The remaining solution after treatment was topped up with the required amount of preservative concentrate and water to achieve a solution concentration needed for the next highest retention. For solvent borne preservatives the undiluted concentrate was used first and the remaining solution was diluted with either toluene (chlorothalonil plus chloropyriphos) or Mobile dry cleaner solvent (creosote/oil).

Stakes were weighed immediately before and after preservative treatment to enable calculation of solution uptake. After treatment, stake dimensions were measured at the epicentre using digital callipers to determine stake volume and enable calculation of wood density. For water borne treatments, stakes were held for 6 weeks in block stack, wrapped in plastic, to allow fixation reactions to occur. Solvent borne preservative treated stakes were held in block stack in plastic for 2 weeks. All stakes were then stacked using spare 20 x 20 x 500 mm untreated stakes allowing a 10 mm gap between stakes in a layer. Stacks were built in an out-building with slatted sides and electric fans were used to encourage a steady but low air-flow. Care was taken to ensure stakes dried slowly, over a period of 2 weeks. Mould growth was observed on the 3 lower retentions of CuAz and ACQ treated pine stakes (*Penicillium* sp.) and on stakes treated with the two lowest CCA retentions (*Penicillium* sp and *Cladosporium* sp.).

A minimum of six spare stakes (pine and beech) treated with the highest retention (from the 30% reject sample) of CCA, ACQ, CuAZ and chlorothalonil were analysed to enable comparison of predicted preservative retention (based on grams of preservative solution taken up during treatment) and actual retentions were determined by chemical analysis (Section 2.4).

Table 2.1: Preservative treatments and target preservative retentions

Treatment N°	Wood / Preserva-tive / retention	No. of Stakes at each of 13 sites	Preservative								
			Site ID (Stake label)		Target concentration (a.i. kg/m^3 active ingredient)						
					CCA			Creosote /oil	ACQ	CuAz (CAZ)	CC
			N°	Reten-tion	Cu	Cr	As	creosote (C)	Cu	Cu	Ctln*
1	Pine (P)	40	1	-	Untreated control						
2	Beech (B)	30	2	-	Untreated control						
3	PC1	22	3	1	-	-	-	47	-	-	-
4	PC2	20		2	-	-	-	71	-	-	-
5	PC3	18		3	-	-	-	107	-	-	-
6	PC4	18		4	-	-	-	160	-	-	-
7	PACQ1	22	4	1	-	-	-	-	0.76	-	-
8	PACQ2	20		2	-	-	-	-	1.14	-	-
9	PACQ3	18		3	-	-	-	-	1.71	-	-
10	PACQ4	18		4	-	-	-	-	**2.57**	-	-
11	PCAZ1	22	5	1	-	-	-	-	-	0.89	-
12	PCAZ2	20		2	-	-	-	-	-	1.33	-
13	PCAZ3	18		3	-	-	-	-	-	2	-
14	PCAZ4	18		4	-	-	-	-	-	**3.0**	-
15	PCCA1	22	6	1	0.2	0.36	0.26	-	-	-	-
16	PCCA2	20		2	0.3	0.53	0.39	-	-	-	-
17	PCCA3	18		3	0.45	0.8	0.58	-	-	-	-
18	PCCA4	18		4	0.67	1.2	0.87	-	-	-	-
19	PCCA5	18		5	**1.0**	**1.8**	**1.3**	-	-	-	-
20	PCC1	22	7	1	-	-	-	-	-	-	1.42
21	PCC2	20		2	-	-	-	-	-	-	2.13
22	PCC3	18		3	-	-	-	-	-	-	3.2
23	PCC4	18		4	-	-	-	-	-	-	**4.8**
24	POIL1	22	8	1	50:50 Oil/Mobil dry cleaner (CC solvent blank 1)						
25	POIL2	22		2	Oil (CC solvent blank retention 2)						
26	SOLV	22	9	1	Mobile dry cleaner (solvent blank)						
27	BCCA1	20	10	1	0.3	0.53	0.39	-	-	-	-
28	BCCA2	20		2	0.67	1.2	0.87	-	-	-	-
29	BCCA3	15		3	1.5	2.7	1.89	-	-	-	-
30	BCAZ1	20	11	1	-	-	-	-	-	2	-
31	BCAZ2	20		2	-	-	-	-	-	4.5	-
32	BCC1	20	12	1	-	-	-	-	-	-	2.13
33	BCC2	20		2	-	-	-	-	-	-	4.8
34	BOIL	20	13	1	Oil (CC blank)						

CAZ = CuAz. Retentions recommended by manufacturer (for CCA, New Zealand Timber Preservation Council (MP3640, 1992)) in-ground (Hazard class 4) minimum retention requirement was used) are in bold. For ACQ and CuAz treated pine these were the recommended retentions in 1993 and are higher than retentions specified in current national standards (Section 3.2). Other retentions were included to ensure early decay. A geometric concentration series of 1.5 was used for pine treatments and BCC. For BCCA and BCAZ the highest retention was 1.5 times as high as for pine and a geometric series of 2.25 (1.5x1.5) was used. *ctln = chlorothalonil.

Section 2.1.2

Comparison of predicted preservative retention based on preservative solution uptake and actual retentions determined by chemical analysis

Predicted and actual preservative retentions for each preservative solution are given in Tables 2.2 to 2.10.

Preservative uptake as grams (g) of active ingredient (a.i.) per 100 grams of wood (% mass/mass a.i.) was calculated using the following formula:

(SU x C/100) x 100/WS

where SU = solution uptake (grams), C* = concentration of working solution as grams per 100 grams of water or solvent and WS = weight of stake in grams.

Uptake expressed as kg of a.i. per cubic meter (m^3) of wood was calculated as follows:

(d x %m/m a.i.)/100

where d = wood density (kg/m^3) calculated as WS x (1,000,000/volume of stake)

For example for stake 1215 (Table 2.2) predicted preservative retention as % mass/mass (m/m) a.i. was calculated as follows

(114.26 g x 0.56% m/m a.i. CCA/100) x 100/106.73 = 0.599% m/m a.i. CCA.

Where 106.73 = weight of stake (g) before treatment and 114.26 = weight (g) of stake after treatment.

Wood density = 106.73 x 1,000,000 / 20.75 x 20.4 x 500 = 504.276 kg/m^3

Where 20.75 x 20.4 x 500 were stake dimensions in millimetres (mm).

Predicted preservative retention expressed as kg/m^3 a.i. was calculated as follows

(504.276 x 0.599)/100 = 3.021

Preservative retention expressed as Kg/m^3 of CCA salt = 3.021/0.361 = 8.368 kg/m^3

Where 0.361 is the active ingredient fraction of Tanalith C i.e. 36.1% Cu, Cr and As. (This step was missed out for the other preservatives).

* The treatment solutions retained for chemical analysis were mistakenly discarded and therefore predicted retentions were based on the solution concentration aimed for i.e. the actual concentration determined by chemical analysis was not available. For BCCA5 the unexpectedly low actual retentions for stakes (Tables 2.6 and 2.7) suggested an error had been made with the quantity of Tanalith C added to the treatment solution. Unfortunately, the logbook sheets for beech treatments were lost so the source of the error could not be identified. The additional analysis of lower retention stakes (only carried out for BCCA) showed that the error was not consistent over the retention range used (Table 2.7).

Table 2.2: Predicted and actual CCA retentions in pine (retention 5 (PCCA5))

Stake N°	Wood density Kg/m^3	Predicted			Actual*
		g/100g a.i.(%)	a.i. kg/m3	kg/m3 CCA salt	g/100g a.i.(%)
1215	504.28	**0.60**	3.02	8.37	**0.66**
1244	586.13	**0.54**	3.17	8.78	**0.60**
1256	447.60	**0.87**	3.90	10.81	**0.87**
1272	595.33	**0.52**	3.12	8.66	**0.56**
1318	441.91	**0.89**	3.93	10.89	**0.92**
1365	406.15	**0.99**	4.02	11.13	**1.04**
Mean	***496.90***	***0.74***	***3.53***	***9.77***	***0.78***

*Actual retention determined by chemical analysis

The variability of retentions shown in Table 2.2 was a reflection of having analysed stakes from the reject portion that represented extremes of preservative solution uptake, a factor largely determined by wood density i.e. void volume of wood cell luminae.

Table 2.3: Predicted and actual Cu retentions in ACQ treated pine (retention 4 (PACQ4))

Stake N°	Wood density Kg/m^3	Predicted		Actual
		g/100g a.i.(%)	a.i. kg/m3	g/100g a.i.(%)
987	559.18	**0.55**	3.08	**0.61**
1057	439.40	**0.86**	3.77	**0.89**
1061	412.30	**0.92**	3.80	**0.89**
1066	366.39	**1.08**	3.95	**0.96**
1069	451.52	**0.83**	3.74	**0.90**
1081	437.69	**0.87**	3.79	**0.95**
1096	424.89	**0.88**	3.73	**0.94**
1115	562.60	**0.54**	3.04	**0.57**
Mean	***456.75***	***0.82***	***3.61***	***0.84***

Table 2.4: Predicted and actual DDAC retentions in ACQ treated pine (retention 4 (PACQ4))

Stake N°	Wood density Kg/m^3	Predicted		Actual
		g/100g a.i.(%)	a.i. kg/m3	g/100g a.i.(%)
987	559.18	**0.34**	1.92	**0.88**
1057	439.40	**0.54**	2.35	**0.95**
1061	412.30	**0.58**	2.37	**0.92**
1066	366.39	**0.67**	2.47	**0.83**
1069	451.52	**0.52**	2.34	**0.96**
1081	437.69	**0.54**	2.37	**0.97**
1096	424.89	**0.55**	2.33	**0.87**
1115	562.60	**0.34**	1.90	**0.77**
Mean	***456.75***	***0.51***	***2.26***	***0.89****

Actual retention was probably high due to an incorrect working solution concentration for DDAC

Table 2.5: Predicted and actual Cu retentions for CuAz treated pine (retention 4 (PCAZ4)) and predicted tebuconazole

Stake N°	Wood density Kg/m^3	Predicted				Actual	
		g/100g a.i.(%)	kg/m3 a.i.	g/100g a.i.(%)	kg/m3 a.i.	g/100g a.i.(%)	
		Cu		**Tebuconazole***		**Cu**	**Tebuconazole**
1036	425.66	**0.71**	3.04	0.028	0.122	**0.68**	ND
1037	446.07	**0.66**	2.97	0.026	0.119	**0.7**	ND
1041	439.38	**0.67**	2.96	0.027	0.118	**0.71**	ND
1065	434.45	**0.69**	2.99	0.028	0.120	**0.7**	ND
1066	443.28	**0.66**	2.94	0.026	0.118	**0.66**	ND
1094	440.65	**0.67**	2.95	0.027	0.118	**0.71**	ND
Mean	***438.25***	***0.68***	***2.98***	***0.027***	***0.119***	***0.69***	ND

*Predicted tebuconazole retention was calculated on the basis that the CuAz formulation used (Tanalith E) has a ratio of Cu:tebuconazole of 25:1. ND = repeated HPLC breakdowns and associated calibration difficulties resulted in results not being available.

Table 2.6: Predicted and actual CCA retentions for beech (retention 3 (BCCA3))

Stake N°	Wood density Kg/m^3	Predicted			Actual
		g/100g a.i.(%)	a.i. kg/m3	kg/m3	g/100g a.i.(%)
1633	547.41	**0.89**	4.89	13.54	**0.56**
1634	637.23	**0.72**	4.61	12.78	**0.45**
1691	611.20	**0.74**	4.51	12.51	**0.44**
1793	658.71	**0.66**	4.37	12.12	**0.42**
1633	726.98	**0.56**	4.05	11.22	**0.34**
1814	678.48	**0.64**	4.32	11.98	**0.39**
Mean	***643.34***	***0.70***	***4.46***	***12.36***	***0.43****

* It was believed that an error was made with treatment solution concentration used, hence the low actual uptake value.

Table 2.7: Predicted and actual CCA retentions for beech (retentions 1, 2 and 3*)

Stake N°	Wood density Kg/m^3	Predicted			Actual
		g/100g a.i.(%)	a.i. kg/m3	Salt kg/m3	g/100g a.i.(%)
Retention 1					
862	612.37	**0.19**	1.18	3.27	**0.11**
872	639.31	**0.18**	1.13	3.12	**0.11**
934	598.49	**0.20**	1.19	3.29	**0.11**
1034	627.55	**0.18**	1.16	3.21	**0.14**
1092	589.61	**0.19**	1.14	3.17	**0.15**
1131	621.35	**0.19**	1.18	3.27	**0.11**
Mean	***614.78***	***0.19***	***1.16***	***3.22***	***0.12****
Retention 2					
1224	620.89	**0.36**	2.23	6.19	**0.22**
1248	545.25	**0.44**	2.41	6.69	**0.26**
1260	630.59	**0.36**	2.24	6.20	**0.2**
1387	529.65	**0.46**	2.45	6.78	**0.31**
1508	595.87	**0.39**	2.33	6.44	**0.22**
1510	607.00	**0.38**	2.28	6.31	**0.23**
Mean	***588.21***	***0.40***	***2.32***	***6.44***	***0.24****
Retention 3					
1644	594.59	**0.80**	4.73	13.11	**0.41**
1709	605.64	**0.76**	4.61	12.78	**0.45**
1725	674.70	**0.66**	4.44	12.29	**0.33**
1769	665.86	**0.66**	4.37	12.11	**0.37**
1830	606.09	**0.78**	4.71	13.04	**0.42**
1837	571.53	**0.85**	4.83	13.39	**0.39**
Mean	***619.74***	***0.75***	***4.62***†	***12.79***	***0.40****

* Analysis was repeated for retention 3 of CCA treated beech because of the lower than expected values. Retentions 1 and 2 were also analysed to check relative values between retention groups. †= predicted retention was lower than aimed for in Table 2.1 (4.62 compared to 6.09 (1.5 Cu + 2.7 Cr + 1.89 As)) because the solution uptakes were on average 25% lower than expected. **Errors with the solution concentration used for BCCA treatments resulted in lower actual retentions.

Table 2.8: Predicted and actual Cu retentions for CuAz treated beech (retention 2), and predicted tebuconazole (BCAZ2)

Stake N°	Wood density Kg/m^3	Predicted				Actual	
		g/100g a.i.(%)	kg/m3 a.i.	g/100g a.i.(%)	kg/m3 a.i.	g/100g a.i.(%)	
		Cu		Tebuconazole**		Cu	Tebuconazole
526	667.50	**0.48**	3.20	**0.019**	0.128	**0.49**	ND
559	627.79	**0.54**	3.42	**0.022**	0.137	**0.56**	ND
570	643.46	**0.52**	3.32	**0.021**	0.133	**0.49**	ND
667	636.69	**0.53**	3.37	**0.021**	0.135	**0.51**	ND
704	628.42	**0.54**	3.36	**0.022**	0.134	**0.51**	ND
716	599.30	**0.58**	3.47	**0.023**	0.139	**0.56**	ND
Mean	***633.86***	***0.53***	***3.36****	0.021	0.134	***0.52***	ND

*predicted uptake was lower (3.36 compared to 4.5) than the aimed for retention in Table 2.1 because the solution uptakes were on average 25% lower than expected.
**Predicted tebuconazole retention was calculated on the basis that CuAz formulation used (Tanalith E) has a ratio of Cu:tebuconazole of 25:1. ND = repeated HPLC breakdowns and associated calibration difficulties resulted in results not being available.

Table 2.9: Predicted chlorothalonil retentions for pine (retention 3* (PCC3))

Stake N°	Wood density Kg/m^3	Predicted		Actual
		g/100g a.i.(%)	a.i. kg/m3	g/100g a.i.(%)
3290	425	**0.46**	1.94	ND
3294	542	**0.30**	1.62	ND
3296	535	**0.30**	1.61	ND
3297	457	**0.34**	1.56	ND
3299	422	**0.41**	1.71	ND
3300	461	**0.35**	1.61	ND
Mean	**474**	**0.36**	**1.68**	ND

*Retention 4 spares were mislaid. The mean value of 1.9 kg/m3 was less than the target 3.33 due to inadequate uptake (approximately 50 instead of the target of 90 litres/m^3). ND = repeated HPLC breakdowns and associated calibration difficulties resulted in results not being available.

Table 2.10: Predicted chlorothalonil retentions for beech (retention 2) (CBCC2)

Stake N°	Wood density Kg/m^3	Predicted		Actual
		g/100g a.i.(%)	a.i. kg/m3	g/100g a.i.(%)
2375	689	**1.08**	7.44	ND
2442	725	**1.03**	7.44	ND
2463	710	**0.86**	6.09	ND
2472	729	**0.85**	6.19	ND
2502	704	**1.06**	7.43	ND
2520	738	**0.89**	6.56	ND
Mean	***716***	**0.96**	**6.86**	*ND*

ND = repeated HPLC breakdowns and associated calibration difficulties resulted in results not being available.

The mean value of 7.54 kg/m3 was greater than the target 5.0 due to excessive uptake (approximately 150 instead of the target 90 litres/m^3)

Section 2.1.3

Mean predicted retentions of stakes exposed at test sites (highest retention only)

Table 2.11: Example of PCCA5 predicted retentions at one site

Stake Number	Weight Before	Weight After	Dimensions		Density Kg/m^3	CCA Retention	
						% m/m a.i.	Kg/m^3 a.i.
1126	105.00	233.77	20.45	20.35	505	0.69	3.466
1131	95.69	230.61	20.3	20.1	469	0.79	3.703
1156	108.33	234.76	20.05	20.45	528	0.65	3.454
1161	95.42	232.91	20.3	20.1	468	0.81	3.774
1165	98.72	229.78	20.2	20.25	483	0.74	3.588
1170	99.97	232.39	20.35	20.1	489	0.74	3.626
1179	114.52	236.20	20.3	20.1	561	0.6	3.34
1185	97.20	231.79	20.1	20.4	474	0.78	3.676
1194	96.05	232.16	20.3	20.25	467	0.79	3.708
1248	104.58	234.65	20.4	20.15	509	0.7	3.544
1267	114.79	250.32	20.69	20.73	535	0.66	3.539
1287	96.75	227.08	20.12	20.23	475	0.75	3.586
1319	119.74	244.73	20.47	20.44	572	0.58	3.346
1331	104.37	237.26	19.93	20.34	515	0.71	3.672
1334	87.67	230.97	20.28	20.35	425	0.92	3.889
1344	96.31	234.60	20.4	20.13	469	0.8	3.772
1351	102.12	236.30	20.48	20.21	493	0.74	3.631
1363	136.40	257.42	20.81	20.25	647	0.5	3.216
Mean						**0.72**	**3.585**

Table 2.12: Example of predicted copper retentions for PACQ4 at one site

Stake Number	Weight Before	Weight After	Dimensions		Density Kg/m^3	Cu Retention % m/m a.i.	
						% m/m a.i.	Kg/m^3 a.i.
895	98.51	235.07	20.35	20.20	479	0.74	3.57
914	96.17	236.97	20.70	20.20	460	0.79	3.62
921	109.73	241.11	20.60	20.20	527	0.64	3.39
927	106.44	237.14	20.10	20.40	519	0.66	3.42
957	96.72	242.91	20.65	20.55	456	0.81	3.7
985	98.22	234.62	20.10	20.45	478	0.75	3.56
992	113.99	245.65	20.90	20.50	532	0.62	3.3
997	108.40	237.26	20.30	20.30	526	0.64	3.36
1011	99.58	233.01	20.58	20.08	482	0.72	3.47
1014	119.18	237.65	20.09	20.19	588	0.53	3.14
1022	104.83	252.33	21.35	21.10	465	0.76	3.52
1038	103.71	236.24	20.51	20.28	499	0.69	3.42
1044	93.89	232.01	20.06	20.44	458	0.79	3.62
1052	106.53	237.09	20.49	20.18	515	0.66	3.39
1054	112.10	243.79	20.44	20.60	532	0.63	3.36
1071	109.55	235.69	20.31	20.23	533	0.62	3.3
1073	102.32	237.05	20.67	20.21	490	0.71	3.46
1094	99.26	233.75	20.27	20.26	483	0.73	3.52
Mean						**0.693**	**3.45**

0.693 Cu is equivalent to 0.867 CuO. Based on the 1:0.8 ratio of CuO:DDAC determined by chemical analysis (Tables 2.3 and 2.4), the mean retention of ACQ exposed at sites was 1.56% m/m a.i. (7.77 kg/m^3 a.i.).

Table 2.13: Example of PCAZ4 predicted retentions at one site

Stake Number	Weight Before	Weight After	Dimensions		Density Kg/m^3	Cu Retention % m/m a.i.	
						% m/m a.i.	Kg/m^3 a.i.
873	105.26	241.36	20.45	20.7	497	0.53	2.649
880	90.56	231.29	20.3	20.45	436	0.64	2.793
883	88.45	230.56	20.2	20.25	432	0.66	2.863
896	100.14	229.91	20.25	20.35	486	0.53	2.595
910	98.19	235.82	20.4	20.35	473	0.58	2.732
927	98.47	233.16	20.35	20.2	479	0.56	2.700
939	114.55	239.75	20.05	20.6	555	0.45	2.498
983	103.43	235.19	20.1	20.45	503	0.52	2.641
984	112.57	242.48	20.65	20.1	542	0.48	2.579
1029	88.46	233.15	20.12	20.22	435	0.67	2.931
1033	108.79	254.93	20.7	20.95	502	0.55	2.777
1046	97.47	234.59	19.92	20.03	489	0.58	2.832
1056	97.21	233.77	20.02	20.1	483	0.58	2.796
1075	95.78	238.32	20.25	20.29	466	0.61	2.859
1084	102.39	238.25	20.36	20.11	500	0.55	2.734
1086	102.67	235.35	20.06	20.17	508	0.53	2.702
1107	93.21	235.25	20.13	20.57	450	0.63	2.827
1115	100.79	236.93	20.05	20.2	498	0.56	2.770
Mean						**0.568**	**2.738**

0.568 Cu is equivalent to 0.59% m/m a.i. (2.84 kg/m^3 a.i.) CuAz (25:1 Cu:tebuconazole).

Table 2.14: Example of PC4 predicted retentions at one site

Stake Number	Weight Before	Weight After	Dimensions		Density Kg/m^3	Creosote Retention	
						% m/m a.i.	Kg/m^3 a.i.
868	109.65	158.67	19.98	19.9	552	27	148
884	106.69	171.05	19.67	19.8	548	36	198
891	94.11	168.51	19.85	19.95	475	47	225
898	107.92	151.44	20.09	19.91	540	24	131
930	86.13	154.54	19.6	19.93	441	48	210
962	110.26	164.05	19.56	19.94	565	29	165
967	97.39	156.67	19.97	20.11	485	37	177
978	101.83	154.16	19.98	20.1	507	31	156
989	109.14	171.38	20	19.83	550	34	188
1027	99.4	152.9	19.61	19.72	514	32	166
1033	110.14	181.74	20.14	20.04	546	39	213
1034	99	175.27	19.98	20.15	492	46	227
1041	96.7	145.29	19.9	19.86	489	30	148
1050	91.6	142.98	19.75	19.84	468	34	157
1059	92.19	180.42	19.96	19.87	465	57	267
1069	96.68	154.15	19.85	19.8	492	36	175
1079	113.59	180.6	20.21	20.11	559	35	198
1089	103.35	165.52	19.88	20.14	516	36	186
Mean						**37**	**185**

Predicted retention of 185 Kg/m^3 a.i. compared to the target of 160 was due to higher than expected uptakes (approximately 230 $litres/m^3$ compared to the target of 200)

Table 2.15: Example of PCC4 predicted retentions at one site

Stake Number	Weight Before	Weight After	Dimensions*		Density Kg/m^3	Chlorothalonil Retention	
						% m/m a.i.	Kg/m^3 a.i.
co4001	96.585	119.791	20.32	20.32	468	1.093	5.114
co4028	90.825	114.008	20.32	20.32	440	1.161	5.109
co4044	91.788	129.847	20.32	20.32	445	1.887	8.388
co4058	106.801	136.901	20.32	20.32	517	1.282	6.634
co4078	107.803	129.503	20.32	20.32	522	0.916	4.782
co4087	103.02	120.404	20.32	20.32	499	0.768	3.831
co4105	113.066	143.13	20.32	20.32	548	1.210	6.626
co4152	91.408	118.924	20.32	20.32	443	1.370	6.064
co4175	105.83	124.353	20.32	20.32	513	0.796	4.082
co4184	103.469	128.155	20.32	20.32	501	1.086	5.441
co4189	103.489	123.515	20.32	20.32	501	0.880	4.414
co4228	85.943	110.721	20.32	20.32	416	1.312	5.461
co4237	112.8	131.33	20.32	20.32	546	0.747	4.084
co4251	93.025	112.994	20.32	20.32	451	0.977	4.401
co4272	111.624	127.375	20.32	20.32	541	0.642	3.471
co4278	101.174	122.994	20.32	20.32	490	0.981	4.809
co4281	87.604	105.037	20.32	20.32	424	0.905	3.842
co4306	100.567	127.932	20.32	20.32	487	1.238	6.031
Mean						**1.070**	**5.1436**

* = actual dimensions of chlorothalonil treated stakes were lost from the laptop computer hard drive during transfer to desk top computer. An average dimension of all stakes used for other pine treatments was calculated on the basis that all pine stakes were from the same batch.

Table 2.16: Example of BCCA3 predicted retentions at one site

Stake Number	Weight Before	Weight After	Dimensions		Density Kg/m^3	CCA Retention	
						% m/m a.i.	Kg/m^3 a.i.
1610	139.605	262.36	21.00	21.64	614.4	0.739	4.538
1633	122.181	252.06	20.84	21.42	547.4	0.893	4.888
1634	139.549	259.79	21.21	20.65	637.2	0.724	4.612
1644	131.798	256.66	20.61	21.51	594.6	0.796	4.732
1691	136.748	256.99	20.64	21.68	611.2	0.739	4.514
1709	137.636	262.42	20.83	21.82	605.6	0.762	4.612
1722	137.458	264.49	22.18	20.57	602.6	0.776	4.678
1725	150.761	268.76	21.20	21.08	674.7	0.657	4.436
1769	156.845	279.40	22.18	21.24	665.9	0.656	4.37
1793	145.613	260.72	21.63	20.44	658.7	0.664	4.374
1796	152.834	268.04	20.89	22.02	664.5	0.633	4.208
1807	164.904	274.32	21.15	21.45	727	0.557	4.052
1814	155.5	273.50	22.36	20.50	678.5	0.637	4.325
1830	137.435	264.54	20.68	21.93	606.1	0.777	4.709
1837	129.16	259.16	20.61	21.93	571.5	0.845	4.832
Mean						**0.724***	**4.525**

* On the basis that the actual mean retention determined by chemical analysis was 0.43 instead of the predicted 0.75 for the stakes analysed (see Table 2.6), an adjusted predicted retention was calculated for exposed stakes as 0.724 x 0.43/0.75 = 0.42% m/m a.i.

Table 2.17: Example of BCAZ2 predicted retentions at one site

Stake Number	Weight Before	Weight After	Dimensions		Density Kg/m^3	Cu Retention	
						% m/m a.i.	Kg/m^3 a.i.
403	148.95	272.31	21.95	20.90	649	0.512	3.324
464	142.15	267.00	21.78	20.88	625	0.543	3.393
480	141.99	266.85	21.71	20.88	626	0.543	3.405
491	140.54	266.18	20.73	21.77	623	0.552	3.441
501	133.58	261.04	21.89	20.51	595	0.590	3.509
508	140.62	261.81	20.66	21.60	630	0.533	3.357
526	150.63	267.52	21.73	20.77	667	0.480	3.201
538	145.92	264.18	21.72	20.61	652	0.501	3.265
547	137.05	263.40	21.77	20.62	611	0.570	3.479
558	150.32	268.52	20.76	21.79	665	0.486	3.229
559	142.55	268.19	21.76	20.87	628	0.545	3.42
570	148.60	272.63	22.11	20.89	643	0.516	3.319
595	138.47	258.40	20.80	21.68	614	0.535	3.287
622	143.14	264.17	21.34	21.04	638	0.522	3.332
667	143.93	267.29	20.57	21.98	637	0.530	3.372
673	140.65	264.69	21.81	20.78	621	0.545	3.383
704	141.09	263.30	21.63	20.76	628	0.535	3.364
716	134.89	261.30	21.59	20.85	599	0.579	3.471
739	140.21	260.22	20.67	21.58	629	0.529	3.325
757	136.12	258.25	21.54	20.86	606	0.555	3.36
Mean						**0.535**	**3.362**

Table 2.18: Example of BCC2 predicted retentions at one site

Stake Number	Weight Before	Weight After	Dimensions		Density Kg/m^3	Chlorothalonil Retention	
						% m/m a.i.	Kg/m^3 a.i.
2096	145.70	169.50	19.96	19.90	734	0.743	5.452
2201	152.29	180.48	20.03	19.64	774	0.842	6.522
2228	137.16	162.59	19.92	20.03	688	0.844	5.799
2254	126.44	157.30	19.99	19.88	636	1.111	7.067
2256	131.51	162.32	19.88	19.85	666	1.066	7.106
2268	132.32	166.29	20	20.00	662	1.168	7.728
2270	145.83	175.76	19.93	19.94	734	0.934	6.853
2280	119.85	149.06	19.96	19.86	605	1.109	6.705
2286	138.37	172.32	19.89	20.38	683	1.116	7.621
2292	150.08	178.27	19.89	19.81	762	0.855	6.510
2307	149.88	185.49	20.01	19.78	757	1.081	8.188
2310	127.10	156.99	19.8	19.86	646	1.070	6.917
2312	141.02	166.35	19.66	20.05	715	0.817	5.848
2341	149.63	182.13	19.98	19.85	755	0.988	7.458
2375	136.81	169.29	19.92	19.94	689	1.080	7.442
2442	140.96	172.73	19.76	19.67	725	1.025	7.438
2463	143.12	170.11	20.15	20.02	710	0.858	6.088
2472	144.20	171.12	19.85	19.93	729	0.849	6.192
2502	137.07	168.90	19.71	19.77	704	1.057	7.433
2520	149.52	178.71	19.89	20.37	738	0.888	6.556
Mean						**0.975**	**6.846**

Section 2.1.4

Preparation and installation of field test stakes

Using a Microsoft Excel spreadsheet stakes of each preservative retention group (Table 2.1) (including solvent and oil blanks) were sorted separately according the following procedure.

1. Stakes were sorted by preservative uptake value (weight after treatment - weight immediately before treatment) and the extremes of uptake either side of the total number required for all 13 sites (13 x replicate number given in Table 2.1) were discarded.
2. Iterative numbering with the sequence 1 – 13 followed by 13 – 1 from top to bottom of the spreadsheet ensured that each site was allocated stakes evenly across the range of uptakes i.e. each site had the same mean retention for each treatment group.

A separate spreadsheet for each site was compiled using a replicate set of stakes with the same mean preservative retention for each preservative retention group. The initial experiment included CCA, ACQ, CuAz and creosote treated pine and untreated pine and beech, a group of 400 stakes at each site (treatments 1 -19 of Table 2.1). A random number sequence provided by the Excel spreadsheet was used to sort these stakes into a random sequence, using a different set of random numbers for each site. Stakes were numbered A1-20, B1-20 and C1-20 etc. up to T20. At each site stakes were placed vertically in the ground with half of the length underground, in 20 rows (A - T) of 20, except at those sites where obstacles (e.g. trees) required a modification to the grid allocation. Rows were 500 mm apart and stakes within a row were 300 mm apart. At Esk valley (ESK) and Tombleson's mandarin orchard (TOM) stakes were placed equidistant between stems of vines and trees respectively (approximately 1 meter apart at TOM and 2 meters at ESK). At the peat site (PT) stakes were placed within 2 rows 500 mm apart (300 mm between stakes within rows) between a drainage ditch and a row of blueberry bushes (blueberries are one of the few crops that tolerate the low pH of peat).

The sorting and numbering procedure used for the first set of 13 x 400 stakes was also used for the remaining treatments (20 – 34 Table 2.1). These treatments were added to the outer edges of the existing 20 x 20 plot using the same spacing regime. Except at the peat site where the ground was soft enough to allow stakes to be pushed in by hand, a 23 mm masonry drill bit attached to a converted chainsaw rig was used to drill a hole for each stake.

The first 400 stakes were installed at all 13 sites (Table 2.9) over a 12 month period to facilitate regular assessment at least once a year. Of the remaining 299 stakes, all pine treatments were added within 3 months and beech treatments were added 12 months later (plus or minus 3 months) (beech was not installed at Mt. Mee).

Table 2.19: Test site characteristics

Test site	**Soil - Vegetation**	**Climate**
Hanmer (HAN), Central Cantebury, South Island (SI) of New Zealand (NZ)	Stony, sandy loam, very good drainage. Grazed by sheep. Drought in summer.	Moderately dry (<1m rain), alpine, hot summers / cold winters
Whakarewarewa (WKA), Rotorua, Bay of Plenty, North Island (NI) of NZ	Pumice sand loam, very good drainage. Mown lawn.	Moderate rain (approximately 1.5m), temperate
Hari Hari (HH), near Franz Josef Glacier, West Coast, SI NZ	Silty sand loam, prone to waterlogging. Originally podocarp swamp forest. Pastoral. Grazed by sheep.	Very wet (4 – 6 m), temperate
Totara flat, mixed species *Nothofagus** forest (TOT), near Greymouth,West Coast, SI NZ	Very acid litter, stony-sandy soil, water-logging. Surrounded by 120 year old gold mine shafts.	Wet (2.5-3 m) rain, cool temperate.
Esk valley vineyard, Poverty Bay, Napier, East Coast, NI NZ (ESK)	Horticultural, sandy, shaley loam (raised seabed), good drainage.	Spray irrigated, warm temperate, hot summers
Tombleson's mandarin orchard (TOM), Poverty Bay, Gisborne NI NZ	Horticultural, silty clay loam, slightly alkaline, prone to floods in winter.	Spray irrigated, warm temperate
Glenbervie (pastoral) (GB), near Whangarei, Northland, NI NZ	Sandy clay loam, poor drainage (flat), water-logging.	Moderate rain, warm temperate
Glenbervie radiata pine forest (GBF), 200 meters from GB	Pine litter, sandy clay loam, good drainage (slope).	Moderate rain, warm temperate

Table 2.19: Continued

Test site	Soil - Vegetation	Climate
Cheviot hills oak (*Quercus**) forest (OAK), some ash (*Fraxinus**) North Cantebury, SI NZ	Oak & ash litter, clay loam, poor drainage.	Dry (<0.6m), hot summers / cool winters. Drought in summer.
Tapanui (TAP), Southland, SI NZ	Clay loam, poor drainage. Grazed by sheep.	Moderate rain, cool temperate
Peat, blueberry orchard (PT), near Hamilton, Waikato, NI NZ	Peat, very acid, high water table above base of stake in winter.	Moderate rain, warm temperate
Canal Creek (CK), Near Cairns, North Queensland, Australia	Red, tropical iron oxide clay loam, prone to flooding. Grassland adjacent to rainforest.	Wet (>3m), tropical
Mt. Mee (MM), Near Brisbane, Queensland, Australia	Stoney, sandy, good drainage, prone to forest fires.	Moderately dry, sub-tropical

*species unknown.

Section 2.1.5

Decay assessment for test stakes

A separate soundness rating for distinguishable decay types was given to each stake using the following procedure.

1. All stakes were removed from the ground and soil was removed using a paint scraper.

2. A 6 mm-blunt steel probe was used to compare the toughness of obviously sound wood near the base and top of the stake with wood in any region suspected of harbouring decay (based on colour), paying particular attention to the ground-line region.

3. All regions showing softness were characterised according to colour and texture using the chart illustrated in Table 2.20.

4. Any region possessing unique macroscopic features and showing signs of surface softness was given a rating according to Table 2.21. Soundness refers

to an estimated % of remaining wood cross-section that is sound in that region where decay is most advanced. A rating was recorded for the most severe example of each decay type, and the stake was then replaced in the ground.

5. Examples (at least 5) of all decay types characterised by colour and texture were removed for all treatment groups (all retentions of a preservative were dealt with as a single preservative treatment group for this purpose) were taken to the laboratory for microscopic analysis. For example, for the 4 commonly encountered decay types identified in Table 2.20, at least 5 stakes of each treatment group with each decay type were removed (5 x 13 x 4 = 260 stakes). Stakes usually had several decay types so the total number of stakes removed was typically around 150 – 200.

6. Stakes were washed unless adhering mycelium or other features of interest were present, prior to microscopy. Typically, sections (15 – 30 micrometers thick) were removed directly from the stake using a hand-held disposable microtome blade. The majority of sections were taken from around the edge of decay pockets so that the interface of sound and decayed wood could be examined. Decay pockets on stake surfaces of different grain orientation were selected to examine any effects this may have had on micromorphology (e.g. movement of hyphae between radial parenchyma and axial wood cells was typically easier to see in radial sections cut across the wood grain. However in order to simplify examination of a large number of sections, tracheids in pine and fibres and fibre tracheids in beech were the only wood cells examined in detail. Transverse sections were rarely required because of the depth of field and resolution achieved across longitudinal sections, as explained in Section 4.2.1. Micromorphology was recorded for each decay type, for each treatment.

7. After microscopy had identified micromorphology associated with macroscopic features the majority of stakes were returned to the site within 2 weeks and others were held permanently for further analysis. Stakes were held at 4°C in bundles in polyethylene bags. A repeat wood soundness assessment was carried out at the same time stakes were returned. At geographically

remote sites preliminary microscopy was carried out at a base near the site. Care was taken not to remove excessive amounts of wood during sectioning, an occurrence that could influence long-term soundness. This was particularly important for the highest retention of each preservative treatment.

8. After all sites had been assessed, knowledge of different macroscopic types encountered at all sites was used to develop a macroscopic decay type nomenclature versatile enough to describe all decay types encountered across all the sites. The classification system derived is described in detail in the results section of chapter 5.

9. Sites were assessed annually to determine overall stake soundness but the first 2 - 3 years was spent consolidating the macroscopic decay type classification system (Table 5.1) a process that involved iterative correlative analysis of macroscopic decay features (colour and texture) and micromorphology (Section 5.2.1). Therefore the final system was used in the last 2 assessments for beech (4.5 and 5.5 years) and the last 3 assessments for pine (4.5, 5.5 and 6.5 years). All decay soundness data analysed was from the final assessment (5.5 years for beech and 6.5 years pine, plus or minus 3 months). Affects of time on decay type and rate, as determined by wood soundness, were not tested statistically since it was assumed that 2-3 years was insufficient for this purpose.

Table 2.20: Example of commonly encountered macroscopic features of 4 decay types (actual decay types common across all sites is given in Table 5.1)

Macroscopic features		Decay type				
Colour		1	2	3	4	Etc....
1	White / bleached				✓	
2	Bleached					
3	Brown	✓				
4	Slight lightening		✓			
5	Unchanged		✓			
6	Slight darkening		✓			
7	Grey - brown					
8	Grey - black			✓		
Etc.....						
Texture						
1	Powdery	✓(dry)				
2	Fibrous		✓		✓	
3	Spongy		✓	✓		
4	Cheesy	✓(wet)	✓			
Etc.....						

⌐ = merged features; (wet) = brown rot had a cheesy texture when wet but became powdery and brittle when dry.

Table 2.21: Wood Soundness Rating

Rating		% Soundness
System used	AWPA equivalent*	
1	1	100
2	2	90
$^{+}3$	3	80
3	4	70
$^{-}3$	5	60
4	6	40
5	7	0

*AWPA equivalent = American Wood-Preservers'Association standard method E7-93 (AWPA 1999).

Section 2.2

Analysis of mean soundness data

1. Each stake had a separate percent soundness value for each decay type present. Only the lowest soundness value was used for calculation of overall mean soundness (MS) of each treatment group as follows:

Sum of soundness values of all stakes remaining / number of stakes.*

* Remaining stakes are those assessed at the final assessment i.e. total number of replicates for that treatment group minus the sacrificial stakes removed for microscopic analysis during preceding assessment periods. Typically, no less than 10 stakes remained for each group. The exception was for untreated control and solvent blank stakes, most of which had failed earlier. At the more severe decay hazard sites this was also the case for lower retention groups. In such cases the zero soundness data and associated decay type information was carried over from the assessment where failure was recorded.

All MS values were converted to MS reduction values (MSR) (Table 2.22) by subtraction from 100. This was to place emphasis on the decay type that **caused** an MSR.

An adjusted percentage contribution of each decay type to overall MSR for each treatment group was calculated as follows:

decay type MSR/sum of decay type MSRs x overall treatment MSR

Table 2.22: Decay type MSR values and percentage contribution of each decay type to overall MSR for all treatments at Hari Hari – an example of one site

Treatment		Decay type MSR*				Overall MSR	% Contribution of each decay type to overall MSR			Sum (Overall MSR)
		2	2a	3	Sum		2	2a	3	
1	Pine	8.00	77.50	16.25	101.75	100.00	7.86	76.17	15.97	100.00
2	Beech	49.33	58.67	29.33	137.33	100.00	35.92	42.72	21.36	100.00
3	PC1	60.00	–	13.64	73.64	75.00	61.11	0.00	13.89	75.00
4	PC2	27.00	–	–	27.00	39.23	39.23	0.00	0.00	39.23
5	PC3	12.22	–	–	12.22	21.82	21.82	0.00	0.00	21.82
6	PC4	5.56	–	–	5.56	7.14	7.14	0.00	0.00	7.14
7	PACQ1	39.09	4.55	48.18	91.82	82.67	35.19	4.09	43.38	82.67
8	PACQ2	20.00	–	18.00	38.00	46.67	24.56	0.00	22.11	46.67
9	PACQ3	12.78	–	13.33	26.11	27.33	13.38	0.00	13.96	27.33
10	PACQ4	8.89	–	3.89	12.78	15.45	10.75	0.00	4.70	15.45
11	PCAZ1	22.73	10.00	28.64	61.36	68.00	25.19	11.08	31.73	68.00
12	PCAZ2	23.50	1.50	20.00	45.00	45.33	23.67	1.51	20.15	45.33
13	PCAZ3	5.56	5.56	17.78	28.89	29.29	5.63	5.63	18.02	29.29
14	PCZ4	7.78	1.67	8.33	17.78	20.00	8.75	1.88	9.38	20.00
15	PCCA1	38.18	40.00	41.82	120.00	100.00	31.82	33.33	34.85	100.00
16	PCCA2	40.00	16.00	30.00	86.00	78.46	36.49	14.60	27.37	78.46
17	PCCA3	14.44	38.40	27.11	79.96	82.31	14.87	39.53	27.91	82.31
18	PCCA4	15.00	15.00	26.11	56.11	46.00	12.30	12.30	21.41	46.00
19	PCCA5	5.00	–	6.67	11.67	23.64	10.13	0.00	13.51	23.64
20	PCC1	2.27	20.91	30.45	53.64	44.38	1.88	17.30	25.20	44.38
21	PCC2	5.00	7.00	31.00	43.00	45.83	5.33	7.46	33.04	45.83
22	PCC3	3.33	7.78	7.78	18.89	24.44	4.31	10.07	10.07	24.44
23	PCC4	2.22	–	9.44	11.67	7.50	1.43	0.00	6.07	7.50
24	POIL1	21.82	41.67	35.91	99.39	100.00	21.95	41.92	36.13	100.00
25	POIL2	9.70	23.48	18.91	52.09	65.00	12.10	29.30	23.60	65.00
26	PSOLV	27.73	36.36	26.09	90.18	100.00	30.75	40.32	28.93	100.00
27	BCCA1	13.00	55.00	16.50	84.50	100.00	15.38	65.09	19.53	100.00
28	BCCA2	17.50	27.00	36.00	80.50	65.00	14.13	21.80	29.07	65.00
29	BCCA3	10.67	–	20.67	31.33	36.00	12.26	0.00	23.74	36.00
30	BCAZ1	10.00	36.50	40.00	86.50	86.36	9.98	36.44	39.94	86.36
31	BCAZ2	10.50	19.00	11.50	41.00	30.00	7.68	13.90	8.41	30.00
32	BCC1	28.00	–	36.50	64.50	93.00	40.37	0.00	52.63	93.00
33	BCC2	4.00	2.00	5.00	11.00	18.33	6.67	3.33	8.33	18.33
34	BOIL	8.50	13.00	10.00	31.50	52.00	14.03	21.46	16.51	52.00

– = decay type absent. * No decay types 1 and 4 were present at Hari Hari

For the purposes of statistical analysis, percent contribution of each decay type to overall MSR was analysed. For example 25% type 2, 50% type 2a and 25% type 3 were % contributions of the 3 decay types to overall MSR (as used for statistical analysis in chapter 5), whereas the decay type MSR values used in all Figures in chapter 5 were 12.5, 25 and 12.5% for a treatment group of stakes with an overall MSR of 50%.

Section 2.3

Statistical analysis

1. In Chapter 3 the aim was to determine if site location and treatment had a significant affect on MSR. A one-way analysis of variance (ANOVAR) followed by a least significant difference test (LSD's) was used to determine significant differences at a 5% level of probability (SAS Institute, 2000).

2. In Chapter 5 two-way ANOVAR were performed to test the significance of site and treatment on decay type. An overall MSR value for each decay type was calculated for each site across all treatments and for each treatment across all sites and this value was then expressed as a percentage of the sum of soundness reduction values for all decay types. Least significant different (LSD) tests were then used to provide pair-wise comparisons between individual sites and preservatives at the 95% level of significance. The SAS procedure GLM (General Linear Model) was used for these analyses (SAS Institute, 2000). This procedure was carried out for the highest retentions of each preservative (and untreated controls, oil and solvent blanks) and for the sum of MSR across all retentions.

3. In chapter 6 the aim was to determine if site location and wood species had a significant effect on preservative depletion. A one-way analysis of variance (ANOVAR) followed by a least significant difference test (LSD's) was used to determine significant differences at a 5% level of probability (SAS Institute, 2000).

Stakes containing didecyldimethyl ammonium chloride (DDAC) were analysed using high performance liquid chromatography (HPLC) according to AWPA A16-93. Stakes containing tebuconazole and stakes containing chlorothalonil were analysed using HPLC according to AWPA standard methods A28 and A20-97 respectively.

Section 2.4

Preservative retention analysis

All Cu and CCA treated stakes were digested according to the American Wood Preservers Association (AWPA) standard method AWPA A7-93 (method 1) (AWPA,

1999). Digested samples from CCA treated stakes were analysed using inductively coupled plasma optical emission spectroscopy (ICP-OES) according to AWPA A21-00. Samples requiring Cu determination only (CuAz and ACQ treated stakes) were analysed by atomic absorption spectroscopy (AAS) according to AWPA A11-93 (AWPA, 1993). Stakes containing didecyldimethyl ammonium chloride (DDAC) were analysed using high performance liquid chromatography (HPLC) according to AWPA A19-93 (AWPA, 1999). Stakes containing tebuconazole and stakes containing chlorothalonil were analysed using HPLC according to AWPA standard methods A28 and A20-97 respectively (AWPA, 1999).

Section 2.5

Light microscopy

The majority of wood sections were cut from stakes using a disposable hand-held microtome blade and mounted in glycerol on a microscope slide. Wood stained with aniline blue was removed as a sliver approximately 1-2 mm thick prior to staining and further sectioning. Slivers were soaked in a drop of aniline blue on a glass topped bench for 5 – 10 minutes prior to cutting sections and mounting in glycerol. Lactophenol aniline blue was prepared by mixing the following constituents: 50 ml of 0.5%w/v aqueous aniline blue, 150g of glycerol, 100g of food grade concentrated lactic acid and 100g of phenol. Bright field, phase contrast and polarised light techniques were employed using a Zeiss Axioplan II light microscope. The optical pathway used consisted of a 10X eyepiece lense plus an optovar intermediate tube (1, 1.25, 1.6, 2 and 2.5X) and a 40X/1,30 oil immersion Plan Neofluar lens.

Section 2.6

Confocal laser scanning microscopy (CLSM)

Section 2.6.1

Sample preparation

A 100 mg ml^{-1} solution of fluorescein conjugated wheat germ agglutinin (WGA) (excitation 494 nm, emission 518 nm) obtained from Molecular Probes (Eugene, Oregon, USA) dye was prepared in phosphate-buffered saline (PBS) (pH 7). This stock solution was divided into small vials and stored at -20°C in the dark. For use,

the stock solution was diluted into 0.1-0.5 mg ml^{-1} in PBS (pH 7.4). A rhodamine conjugated WGA stain (excitation 555 nm, emission 580 nm) (Molecular Probes) was prepared in the same way.

Nitrobenzoxadiazole glycerophosphoethanolamine (NBD-PE, excitation 463 nm, emission 536 nm, Molecular Probes) was dissolved in chloroform and divided into small vials. After the chloroform evaporated, the vials were stored at -20°C in a desiccated condition, protected from light. For use, the stain was dissolved in ethanol at about 1 mg ml^{-1}, and 10 µl of this solution was diluted into 5 µg ml^{-1} solution in PBS (pH 7.4). Lissamine rhodamine labeled phospholipid (*N*-Rh-PE, excitation 560 nm, emission 581 nm, Molecular Probes) was prepared in the same way.

All wood materials were sectioned longitudinally at a thickness of 20-50 µm using a microtome. Fungal-degraded wood sections (from the Peat site) were fixed in 3% (v/v) glutaraldehyde in PBS (pH 7.2) overnight. After rinsing in the PBS solution 4 times (10 min each), wood sections were immersed in 0.1-0.5 mg ml^{-1} solution of green WGC for 10-15 min in the dark. Bacterial-degraded wood sections (from Tombleson's mandarin orchard) were stained in 5 µg ml^{-1} *N*-Rh-PE for 1 h in the dark after fixing in glutaraldehyde. The stained sections were sequentially washed in a series of 75%, 90% and 100% ethanol solution (5 min in each solution), to remove excess stain. Sections cut from ponded wood samples were stained in 5 µg ml^{-1} NBD-PE for 1 h in the dark, and then washed in a series of 75%, 90%, and 100% ethanol solution (2 min each). After being washed in ethanol, some sections were counter-stained in 0.5-1 mg ml^{-1} solution of red WGA for 10-15 min in the dark.

After staining, samples were washed with water 4 times at 10 min intervals and mounted in glycerol or in immersion oil after dehydration. Cover slips were applied to the slides and the edges sealed using nail varnish. Three wood sections were treated for each staining procedure. Additional wood sections were examined directly after microtome sectioning without further preparation. Other glutaraldehyde-fixed sections with or without fluorescent staining were embedded in Spurr's resin (Spurr, 1969). The embedded samples were further sectioned at a

thickness of 3-5 μm longitudinally or transversely using glass knives with an ultra-microtome and then mounted in immersion oil with a cover slip.

Section 2.6.2

CLSM operation

Prepared slides were examined using a Leica TCS/NT confocal microscope using an epi-fluorescence technique. Confocal images acquired at different wavelengths were displayed on separate display channels representing green, red, and far red wavelengths. A fourth channel was used to display an overlay or combined image of all three wavelengths. An argon/krypton (Ar/Kr) laser with excitation wavelengths of 488/568/647 nm was used for illumination. Images were captured in the green channel through a band-pass filter (530/30 nm) and in the red channel through a long-pass filter (590 nm). Objective lenses were 20 x air/dry, 25 x oil, 40 x oil, and 63 x oil, with numerical aperture settings of 0.60, 0.75, 1.00 and 1.40 respectively.

A series of confocal images was collected at a resolution of either 512 x 512 or 1024 x 1024 pixels. Eight, 16 and 32 scans were taken and averaged for each single image. Quick examinations were carried out by mounting samples in water and collecting images at a resolution of 512 x 512 pixels with 8- or 16-scan averaging. High-quality images were acquired by mounting dehydrated samples in immersion oil and collecting images at a resolution of 1024 x 1024 pixels with 16- or 32-scan averaging.

Section 2.6.3

Image processing

The digital image series obtained by CLSM was transferred to an SGI Indy workstation and processed using Image Space software (Molecular Dynamics, Sunnyvale, CA, USA). Importing the data set into Volume Workbench (part of Image Space) allows construction of a 3D model. This model can then be processed for different objectives.

A maximum intensity technique, which recorded the pixel of the maximum intensity along an imaginary ray through the image stack from back to front, was performed to construct a projected image that combined the information from original serial

images. Projections for each channel were performed separately, and then a dual-channel projection was applied to combine the projections into one red-green-blue (RGB) image, in which the image obtained in a confocal channel (356 grey-level) was treated as one colour. All colour images in this study were made in the same way.

Another technique, slice viewing, revealed internal details of decayed wood cell walls. The xy, xz, and yz views of a selected location were simultaneously displayed by slicing through the 3D model and creating lateral views of the model.
The images were then transferred to a personal computer and adjusted for final printing using Adobe Photoshop 5.0 (Adobe Systems, Mountain View, CA, USA).

Section 2.7

Scanning electron microscopy (SEM)

Sections approximately 100 microns in thickness were cut using a microtome and then fixed in 3% glutaraldehyde overnight. After being dehydrated in acetone and air dried, sections were coated using a gold sputter coater and examined with a Cambridge Stereoscan SEM.

Section 2.8

Transmission electron microscopy (TEM)

Small sections were fixed in 3% glutaraldehyde (in 0.05M sodium cacodylate buffer) and post-fixed in 2% osmium tetroxide also prepared in the same buffer. They were then dehydrated in acetone and embedded in Spurr's low-viscosity resin (Spurr 1969). Ultrathin sections were cut on an LKB ultramicrotome, stained with 1% potassium permanganate (in 0.1% citrate buffer) and examined using a Philips 300 TEM.

Section 2.9

Calculation of preservative depletion

For each site approximately 5 stakes of treatments PCCA5 and PCAZ4 were removed after 5.5 years and 5 stakes of treatments BCCA3 and BCAZ2 were removed after 4.5 years (plus or minus 3 months). Stakes with the lowest % MSR were selected

(typically zero). These stakes were cut into two 250 mm long sections to give above and below ground portions, which were then analysed separately according to section 2.4. Depth of stakes in soil ranged from 240 and 260 mm. It was decided not to measure this depth precisely on the basis that depth would probably have varied over a range spanning approximately 20 mm during the exposure period. The predicted preservative retention as percentage mass/mass (g/100g) was calculated according to Section 2.2, i.e. based on preservative treatment solution concentration (g/100g), weight of solution uptake, wood density and stake volume. A close correspondence of predicted and actual retention was demonstrated for unexposed stakes of PCCA5 (5% deviation), PCAZ4 (0.7% deviation) and BCAZ2 (1.8% deviation) (Tables 2.2, 2.5 and 2.8). This established a high degree of confidence that predicted retentions for exposed stakes chosen for depletion analysis were close to the actual retentions at the commencement of exposure.

As explained in section 2.1.2, the quantity of Tanalith C added to the treatment solution used for BCCA was not known and the discrepancy between predicted and actual suggested a mistake had been made during preparation of the treatment solution. On the basis that the actual mean retention determined by chemical analysis was 0.43 instead of the predicted 0.75 for the stakes analysed (see Table 2.6), an adjusted predicted retention was calculated for exposed stakes as 0.72 x 0.43/0.75 = 0.42% m/m a.i. where 0.72 was the predicted retention for exposed stakes. The residual preservative concentration in wood (g/100g) determined by chemical analysis after exposure was expressed as a percentage of the predicted retention for each stake.

CHAPTER THREE

In-ground performance of *Pinus radiata* and *Fagus sylvatica* treated with modern and traditional wood preservatives at 13 field test sites

Section 3.1

Experimental

The objective was to determine the effect of site on the performance of variously preservative treated *P. radiata* and *F. sylvatica* stakes and compare performance of traditional preservatives with candidate replacement preservatives after 5.5 and 6.5 years in ground contact for beech and pine respectively. Sites (Table 2.19) were selected to ensure that wood was exposed to a highly diverse set of field conditions (photographs of some sites are given in Section 3.5). The following site selection criteria were of particular importance.

1. Cool temperate, warm temperate, sub-tropical and tropical sites were included to test the effects of temperature.

2. In an attempt to select sites that harbour different decay microorganisms, the following site parameters were identified as important: soil type, vegetation and litter coverage and water availability. Sites known to encourage decay types of particular significance were also included (e.g. sites harbouring tunnelling bacteria or brown rots previously suspected of possessing preservative tolerance).

3. The significance of preservation depletion was tested by including sites with highly variable conditions of leaching, as affected by rainfall, drainage, soil type (e.g. pH and cationic exchange capacity), and microbial activity that might cause biodegradation of preservative.

Section 3.2

Results

Percentage mean wood soundness reduction (MSR) at all test sites for the highest retentions of all preservative treatments and untreated controls are given in Table 3.1. Overall performance of each preservative across 13 sites is given by the treatment mean and overall hazard at each site is given by the site mean. Standard

deviations for each treatment at each site are included as well as significant differences between site and treatment means at a 5% level of probability. Significant differences between preservative treatments within sites and between each site for each preservative treatment are given in Section 3.5 (Appendix 3).

Key Results

1. Mean soundness reduction was significantly affected by site, preservative, and timber species at a 5% level of probability.

2. Mean site soundness reduction was a reliable indicator of the decay hazard for most preservatives. ANOVAR gave a significant site-preservative interaction effect indicating that not all preservatives performed as expected according to mean site MSR. An example of this occurred for ACQ treated pine which gave poorer performance at Glenbervie forest (GBF), Tapanui (TAP) and Hanmer (HAN) compared to Canal Creek (CK) and Tomblesons (TOM) which were ranked as more severe overall (see shaded cells in Table 3.1).

3. Based on overall performance across all sites pine treated with chlorothalonil plus chlorpyriphos (PCC4) (11% MSR), creosote/oil treated pine (PC4) (14% MSR) and PCAZ4 (15%MSR) all gave significantly better protection (5% P) than pine treated with CCA (19% MSR) and all other treatments. ACQ treated pine (PACQ4) gave equivalent performance to CCA treated pine.

4. Beech treated with CAZ (27% MSR) and CC (29% MSR) performed significantly better (5% P) than beech treated with CCA (40% MSR).

Table 3.1 MSR (bold) and standard deviation for 13 treatments at 13 test sites

Treat-ment a.i. % m/m	GB	CC	TOM	GBF	HH	OAK	ESK	WKA	TAP	PT	TOT	HAN	MM	*Treat-ment mean*
PCC4 1.07	**14**	**32**	**36**	**6**	**8**	**6**	**12**	**10**	**0**	**4**	**4**	**2**	**8**	**11a**
	25	30	28	18	27	24	40	30	0	24	5	39	30	
PC4 37	**18**	**28**	**19**	**19**	**7**	**15**	**21**	**5**	**9**	**11**	**5**	**10**	**12**	**14a**
	28	27	28	36	30	25	34	24	40	21	5	10	30	
PCAZ4 0.59[rr]	**27**	**29**	**20**	**18**	**20**	**14**	**19**	**1**	**9**	**15**	**18**	**0**	**11**	**15a**
	36	27	25	27	29	33	34	40	26	21	0	0	30	
PACQ4 1.56[r]	**31**	**19**	**17**	**28**	**15**	**20**	**11**	**21**	**30**	**19**	**5**	**23**	**4**	**19b**
	38	28	22	37	23	33	35	33	48	26	5	50	28	
PCCA5 0.72	**30**	**23**	**27**	**33**	**24**	**18**	**20**	**25**	**14**	**15**	**15**	**3**	**6**	**19b**
	22	25	22	33	29	28	29	38	40	31	5	39	34	
BCAZ2 0.56	**47**	**57**	**32**	**28**	**30**	**16**	**15**	**20**	**23**	**26**	**20**	**14**	**ND**	**27c**
	27	32	33	38	25	22	16	26	25	10	5	8		
BCC2 0.98	**46**	**55**	**54**	**25**	**18**	**36**	**21**	**25**	**15**	**16**	**14**	**23**	**ND**	**29c**
	15	30	39	29	22	32	23	24	30	13	5	39		
BCCA3 0.42	**72**	**61**	**58**	**66**	**36**	**26**	**33**	**21**	**26**	**30**	**18**	**28**	**ND**	**40d**
	23	27	30	34	36	18	24	27	30	22	4	34		
POIL2	**53**	**40**	**82**	**58**	**100**	**37**	**66**	**39**	**32**	**16**	**8**	**18**	**ND**	**46d**
	30	11	27	29	0	11	23	24	34	12	13	28		
BOIL -	**88**	**64**	**81**	**52**	**52**	**100**	**46**	**33**	**44**	**38**	**83**	**34**	**ND**	**60e**
	25	24	29	26	27	0	27	13	31	25	5	31		
Solvent -	**100**	**100**	**100**	**100**	**100**	**91**	**90**	**100**	**89**	**88**	**85**	**88**	**ND**	**94f**
	0	0	0	0	0	18	0	0	30	19	21	24		
Beech -	**100**	**100**	**84**	**100**	**100**	**89**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**98f**
	0	0	30	0	0	18	0	0	0	0	0	0	0	
Pine -	**100**	**100**	**94**	**100**	**100**	**91**	**100**	**100**	**98**	**100**	**100**	**100**	**88**	**98f**
	0	0	20	0	0	17	0	0	9	0	0	0	26	
Site Mean	**56 a**	**54 a**	**54 a**	**49 b**	**47 b**	**43 bc**	**43 bc**	**39 cd**	**38 cde**	**37 def**	**36 def**	**34 ef**	**33 f**	**44***

% m/m = % mass/mass or g/100g (see Tables 2.11 – 2.18 for retentions as a.i. kg/m^3). ND = no data. Treatment means are ranked with the best at the top of the table and treatments with the same letter in a column do not differ significantly at a 5% level of probability. Site means are ranked with the most severe on the left of the table and treatments with the same letter in a row do not differ significantly at a 5% level of probability. * = overall mean for all treatments across all sites. Shaded cells = examples of site-preservative interaction effects where a preservative did not perform as expected according to the overall site MSR. r = retention of ACQ in pine was 1.56% m/m a.i. (1:0.8 ratio of CuO:DDAC). rr = retention of CuAz in pine was 0.59% m/m a.i. (25:1 ratio of Cu:tebuconazole), (the boric acid (BA) constituent was not included in the retention value given (Cu:BA was 25:10)). CCA retention is based on total a.i. as Cu + Cr + As. CC was based on chlorothalonil only (chlorpyriphos concentration in concentrate as supplied by manufacturer was unknown). Creosote (PC4) retention was as undiluted creosote. All retentions shown were based on mean retention of the treatment group of stakes at each site (Tables 2.11 – 2.18). Closeness of predicted and actual retentions was determined by chemical analysis of a sub-set of unexposed stakes of each treatment group (Tables 2.2 – 2.10).

5. The OIL treatment for pine (46% MSR) and beech (60% MSR) performed significantly better (5% P) than the untreated pine (98% MSR) and beech (98% MSR) but performed significantly poorer (5% P) than OIL plus chlorothalonil and chlorpyriphos treated pine (PCC4) (11% MSR) and beech (BCC2) (29% MSR).

6. Large standard deviations (SDs) were the product of two factors. Firstly, stake soundness is based on a rating scale of broad bands. For example there is a large difference between a rating of 5 (zero soundness (100% MSR)) and the preceding rating 4 (40% sound (60% MSR)). Therefore small samples of stakes of a mixture of these two ratings will have large SDs. Secondly, abrupt reduction in soundness sometimes occurred between assessments so that stakes of the same treatment group contained stakes of widely different ratings. This was especially common at sites where brown rot was severe and where a stakes soundness reduction sometimes changed from zero to 100% within 1 year. This resulted in a treatment group with a mixture of stakes at opposite ends of the rating scale i.e. rating 1 (zero soundness reduction) and rating 5 (100% soundness reduction) and this resulted in a treatment group having a large SD.

ACQ, CuAz and CCA treated pine exhibited a clear dose response at the majority of sites (Table 3.2). Across all sites:

- stakes treated with 1.56, 1.04, 0.69 and 0.46 % a.i. m/m ACQ (ratio of 1:0.8 CuO:DDAC) had MSR values of 19, 41, 61 and 74;
- stakes treated with 0.59, 0.39, 0.26; and 0.18 % a.i. m/m CuAz (ratio of 25:1 Cu:tebuconazole) had MSR values of 15, 32, 47 and 61;
- stakes treated with 0.72, 0.48, 0.32, 0.21 and 0.14 % a.i. m/m CCA had MSR values of 19, 38, 54, 70 and 82.

Table 3.2: MSR (bold) and standard deviation for all retentions of ACQ, CuAz and CCA treated pine at 13 test sites

Treatment / Retention a.i. % m/m*	Site													Mean across sites
	GB	CC	TOM	GBF	HH	OAK	ESK	WKA	TAP	PT	TOT	HAN	MM	
ACQ														
1.56	**31**	**19**	**17**	**28**	**15**	**20**	**11**	**21**	**30**	**19**	**5**	**23**	**4**	**19a**
	18	8	5	16	7	13	9	18	35	5	8	28	5	13
1.04	**49**	**70**	**26**	**34**	**27**	**48**	**46**	**49**	**40**	**35**	**17**	**63**	**24**	**41b**
	27	14	5	29	9	29	26	20	37	9	5	41	5	20
0.69	**78**	**95**	**36**	**76**	**47**	**88**	**64**	**76**	**61**	**53**	**25**	**63**	**36**	**61c**
	30	14	5	33	19	32	31	32	46	17	8	47	10	25
0.46	**88**	**95**	**64**	**79**	**83**	**77**	**83**	**83**	**80**	**63**	**29**	**75**	**57**	**74d**
	38	28	22	37	23	33	35	33	48	26	5	50	28	31
CuAz														
0.59	**27**	**29**	**20**	**18**	**20**	**14**	**19**	**1**	**9**	**15**	**18**	**0**	**11**	**15a**
	6	0	5	6	8	9	9	3	5	7	7	0	8	6
0.39	**39**	**80**	**34**	**26**	**29**	**49**	**25**	**27**	**12**	**22**	**18**	**31**	**16**	**32b**
	26	6	5	22	11	29	12	34	6	7	5	41	22	17
0.26	**57**	**93**	**52**	**32**	**45**	**66**	**66**	**34**	**16**	**41**	**26**	**54**	**36**	**47c**
	35	16	10	25	29	32	22	37	8	12	8	48	24	24
0.18	**68**	**100**	**74**	**18**	**68**	**79**	**85**	**71**	**31**	**45**	**29**	**54**	**69**	**61d**
	36	27	25	27	29	33	34	40	26	21	0	48	30	29
CCA														
0.72	**30**	**23**	**27**	**33**	**24**	**18**	**20**	**25**	**14**	**15**	**15**	**3**	**6**	**19a**
	0	0	7	17	0	8	8	10	5	8	26	0	6	7
0.48	**47**	**62**	**43**	**39**	**46**	**44**	**43**	**42**	**26**	**31**	**35**	**25**	**17**	**38b**
	13	19	12	21	8	11	14	16	30	10	13	9	7	14
0.32	**62**	**84**	**51**	**44**	**82**	**76**	**45**	**61**	**35**	**42**	**39**	**46**	**33**	**54c**
	20	21	13	24	25	19	16	21	33	12	9	27	8	19
0.21	**86**	**100**	**73**	**56**	**78**	**81**	**68**	**85**	**46**	**60**	**50**	**63**	**69**	**70d**
	21	24	21	28	28	25	29	23	38	29	7	31	27	25
0.14	**100**	**94**	**80**	**81**	**100**	**89**	**84**	**89**	**60**	**69**	**69**	**77**	**79**	**82e**
	22	25	22	33	29	28	29	38	40	31	5	39	34	29

MSR across all sites with the same letter in a column were not significantly different (5% level of probability) within different retentions of a preservative treatment. Shaded cell contains that retention which meets the minimum requirement of NZS3640 (ACQ = 1.02; CuAz = 0.46; CCA = 0.72% m/m a.i.). For stakes treated with ACQ, 1.56 % m/m CuO + DDAC (1:0.8 CuO:DDAC) contained 0.87 CuO and 0.69 % m/m DDAC compared with the required 0.68 CuO
and 0.34 % m/m DDAC.

Section 3.3

Discussion

This section is divided into three sub-sections.

- Interpretation of MSR data for 20 x 20 x 500 mm stakes (section 3.3.1).
- Relative performance of CCA, ACQ and CuAz treated radiata pine (section 3.3.2).

- Selection criteria for field test sites for testing preservative treated wood in ground-contact (section 3.3.3).

Section 3.3.1

Interpretation of MSR data for 20 x 20 x 500 mm stakes

The rate of decay for 20 x 20 x 500 mm radiata pine stakes treated with the 3.59 and 2.39 kg/m^3 a.i. of CCA was greater than rates encountered in previous studies conducted at *Forest Research* (Table 3.3).

Table 3.3: Comparison of performance of 25 x 50 x 500 mm (IUFRO) radiata pine stakes treated with Tanalith NCA (Hedley *et al.*, 1996) after 16 years and 20 x 20 x 500 mm stakes treated with Tanalith C after 6.5 years at four test sites

Treatment / Retention a.i. Kg/m^3		% Mean Soundness Reduction							
		Site							
		Glenbervie		Wakarewarewa		Hanmer		Hari Hari	
Tan NCA	Tan C	Tan NCA	Tan C	Tan NCA	Tan C	Tan NCA	Tan C	Tan NCA	Tan C
6.2	-	20	-	4	-	1	-	20	-
4.5	-	29	-	14	-	2	-	27	-
3.3	3.59	43	30	26	25	8	3	32	24
2.4	2.39	57	47	34	42	30	25	34	46
-	1.6	-	62	-	61	-	46	-	82
-	1.06	-	86	-	85	-	63	-	78
-	0.71	-	100	-	89	-	77	-	100

IUFRO = International Union of Forest Research Organisations

Comparison of the performance data reported by Hedley *et al.* (1996) for IUFRO radiata pine stakes treated with Tanalith NCA (AWPA Type B CCA) and performance of Tanalith C treated stakes gives some perspective to the relative durability of CCA treated radiata pine stakes in two different studies conducted at some of the same sites. It is known that performance of AWPA Type B and C CCA preservatives can differ (Hedley, 1984) but both types have an established track record of in-service performance (Richardson, 1993) and are therefore useful reference standards. Where there is overlap between the retention ranges used in each study (shaded cells in Table 3.3), the MSR values were similar. For 2.4 kg/m^3 a.i. Tanalith NCA the average MSR across the 4 sites was 38.75, compared to 40 for 2.39 kg/m^3 a.i. Tanalith C. However the IUFRO stakes had been in the ground for nearly 10 years longer. MSR values for earlier assessments were not reported by Hedley *et al.* (1996) but clearly the onset of decay was more rapid in the current study.

At some sites the explanation may lie in the prevalence of certain decay types within sub-plots of the same site and whilst this subject is dealt with in detail in chapter 5 it is important to consider some of its implications here. For example, at Hanmer (HAN) and WKA the plots used in this study had a high brown rot hazard but the plot at WKA used by Hedley *et al.* (1996) had no brown rot and at HAN the Hedley *et al.* plot had a lower brown rot hazard (Wakeling, 1991). All stakes with brown rot at HAN in this study, were in one area of the plot that covered approximately one third of the total area. Within the plot, brown rot caused rapid failure (within 12 months) of radiata pine treated with 0.72 % m/m a.i. CCA (highest retention tested), 0.39 % m/m a.i. CuAz (second highest retention tested) and 1.56 % m/m a.i. ACQ (above the NZS3640 minimum requirement of 1.02% m/m a.i.). The ACQ used in this study had a lower CuO ratio (1:0.8 CuO:DDAC) than the NZS3640 requirement (2:1 CuO:DDAC) but both the copper oxide and DDAC retentions for PACQ4 treated stakes were higher than the minimum requirement of NZS3640. Sudden failures in stakes treated with these retentions started to occur after the fourth year of exposure, but lower retentions failed earlier.

Outside the area with brown rot the rate of decay was much lower and consisted of a shallow layer of erosion caused by soft rot and several other decay types discussed in chapters 4 and 5. At HAN, if the brown rot hazard had been evenly spread across the plot then the MSR values would have been much higher. It may have been important that in this study a much higher percentage of untreated stakes and stakes with low preservative retentions were used than is typical of in-ground field trials. This wood may have increased the inoculum potential of decay fungi in the soil around untreated and poorly treated stakes. Untreated radiata pine marker stakes were also placed in all holes left by stakes removed for microscopic examination and whilst the majority of the test stakes were replaced within 2 weeks, at remote sites like HAN, proportionately more test stakes were removed permanently. In such cases the untreated marker stakes were left in position, and at HAN, within the areas with brown rot, they all failed rapidly (within 6 – 12 months) to brown rot. Marker stakes within the area where brown rot was absent did not fail to brown rot but were decayed more slowly by other decay types. This suggested that soil around the stakes contained brown rot fungal mycelium or spores (chlamydospores were seen in wood with brown rot: chapter 4). Untreated

marker stakes may have added to an already elevated soil inoculum potential for brown rot.

At WKA, brown rot was also very active across the whole plot but was absent from most other areas of the main test site. The main difference between the WKA plot used in this study (apart from use of marker stakes) and the main test site was that the grass was not cut in an attempt to reduce inter-site variability, it being impractical to mow the more remote pastoral sites. It was clear that moisture at the ground-line was higher in the non-mown mini plot used in this study, during the summer period. This was tested by touch and by rough measurement of the quantity of condensate on the underside of a sheet that had been tightly secured over the ground for a defined period. Ironically the higher temperatures of summer would normally accelerate decay but at WKA moisture was probably limiting during the summer. Possibly the accumulation of thicker plant stems with a higher ligno-cellulose content than grass, that occurred amongst the more diverse plant community in the non-mown plot, favoured brown rot decay. Several other important types of decay were also common in the mini test plot but were not encountered in a previous study of decay type in variously preservative treated pine from the main site (Wakeling, 1991).

Therefore at HAN and WKA intra-site variability caused by differential brown rot occurrence and possibly other decay type differences had a major impact on decay rates within the 6.5 year study period and was probably the cause of similar MSR values ten years earlier in this study compared to the study of Hedley *et al.* (1996). Brown rot was of moderate importance at Glenbervie (GB) in this study but was completely absent in a previous study (Wakeling, 1991). Possibly an artificially elevated brown rot inoculum potential was also important at this site, even though the high moisture content of soil at GB probably did not favour its occurrence. However at GB and Hari Hari (HH) there may have been another important contributing factor to account for the rapid onset of high MSR values. Chapter 6 discusses the high variability of copper, chromium and arsenic loss from CCA treated pine and beech across test sites in detail but it is useful to consider some of its possible implications here.

20 x 20 x 500 mm stakes have a larger surface area to volume ratio than the 50 x 25 x 500 mm IUFRO stakes used by Hedley *et al.* (1996). For stakes treated with preservative concentrations that are close to the minimum required for in-ground exposure such as the highest retention of CCA used in this study (0.72% m/m a.i.), and where they are exposed to a high depletion hazard, this is likely to result in earlier decay and higher MSR values early on in exposure. For example, the outer 5 mm of wood for a 20 x 20 x 500 mm stake accounts for 75% of the total volume compared to 52% for IUFRO stakes. The outer 5 mm is not entirely an arbitrary figure since during assessment of stakes this is typically the depth of decay pockets associated with a soundness reduction of up to 40% for 20 x 20 x 500 mm stakes. This is because decay within the outer 5 mm of all faces is equivalent to a reduction of cross section of 50% which equates to a rating of 3^- with an associated soundness reduction of 40%.

It is to be expected that this outer wood is more susceptible to decay due to indirect below ground exposure effects such as increased rates of preservative depletion and direct effects of greater interaction with soil factors that contribute to onset of decay such as increased inoculum potential of decay microorganisms associated with soil water, decomposing material in soil and associated litter. Harrow (1959) reported that leaching operates by the square law with respect to time such that if a given quantity is removed in a certain time it will take four times as long to remove double the amount. Cockcroft and Laidlaw (1978) pointed out that earlier higher leaching rates from preservative treated timber was partly due to the greater accessibility of outer wood. This suggests that the outer wood acts as a buffer zone, reducing leaching from deeper wood by isolating it from the effects of close association with soil.

The statements of Harrow (1959), and Cockcroft and Laidlaw (1978) were made within the context of preservative treated wood in service which typically has a much higher proportion of inner wood. Therefore, it is to be expected that the early decay ratings for small stakes are likely to be unrepresentative of overall performance because they are largely a reflection of decay within a narrow outer wood zone exposed to conditions unrepresentative of those encountered by the greater part of the cross section of larger commodities. Soft rot and bacterial decay that causes a gradual erosion of the wood's surface layers, and where the

depth of decayed wood remains constant, appears to be a common occurrence in certain situations (Eaton and Hale, 1993; Zabel and Morrell, 1992). For a shallow (1-5mm) outer region of unknown depth, this erosion phenomenon may be partly a reflection of a similar square-law to that reported by Harrow (1959) i.e. as the distance from the centre of the stake doubles (within the outer zone), the decay hazard increases four fold. In very wet environments such as waterlogged soil or for wood used in an industrial cooling tower, low oxygen availability is likely to reduce decay hazard as depth from the surface increases, but in the majority of soils, where moderate moisture conditions prevail and where oxygen is not limiting, preservative depletion, fungal inoculum potential and other factors are likely to be more important.

Drysdale (1983) showed that CCA was more mobile in wood than benzalkonium chloride and was redistributed within the outer 6mm of wood wafers (sealed on all faces bar one) subjected to an 18 week leaching regime. It would therefore be expected that movement of CCA from the centre of stakes at a rate determined by Harrow's square-law would interact with soil effects to determine a defined boundary of wood susceptible to decay i.e where the toxic threshold of preservative for decay occurred. Whilst a 5 mm deep zone of decay is of little consequence to a 300 mm diameter telegraph pole, it would result in an MSR value of approximately 40% for a 20 x 20 x 500 mm stake. Irrespective of the importance of the putative "buffer" zone, it is inevitable that early assessments of 20 x 20 x 500 mm stakes will result in higher MSR values than for larger wood samples, including IUFRO stakes, and this may partly explain the more rapid onset of decay for the Tanalith C treated pine stakes used in this study compared to those treated with Tanalith NCA, reported by Hedley *et al.* (1996).

The importance of Harrow's square-law to the durability of preservative treated wood is likely to be inversely proportional to stake size. This is partly because the size of "reservoir" of preservative provided by the internal wood is determined by the square law. The large copper and arsenic depletion values for CCA treated pine and beech (more than 50% at some sites) for the below ground portion of stakes (chapter 6), supported the concept of preservative movement from the centre of a stake to the surface. Hypothetically, based on the outer 5 mm zone model, a 20 x 20 x 500 mm stake had an internal "reservoir" of preservative half

the volume of the IUFRO stakes (25/52). It is to be expected that this would affect durability if, as demonstrated in chapter 6, CCA movement out of stakes increases greatly at wet sites like HH and GB or moderately wet but highly acidic sites like Totara flat *Nothofagus* forest (TOT). The CCA retention of 0.72 % m/m a.i. (3.59 kg/m^3) used in this study was the same as the NZS3640 minimum requirement and typically H4 CCA treated radiata pine entering service would contain between 30 and 50% more preservative than the minimum requirement. This was reflected in the retentions used by Hedley *et al.* (1996) which were based on IUFRO guidelines (Becker, 1972). The ground contact retention chosen was 4.5 kg/m^3 a.i. (approximately 1 % m/m a.i.) and the other retentions were based on the IUFRO guidelines for field trials where one higher and two lower retentions in a geometric series of 1.4 are stipulated.

The importance of Harrow's square-law is also likely to be inversely proportional to the original retention of exposed stakes, for several reasons. Firstly, it is known that initial preservative solution concentration affects subsequent susceptibility to leaching due to efficiency of fixation reactions (Cockcroft and Laidlaw, 1978). It has been suggested that if components of the CCA mixture react prematurely, for example with lumen wood extractives or tannins (Kamden *et al.*, 1996), before reaction with sites across the wood cell wall, fixation is compromised. Clearly, the potential for this problem is greater for the weaker treatment solutions used to achieve lower retentions. This may be especially problematic for highly treatable wood species like radiata pine, since retentions can be achieved with lower treatment solution concentrations than for species of inherently lower uptakes. Conversely, the greater the solution concentration, the greater the efficiency of fixation, at least until the number of "reaction sites" on and within the cell wall become limiting.

Secondly, the reservoir of preservative within inner wood that is protected by the outer "buffer" zone is greater for stakes treated with higher retentions. Since leaching is determined by Harrow's square-law, the significance of retention in terms of long-term durability is similarly governed by this square-law i.e if the concentration of preservative within the inner "reservoir" is doubled, it will take four times as long to achieve toxic thresholds for decay. The relatively high losses of copper and arsenic from radiata pine treated with the 0.72 % m/m a.i. of CCA

at many of the test sites used in this study and its significance are further discussed in chapter 6.

Site decay hazard for CCA and CuAz treated pine and beech (Table 3.1) was not clearly linked to preservative depletion hazard. Table 3.4 was constructed to illustrate this point for pine treated with 0.72% m/m a.i. CCA. For example Totara (TOT) was the most severe copper depletion site but had only a low to moderate decay hazard. Similarly Tombleson (TOM) had a low depletion hazard but a high decay hazard. Conversely GB and HH both had moderately high depletion and decay hazards. As discussed in chapter 5 preservative treated wood was affected by a complex mixture of decay types that was affected by preservative type, wood species, site factors and their interactions. It is therefore

Table 3.4: Percentage residual copper (Cu), chromium (Cr) and arsenic (As) in 20 x 20 x 500 mm radiata pine stakes treated with 0.72% m/m a.i. Tanalith C after 5.5 years, and mean soundness reduction (MSR) after 6.5 years, at 13 Sites

Mean copper, chromium and arsenic remaining in stakes at each site													
	TOT	HH	CC	GB	PT	TAP	HAN	WKA	OAK	GBF	TOM	ESK	MM
Cu	27	39	45	48	57	63	81	82	82	85	94	97	104*
Cr	78	85	77	88	96	87	90	101*	97	86	100	88	106*
As	59	68	58	71	79	83	81	87	88	77	87	80	97
MSR at Each Site													
	GBF	GB	TOM	WKA	HH	CK	ESK	OAK	PT	TOT	TAP	MM	HAN
MSR	33	30	27	25	24	23	20	18	15	15	14	6	3

The top row of site names are in order of copper depletion severity, the site with the least copper remaining being on the left. The bottom row of site names are in order of decay hazard severity with the greatest MSR value on the left. *It was not clear why copper and chromium values were occasionally higher for unexposed stakes (hence % residual greater than 100%). Possibly the calibration carried out for CCA retention analysis for unexposed and exposed stakes was slightly different for each. Repeat analysis of unexposed CCA treated beech stakes (Section 2.1.2) produced very similar results. Loss of wood density due to slight decay (essentially sound stakes were selected for analysis) may also have caused a residual value greater than 100%.

not surprising that decay hazard was not clearly related to a single phenomenon such as preservative depletion at all sites. Preservative depletion was however clearly linked to site conditions as discussed in chapter 6. Whilst a high depletion and decay hazard were not always present at the same site, GB and HH were

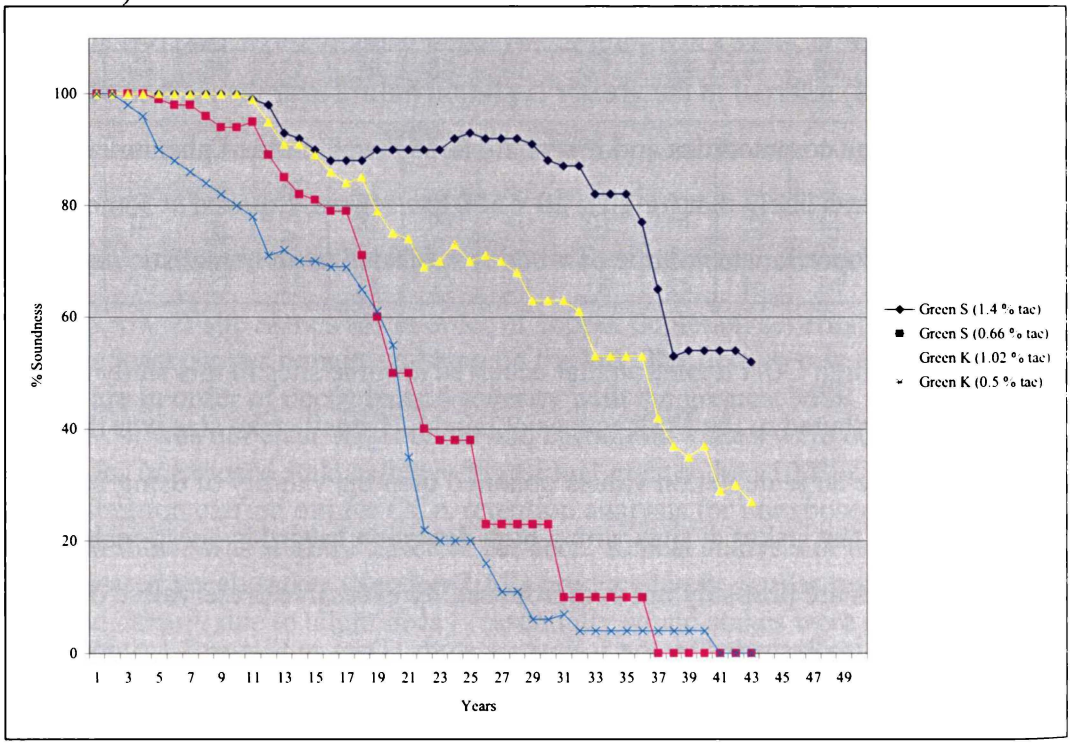
exceptions and therefore preservative depletion may have been a primary causative factor for high decay hazard at these sites. This may partly explain the more rapid onset of decay found in this study compared to the study of Hedley *et al.* (1996) because of a greater leaching hazard for the smaller 20 x 20 x 500 stakes compared to IUFRO stakes.

The implications of stake size on preservative depletion demonstrated in this study and prediction of long-term performance of preservative treated wood are important. Results of chapter 6 showed that for 20 x 20 x 500 mm radiata pine and European beech stakes, preservative depletion was very high at wet and/or acid sites. The mean copper, chromium and arsenic depletion for pine across all sites was 30% but was as high as 73% at the most severe site. Losses of active ingredients of this order of magnitude occurred for CCA and CuAz treated pine and beech and since over 200 stakes were analysed, the data was compelling. Furthermore, it was clear that above ground depletion was much less, illustrating that in-ground affects were the cause of depletion. The fact that at low depletion hazard sites, the percentage reduction was close to zero below ground, further illustrated that the range of depletions were clearly linked to site conditions. The review of factors affecting preservative depletion by Cockcroft and Laidlaw (1978) referred to the greater depletion hazard near the surface of preservative treated commodities and it appears to be a well defined phenomenon. Furthermore it seems likely that for 20 x 20 x 500 mm stakes, exposed at some sites, a disproportionate volume of wood is subjected to an unrealistic depletion hazard.

The high CCA depletion that occurred at some sites in this study almost certainly contributed to the MSR values obtained. If small stake size was the primary cause of the large depletion values obtained then the validity of using 20 x 20 x 500 mm field test stakes at sites with a high depletion hazard is questionable. That small stakes are probably not compromised by excessive preservative depletion at some sites is illustrated by the following study. Data in Figure 3.1 (unpublished data of Hedley, *Forest Research*) shows that 20 x 20 x 500 mm stakes treated with 0.5% m/m a.i. Greensalt K (AWPA type A) had an MSR value of 31% after 16 years which is similar to the value of 34% obtained for IUFRO stakes treated with 0.53% m/m a.i. Tanalith NCA at 16 years (Hedley *et al.* 1996). This suggests that small stake size did not cause premature decay in these two studies at WKA

possibly because it is not a severe preservative depletion site (chapter 6). This suggests that different stake size did not affect performance of CCA treated radiata pine in these two studies. The sudden increase in MSR for 0.5% m/m a.i. Greensalt K and 0.66% m/m a.i. Greensalt S over the six year period between years 18 and 23 years may have been caused by CCA depletion having reduced the retention to a threshold required for more rapid decay. The soundness dropped 47% over this period which was similar to the 42% MSR obtained for 20 x 20 x 500 mm stakes treated with 0.48% m/m a.i. Tanalith C in the current study over a similar time frame (6.5 years). The reason why this rate of decay occurred for Tanalith C stakes immediately (within the 6.5 years of the study) whereas for stakes treated with a similar retention of Greensalt, this rate of decay did not occur for 10 years, is probably due to the brown rot hazard within the plot used in this study but which was absent in the plot used by Hedley *et al.* (1996). Higher moisture retention in the WKA plot used in this study may also have caused an elevated depletion hazard.

Figure 3.1: Performance of 20 x 20 x 400 mm radiata pine stakes treated with Greensalt S and K (AWPA Type A CCA) (unpublished data of Hedley, *Forest Research*)



Green = Greensalt; tae = total active element.

Section 3.3.2

Relative performance of CCA, ACQ and CuAz treated radiata pine

Minimum retentions of ACQ and CuAz currently required by NZS3640 for commercial use are 1.02 (0.68 CuO) and 0.46% (0.44 Cu) m/m a.i. respectively. Average MSR data across all sites in Table 3.2 shows that radiata pine stakes treated with 1.56 % m/m a.i. ACQ (0.88 CuO) gave equivalent performance to 0.72% m/m a.i. CCA whereas 1.04 % m/m a.i. ACQ (0.58 CuO) gave inferior performance to 0.72% m/m a.i. For CuAz treated pine, 0.59% m/m a.i. gave superior performance to 0.72% m/m a.i. CCA and 0.39 % m/m a.i. CuAz gave inferior performance to 0.72% m/m a.i. CCA (see Appendix, Figure 3.8 to 3.12, for photographs of stakes after treatments). These results showed that pine treated with CuAz retentions 28% above the NZS3640 minimum requirement gave superior performance to the NZS3640 minimum requirement for CCA, but that a CuAz retention 15% below the minimum requirement gave inferior performance compared to CCA. For pine treated with ACQ, a CuO retention 29% above the NZS3640 minimum requirement gave equivalent protection to CCA but CuO 15% below the minimum requirement gave inferior control. It is difficult to extrapolate the minimum retentions of CuAz and ACQ that would have given equivalent control to CCA.

However in general the results are encouraging, suggesting that a retention close to the NZS3640, is likely to give equivalent performance to CCA. Some of the sites had severe depletion hazards (as shown by the results of chapter 6) and since CCA is generally regarded as a highly fixed preservative, particularly suited to exposure situations that harbour a severe depletion hazard, the performance given by ACQ and CuAz was encouraging. There was a suggestion that the performance of ACQ treated pine, above the NZS3640 minimum retention, increased more slowly as retention increased compared to CuAz treated pine. The relatively poorer performance of ACQ treated pine at some of the sites with a high brown rot hazard also suggested that performance against copper tolerant brown rots may be difficult to achieve unless retentions considerably above the NZS3640 requirement are used. However 1.56% m/m a.i. ACQ did not always give poorer performance than 0.59% m/m a.i. CuAz. At Canal Creek (Appendix, Figures 3.2 and 3.3) ACQ (1.56% m/m a.i) treated pine performed significantly (5% level of probability) better than CCA (0.72% m/m a.i) and gave equivalent performance to

CuAz (0.59% m/m a.i). Possible explanations for such irregularities in performance are discussed in chapter 5.

Little published information is available concerning long-term in-ground performance of pine treated with commercially available ACQ and CuAz. CuAz treated wood has been tested in field trials for almost 10 years but little has been published, possibly because of commercial sensitivity during the current period of introduction to service. Earlier trials of ACQ treated pine have been conducted but these did not involve use of now favoured copper amine systems. Hedley *et al.* (1995) reported field trials of 20 x 20 x 400 mm radiata pine stakes treated with 10.99 kg/m^3 ACQ (2.5:1 ratio of DDAC:$CuCl_2$ plus an unspecified concentration of NH_3, described as cupramine chloride) at two sites in Japan. After 12 – 13 years, ACQ treated stakes had MSR values of 10 and 60 at the two sites, compared to zero and 30 respectively for stakes treated with 9.44 kg/m^3 of Boliden K33 (AWPA Type B CCA). Whilst it was not clear what the % m/m a.i. retentions were, it was apparent that this type of ACQ had a lower CuO:DDAC ratio than the ACQ used in the current study and that recommended by NZS3640. It is now generally considered that higher CuO:DDAC ratios of 2:1 perform better. The significance of different CuO:DDAC ratios and the use of ammonia in solution to dissolve $CuCl_2$ instead of ethanolamine was not clear. The ACQ used in the current study had a 1:0.8 CuO:DDAC ratio and used ammonia solution to form the cuprammonium complex. A direct comparison of the 12 – 13 year old data of Hedley *et al.* (1995) with the 6.5 year data of this study is difficult as is prediction of the likely effects of having used an ammonia solution in this study instead of ethanolamine used in current formulations.

Creffield *et al.* (1996) reported that the performance of radiata pine treated with Tanalith E at retentions of 1.54, 2.08, 2.92 and 4.30 kg/m^3 of copper, gave equivalent performance to Tanalith C at 1.18 kg/m^3 a.i., against termites and decay at a tropical site in Northern Australia. However the method used was primarily designed to simulate a high termite hazard and used 35 x 35 x 500 mm samples placed horizontally on veneers in a pit and were not therefore in direct soil contact (apart from soil introduced by termites), although the term "in ground contact" was used. At that time the results were used to recommend use of pine treated with 2.0 – 2.5 kg/m^3 of copper for above ground situations in Australia. It is

interesting therefore that NZS3640 now specifies a requirement of 0.46 copper (approximately 2 kg/m^3) for in-ground contact.

In this study, raised inoculum potential of decay fungi caused by the experimental design probably had a significant effect on overall performance and this was especially important for increasing the brown rot hazard. Since ACQ was more susceptible to brown rot than CCA and CuAz over the retentions tested, the conditions of the current study may have favoured CCA and CuAz, a result that may not reflect the general hazard encountered in most service situations. Furthermore, the small stake size used probably caused the high preservative depletions recorded (CCA and CuAz) at wet and/or acid sites and this also probably exacerbated the decay hazard beyond what is typical in field test sites. This may not have had a major impact on performance of pine treated with the highest retentions within the 6.5 years of the study because threshold preservative retentions for decay may not have been reached in the majority of stakes exposed across all sites. However it is likely that had the study continued, the results would increasingly fail to reflect long term performance of larger commodities in service that are exposed to similar high depletion hazards. At sites with a lower depletion hazard this effect of time of exposure is likely to be much less, as dictated by the square law of Harrow (1959) and as suggested by the results of Hedley *et al.* (1996).

CCA treated pine performs well across diverse in-ground exposure situations but is susceptible to rapid failure caused by brown rots in some situations (Wakeling and Singh, 1993). Since ACQ was more susceptible to brown rot than both CCA and CuAz, it is likely that weakness against copper-tolerant brown rots could be a significant issue in a much broader range of exposure situations than for CCA, as occurred for acid-copper-chromate (ACC) treated pine (Richardson, 1993). In contrast, the CuAz preservative was a highly effective treatment against copper tolerant brown rots. This has been demonstrated in laboratory trials before but the finding that it is also the case over a diverse range of field conditions some of which harboured very aggressive brown rot fungi, was encouraging.

The CuAz supplied for this study in 1993 was referred to as Tanalith 3485 (now known as Tanalith E), however commercial sensitivity at that time prevented

keeping accurate records of its precise composition. It has been suggested that the nature of the tebuconazole microemulsion system has been altered and since formulation type is known to affect performance (Preston, 1999), especially sensitive microemulsion systems, it is possible that the Tanalith E that is now commercially available and the prototype used in this study were not identical in terms of performance. The ACQ supplied by the manufacturer was described as ACQ-C, a formulation that is not currently supplied for commercial use. Shortly after this study was initiated (1993) the manufacturer of the ACQ supplied, changed from using ammonia solution to dissolve the copper salt (oxide or salt unkown) to use of ethanolamine as the solvent. The significance of these changes on performance is not known and therefore a degree of caution should be exercised during comparisons of performance between CCA treated pine and ACQ and CuAz treated pine used in this study and currently available formulations. The primary emphasis of the data provided in this chapter is on the relative performance of different preservatives between sites and its significance for in-ground preservative performance and test site selection.

Possibly one of the reasons the oil based preservatives creosote and chlorothalonil performed well in this study was because depletion was less significant under the experimental conditions employed. Creosote has been reported to perform poorly in stake tests compared to performance in posts and poles (Richardson, 1993). The rationale was that creosote was more mobile than a fixed preservative like CCA and therefore replacement of outer depleted wood by inner reserves of creosote deep within a post ensured long term performance. Possibly the 6.5 years employed in this study was inadequate for this type of depletion to become significant for the highest creosote retention used (35% m/m a.i.).

It is difficult to comment on the relative performance of beech treated with CuAz and chlorothalonil plus chlorpyriphos because the retention achieved for the reference standard, CCA treated beech (0.42 % m/m a.i.) was below what was aimed for and what would typically be regarded as adequate for a hardwood. European beech is not usually used in ground contact so recommended retentions are not available but the New Zealand Timber Preservation Council specifications (MP3640, 1992) required a minimum of 30 kg/m^3 (approximately 6.6 % m/m a.i) of CCA salt in the sapwood of Australian eucalypt poles. As discussed in

chapter 5 this high retention requirement is due to the increased susceptibility of preservative treated hardwoods to soft rot decay which is related to the difficulty of obtaining even preservative uptake (macro-distribution) and adequate preservative concentration across fibre walls (micro-distribution) (Eaton and Hale, 1993) that are inherently more susceptible to soft rot than pine tracheids because of a high syringyl:guaiacyl lignin ratio. It is possible that the reason CuAz treated beech and Chlorothalonil-chlorpyriphos treated beech performed significantly better than CCA treated beech was related to their more even distribution across fibre cell walls. Hedley *et al.* (1996) suggested that improved distribution of copper across the cell wall of pine treated with copper-chromium-boron (CCB) resulted in improved resistance to soft rot at wet sites.

Section 3.3.3

Selection of field test sites for testing preservative treated wood in ground-contact

Site location had a major impact on performance of preservative treated wood in ground contact. In general, this could be attributed to the effects of temperature, water availability, soil type, and to a lesser extent vegetation and litter type. A warm, moderately wet climate, in combination with a loam soil, resulted in a severe decay hazard. At 2 of the warm temperate test sites, decay hazard was as severe as occurred at a wet tropical site. In general, drier sites had lower mean soundness reduction values but not all preservatives gave better performance at these sites. For example at HAN, which had one of the lowest mean soundness reduction values, ACQ treated pine performed relatively poorly. This effect and other site-treatment interaction effects were determined by inter-site decay type variability and are discussed in chapter 5.

The effects of site conditions on decay type and their implications for performance of preservative treated wood is discussed in detail in chapter 5, however it is useful to cover some of the implications of these effects for selection of an optimal range of test sites for in-ground testing of preservative treated wood. Whilst it is impossible to test all the permutations of decay hazard likely to be encountered in service, it should be possible to select test sites that adequately represent the majority of decay hazards encountered in service. Based on site conditions and the affect these have on decay hazard, preservatives do not always

perform as expected. Therefore it is probably unwise to rely on data from a single site, even if it is known to be a severe site in general terms, and a similar conclusion has been reached by others (Hedley *et al.*, 1996; Edlund, 1998;). Based on the data from this study and a knowledge of decay types encountered (chapter 5) it was possible to select 4 sites that collectively pose a diverse decay hazard designed to test different types of preservative and preservative-wood species combination. These sites would cover the majority of situations encountered by wood in service: GB, HH, Tapanui (TAP) and TOM. These sites include very active soft rot, brown rot, bacterial decay and several other important types (accounting for 50% of decay – chapter 5) partly attributed to basidiomycete white rot fungi (chapter 4). They are also representative of warm temperate and cool temperate conditions and so the effects of temperature are incorporated. All these sites have at least moderate water availability since the results suggested that inclusion of arid sites (HAN and Mt. Mee (MM)) is of little value.

Whilst HAN had a high brown rot hazard this was largely because other decay types were excluded by the arid conditions and since a high brown rot hazard can be achieved at sites that also harbour a high hazard from other important decay types they are more cost-effective (e.g. GB and TAP). Sites that drain quickly like the pumice soil at WKA are not ideal because they are too arid for some types of decay (chapter 5), probably do not promote preservative depletion mechanisms (chapter 6) and is therefore not the most versatile type of site to include within a small group. Ironically, *Forest Research*, located at Whakarewarewa, is gradually loosing remote test sites and the great bulk of test material is exposed at the local site only.

The decay hazard at WKA is lower than at many of the sites studied and therefore it takes longer to provide performance data. It could be argued that performance data derived from a moderate to low hazard site is important because much of the preservative treated wood commodities in service are also exposed at low hazard sites. However, the results of this study suggested that more is achieved by selecting a low to moderate hazard site which has a high water holding capacity such as occurred at TAP, because decay type is more diverse and decay is not interrupted by periods of low moisture during dry spells caused by excessive drainage. Therefore a low temperature site with a clay loam is more likely to

promote diverse decay types whilst maintaining low to moderate decay rates due to the lower temperatures. A similar effect was achieved at the Oak site (OAK) which had very low annual rainfall (<500 mm) and long periods of drought over the summer, but because of the tree canopy coupled with a heavy clay soil and associated litter, the groundline region was rarely dry. This site was on a slope and did not appear likely to have been affected by ground-water but this possibility cannot be ruled out.

A disadvantage of the main WKA site compared to the WKA plot used in this study was that vegetation was not cut in the current study and this promoted higher moisture at the groundline, especially during hot summer months when higher temperatures are likely to accelerate decay rates. Intra-site decay type and associated decay hazard variability is an important phenomenon and can potentially be manipulated to encourage a diverse decay type within different plots of the same site. This may be a way of achieving some of the benefits of multi-site testing at one location whilst increasing cost effectiveness of testing.

Hari Hari was a useful site because the prevalence of a water-logged soil excluded brown rots, whilst encouraging other important decay types (Appendix, Figure 3.10). Brown rot can cause rapid failure (within 12 months) of radiata pine treated with 0.72 % m/m a.i. CCA (highest retention tested), 0.39 % m/m a.i. CuAz (second highest retention tested and 15% below the minimum requirement of NZS3640) and 1.56 % m/m a.i. of ACQ (highest retention tested and above the minimum requirement of NZS3640). Such sudden failures started to occur after the fourth year of exposure. Clearly it is important to include sites with a high brown rot hazard but it is also important to include a site that excludes brown rot, otherwise the differential effects of a range of decay types is likely to be missed. The water-logged conditions at Hari Hari also caused high CCA depletion. It is clearly an advantage to test candidate preservative systems under severe leaching hazard conditions. This study has shown that even CCA treated pine, a combination widely accepted as a highly "fixed" treatment, is susceptible to rapid loss from test stakes at very wet and, or acid sites (possibly not for larger preservative treated commodities). Results of chapter 5 suggested that the low pH sites PT and TOT had a narrower range of decay types. In particular, the soft rot hazard was diminished and brown rot and bacterial attack was absent. Therefore

whilst low pH promotes rapid loss of CCA from pine and beech and copper from CuAz treated pine and beech, it is probably advisable to select a wet site with a pH within the normal range for horticultural and pastoral soils (4.5 – 6.5) thereby achieving a high depletion hazard as well as encouraging a greater decay type diversity.

Tombleson's mandarin orchard (Appendix, Figures 3.5 and 3.6) was a useful site because it combined a very low preservative depletion hazard and excluded brown rot. The result was that preservative tolerance exhibited by tunnelling bacteria and soft rot could be tested. This type of tolerance is different to brown rot tolerance. Within the 6.5 year period of the study, the ability of brown rot to decay pine (and sometimes beech) treated with the highest retentions of ACQ and CCA, and the second highest retention of CuAz did not appear until the fourth year, and the great majority of failures, especially for ACQ, did not occur until the final years assessment. This factor coupled with the resistance of the highest retention of CuAz suggested that preservative depletion was required before the threshold retention for decay was achieved. Alternatively this phenomenon could have been related to build-up of an inoculum potential in the soil. However, the latter seems unlikely because at Esk Valley vineyard (Appendix, Figure 3.7) and Glenbervie Forest (GBF) (Appendix, Figure 3.4), stakes were touching woody material in the soil that contained brown rot. This did not appear to cause more rapid onset of brown rot compared to WKA, TAP, HAN and GB, which also had high brown rot hazards. Therefore, preservative depletion appeared to be a more likely contributing factor to the timing of brown rot initiation (unless soil mycelium of brown rot fungus at pastoral sites is of similar significance in fungal-spread as it is known to be for cord-forming forest floor fungi). At TOM, a combination of low preservative depletion and presence of a compact silty clay loam encouraged preservative tolerant bacteria and soft rot. This type of decay arrived earlier than brown rot suggesting it had a higher tolerance than brown rot. Alternatively tunnelling bacteria and soft rot may only be able to decay wood in an outer zone where proportionately more preservative depletion has occurred. Fine clay soils that compacted easily and a had a high water holding capacity appeared to be more important for encouraging bacterial decay (TOM, OAK, TAP and CC) than high moisture and soil fertility factors like high mineral availability (e.g calcium and magnesium) (discussed in more detail in chapter 5).

Section 3.4

Conclusions

1. Pine stakes treated with 0.59% m/m a.i. CuAz (28% above the NZS3640 minimum requirement for CuAz of 0.46% m/m a.i.) gave superior performance compared to pine treated with 0.72% m/m a.i CCA (NZS3640 minimum requirement), but pine treated with a CuAz retention (0.39% m/m a.i.) 15% below the minimum requirement gave inferior performance compared to CCA.

2. Pine treated with 1.59% m/m a.i. ACQ (CuO:DDAC of 1:0.8) (CuO retention 29% above the NZS3640 minimum requirement of 0.68% m/m a.i.) gave equivalent protection to the NZS3640 minimum requirement for CCA but 1.02% m/m a.i. ACQ (CuO 15% below the minimum requirement) gave inferior performance.

3. In general, the results for the new preservatives CuAz and ACQ were encouraging, suggesting that at retentions close to the minimum requirements of NZS3640, both gave equivalent performance to CCA across widely varying conditions, including sites with extreme preservative depletion hazards (as shown in Chapter 6). 6.5 years is however too short an exposure period for prediction of long-term performance.

4. ACQ (1.56% m/m a.i.) and CCA (0.72% m/m a.i.) treated pine were susceptible to sudden failure at some of the sites that had a high brown rot hazard whereas pine treated with CuAz (0.59% m/m a.i.) was not affected, suggesting that CuAz was particularly effective against brown rots. At the most severe brown rot sites ACQ treated pine was more susceptible than CCA and its performance in service may be compromised as a result, as previously occurred for pine treated with acid copper chromate (ACC).

5. The decay hazards encountered, as determined by mean soundness reduction after 6.5 years, were more severe than encountered in previous studies at some of the same sites and this was linked to differences of intra-site decay type between test plots and associated decay hazard

differences. It was also likely that the presence of a high percentage of untreated stakes and stakes with low preservative retentions raised the inoculum potential of surrounding soil thereby enabling decay fungi, brown rot in particular, to decay wood containing the highest retentions used. This elevated hazard may have been too high to enable accurate prediction of in-ground performance across the majority of likely in-service (in-ground) situations.

6. Greater decay rates encountered in this study were, in part attributed to high preservative depletion (chapter 6). At very wet sites, particularly those likely to have an acid soil 20 x 20 x 500 stakes are probably too small for accurate interpretation of long-term durability of wood treated with CCA and CuAz preservatives. This was believed to have been linked to their high surface area to volume ratio (75% of stake volume is in the outer 5 mm zone) causing preservative depletion greatly in excess of what is likely to occur for larger preservative treated wood commodities.

7. The oil based preservatives creosote and chlorothalonil plus chlorpyriphos performed significantly better (5% level of probability) across all sites compared to all water based treatments except CuAz, and this was attributed to the high leaching hazard having a proportionally greater effect on water based preservative formulations.

8. Site had a significant (5% level of probability) effect on preservative performance and a significant site-preservative interaction effect meant that overall site hazard index did not always reflect performance of preservative treated wood. However, overall site hazard index was in general a reliable indicator of preservative performance. An important exception occurred at those sites with a high brown rot hazard. At these sites, pine stakes treated with the highest retentions of CCA (0.72% m/m a.i.) and ACQ (1.56 % m/m a.i) and the second highest retention of CuAz (0.39 % m/m a.i.) started to fail suddenly (within 12 months) after the fourth year of exposure.

9. Knowledge of the distribution of different decay types across sites tested, coupled with associated decay hazards and preservative depletion hazard, suggested that 4 test sites of clearly defined features would enable comprehensive field testing of preservative treated wood. Selection of sites is not straightforward and requires a rudimentary knowledge of soil type, geology, vegetation and climate, or comprehensive knowledge of the decay types present. In view of the cost of maintaining test facilities, adequate multi-site testing of new wood preservatives may be achievable through cooperation between research establishments. Possibly, current test site selection criteria fall short of ensuring adequate test site parameters are incorporated and maximum cost effectiveness of testing may not always be achieved through duplication of test site parameters between sites of similar but unrecognised properties

Section 3.5

Appendix 3

Table 3.5: Significant differences at a 5% level of probability between preservative treatments within sites

Treatment	HH		MM		OAK		ESK		GB		GBF		HAN		CC		PT		TAP		TOM		TOT		WKA	
Pine	100	f	100	c	91	d	100	f	100	f	100	g	100	i	100	e	100	g	98	f	94	g	100	f	100	f
Beech	100	f	88	b	89	d	100	f	100	f	100	g	100	i	100	e	100	g	100	f	84	f	100	f	100	f
PC4	7.1	a	12	a	15	ab	21	b	18	a	19	bc	10	bc	28	ab	11	ab	8.6	ab	19	a	5	ab	5.5	a
PACQ4	15	b	11	a	20	abc	11	a	31	b	28	cd	23	def	19	a	19	bc	30	d	17	a	5	ab	21	cd
PCAZ4	20	b	11	a	14	ab	19	ab	27	b	18	ab	0	a	29	b	15	b	9	a	20	a	18	d	0.9	a
PCCA5	24	bc	5.9	a	18	abc	20	ab	27	b	33	de	2.7	ab	23	ab	15	b	9.3	ab	27	ab	15	cd	25	d
PCC4	7.5	a	3.8	a	6	a	12	ab	30	b	5.7	a	2.4	ab	32	bc	4	a	14	b	36	bc	3.8	a	10	ab
POIL	100	f	ND		37	c	66	e	53	c	58	e	18	cde	40	c	16	bc	32	de	82	f	8.3	abc	39	e
Solvent	100	f	ND		91	d	90	f	100	f	100	g	88	h	100	e	88	f	89	f	100	g	85	e	100	f
BCCA3	36	d	ND		26	c	33	c	72	d	66	f	28	fg	61	d	30	de	26	d	58	e	18	d	21	cd
BCAZ2	30	cd	ND		16	ab	15	ab	47	c	28	cd	14	cd	57	d	26	cd	23	cd	32	bc	20	d	20	bcd
BCC2	18	b	ND		36	c	21	b	46	c	25	bcd	23	def	55	d	16	bc	15	bc	54	e	14	bcd	25	d
BOIL	52	e	ND		100	d	46	d	88	e	52	f	34	g	64	d	38	e	44	e	81	f	83	e	33	e
Mean	*47*		*33*		*43*		*43*		*56*		*49*		*34*		*54*		*37*		*38*		*54*		*36*		*39*	

Within each column, values followed by the same letter do not differ significantly. ND = no data (beech treatments not exposed).

Table 3.6: Significant differences at a 5% level of probability between sites for each preservative treatment

Site	Pine		Beech		PC4		PACQ4		PCAZ4		PCCA5		PCC4		POIL		Solvent		BCCA3		BCAZ2		BCC2		BOIL	
HH	100	c	84	a	21	de	15	bcd	20	d	2.7	a	4	a	100	h	100	b	36	e	30	e	18	abc	52	d
MM	100	c	88	a	12	abc	11	a	11	b	5.9	a	3.8	a	ND		ND		ND		ND		ND		ND	
OAK	91	a	89	a	15	bcd	20	de	14	bc	9.3	ab	5.7	ab	37	cd	91	a	26	bc	16	ab	36	e	100	h
ESK	100	c	100	b	18	cd	11	ab	19	cd	15	bc	2.4	a	66	f	90	a	33	de	15	a	21	bcd	46	cd
GB	100	c	100	b	19	cd	31	g	27	bc	15	bc	36	f	53	e	100	b	72	h	47	f	46	f	88	g
GBF	100	c	100	b	19	ab	28	fg	18	cd	18	cde	10	bcd	39	d	100	b	66	g	28	de	25	d	52	d
HAN	100	c	100	b	28	e	23	ef	0	a	20	cde	7.5	abc	18	b	88	a	28	cde	14	a	23	cd	34	a
CC	100	c	100	b	11	ab	19	de	29	e	23	ef	32	f	40	d	100	b	61	fg	57	f	55	g	64	ef
PT	100	c	100	b	8.6	ab	19	de	15	cd	24	ef	12	cd	16	b	88	a	30	cd	26	de	16	ab	38	ab
TAP	98	bc	100	b	19	cd	30	g	9	a	25	ef	6	ab	32	c	89	a	26	bc	23	cd	15	a	44	bc
TOM	94	ab	100	b	7.1	a	17	bcde	20	d	27	f	3.8	a	82	g	100	b	58	f	32	e	54	g	81	f
TOT	100	c	100	b	5	a	5	a	18	a	27	f	14	d	8.3	a	85	a	18	a	20	b	14	a	33	a
WKA	100	c	100	b	5.5	a	21	de	1	cd	33	g	30	e	39	d	100	b	21	ab	20	b	25	d	83	f
Mean	*98*		*98*		*14*		*19*		*15*		*19*		*11*		*46*		*94*		*40*		*27*		*29*		*60*	

Within each column, values followed by the same letter do not differ significantly. ND = no data (beech treatments not exposed).



Figure 3.2: Canal Creek tropical site, showing 8 foot high grass. Inset shows stake after long grass had been pushed and cut away.



Figure 3.3: Canal Creek tropical site, after grass had been cut.



Figure 3.4: Glenbervie radiata pine forest, showing thick layer of pine needles and slash around stakes.



Figure 3.5: Tombleson's mandarin orchard.



Figure 3.6: Tombleson's mandarin orchard: stake situated between mandarin trees. Dashed circle = irrigation spray.



Figure 3.7: Esk Valley vineyard. Stakes were in rows located between grape vines as for Tombleson's mandarin orchard.



Figure 3.8: Below ground portion of pine stakes treated with the highest retention of CCA (0.72% m/m a.i.) after 6.5 years at the severe test site Glenbervie.

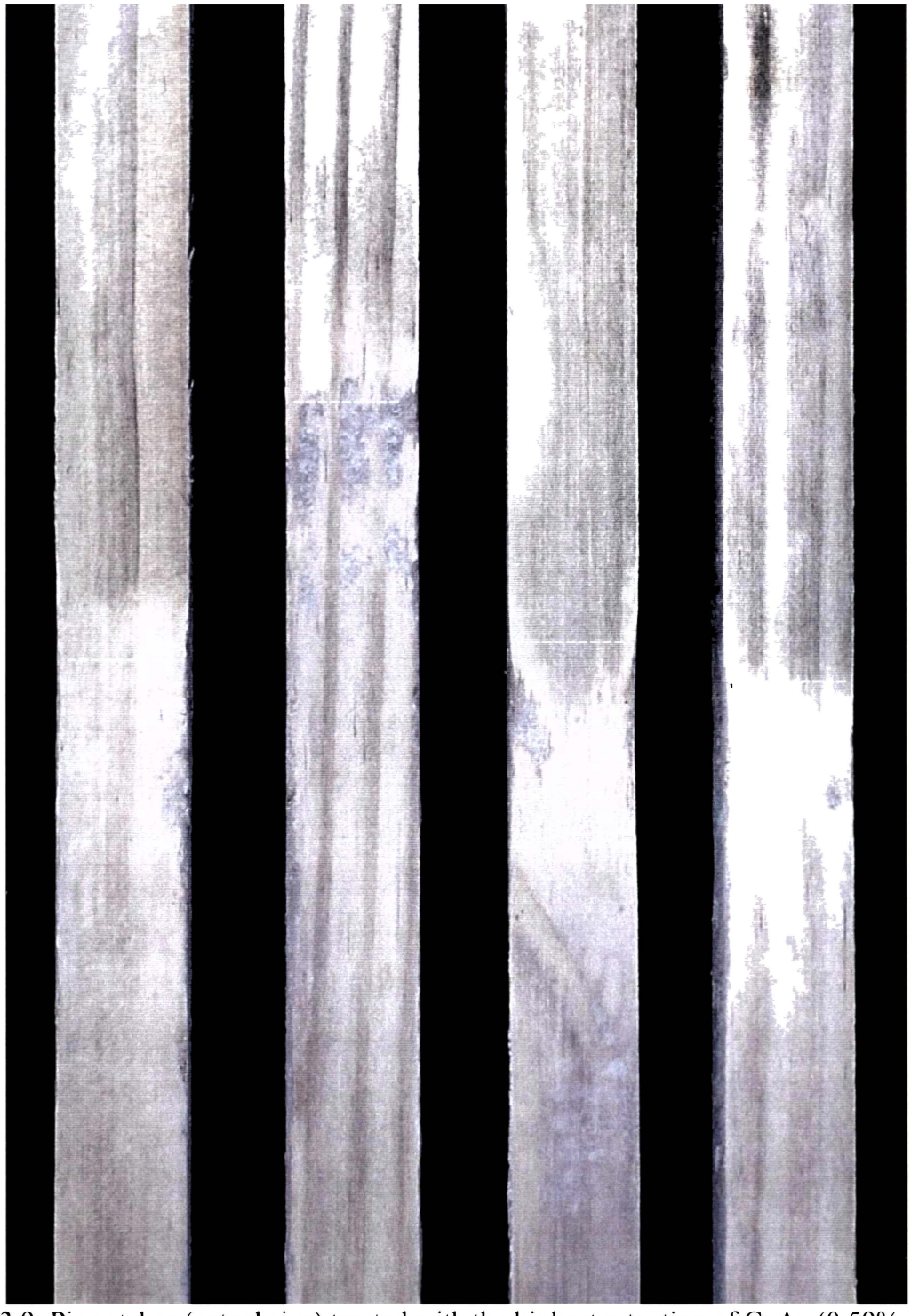


Figure 3.9: Pine stakes (actual size) treated with the highest retention of CuAz (0.59% m/m a.i.) after 6.5 years at the severe test site Glenbervie. Dotted line indicates approximate ground line position, below ground portion located at bottom of figure.



Figure 3.10: Stakes from Hari Hari, a moderately severe site, after 5.5 and 6.5 years for beech and pine respectively: a) beech treated with CuAz (0.56% m/m a.i.); b) beech treated with CCA (0.42% m/m); c) pine treated with CCA (0.72% m/m); d) pine treated with CuAz (0.59% m/m a.i.). Dotted line indicates approximate ground line position, below ground portion located at bottom of figure.



Figure 3.11: Below ground portion of stakes from the oak forest site, a moderately severe site, after 5.5 and 6.5 years for beech and pine respectively: a) beech treated with CCA (0.42% m/m a.i.); b) beech treated with CuAz (0.56% m/m a.i.); c) pine treated with CCA (0.72% m/m); d) pine treated with CuAz (0.59% m/m a.i.).
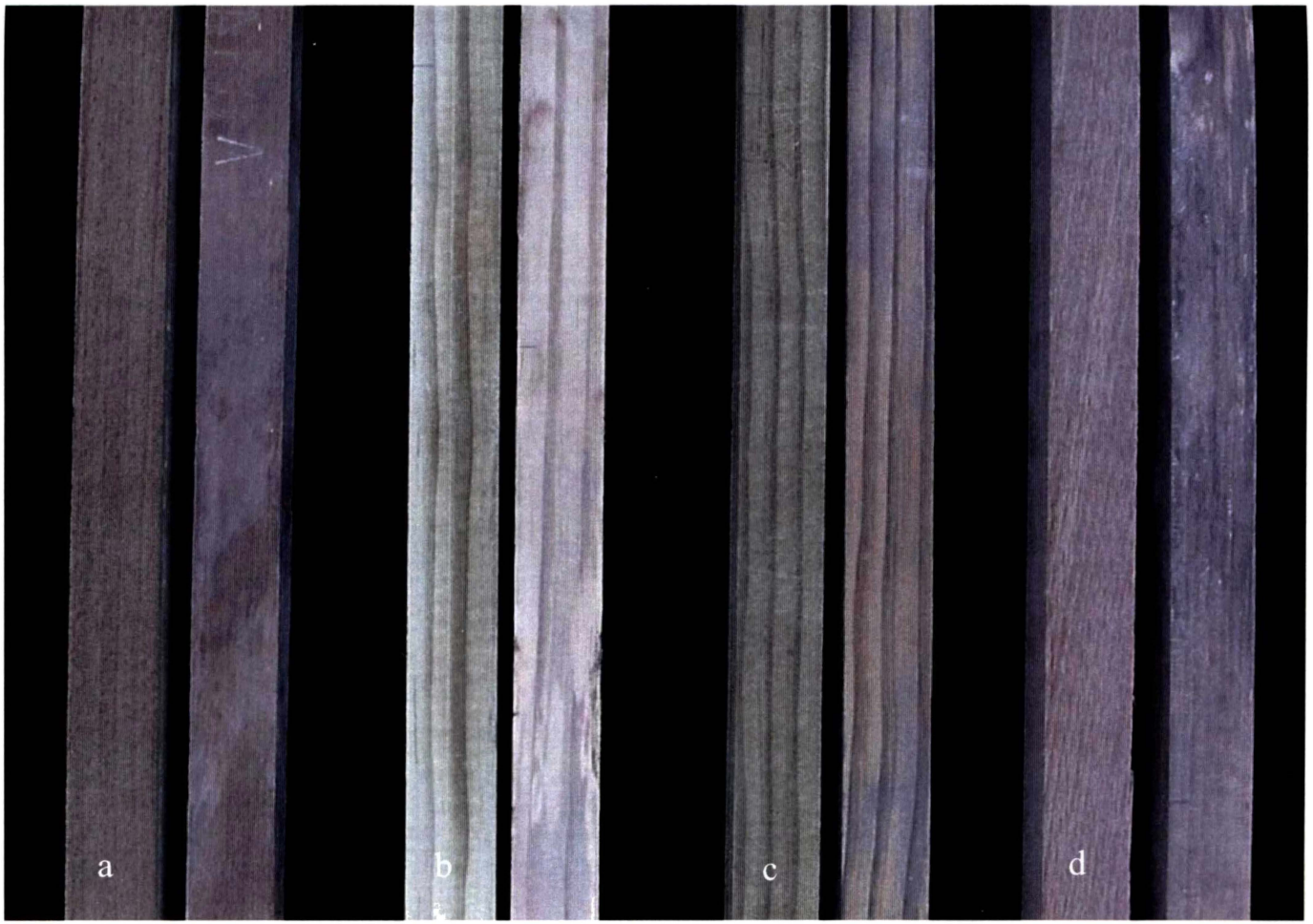


Figure 3.12: Below ground portion of stakes from the Totara Flat *Nothofagus* beech forest, a low hazard site. Stake on left of each pair was unexposed and the stake on the right had been in the ground for 5.5 and 6.5 years for beech and pine respectively: a) beech treated with CCA (0.42% m/m); b) pine treated with CCA (0.72% m/m); c) pine treated with CuAz (0.59% m/m Cu); d) beech treated with CuAz (0.56% m/m Cu).

CHAPTER FOUR

Wood decay micromorphology in *Pinus radiata* and *Fagus sylvatica*

Section 4.1

Experimental

The objective was to determine decay micromorphology diversity in variously preservative treated and untreated *Pinus radiata* and *Fagus sylvatica* wood in ground contact and evaluate the ability of current decay classification nomenclature to describe it.

1. For non-durable wood species, the different wood cell wall chemistry and structure between hardwoods and softwoods has a major effect on decay type. Therefore, exposure of hardwood and softwood stakes was designed to increase the likelihood of a more diverse group of decay types developing. The softwood *P. radiata* and the hardwood *F. sylvatica* were used in this study because they have been extensively studied within the wood preservation field and are therefore useful reference species.

2. Preservative type (chemistry) and concentration within biodegradable materials are widely known to influence microbial species selection. This is in part due to suppression of susceptible species that are therefore excluded from the wood surface. Preservative could also have an indirect effect on species selection by altering the competitive dynamics of microbial populations on the wood surface. By exposing wood treated with different preservatives it was expected that this would select different decay microorganims possessing the appropriate tolerance mechanisms. To ensure that the results were representative of in-service preservative treated wood, retentions approximating to those specified by NZS3640 for ground contact were included (for pine). Lower concentrations were included to ensure advanced decay occurred within the timeframe of the study. Preservative-wood species interaction effects also influence decay type. For example differential preservative distribution across hardwood and softwood cell walls are believed to affect susceptibility to soft rot (SR).

3. Field test site location is known to affect decay type. One of the most important site factors that influence decay type are soil and hence wood moisture content and possibly local microorganism species. Both these factors are affected by rainfall, water-holding capacity of the soil, site drainage characteristics, the type of vegetation and other local factors that influence arrival of spores of decay fungi. Soil chemistry and fertility may affect decay microorganism species occurrence directly, or indirectly by altering wood properties or by affecting preservative depletion. Sites were chosen to increase the probability that all these factors were affected to varying degrees. Since decay fungi play an important role in wood decay in forest ecosystems it was decided to include three forest sites. Since hardwoods and softwoods have different decay type susceptibilities it was decided to include a softwood forest and a hardwood forest. It was considered that an ideal experimental design would include a *P. radiata* and an *F. sylvatica* forest as this would increase the likelihood of encountering natural populations of decay microorganism species adapted to decay the *P. radiata* and *F. sylvatica* stakes used. Unfortunately *F. sylvatica* forests do not occur in New Zealand and so a southern-hemisphere beech forest containing mixed *Nothofagus* species was selected instead. As well as the *P. radiata* forest, a mixed oak (unknown *Quercus* sp.) and ash (unknown *Fraxinus* sp.) forest was selected. Temperature has a major effect on the rate of decay and may also affect decay type and therefore site latitude and altitude were selected to ensure a diverse temperature range and a wide diurnal and seasonal temperature fluctuation.

4. Wood species, preservative type and site location effects on decay type selection are likely to combine to produce interaction effects.

This study was conducted in parallel with the objectives of chapter 5 where it was necessary to establish a correlation between macroscopic and microscopic features as part of determining the effects of site, preservative and wood species on decay type occurrence. The requirement explained in chapter 5, for microscopic examination of a representative sample of stakes of all macroscopic types for all treatments exposed at all sites, was also an effective method of ensuring that samples examined

microscopically contained a broad diversity of decay micromorphology. Whilst a small sub-sample of decay types were examined using SEM, TEM and CLSM, LM was the most suitable technique for examining large numbers of wood samples generated by a field study of this type (Anagnost *et al.*, 1998).

Section 4.2

Results

Each decay type was described in a separate section. To facilitate efficient referral to the micrographs whilst reading the text of this chapter, micrographs are presented in a separate binding (Volume II, Sections 4.5.1 – 18). Diagrams illustrating the mechanism of formation of decay types are given in Section 4.6 of Volume II.

Terminology for describing the orientation of micromorphological features in wood cells

Within the field of wood science the term radial section usually refers to a cut along the radius of the tree. On the assumption that a tree is a perfect cylinder, a hypothetical tangential section is a cut at right angles to the radius of the tree. Since a tree is not a perfect cylinder, the tangent of the curvature of the tree or its growth rings is not exactly at right angles to the radius. A wood section intended for microscopy is usually described in terms of its orientation relative to the radius or hypothetical tangent of the tree. However, for the purposes of describing the orientation of micromorphological features within a single wood cell, as was the focus of this study, the terms radial and tangential refer to the radius and tangent of an essentially cylindrical wood cell, not the tree. Therefore, a cut through the radius of a wood cell is a right-angled cut through the vertical (longitudinal) axis of the cell wall. A tangential cut passes through the cell wall at right angles to the radius i.e it is parallel to the tangent of the curvature of the cell. A tangential cut does not therefore expose the cell lumen. For example, a tangential T-branch produced by the SR fungus *Chaetomium globossum* in bamboo (*Phyllostachys viridi-glaucescens*) fibres was described by Sulaiman and Murphy (1995) as tangential because the "hat" of the "T" was within (parallel to) the tangential plane of the wood cell wall (Figure 4.1) i.e. it was at right angles to the radial plane of the cell and the stem of the "T" (not the radial plane of the tree). In this study, micrographs were labeled using the same

terminology as used by Sulaiman and Murphy (1995) (Figure 4.1). Adjacent wood cells are typically staggered within woody tissues and therefore within a single plane of a wood section it is possible to achieve both radial sections (faces) of cells and tangential sections in different wood cells. If a wood section is not cut parallel to the long axis of the cell i.e. it is at an oblique angle, this also results in exposure of cell wall faces that are intermediate between tangential and radial. All three types of section (radial, tangential and oblique) are useful because they provide insight into the orientation of the decay micromorphology as observed from different angles.

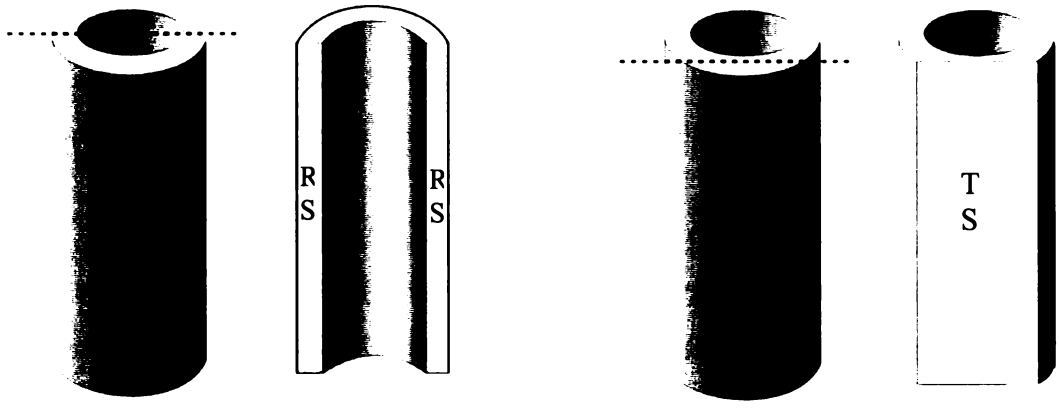


Figure 4.1: Radial (RS) and tangential (TS) sections through wood cell wall

Section 4.2.1

Rationale for separation of decay micromorphology using established and new decay type nomenclature

Current decay classification lacked the nomenclature to describe several decay types frequently encountered. This is not a new situation since some of the decay types reported in the literature (Table 1.3) also do not fit neatly within any one of the recognised types (Table 1.2). However the general impression gained from the literature is that the current classification system accounts for the majority of decay types that affect wood and that those which do not fit are a small minority. However the results of this study suggested that decay types that do not fit were amongst the most common types and were widely distributed across different in-ground field

conditions, and it was therefore necessary to invent nomenclature to describe them in a way that enabled their separation from other types. New nomenclature was employed as a mechanism to separate types primarily on the basis of a defined group of micromorphological features and was not in general representative of taxonomic groupings of fungi or bacteria since this was typically unknown. However identification of basidiomycetes (by observation of clamp connections) associated with several important types of micromorphology that might otherwise be considered soft rot (SR) or closely related to SR, was common and this influenced the classification system derived.

The most robust nomenclature developed was primarily a product of deduction of the mechanism of decay formation through iterative examination of many samples at varying stages of decay. At the core of the process was construction of three-dimensional (3D) imagery of wood cell wall destruction (erosion, cavitation, tunnelling etc.). Construction of 3D imagery was achieved by examining many sections of wood of different orientations at varying stages of decay. Where a high degree of confidence was established, diagramatic representations of decay micromorphology were produced (Section 4.6). Most information was obtained from longitudinal radial and longitudinal tangential sections of pine tracheids and beech fibres. The depth of field and resolution achieved with the Zeiss Axioplan II LM optics represented a considerable improvement over some of the observations reported in the literature and this enabled new interpretations to be made. Transverse sections (TS) typically did not provide information beyond that provided by longitudinal sections and therefore micrographs of TS sections were rarely produced. TS sections provide knowledge of the position of decay micromorphology in the radial plane (across the depth of the wood cell wall), however the orientation of radial features was best visualised in LS in this study.

Other, less robust nomenclature was not representative of a single well-defined sequence of decay events, but described unusual micromorphology, the cause of which was unclear but which did not appear to fit with decay mechanisms and features described for classical decay nomenclature. In some cases it was likely that some groups of features were representative of more than one decay type, but were

placed together because of similarities. Failure to describe a detailed sequence of decay events was usually a reflection of the difficulty of finding early stages of attack. This was probably related to the ephemeral nature of some early features and the awkward orientation of initiatory decay structures, such as tangential T-branching of proboscis hyphae that made observation in longitudinal sections difficult. It was not practical to elucidate these decay types in greater detail using more time consuming microscopy techniques since the primary focus was on collecting information from a large number of samples exposed to diverse field conditions. There is however considerable scope for further studies in this area.

Very few micromorphological features were unique to single decay types. Instead, the great majority of features were widely spread across several types and therefore there was an inevitable overlap between types. Another way of explaining this is to consider decay micromorphology as a continuum rather than a series of neat subdivisions. Positioning within the continuum is likely to shift according to the precise combination of environmental, wood, preservative and microorganism factors and the nature of their interactions. Therefore, segregation was not intended to identify fixed groupings of decay micromorphology but it enabled the initial focus, on groups of micromorphological features, that is required to understand the underlying sequence of decay events that link them.

Section 4.2.2

Brown rot (micrographs in Section 4.5.1, Volume II)

Decayed wood cell walls contained no cavities or tunnels and cell walls did not shrink unless decay was advanced, although this did occur for wood with earlier attack if wood had dried. Loss of cellulose birefringence of the wood cell wall was not a reliable indicator of brown rot (BR) since it often did not occur for wood with advanced BR (micrograph 6 (M6-BR)), as determined by highly distinctive macromorphology (Section 5.5.4). Furthermore, loss of birefringence was common for some types of frequently observed cavitation decay, and whilst other distinctive features were present, it is possible that they would be missed if the observer was not thoroughly familiar with their significance (Sections 4.2.6.6 and 4.2.6.7). Bore holes (BH) were often absent or infrequent. However, whilst no single micromorphological

feature enabled reliable identification of BR, one or more of a group of features were usually present and in combination with a highly distinctive macromorphology. BR was typically readily identifiable (exceptions that occurred for wood possessing a mixture of white rot (WR) and brown rot (BR) micromorphology is covered in Section 4.2.3). Common micromorphology associated with BR included:

1. presence of distinctive chlamydospores (M1-BR) and M2-BR);
2. intense brown staining of the wood cell wall in the absence of SR cavities (similar staining of wood with SR was common) (M3-BR);
3. characteristic fracturing of the wood cell wall believed to occur during sectioning (M4-BR, M5-BR and M6-BR);
4. copious uptake of aniline blue dye by an apparently intact cell wall i.e. no visible damage under LM (M8-BR);
5. dense crystalline deposits in wood cell luminae and on hyphal walls, believed to be oxalic acid or an oxalate salt (M9-BR and M10-BR).

Absence of bore holes in many sections and degradation of parenchyma ray cells (M1-BR) suggested colonisation typically occurred via the rays and that entry into the luminae of pine tracheids and beech fibres occurred through connecting bordered pits. Ray parenchyma cells were usually destroyed and the resultant voids typically contained hyphal remnants and, or chlamydospores (M1-BR). Wood cell walls with advanced decay shrunk, leaving thinner wall remnants of an irregular width (M9-BR).

BR was rare in beech compared to pine and this factor coupled with a lack of beech wood samples with early decay, resulted in few samples being examined microscopically. Typically, a thorough understanding of wood decay micromorphology using LM requires that the bulk of microscopy effort be focused on wood with early attack, since this is when discrete defining micromorphology is present. BR typically advances rapidly compared to other decay types, and therefore the window of opportunity for examination of early decay is less i.e. during infrequent site visits, BR was typically either absent altogether or was advanced. Unlike most other decay types, brown rot typically has an abrupt decayed-sound wood boundary. An advantage with pine was that BR commonly attacked pine

treated with the highest preservative retentions used in this study, but at a reduced rate, thereby increasing the window of opportunity for collecting samples with early decay. There was therefore not the opportunity for studying early BR in beech in detail within the time constraints and design of this study. This was not considered to be a problem because the occurrence of BR in preservative treated beech was insignificant (chapter 5) and therefore it was not a primary focus of this study.

Unlike most other decay types beech and pine with advanced BR had similar micromorphology, including darkly stained and shrunken cell walls and associated chlamydospores. Sometimes the lumen wall remained birefringent after the S2 had lost all birefringence (M7-BR). Possibly a higher lignin content of the lumen wall compared to the S2 layer had a protective effect on crystalline cellulose. The high lignin containing middle lamella (ML) may have remained intact but this was difficult to detect under polarised light since the ML does not contain crystalline cellulose and is therefore not birefringent. Vessels often remained birefringent and unstained when surrounding fibres, fibre tracheids and axial parenchyma were in an advanced stage of decay (M7-BR), suggesting that vessels might be more resistant to BR, perhaps due to a higher guaiacyl:syringyl lignin ratio.

Intense staining of wood cell walls may have been caused by acid hydrolysis of the wood cell wall resulting from luminal deposits of oxalic acid. Hydrolysis of hemicellulose that occurs early in BR may in this instance have caused sufficient increase of wood pore size to enable uptake of aniline blue. Deep penetration of cell walls by aniline blue, within the short time frame in which hand cut sections were mounted and examined (5 – 10 minutes), was only observed for pine with BR from Tapanui and Whakarewarewa, suggesting that oxalic acid production was fungal species specific. Apart from refractive resin in some wood sections, refractive crystalline deposits were not seen in wood with other decay types.

Section 4.2.3

Wood with shared or closely associated BR and WR micromorphology (BR-WR) (micrographs in Section 4.5.2, Volume II)

Bleached treated pine with a cheesy texture typical of wet wood with BR often

contained BR micromorphology only, suggesting that bleaching is sometimes a feature of BR. However, wood of a very similar colour and texture sometimes contained micromorphology intermediate between BR and white rot (WR). Two combinations of micromorphology were particularly common.

1. Tracheid lumenae contained a mixture of degraded wood fibres that appeared to slough off the lumen wall, and a granular material that may have been a further breakdown product (M1-BR/WR). Typical WR cell wall thinning or erosion troughs were absent. Erosion resulting from sloughing off of degraded wood fibres continued until only the ML remained (M2-BR/WR). Disintegration of the wall into a fibrous material suggested that the wood cell wall was modified by a diffusible degradative product before further breakdown into the observed granular material. No consistent presence of fungal hyphae was observed.

2. Intact wood cell wall with loss of birefringence was closely associated with stripy wall erosion (M3-BR/WR and M4-BR/WR). Erosion and loss of birefringence appeared to be associated with continuous lumen hyphae. It is possible that decayed wood of this type had been attacked simultaneously or sequentially by both BR and WR fungi. However, distinctive WR and BR on the same stake were always within discrete pockets that did not merge. They were either separated by sound wood or wood with other types of decay or produced dark interaction zones at the interface. In contrast, wood with BR-WR decay had a homogenous colour and texture and was distributed evenly across stakes. Furthermore, the environmental conditions and the wood-preservative combinations that promoted WR and BR were essentially different and therefore they did not typically occur in close association. These factors suggested the occurrence of a fungus capable of producing a decay type possessing a mixture of WR and BR, suggesting possession of a dual or intermediate decay type capability.

Section 4.2.4

Micromorphology common to cavitation, tunnelling and erosion decay types

The majority of tunnelling and cavitation decay types and some erosion types

contained micromorphological features that were closely aligned with the cellulose microfibrils (CM) of the wood cell wall. Orientation of tunnels and cavities relative to the long axis of the wood cell depended on position within the cell wall i.e. depth in a radial plane. Tunnels, cavities, hyphae and tunnelling bacteria (TB) close to the middle lamella (ML) region were close to perpendicular to the radial plane. This was probably due to alignment with the CM in the S1 layer or alignment with an interface between the ML and the S1 layer. CM angle for the S1 layer is typically about 90 degrees (relative the vertical axis of the wood cell) in most wood species. Unpublished results produced by Lloyd Donaldson at *Forest Research* showed that CM angle in the S1 layer of radiata pine typically varies from 80 – 110 degrees relative to the vertical (long axis) of the wood cell. No specific information on CM angles in S2 layer of European beech was available but they are probably similar. In some cases tunnels or cavities changed direction abruptly and this appeared to be related to changing position within the cell wall.

S2 layer CM angles in New Zealand grown radiata pine are extremely variable depending on sampling position within the tree and between adjacent tracheids. Donaldson (unpublished data of *Forest Research*) reported that an angle of 40 degrees was typical for juvenile wood and 20 degrees was typical for mature wood. Angles decline from pith to bark and from butt to top log. Angles typically vary by 20 - 30 degrees among adjacent tracheids. No data on CM angles in the S2 layer of *F. sylvatica* could be found but hardwoods typically have low angles of 10 - 15 degrees. The majority of tunnels and cavities observed that were not close to perpendicular, were at an angle within the range 20 – 50 degrees for pine and 5 – 15 degrees for beech, suggesting that they were within the S2 layer. The central positioning of features between the lumen wall and the ML, within radial sections also suggested positioning within the S2 layer. Some micrographs of decayed pine contained tunnels or cavities at steeper angles up to 75 degrees. However, in pine tracheids it was usually possible to observe their helical alignment (Section 4.5.8, M3-SR), suggesting that they were aligned with the helical CM of the S2 layer. What appeared to be steeper angles may have been misleading due to the distortion sometimes caused by oblique sectioning or diffraction of light by voids (e.g. cavities or tunnels) and other features across the cell wall or focal plane.

Section 4.2.5

Tunnelling (T) decay types

Section 4.2.5.1

T1 (pine only) (micrographs in Section 4.5.3, Volume II)

Tunnels with rounded ends contained fungal hyphae that typically penetrated the wood cell wall to form a dense network of tunnels (diameter. 1 - 4 μm). Tunnels shown in M1-T1 had a random orientation compared to those of M2-T1 that were a mixture of tunnels aligned with the CM of the S2 layer and side branches were close to perpendicular to the tracheids long axis. In other sections tunnels were exclusively close to perpendicular to the long axis of the wood cell and were closely associated with the ML region, suggesting that they were aligned with the CM in the S1 layer (M7-T1). Hyphae in tunnels were connected to hyphae with clamp connections in the cell lumen (M3-T1, M4-T1, M5-T1, M6-T1, M8-T1). Hyphae were not observed in most tunnels suggesting that they were short lived, whereas the cell lumen hyphae were considerably more common, suggesting that they persisted for longer periods. Alternatively, uptake of aniline blue stain may have been impeded by surrounding sound wood cell wall, although this did not appear to impede staining of SR (SR) cavity hyphae (Section 4.2.6.1). Tunnels aligned with the S1 CM penetrated the cell wall concentrically i.e. around the circumference of the wood cell wall, possibly in response to a plane of resistance offered by the middle lamella (M7-T1 and M8-T1). The presence of continuous tunnels between wood cells suggested hyphae move between wood cells via the wood cell wall (M2-T1 and M9-T1) although bore holes (BH) were sometimes present (M1-T1), suggesting hyphae also follow the shortest route between tracheids. Unlike the cavity space between SR cavity hyphae and the cavity wall that was always stained by aniline blue, the space between T1 hyphae and the tunnel wall was not (only hyphae were stained).

Section 4.2.5.2

T2 (micrographs in Section 4.5.4, Volume II)

Tunnels with rounded ends were initiated by T-branching (M1-T2) within the S2 layer of pine tracheids. Tunnel expansion around the stem of the T-branch formed a BH and expansion around the hat formed two diametrically opposed tunnels at right

angles to the stem. Even expansion around the hat formed a cylindrical tunnel with a rounded cone at the apex. T-branching was not restricted to alignment with the helical CM (M1-T2, M6-T2 and M7-T2). Possibly, tangential T-branching is in response to alignment with CM of the S1 layer. Whilst tangential T-branching is typically difficult to observe in longitudinal sections it was deducible from the presence of bore holes located underneath T-branch hats orientated perpendicular to the wood cells long axis (M2-T2).

Maximum tunnel and BH diameter was 3 – 4 μm. BH angle within the cell wall was variable (M2-T2, M3-T2, M4-T2), suggesting that a T-branch stem penetrated at an oblique angle i.e. it was not always perpendicular to the lumen wall as typically occurs for SR. The term BH is misleading when their angle relative to the lumen wall was very shallow (close to tangential) since their length greatly exceeded the width and they resemble a tunnel (M5-T2). These tunnels appeared to penetrate concentrically around the circumference of the wood cell, after initiation by tangential T-branching. Failure to observe penetration pegs (T-branch stems), could have been due to rapid BH formation, and/or oblique wall penetration causing deviation of their path from a single focal plane. BHs often do not sit directly below the hat of the T-branch and were curvaceous, suggesting that the penetration hypha is curved and penetrated the cell wall in a random fashion, similar to T1 tunnelling.

BHs frequently connected tunnels within the S2 layers of two adjacent cells (M6-T2). This feature shows that T-branching can be initiated from hyphal branches within the cell wall i.e. independently of lumen hyphae. This suggestion corroborates with a lack of lumen hyphae in close proximity to T2 tunnelling. BHs were frequently cone shaped with the narrow end closest to the lumen wall (M1-T2).

Several micromorphological features were closely associated with T-shaped tunnels and appeared to be later stages of a decay sequence initiated by T-branch formation. It is possible that several different species of fungi produce T-branches and T-shaped tunnels but subsequent steps and associated features vary between species. The most common micromorphology to follow formation of T-shaped tunnels was extension and lateral expansion of tunnels. Whilst tunnel alignment with the CM was often

maintained during extension and expansion, either aligned with the helical CM of the S2 layer (M6-T2) or the CM of the S1 layer (M2-T2, M7-T2), wavy tunnel extensions were also common (M9-T2, M10-T2). Wavy tunnels were most likely initiated by wavy proboscis hyphae (M1-T2). Proboscis hyphae were produced after expansion of the T-shaped tunnel and BH to a diameter of 1.5 - 2.5 μm, extending from the tunnel apex for up to 20μm. Proboscis hyphae were observed considerably less often than is typical for classical SR. Possibly its wavy form resulted in a penetration pathway that deviated too far from a single focal plane to enable easy detection. However the presence of a proboscis hypha, approximately 20μm long in M1-T2, suggested that the direction of penetration was variable only within the tangential plane, otherwise a continuous tunnel of such a narrow diameter (approximately 0.2 – 0.4μm) would not have been seen. The wavy path of tangential penetration may have been a response to hyphal extension within a plane of lesser resistance, perhaps at the interface of concentric lamellae in the cell wall.

Another micromorphological feature that occurred after T-branching, was formation of stepped tunnels (M10-T2, M11-T2, M12-T2, M13-T2), suggesting that the causative fungus had an oscillatory growth pattern involving apical extension of an essentially straight proboscis hypha aligned with the helical CM of the S2 layer, as occurs for SR fungi. Tunnels containing a hypha with clamp connections and, or a hypha connected to a lumen hypha with clamp connections (M13-T2) was a common observation, demonstrating that a basidiomycete was the causative fungus.

Section 4.2.5.3

T3 and C3 (micrographs in Section 4.5.5, Volume II)

Micromorphology of types T3 and C3 suggested they were sub-types of T1 and SR respectively, which develop when frequency of hyphal branching increases dramatically to form a dense reticulum of fine tunnels. Expression of similar multiple branching patterns by two otherwise distinctive decay types suggested that it was, to a large degree, a response to exogenous factors (e.g. variations of wood properties and environmental factors) rather than inherent fungal characteristics alone. It also suggested that the mechanism of formation of the defining micromorphology was similar. Multiple branching patterns (T3 and C3) are therefore described together.

Micromorphology of type C3 consisted of multiple T and L-branching of apical proboscis hyphae from SR cavities. Lateral expansion of apical extensions and their subsequent coalescence often resulted in voids, of non-discernible micromorphology, within the wood cell wall. Therefore, frequency of occurrence of observable connectivity between SR cavities and multiple branching was higher where the closeness of branching was less, allowing subsequent cavity expansion to occur without loss of form through coalescence with neighboring cavities. In other examples lateral branching was suppressed to a greater degree allowing detection of a reticulum of very fine tunnels.

Type T3 also consisted of a network of highly branched fine tunnels of similar initial diameter (0.2 – 0.3 μm) to those produced by proboscis hyphae of type C3. However, lateral expansion resulted in tunnels, of even diameter along the length, and with rounded ends (as for type T1). Branching patterns included T-, L- and Y-branches. Whereas type T3 produced "stacks" of T-branches where the stem was directly in line with the preceding stem, with type C3 the stem of each T-branch within the stack was always slightly offset (e.g. M10-T3). It was difficult to ascertain if this was the result of an intervening L-branch between each T-branch, or whether the stem of the next branch was a lateral side branch of the hat of the preceding branch. An L-branch is distinct from an L-bend (Eaton and Hale, 1993) since the branch forms to the side of the parent proboscis hyphae whereas a bend is a continuation of the parent hypha.

Linkage between C3 and SR was established by showing that apical proboscis hyphae of SR cavities were connected to lateral or apical branches that then continued to branch in a multiple fashion identical, or similar to isolated reticular patterns (M1-C3, M2-C3, M3-C3, M4-C3 and M5-C3). Isolated branching patterns are shown in M6-C3, M7-C3 and M8-C3. Another type of pattern was produced when SR cavities produced long wavy proboscis hyphae that branched infrequently (M8-C3 and M9-C3). Whereas highly branched patterns were typically aligned with the CM of the S2 layer, patterns involving wavy proboscis hyphae were more frequently aligned with the CM of the S1 layer.

Whilst micrographs also suggested a similar linkage between types T1 and T3 (M1-T3, M2-T3, M3-T3, M4-T3, M5-T3) it was found to be much more difficult to produce good micrographs of the linking micromorphology. This was because, under LM, SR and the aberrant type C3, produced micromorphology (cavities, proboscis hyphae, reticular branching) within the same focal plane. This feature was caused by alignment of cavities, apical proboscis hyphae, and the connected reticular branching, with the helical CM of the S2 or S1 layer. They were therefore located at a similar depth within the cell wall, probably at the interface of concentric lamellae. In contrast, tunnels of T1 typically follow a more random path within the cell wall. Consequently there was a much lower probability of observing a tunnel, its apical proboscis hypha (M9-T3) and connected reticular branching, all within the same focal plane. However, micromorphologies of T1 and T3 were almost invariably closely associated within the same layer of the wood cell wall (M1-T3 and M2-T3), although this was often hard to observe because of coalescence of the two types (M5-T3). It is possible that due to the similar appearance of T3 and C3, in the absence of closely associated parent types, T3 and C3 could be mistaken for each other.

It is possible that under certain environmental conditions, or in response to particular wood-preservative-site combinations, the parent type T1 or SR was suspended, or prevented. It is further suggested that two common scenarios account for the subsequent micromorphology observed. In the first scenario T3 and C3 are an early form of T1 and SR respectively, that is eventually overtaken by the parent decay type i.e. the parent type is more effective at wood cell wall removal and overruns the aberrant form. Secondly, the aberrant form persists until lateral coalescence of branches within the reticulum completes the decay sequence. The first scenario appeared to be more common for C3 (M2-C3 and M4-C3) and the second scenario appeared more common for T3 (M3-T3, M6-T3 and M8-T3).

An alternative hypothesis to account for the connecting micromorphology of SR and C3 would be that it is a rare feature, of similar appearance, to a much more common reticular form not produced by SR fungi. However the close association of SR with C3 and T1 with T3 within the cell wall, and the frequency of their coexistence at sites

and within treatments (preservative-wood combination), suggested that this hypothesis was not correct. Type C3 was only observed in pine and type T3 was considerably rarer in beech than pine. This could be related to the high guaiacyl:syringyl lignin ratio of pine and distribution acting as a more potent trigger for hyphal branching.

Another multiple branching pattern with features of both types T3 and C3 is shown in M11-T3, M12-T3 and M13-T3. Tunnels produced long straight apical proboscis hyphae (M11-C3) aligned with CM of the S2 layer. Lateral expansion resulted in tunnels of even diameter along their length (approx. 1μm), with either angular (M13-T3) or rounded ends (M11-T3). Branching of apical proboscis hyphae that occurred for types T3 and C3 was absent. Branching was restricted to lateral T- (M12-T3 and M13-T3) or L- branching (M13-T3) from the parent cavity. Apart from the presence of proboscis hyphae and the bulbous shape of some tunnel apices, this branching pattern was similar to branching produced by TB. Frequency of occurrence of proboscis hyphae was not always high enough to be certain that this branching pattern was not TB tunnelling (M1-TB and M2-TB) i.e. the mixture of micromorphology observed in M11-T3, M12-T3 and M13-T3 may have been caused by closely associated micromorphology of TB and a fungus.

Section 4.2.5.4

T4 (beech only) (micrographs in Section 4.5.6, Volume II)

Large BHs (diameter 4-10 μm) and concentric tunnels were closely associated in fibre walls. BHs appeared to penetrate adjacent walls of two fibres at approximately 90° to the lumen wall. Tunnels penetrated the cell wall concentrically, moving around the perimeter of the wall between the ML and the lumen wall (M1-T4). Whilst this type of decay was frequently encountered, hyphae within tunnels or within the cell lumenae adjacent to BHs, were rarely observed (M2-T4). Rarely, a fine BH (0.5μm) was seen to connect the lumen with an early stage of a concentric tunnel and this was believed to be the remnants of a T-branch stem. Possibly a tangential T-branch initiated a concentric tunnel that then enlarged to fill the entire space available between the lumen wall and the ML.

An intriguing aspect of type T4 was radial expansion of concentric tunnels beyond the ML, leaving remnants of it intact (M2-T4 and M3-T4). M1-T4 suggests that the ML was partially degraded and this could have enabled diffusion of degradative products. However the presence of fine bore holes between the concentric tunnel and the lumen, on both sides of the ML (M1-T4) suggested that a hypha penetrated the ML and this is believed to be a more likely explanation than diffusion of degradative products across the ML. After the ML, the lumen wall appeared to be the most resistant part of the fibre wall (M2-T4) but was eventually degraded leaving a void that spanned adjacent fibre lumenae and which could easily be mistaken for a large BH if seen in tangential sections. The BHs observed for type T4 may have formed around a T-branch stem or they may have been adjoining concentric tunnels, or a mixture of both. The suggested method of concentric tunnel formation would account for the lack of observable hyphae since bisection of the fibre wall would result in transverse sectioning of tunnelling hyphae.

Section 4.2.5.5

Tunnelling bacteria (TB) (micrographs in Section 4.5.7, Volume II)

Tunnels aligned with the helical CM within the S2 layer of pine tracheids can be seen in micrographs M1-TB, M2-TB, M3-TB and M4-TB. Tunnels approximately perpendicular to the long axis of tracheids, believed to be aligned with the S1 layer, are presented in micrographs M10-TB, M11-TB, M13-TB and M14-TB. Tunnels in beech fibres of an apparently random orientation are shown in M5-TB and M7-TB.

Whilst tunnel shape was sometimes distorted by differential light diffraction and the angle of the tunnel within the wood cell wall, it is believed that some of the micrographs presented possess sufficient clarity to detect a consistent linkage between tunnel shape and bacterium morphology. Bacteria in close association with the tunnel wall (i.e. no detectable gap between bacterium and wall) were typically "squeezed" into a narrow pointed tunnel apex with steep sides (M6-TB and M8-TB). Bacteria in rounded pear shaped apices were typically more globose and a detectable gap between the tunnel wall and the bacterium was present. It is possible that these differences provide clues for the mechanism of tunnel extension.

It is generally accepted that tunnel-forming bacteria are pleomorphic and this feature has been discussed in most detail in relation to the mechanism of lumen wall penetration and tunnel initiation. Bacterium motility within tunnels is poorly understood. It has been suggested that TB are gliding bacteria. If correct, then it is reasonable to assume that the bacterium must obtain sufficient traction between its outer surface and the tunnel wall to facilitate forward motion. It is possible that bacteria within narrow pointed apices may be actively penetrating the cell wall and bacteria in tunnels with rounded apices may be undergoing a stationary phase or cell division. The putative but generally accepted stationary phase is generally believed to be linked to cross wall formation i.e. cross walls are laid down at the distal end of the bacterium surface whilst it is in a stationary phase. The bacterium visible at the tunnel apex in M13-TB is attached to a cross wall and is separated from the tunnel wall by a gap in the tangential plane. The bacteria that are tightly squeezed into the tunnel apices presented in M6-TB and M8-TB have no visible cross wall behind them.

Those tunnels aligned with CM in the S1 layer of pine tracheids always had a wider apex with convex sides, similar to the proposed "resting" shape of tunnels aligned with helical CM in the S2 layer. These differences are likely to be linked to cell wall chemistry. A similar phenomena was observed for SR cavities which had rounded ends, or pointed ends with convex sides in the S1 layer of pine tracheids but had angular apices in the S2 layer (some were clearly connected and caused by the same fungus suggesting exogenous factors (probably cell wall chemistry) were the cause of the different cavity shapes).

The cross wall may be invisible during active penetration because of its close association with a bacterium, that completely fills the available space at the apex. The later scenario fits better with the hypothesis that the cross wall is important for forward traction since the cross wall may provide a surface that aids a gliding motion. If, as generally believed, the cross wall assists wood cell wall degradation by encapsulating and concentrating bacterial degradative products, it is likely that it would also offer a physical resistance.

The absence of apical growth by a tubular hypha in TB, may be compensated for by production of a cross wall. The cross wall, in combination with the tunnel wall, would be expected to physically restrain a flexible bacterium, achieving an effect similar to the containment of fungal cytoplasm by the rigid hyphal wall. It is therefore suggested that the primary function of cross walls is provision of a surface that provides resistance to the gliding motion (or another form of motion) of the bacterium surface. Apical pressure exerted by cytoplasmic flow as must occur for amoebae that overcome interfacial tensions during forward motion, or localised apical pressure via exocytosis or cross-wall/cross-membrane solute "pumps", cannot be ruled out as a contributing degradative mechanism.

All micrographs showing tunnels containing a discernible bacterium, a distal cross wall and a gap between the bacterium and the tunnel wall, clearly showed that only the bacterium and the cross wall were stained. Previous suggestions that the entire bacterium surface is encapsulated by a mucilage layer of a similar composition to the cross wall may therefore not be valid unless non-cross wall slime is too thin to be seen under LM. Furthermore, distal localisation of bacterial mucilage fits with the suggested gliding motion and physical restraint function of the cross wall. It seems unlikely that a bacterium undergoing a "resting" phase would continue to produce copious amounts of mucilage. It seems more likely that its presence is the result of an active process and that it forms a function beyond encapsulation of degradative products.

Micrograph M3-TB shows a group of closely associated tunnels of similar size that appear to share a point of origin, a configuration typical of early attack in pine and beech. M5-TB and M15-TB show a dense concentration of tunnels in pockets, surrounded by sound wood. These two micrographs suggest that isolated colonies of bacteria caused localised pockets of advanced decay as a result of frequent tunnel branching. Other micrographs show a more spread out tunnelling configuration in which tunnels do not branch as frequently (M4-TB and M9-TB). Highly branched patterns are probably a response to the generally accepted but poorly understood self-evasion mechanism whereas less frequent branching is a response to alignment with CM. These different patterns suggested that the frequency of the bacterial division

varies in different parts of the cell wall. It also suggests a link between the generally accepted self evasion mechanism and branching i.e. bacterium cell division.

Section 4.2.6

Cavitation decay types

Section 4.2.6.1

Soft rot (SR) (micrographs in Section 4.5.8, Volume II)

Biconical or cylindrical cavities with conical ends were typically confined to the S2 and S1 layers of the wood cell wall and were parallel to the CM, as described in Section 4.2.4. In pine, cavities were commonly aligned with both the S2 CM (M3-SR and M4-SR) and S1 CM (M15-SR and M18-SR). In beech fibres, cavities were only seen aligned with the S2 CM. Penetration within the wood cell wall was typically confined to a single wood cell, but in pine continuous cavity chains sometimes crossed the ML into the adjacent tracheid wall. Sister cavities in the S2 layer of adjacent tracheids were typically connected by a proboscis hypha in the vicinity of the ML (M17-SR). However, connected cavities aligned with the S1 layer of adjacent tracheids did not change diameter at the ML (M15-SR). This suggested that the S2-S1/ML interface was a more potent trigger for proboscis hypha initiation than the S1-ML interface.

Lumen hyphae were typically abundant in wood with early decay, and produced penetration hyphae that either traversed the cell wall into an adjacent cell lumen (M2-SR) or produced a T-branch within the cell wall (M1-SR). T-branches aligned with the long axis of the S2 layer CM were most common (M1-SR, M5-SR, M7-SR, M10-SR and M13-SR). T- and L-branches were formed by cavity hyphae side branches (M10-SR, M12-SR and M13-SR) and from apical proboscis hyphae (M5-SR, M7-SR, M18-SR and M19-SR), producing new cavities in close association with the parent hypha. Cavities produced by side branches were typically of the same alignment within the wood cell wall but when a change of alignment occurred between the S1 and S2 or during movement across the ML, it often occurred in the vicinity of branching. Possibly the stimulus for branching is provided by a change in wall chemistry or CM alignment encountered by penetrating proboscis hyphae at a wall layer interface. Where cavity formation around side branches was the predominant

mechanism of cavity proliferation, a large portion of the S2 layer became occupied with parallel adjacent cavity hyphae after complete coalescence of cavities (M20-SR).

Cavity shape was hugely variable and all shapes encountered were not reported. Cavity shape and position within the cell wall was related to wood species. Beech fibres and fibre tracheids typically contained biconical cavities with angular ends and which were often nearly as wide as they were long (M6-SR and M9-SR). Cavities in pine tracheids were typically longer, with essentially straight sides and conical ends (M3-SR, M4-SR and M5-SR), although biconical cavities also occurred (M7-SR and M8-SR). Long cavities (>15 μm) were not seen in beech but were common in pine (M(12-15)-SR and M(17-20)-SR). SR cavities with serrated edges were common in pine tracheids. Serration of cavity walls suggested a different mechanism of formation compared to smooth walled SR cavities. Furthermore, some cavities had very deep serrations containing side branches from the cavity hypha. Once serrated cavities became closely associated in moderate to late decay, the resultant micromorphology was easily confused with decay previously described as SR erosion attack (Corbett's type 2 SR) in the literature. Because this type of cavitation was substantially different from classical SR and because of the potential for confusion with SR erosion, a significant decay type within the literature but thought to be of minor significance in this study, it was decided to describe it under a separate section (type C7). Type C5 produced serrated edged non-SR cavities in beech fibres but the mechanism of formation was clearly different to classical SR.

Where S1 and S2 layer cavities were present in close proximity within the wood cell wall, it was usually possible to observe connecting hyphae, illustrating that these essentially different cavity types were produced by the same fungal species. Change of direction between layers occurred either within the same wood cell or in the vicinity of the ML. It was not always clear which layer the leading cavity hypha moved into but both the S1 and S2 layers contained the leading cavity (M16-SR and M17-SR), suggesting it was the interface that triggered change. Cavities in the S1 layer tended to be wider, have wavy sides and more rounded apices compared to those within the S2 layer. It is likely that this difference of cavity form was caused by differences in wall chemistry (and by inference its structure). Whilst the differences

of cavity form between layers described, accounted for the majority of observations made, in some sections different cavity morphology existed between connected cavities of a similar angle to the long axis of the wood cell (M21-SR). This suggested that they were within the same wall layer and would therefore have been influenced by similar wall chemistry, although under LM it is sometimes difficult to determine where cavities are situated relative to the wall layers. One explanation is that in some wood cells, typical CM angle or wood chemistry was not present, as might occur in reaction wood for example. Another possibility is that some species of SR fungus react to non-wood chemistry stimuli that cause changes of cavity form (e.g. preservative, moisture or oxygen stress). For example it is possible that differential preservative uptake, related and unrelated to differences of wall chemistry between and within layers, may have influenced cavity form. However it would be expected that differential preservative uptake across the wall would typically be related to wall chemistry changes and therefore it might be predicted that fungal reactions to both would have occurred simultaneously or would have been difficult to differentiate. A further possibility was that intra-wall layer differences, for example heterogeneous lignin distribution, may account for the diversity of cavity shape.

SR micromorphology was determined largely by the presence of discrete cavities within wood with early decay, usually at the margins of pockets of severely attacked wood. However, under certain circumstances cavities were obscured. This was particularly common when many closely associated or coalesced serrated cavities particularly at the S2-S3 interface gave rise to multiple wall serrations that, without discernible cavities, appeared to be a type of erosion. This type of attack is dealt with under type C7. A second common occurrence was loss of wood cell wall birefringence within the vicinity of what initially appeared to be SR-like cavities. For example, the presence of remnants of angular cavity apices in radial sections, amongst surrounding wood cells with complete or almost complete loss of birefringence (M7-C5) suggested that SR cavity hyphae were causing loss of birefringence beyond the cavity wall. This might have been taken to suggest that a diffusible degradative product released by cavity hyphae within discrete cavities was involved. However, detailed observation of this common occurrence suggested that loss of birefringence was not related to release of a diffusible degradative product by

cavity hyphae, but was the result of complete removal of the S2 layer by multiple branching cavity hyphae within a "super-cavity". This distinctive decay micromorphology that caused highly efficient concentric removal of the S2 layer, often across several adjacent wood cells, was described as type C5 for beech and type C6 for pine. It is suggested that these are important decay types that have been overlooked or largely misinterpreted within the literature.

Section 4.2.6.2

C1 (pine only) (micrographs in Section 4.5.9, Volume II)

Tangential cavity expansion, perpendicular to the apical proboscis hypha and helical CM (i.e. within the tangential plane) exceeded longitudinal apical extension, resulting in formation of a wide, shallow (radial depth) cavity (M1-C1). An alternative form occurred when apical extension was aligned with the CM of the S1 layer (M2-C1). Cavity shape was intermediate between symmetrical biconical SR cavities (M7-SR) and the irregular cavities associated with types C5 and C6. Branching of hyphae within the cavity was sometimes seen but was not profuse as in types C5 and C6, and typically a single side branch was closely associated with the lateral extensions. In radial section (M1-C1) the cavity wall had an appearance very similar to irregular erosion usually associated with simultaneous WR. It is possible that coalesced cavities could be mistaken for WR erosion.

Section 4.2.6.3

C2 (pine only) (micrographs in Section 4.5.10, Volume II)

The key feature of this cavity type was extensive uneven (diffuse) lateral expansion around anterior portions of the cavity hypha, giving rise to concave cavity walls. The cavity therefore had a tornado shape when seen in tangential longitudinal section (M1-C2 and M2-C2). In contrast, the diffuse uptake of aniline blue by the cavity wall in the tangential plane did not occur in classical SR cavities in which the dye was confined to the cavity (see M4-SR, M8-SR, M9-SR, M12-SR and M12-SR). This difference suggested that SR degradative products act only at the cavity wall whereas those of type C2 modified the cell wall enabling aniline blue dye to penetrate 2 - 5 μm from the cavity wall surface.

Section 4.2.6.4

C3

Type C3 is described with the closely related type T3 in Section 4.2.5.3.

Section 4.2.6.5

C4 (beech only) (micrographs in Section 4.5.11, Volume II)

Cavities seen as V-shaped notches in radial sections, extended from the ML into the S2 layer of beech fibres (M1-C4). Longitudinal expansion of cavities caused adjacent cavities to coalescence resulting in long serrated cavities between the ML and the remaining S2 layer (M2-C4), eventually causing delamination of adjacent fibres. Residual ML, often sandwiched between cavities of adjacent fibres, was less refractive under partially polarised light and under phase contrast compared to intact ML, and was therefore easily overlooked. Possibly the ML had been modified by degradative products released by the hyphae contained within the cavity. The primary feature of advanced decay was a continuous serration of the outer edge (relative to the "inner" lumen wall) of the S2 layer (M2-C4). Small hyphae were visible in the lumen and within cavities (0.3 – 0.5μm diameter) and were therefore of a similar size to SR proboscis hyphae (0.2 – 0.4 μm) but were much smaller than is typical for SR cavity hyphae (0.4 - 5μm diameter) (M1-SR, M2-SR and M4-SR). These diameters were based largely on relative size compared to hyphae of previously known size such as SR proboscis hyphae and this factor coupled with the resolution limits of LM could have resulted in misinterpretation of actual diameter. However the key observation was that the cavity hyphae of type C4 were much smaller than is typical for SR cavity hyphae.

In some sections examined, type C4 micromorphology occurred close to classical biconical SR cavities (M6-SR). The cavity geometry of type C4 was identical to the geometry of one half of the biconical cavities, suggesting a common causative fungus. It was not clear how the fungus gained access to the ML but the most likely scenario was penetration by a proboscis hypha from a parent hypha in the cell lumen or within the cell wall followed by T- or L-branch formation at a plane of resistance offered by the ML. Alternatively, as suggested by micrograph M6-SR, wood cell delamination at the ML may occur prior to initiation of type C4 cavities and this would enable access to the outer S1 layer.

Section 4.2.6.6

C5 (Beech only) (micrographs in Section 4.5.12, Volume II)

Cavities were formed within the S2 layer of beech fibres by multi-branched hyphae (M1-C5 and M2-C5). Cavity expansion was concentric and extensive S2 layer removal occurred before the lumen wall was degraded (M3-C5, M4-C5, M5-C5, M6-C5 and M7-C5). Complete removal or almost complete removal of the S2 layer resulted in loss of birefingence across many cells when decay was advanced (M7-C5).

Hyphae were often closely associated with V-shaped troughs within the cavity wall on the side nearest the ML (seen as notches in radial section). Hyphae in troughs were very difficult to see in radial section because only their cross-section was exposed and possibly also because they tunneled at a shallow angle to the cavity wall so that wood cell wall obscured the hyphal tip. In some instances V-shaped notches of similar size were seen on opposite sides of a fibre. These were believed to result from V-shaped troughs expanding concentrically within the S2 layer around the circumference of the fibre. When troughs were perpendicular to the fibres long axis this resulted in a continuous trough in the cavity wall, seen as two V-shaped notches on either side of the fibre.

The uniformity of depth and geometry of troughs that sometimes occurred is intriguing since it would be expected that as the trough extended concentrically, its depth at the point of initiation would be greater than elsewhere. This suggested an oscillatory growth pattern involving tangential T-branch formation. If a T-branch extended around a substantial portion of the circumference, followed by even lateral cavity expansion, this would account for formation of troughs of equal depth on opposite sides of fibres. However, where hyphae were observed (M1-C5) they appeared to follow a random pathway within the base of troughs which seems inconsistent with a T-branch mechanism. A possible explanation lies in a further observation regarding the relationship between the size of the V-shaped notches and presence of symmetry. Symmetrical cavities were typically much smaller, suggesting that symmetry is retained early during cavity formation but is lost as the cavity expands. Unfortunately it was not possible to observe hyphae in smaller cavities,

possibly due to their small size. Hyphae observed in larger, asymmetrical cavities, had a diameter of approximately 0.3μm and were therefore on the verge of the resolving power limits employed. The small size of hyphae is consistent with their location at the narrow apex of steep V-shaped troughs. The orientation of hyphae within larger cavities suggested that they tunnel into the S2 layer towards the middle lamella at a shallow angle i.e. tunnelling and lateral erosion at the base of troughs occurs simultaneously.

The presence of concentric V-shaped notches in the absence of a thin layer of overlying lumen wall was intriguing since it was not possible to ascertain with certainty whether this type of attack was initiated as erosion, or whether it was initiated as cavitation as described, followed by loss of the overlying lumen wall. In some micrographs the partial remnants of lumen wall overlying cavities containing hyphae, suggested a cavitation mode of attack. It is possible that both cavitation and erosion occur in close association. In all likelihood, type C5 is initiated as a cavitation decay type but once the lumen wall has degraded, it then becomes what is essentially an erosion decay pattern. The choice of terminology in such situations is tricky but it is suggested that the features associated with initiation of decay, in this case a cavity, is the critical phase of decay and it should therefore be termed cavitation.

The concentric mode of cavity expansion was similar to type C6 in pine, and occasionally beech. However with type C6 in pine and beech, removal of the S2 was caused by much larger hyphae (some were seen with very short apical proboscis hyphae) that branch in a random fashion and are not associated with V-shaped troughs.

Section 4.2.6.7

C6 (micrographs in Section 4.5.13, Volume II)

The defining feature of type C6 in pine tracheids was expansive concentric cavities within the S2 layer, containing multi-branched hyphae. S2 layer close to the lumen wall was removed preferentially, possibly at the S3-S2 interface, which resulted in a very thin layer (< 0.5 μm) of overlying lumen wall (presumably the S3 layer). This thin layer was easily sloughed off during sectioning. Retention of lumen wall is a

vital diagnostic feature since this type of decay, as for type C5, would almost certainly be misdiagnosed as WR, or possibly type 2 SR for type C5 in beech, once the overlying lumen wall had been removed.

Initial cavity extension appeared to have been initiated by short stubby apical proboscis hyphae aligned with helical CM within the S2 layer (M5-C6, M6-C6). However, a well-defined oscillatory growth pattern, involving significant visible proboscis extension prior to lateral expansion of the cavity hypha, did not occur. This suggested that the mechanism controlling oscillatory growth had been almost completely suppressed. Rapid cell wall breakdown between apically extending hyphae prevented formation of cross walls between hyphae. Therefore, apical cavity extension by several hyphae occurred across a broad front i.e. a "super" cavity was formed collectively by a group of extending apical hyphae (M1-C6, M2-C6 and M3-C6). Cavity expansion at right angles to the helically aligned apical proboscis hyphae was further facilitated by lateral branches, some of which may have been T-branches (M3-C6). Lateral branches either continued extension across helical CM (M2-C6), or realigned with them, causing further cavity expansion parallel to them.

T-branches had a similar dimension and shape to the parent hyphae i.e. the classical SR T-branches consisting of a narrow straight stem and narrow perpendicular hat, were absent. This suggested that oscillatory growth was either absent or its cycle greatly compressed such that apical proboscis hyphae were too short to be detected using LM. M2-C6 showed that extending hyphae were immediately underneath the lumen wall, perhaps extending at the S3-S2 interface. This factor, coupled with alignment of extending hyphae with helical CM, would have facilitated observation of proboscis hyphae which suggested proboscis hyphae were not wavy but were very short or absent. The bulbous end of hyphal apices was similar to the transpressorium of sapstain fungi, which lends support to the suggestion that proboscis hyphae were a feature of type C6. The distribution of aniline blue around T-branches was diffuse, as described for type C2, suggesting wood cell wall modification beyond the cavity wall. This diffuse attack could account for the rapid wall breakdown around lateral cavity hyphae, causing rapid wall removal between extending hyphae in the tangential (concentric) plane, thereby causing formation of a "super-cavity".

A distinctive form of type C6 occurred when extending hyphae followed a wavy path causing an uneven removal of the S2 layer (M5-C6, M6-C6, M7-C6, M8-C6 and M9-C6). Whilst side branches caused lateral cavity expansion, apical extension was typically greater prior to formation of side branches. When the residual lumen wall remnants were sloughed off the remaining S2 layer had an appearance very similar to WR erosion. The remarkable similarity between WR erosion and cavity wall attack by type C6 had the potential to cause misinterpretation.

This raises an interesting point regarding current interpretation of certain types of decay as Corbett's type 2 (erosion) SR. If erosion SR is as common as suggested by the literature, it was intriguing that it was not common in this study. Reports in the literature of hardwood cell wall erosion caused by lumen hyphae lying above an intact or partially modified lumen wall, are not convincing in terms of what is known about diffusion of degradative products that act locally at the wall surface (as opposed to chemical agents of BR). It is therefore tempting to suggest that what has sometimes been reported as type 2 SR erosion, was cavitation decay. Furthermore, it is to be expected that if the lumen wall is sloughed off during sectioning or is broken down soon after cavity initiation, misinterpretation of cavitation as erosion is almost inevitable

Section 4.2.6.8

C7 (micrographs in Section 4.5.14, Volume II)

Cavities had angular serrated edges (M1-C7 and M2-C7). In some cavities, V-shaped serrations contained lateral branches from the parent cavity hypha (M6-C7). Lateral cavity serrations (M3-C7) and, or angular apices (M5-C7) caused V-shaped notches or serrations along the edge of pine tracheids (M3-C7, M4-C7 and M5-C7). This feature could possibly be mistaken for type 2 SR erosion. Some types of cavitation formed serrations in pine tracheid walls (M8-C7) which later became diffuse as decay progressed (M7-C7). In advanced decay this type of cavitation was also associated with complete loss of birefringence across paired tracheid walls and breakdown of the ML.

Section 4.2.7

Erosion decay types

Section 4.2.7.1

White rot (WR) (micrographs in Section 4.5.15, Volume II)

Typical WR micromorphology comprising cell wall thinning and erosion troughs around lumen hyphae, in the presence of BHs, had a high frequency of occurrence in untreated beech and pine only, and has been thoroughly described in the literature. However, closely related micromorphology, was important in both untreated and treated pine and beech and is described here.

Even thinning along the length of beech fibres was unusual in treated wood, but abrupt boundaries between intact wall and almost completely degraded wall were common (M1-WR). This was related to an oblique alignment of hyphae with the lumen wall, causing micromorphology intermediate between a short, wide erosion trough and an expansive BH (M2-WR, M3-WR, M4-WR and M5-WR). Hyphae parallel to the long axis of the cell wall were typically not associated with cell wall erosion. Instead, erosion occurred around those hyphal branches involved in formation of oblique BHs with tapered, often serrated edges. Hyphae with clamp connections were typically present, confirming a basidiomycete was the cause. When BH's were at a shallow angle to the lumen wall, they appeared more like short expansive erosion troughs. Micrograph M6-WR shows hyphae associated with this type of BH and what appears to be a trough parallel to the cell's long axis. It appears that the fungus involved, produced both long troughs with straight sides and oblique BHs with serrated edges. The longitudinal trough had a rounded apex similar to a wide SR cavity and the possibility that this trough was in fact a cavity where the overlying lumen wall had collapsed cannot be ruled out. Another commonly observed WR of pine (M7-WR) and beech (M8-WR) consisted of irregular cell wall erosion together with wood cell delamination at the ML. Delamination was similar to that reported for preferential WR and which is caused by delignification of the ML. Wood with irregular erosion and cell wall delamination was commonly associated with proboscis hyphae and T-branching, features usually associated with SR. However

clamp connections were also common, showing that a basidiomycete WR fungus also produced these features (M8-WR and M9-WR).

Section 4.2.7.2

Erosion and cavitation bacteria attack (micrographs in Section 4.5.16, Volume II)

Rod-shaped bacteria aligned with the helical CM of the S2 layer of pine tracheids (M1-EB) typically resulted in formation of angular pits, seen as V-shaped notches in both radial and tangential sections (M2-EB). Whilst EB attack was much more common in pine (M3-EB), the shape of pits was similar in both wood species. The main difference between pine and beech was that in beech bacterial alignment was difficult to determine, appearing to be more random (M6-EB), and the long erosion troughs present in pine (M5-EB) were absent. Alignment with CM of the S2 layer in pine, coupled with bacterial division within troughs (known from SEM observations reported in the literature), is likely to have facilitated formation of elongated troughs in pine. Cavitation was always closely associated with erosion caused by EB in beech whereas in pine, erosion in isolation was not unusual (M4-EB, M6-EB). EB attack was straightforward to identify and all micormorphology encountered supported previous observations reported in the literature. In particular the more recent belief that cavitation bacterial attack is caused by EB was strongly suggested by the observations of this study. EB frequently eroded the S2 layer close to the ML or lumen wall (presumably at the S3 interface) faster than the central S2 layer and this resulted in convoluted erosion troughs close to the ML, seen as cavities in radial sections.

Erosion bacteria decay micromorphology (M7-EB and M8-EB) could be confused with other V-shaped erosion in the absence of bacteria. In particular the V-shaped indentation of the cell wall caused by SR cavities with serrated edges looks similar in radial sections. Also the reports in the literature of V-shaped erosion caused by Corbett's type 2 SR in hardwoods might also be confused with EB attack. However in this study use of aniline blue enabled easy identification of EB within troughs and pits.

Section 4.2.7.3

E1 (micrographs in Section 4.5.17, Volume II)

V-shaped troughs, seen as notches in radial longitudinal section of the cell wall beginning at the lumen wall surface and extending into the S2. These troughs often extend concentrically around a large portion of the wood cell circumference and are therefore perpendicular to the long axis of the wood cell (M1-E1). Troughs containing hyphae were not seen, probably because they were beyond the resolving power of the LM optics and hand sectioning techniques employed. However lumen hyphae orientated at right angles to troughs were common (M2-E1). As explained for type C5, if hyphae sit at the bottom of concentric V-shaped troughs, this makes them inherently difficult to observe. This is because in tangential section hyphae do not lie within a single focal plane and in radial section only the small cross section would be detectable and then only if a near perfect bisection had been achieved. The best method for viewing such a configuration would be to use the resolving power of TEM to view wood cell cross-sections. Type E1 was only present in beech and was usually associated with, and was sometimes difficult to separate from, type C5. The major distinction was that for type C5 a thin layer of lumen wall remained intact, overlying the cavity. Their similarities and common association could suggest that both types shared a single causative fungus. Micromorphology of type E1 was similar to micromorphology described as Corbett's type 1 erosion SR in hardwoods.

Section 4.2.7.4

E2 (pine only) (micrographs in Section 4.5.18, Volume II)

Type E2 consisted of unusually large erosion troughs (M1-E2, M3-E2, M8-E2) and oblique, irregularly shaped BHs formed by anastomosing hyphae (diameter 7 – 20 μm) of highly variable shape (M2-E2, M4-E2, M5-E2). Proboscis-like, angular hyphal protuberances (M2-E2, M7-E2) were often intimately associated with angular erosion troughs and BHs. As for type T2, the distinction between BH and erosion trough was not always clear due to the occurrence of oblique BHs at a shallow angle to the lumen wall. When BHs were almost parallel to the wood cell wall they formed wide tunnels that often spanned the entire width of adjacent tracheids (M4-E2).

Unlike classical WR hyphae that are typically separated from the wood cell wall by a gap around most of their circumference, no gap was detectable between type E2 hyphae and the voids created in the wall (M4-E2, M6-E2). The edges of erosion troughs either had well defined smooth edges in the radial plane or diffuse edges in the tangential plane similar to cavity walls of types C2 and C6. The presence of intensely stained hyphal material along the diffuse edges of voids within the wood cell wall was intriguing. Unlike cavitation types of attack where the gap between hyphae and the cavity wall was filled with stained material, most probably of fungal origin (e.g. degradation products and mucilage), there was no detectable gap between hyphae and the wall for type E2 attack. An alternative explanation was that the intensity of the stain prevented detection of thin-walled hyphae and therefore the actual nature of the association between hyphae and the wood cell wall was not visible.

Section 4.3

Discussion

One of the key findings of this study was the frequency of occurrence of decay micromorphology rarely reported, absent, or that was interpreted differently in the literature. Initially, such a situation might appear odd since the wood species and preservatives employed were not unusual, and many of the field exposure situations have been encountered in previous studies. However, the effects of a large number of different combinations of different wood species, preservative and site on decay type have not previously been tested in a single study. Some of these combinations were highly selective, enabling detection and elucidation of single decay types. This is typically only possible in laboratory studies using single decay microorganisms but where the significance of important field factors cannot be tested. Possibly the combination of treatment selectivity and inclusion of field conditions enabled detection of significant decay types that might otherwise be overlooked, in much the same way that SR was overlooked or misinterpreted for many years. Also, the resolution and depth of field of the Zeiss Axioplan II optics helped gain insight into the 3D aspects of the decay patterns involved, for a great many samples containing a wide range of subtle decay variations, which for some decay types helped build up a more complete picture of the significance of the observations made.

Examples of all the major decay types described in the literature were also commonly encountered in this study (Table 1.2). It was clear that amongst the great diversity of decay micromorphology that exists in the field, some of the important types, in terms of frequency of occurrence (chapter 5), contained mixtures of features of more than one of the accepted types. Therefore some of the less familiar but important decay types could easily be mistaken for accepted types because of shared features. Furthermore these shared features were often more easily detected than those which are more important diagnostically i.e. for elucidation of the mechanism of their formation. Observations of this study suggested that this was often related to the early defining features of novel decay micromorphology having been overrun by features associated with later decay that shared characteristics of well known types.

The focus of this discussion is on what is believed to be new insight into mechanisms of formation of decay micromorphology and each decay reported in the results section is discussed within this context.

Section 4.3.1

Brown rot

Loss of birefringence was not a reliable indicator of BR. Kuo *et al.* (1988) examined the progression of the early stages of BR in cottonwood (*Populus deltoides*) caused by *G. trabeum* and found that presence of hyphae was the only feature which distinguished sound wood from wood with a weight loss of 2.7%. At a weight loss of 4.3% loss of wood cell wall birefringence had occurred, and small bore holes were present. Wilcox (1993) reported that loss of birefringence was the first diagnostic feature of attack in Douglas-fir and white fir decayed by *G. trabeum* and *P. placenta*. *G. trabeum* produced a significant amount of weight loss prior to loss of birefringence which was first seen in Douglas-fir at 7% weight loss and 15% for white fir. *P. placenta* produced loss of birefringence in the earliest weight losses recorded of 3% for Douglas-fir and 2% in white fir. Eaton and Hale (1993) and Zabel and Morrell (1992) both reported loss of birefringence as being diagnostic for BR. In this study there was a poor correlation between severity of BR and extent of loss of birefringence. Some stakes with advanced decay, likely to have suffered weight losses in excess of 30% (wood was crumbly to touch) remained birefringent, whilst

others with early BR had lost birefringence. The value of birefringence loss as a diagnostic tool for BR was further questioned by the finding that the common cavitiation types C5 and C6 caused loss of birefringence by complete removal of the S2 layer. Whilst these types typically left an intact lumen wall that usually had some remaining birefringence this might not be a reliable diagnostic feature since Wilcox (1993) reported that typically the S3 remains undegraded until late in BR decay. Whilst removal of the S2 layer that occurs for C5 and C6, does not occur for BR, when viewed under polarized light or when interference contrast optics are used, the effect of BR and these cavitation types on birefringence loss across the cell wall were sometimes similar.

Production of copious amounts of oxalic acid and associated rapid uptake of aniline blue dye appeared to be site, and by inference, fungal species specific. This supports suggestions that the importance of acid hydrolysis in early and extensive wood cell wall modification is species specific amongst BR fungi (Shimada, 1991; Green and Claussen, 2003; Claussen and Green, 2003).

Section 4.3.2

Wood with shared or closely associated BR and WR micromorphology

The finding that wood with advanced WR and BR could not be reliably distinguished on the basis of colour and texture alone, is contrary to the widely held view that wood with WR is bleached and fibrous and wood with BR is brown and powdery (Zabel and Morrell, 1992; Eaton and Hale, 1993). Reference to wood with a mixture of BR and WR features (anatomical features) having been caused by a single fungal species could not be found in the literature. However this may have been because expression of a mixed BR-WR decay type by a putative intermediate (BR-WR) decay fungus was suppressed under laboratory conditions typically employed for studying decay. Furthermore, it is generally accepted that certain wood-preservative interaction effects are highly specific to particular species of fungi. Therefore, it is possible that the combination of wood-preservative-site factors employed in this study resulted in selection of a putative BR-WR fungus.

Examination of some of the theories surrounding evolutionary linkages between different types of decay fungi may provide insight into the possible existence of fungi possessing genes that encode biochemical reactions of more than one decay type. Zabel and Morrell (1992) suggested that decay capability evolved independently in several groups of fungi, and that possession of similar decay patterns by widely divergent taxonomic groupings was a convergence response to exploitation of a unique substrate (wood). However, arguments of Gilbertson (1980) and Worrall *et al.* (1997) concerning a theory that BR fungi evolved from WR fungi were convincing and if correct it would be expected that some fungi might possess attributes of both types.

Some BR fungi produce enzymes typically associated with WR and vice versa. For example some BR fungi belonging to the Coniophoraceae produce an exo-1, 4-ß-gluconase (Highley, 1975 in Worrall *et al.* 1997). Some BR fungi, such as *Oligoporus fragilus*, produce extracellular phenoloxidases which Worrall *et al.* (1997) suggested might be a vestigial feature of an evolutionary linkage with WR fungi. Worrall *et al.* (1997) argued that development of a BR fungus from a WR fungus was a simplification process involving conversion of the bifactorial to a unifactorial mating system and loss of extracellular phenoloxidase and exogluconase.

The WR fungus *P. Chrysosporium* possesses hydrogen peroxide producing enzymes and peroxide dependant enzymes (e.g. lignin-peroxidase) linked to oxidation, hydroxylation and cleavage reactions of lignin (Kirk, 1983; in Eaton and Hale, 1993). The importance of Fenton chemistry in oxidative removal of carbohydrate from wood cell walls by BR fungi has been widely reported. Until fairly recently it was considered that WR fungi caused a much more local modification of wood cell walls compared to BR fungi. The suggestion that wood cell wall modification some distance away from hyphae may be a common phenomena amongst WR fungi, as it is in BR fungi (Zabel and Morrell, 1992) is consistent with the finding that hydrogen peroxide, a low molecular weight and therefore mobile degradative substance, is produced by some WR fungi (Ding, *et al.* 1997; Leonowicz, *et al.* 1997; Hofrichter, 2002). It is possible that during evolution of BR fungi from WR fungi, the

role of hydrogen peroxide and other ROS changed from a primary role in lignin degradation reactions, to a primary role in carbohydrate oxidation reactions.

Section 4.3.3

Micromorphology common to cavitation and tunnelling decay types

Very few micromorphological features were unaffected by cellulose microfibril (CM) angle. Only tunnelling type T1 produced a substantial proportion of tunnels of an essentially random orientation. The great majority of tunnels and cavities and their associated hyphae or bacteria were either aligned with the helical CM of the S2 layer or the essentially perpendicular CM of the S1 layer. The common occurrence of dramatic changes of direction of tunnels and cavities during transition between these two wall layers further supports the supposition that CM orientation is a major influencing factor for the great majority micromorphological features. Typically, alignment appeared to be initiated at the penetration stage of wood cell wall attack and subsequent non-alignment was typically associated with lateral side branching of cavity hyphae, or division of TB. References that refer to alignment of SR cavities with the S1 CM are much less frequent than reports of alignment with the S2 CM (Eaton and Hale, 1993: Zabel and Morrell, 1992). This suggests it is a less frequent occurrence, contrary to what was found in this study where both types occurred with similar frequency. Of particular interest was the finding that the great majority of tunnels formed by TB were aligned with either the S1 or S2 CM. This is in contrast to reports in the literature that describe tunnelling by TB as essentially random with regard to CM orientation.

The only reference to a non-random tunnelling event was the putative avoidance mechanism discussed in a review by Singh and Butcher (1991) that was purported to prevent merging of closely associated tunnels. This may be largely because most studies of TB have been carried out using high magnification TEM micrographs (usually TS) that would not readily allow an appreciation of alignment with the CM. This is partly because many of the micrographs published show very dense tunnelling in wood with advanced decay where the avoidance response would be expected to be of greater significance in determining tunnel orientation. But also the majority of micrographs are of transverse sections where alignment with CM would not be

apparent. In this study, close association of tunnels prevented alignment with CM in situations where several colonies had become established within close proximity of each other so that avoidance tunnelling became the dominant factor. Possibly the reason that alignment of TB with CM was the predominant mode of tunnelling was because it was most commonly observed in wood treated with preservative that might be expected to have slowed colonisation thereby reducing occurrence of closely associated bacterial colonies.

Several of the tunnelling and cavitation types described in this study involved concentric removal of the S2 layer and may have been linked to concentrically arranged wood cell wall layers in pine and beech. Sulaiman and Murphy (1995) described crescent shaped cavities in bamboo caused by *C. globosum* that were initiated by tangential T-branching at the interface of narrow and thick concentric lamellae of bamboo parenchyma cells. Subsequent cavity shape was determined by concentric cavity expansion within the thick lamella between thin lamellae. Nilsson and Daniel (1983a) produced TEM micrographs of concentrically arranged crescent shaped cavities in *H. foetidium* fibres and their argument that radial expansion was restricted by high lignin narrow lamellae was very convincing. The possible significance of concentric wood cell wall layers is discussed in the sections covering tunnelling (4.3.4) and cavitation (4.3.5).

Section 4.3.4

Tunnelling decay types

One of the problems associated with wood decay micromorphology nomenclature and one that requires addressing before embarking on a clearer terminology, is the confusion within the literature concerning the distinction between a tunnel and a cavity, the two having been widely used to describe the same, or similar features. Some authors have also used the term longitudinal bore hole which further confuses the issue. This is of particular relevance for those decay types referred to in Table 1.3 that possess features of more than one of the recognised decay types and which are of special significance to this study because of their frequency of occurrence in preservative treated pine and beech. Also confusing is the assumed distinction between some of the associated hyphal features. For example the terms microhyphae,

used to describe small tunnelling hyphae produced by preferential WR fungi (Nicole *et al.*, 1995), and the more commonly referred to proboscis hyphae of SR fungi, are difficult to distinguish in terms of ultrastructure and orientation within wood cell walls.

According to the Oxford English dictionary both cavities and tunnels are essentially empty spaces in a solid material, but implicit in the definition of a tunnel, is the concept of formation by an object passing through the material. Therefore if one were to apply a strict English definition to what SR fungi do to wood cell walls one could argue that SR fungi do not form cavities but form tunnels with a regularly oscillating diameter. However, the term SR cavity is so entrenched in current decay literature that renaming it would be counterproductive. Anagnost (1998) described distinguishing features of cavities produced by WR fungi from those produced by SR fungi. The WR basidiomycete *A. auricula-judea* produced "longitudinal cavities" originating from T-branching in the S2 layer of birch (*B. alleghaniensis)* fibres and Anagnost (1998) reported that they differed from SR cavities by "1) the transverse bore hole which initiated the cavity expanded, while in SR, the bore hole did not expand, 2) the cavities did not exhibit the oscillatory growth pattern characteristic of SR, and 3) the cavities caused by WR fungi were not angular."

Whilst Anagnost's (1998) distinction was useful for separating cavitation produced by *A. auricula-judea* from classical SR cavitation (some SR fungi produce bore holes), there is still the possibility for confusion arising from the terminology used. Implicit within the terminology was the assumption that WR fungi do not cause SR cavitation. Whilst this is reasonable on the basis of the widely reported definition of SR being decay caused only by deuteromycetes and ascomycetes (Nilsson, 1988) it is now known not to be correct (Worrall *et al.*, 1997). For some time it has been generally accepted that Xylariaceous ascomycetes are a problem for this definition because they cause erosion decay in hardwoods similar to that caused by basidiomycete WR fungi. However the recent review of decay types by Blanchette (1995) pointed out that there are well defined biochemical and anatomical differences between the two and therefore it does not pose a major problem to the definition of SR. However, the possibility that SR cavitation similar to that commonly attributed to

SR fungi belonging to the Deutermycotina and Ascomycotina might be a significant phenomenon for basidiomycetes does not appear to have been widely discussed as a problem for the SR definition. The WR basidiomycete *O. mucida* produced "SR cavities" in baltic redwood (*P. sylvestris)* (Daniel *et al.*, 1992), and *I. hispidus* produced "SR cavities" in ash (*F. excelsior*) and London plane (*P. hispidus*) (Schwarze *et al.*, 2000). The BR fungus *Fistulina hepatica* was reported to produce "SR cavities" in baltic redwood. It was clear that all three of these basidiomycetes produced cavities with an oscillatory growth pattern. In this study, hyphae with clamp connections were commonly observed in SR-like cavities described as tunnelling type T2 (Section 4.2.5.2). For all these examples there was evidence of oscillatory growth, a feature which Anagnost (1998) listed to distinguish SR cavities from WR cavities.

Schwarze and Fink (1998) also reported SR cavities in baltic redwood attacked by the WR basidiomycete *M. giganteus* but an oscillatory growth pattern and T-branching was absent. Similarly Kim *et al.* (1988) reported SR cavities in oak (*Q. serrata*) attacked by the WR fungus *L. edodes*, but it was not possible to deduce if an oscillatory growth pattern was present due to low resolution of LM micrographs presented.

Whilst it is now clear that several basidiomycetes belonging to different orders, produce decay patterns that have been described as SR, in some cases the decay patterns referred to possess a number of features quite different to classical cavitation SR and it could be argued that use of term SR therefore confuses decay nomenclature. A good example of this was described by Daniel *et al.* (1992) in their account of "SR and multiple T-branching by the basidiomycete *O. mucida*". The most significant similarities with SR were production of T- and L- branches, evidence of oscillatory growth and alignment of cavities with the CM, although examples of non-alignment were also referred to. Multiple branching of the type described and the extent and shape of coalescence of expanding cavities and bore holes was different to classical SR. In particular the curved edges of cavity walls resembled eroded lumen walls attacked by WR whereas SR cavities usually have straighter sides and the apices are more angular (Anagnost, 1998). Daniel *et al.* (1992), referred to a report by Hale

(1983) of three microfungi that also produced multiple branching but pointed out that the pattern produced by *O. mucida* was highly distinctive.

Anagnost *et al.* (1998) pointed out that wood decayed by *O. mucida* (Daniel *et al.* 1992) and *A. auricula-judea* both contained enlarged bore holes and uniform cavity width but were different because pine with 25% weight loss decayed by *A. auricula-judea* still had fine cavities whereas wood attacked by *O. mucida* had almost complete loss of the S2 layer. However micrographs of Anagnost (1998) also suggested substantial removal of the S2 had occurred and perhaps allowance had not been made for the possibility that wood sections from blocks with 25% weight loss contained decay representative of various stages of decay. The important point is that there were significant similarities between decay patterns produced by these two fungi but the authors involved used quite different terminology (SR verses WR cavitation).

The decay pattern described as "SR and multiple T-branching" produced by *I. hispidus* in ash and London plane (Schwarze *et al.*, 2000) had fewer departures from classical SR cavitation than decay caused by *O. mucida* (Daniel *et al.*, 1992). Cavities were in chains interconnected by apical proboscis hyphae and cavity apices were more angular in longitudinal cross-section. In 3D, apices of cavities produced by *I. hispidus* would presumably be cone shaped with straight, steep sides as opposed to the rounded or convex cone shape of cavities formed by *O. mucida*. The main departure from classical SR cavitation was production of bore holes which supported the definition of WR cavities given by Anagnost (1998).

One of the micrographs of Daniel *et al.* (1992) showed a serrated cavity the shape of which was quite different to cavities that were initiated as multiple branches but was not referred to as being representative of a significantly different form. This is believed to be an important point because it suggests that *O. mucida* produced two different patterns. One had shared features with WR (bore holes, irregularly eroded cavity walls and a propensity for coalescence of cavities without leaving remnants of inter-cavity walls) was similar to serrated erosion found in wood decayed by both type 2 SR (serrated erosion) and type 1 SR (serrated cavity wall) (Worrall *et al.* 1997)

and type C7 of this study. This is more evidence of a decay fungus exhibiting more than one distinctive decay type. A number of publications have suggested that WR basidiomycetes may gain an advantage by switching from a WR mode to a SR mode i.e. from erosion decay to formation of cavities (Schwarze *et al.*, 1995; Schwarze and Fink, 1998; Schwarze *et al.*, 2000). Whilst these suggestions were made within the context of decay fungi that colonise pathogenic reaction wood containing allopathic chemicals and physical obstructions of lumenae in living trees, it may be a common stress response amongst many decay fungi. This was further supported by many of the references listed in Table 1.4 which included several examples of fungi capable of causing more than one decay type (the combination of types is given in Table 1.3).

Decay type terminology needs to allow for production of oscillatory growth patterns by basidiomycetes but use of the term SR does not adequately describe some of the decay patterns produced. It would therefore seem logical to assign a different term that recognises these differences, as suggested by Anagnost (1998). Furthermore, the finding in this study that decay patterns resembling those that have been attributed to basidiomycetes that are known to produce several decay types, and which are very common over a wide range of field conditions, suggested that it would be advantageous if decay nomenclature is designed to adequately distinguish them from other well established decay types. The term WR tunnelling seems more appropriate than WR cavitation or SR for two reasons. Firstly it avoids the confusion that can arise from the legacy of a decay classification nomenclature that defines SR as decay caused by deuteromycetes and ascomycetes. Secondly, oscillatory growth and multiple branching decay patterns that are caused by basidiomycetes have features that are typically distinguishable from classical SR. Therefore in this study, those decay patterns which were associated with fungi possessing clamp connections and/or which bore resemblances to previously described decay patterns known to have been caused by basidiomycetes, were termed tunnelling decay types (T1, T2, T3 and T4).

Section 4.3.4.1

T1

This type of tunnelling was the only type that contained a high proportion of tunnels of a random orientation and has been reported previously. Singh and Wong (1996), described a type of hyphal tunnelling in fibres of Belian (*Eusideroxylon zwageri*) and Xiao *et al.* (1997) described hyphal tunnelling in radiata pine (*Pinus radiata*), both of which were described as possessing an essentially random orientation within the wood cell wall. Tunnels had rounded ends and straight sides and diameter increased from the apex towards posterior regions. Tunnels reported by Xiao et al. (1997) had a tangential diameter of 0.5 – 5 μm. The diameter of tunnels was not reported by Singh and Wong (1996) but examination of TEM micrographs suggested that they were in the order of 0.5 – 3 μm. All these features were in common with T1 tunnels. The authors did not report the fungal species that caused the hyphal tunnelling but in both cases the macroscopic features of decay were typical of WR, suggesting that a basidiomycete WR fungus was involved.

The presence of two essentially similar types of hyphal tunnelling in a hard wood and a softwood could suggest that the fungi involved are able to attack wood species with a diverse chemical composition. For example it is widely reported that fungi capable of causing types 1 and 2 SR often fail to cause type 2 (erosion) in softwood species because of their higher lignin content and presence of a high guaiacyl-syringyl lignin ratio particularly in the S3 layer. Similarly, WR is believed to be more common in hardwoods for the same reasons and therefore it might be expected that under certain environmental conditions, some species of WR fungi initiate decay in soft woods by tunnelling, thereby bypassing the requirement to erode large tracts of guaiacyl-rich, high lignin containing lumen wall. However, unlike temperate hardwoods the Malaysian tropical hardwood Belian has a high lignin content, a feature in common with softwoods like *P. radiata.*

The absence of T-branching as occurs for T2 and for SR, suggests that a different mechanism of chemical dissolution of the wood cell wall occurs around the tip of the penetrating WR hypha. TEM micrographs (Figure 7.7, Section 7.5.3) of pine tracheids with type T1 tunnelling that were stained with $KMnO_4$, showed a halo of

electron translucent cell wall 0.3 - 0.6μm wide between sound wood cell wall and hyphae, indicative of lignin loss (Hepler *et al.* 1970). Similar loss of electron density in wood cell wall adjacent to lumen hyphae of preferential WR fungi has been considered a diagnostic feature of lignin loss in advance of carbohydrate loss (Eriksson *et al.*, 1990). The presence of a halo is consistent with current theory that lignin removal by preferential WR fungi and possibly early simultaneous WR involves release of diffusible non-enzymatic degradative agents as discussed for *P. chrysosporium* in Section 1.4.2. There was no evidence of wall delignification beyond the halo, or defibrillation caused by extensive ML attack as is typical for many preferential WR fungi and therefore type T1 is probably more closely related to simultaneous WR. Tunnelling through the ML is consistent with this suggestion.

Alignment of tunnels with CM of the S1 was common, either in isolation or continuous with tunnels within the S2 that were parallel to the helical CM. These tunnels were essentially concentric, following the outer perimeter of pine tracheids between the ML and S2. This concentric penetration by tunnels of an even diameter and with smooth sides has not been reported previously, although a pattern described by Courtois (1965) as type 11c SR was very similar. Courtois (1965) was describing decay patterns observed in many wood samples collected from the field over a prolonged period and the causative decay microorganism was unknown, and whilst attributed to SR, Eaton and Hale (1993) suggested that it was likely that other types of decay fungus may have contributed to the diversity of patterns described by Courtois (1965). Confinement of tunnels to the outer cell perimeter may have been a result of the plane of resistance offered by the ML although it is also possible that concentrically arranged wood cell wall layers within the S1 or S2 might be involved, as previously described for *C. globossum* cavitation in bamboo (Sulaiman and Murphy, 1995) (Section 4.2.6.1).

Subtle variations between tunnels described as type T1 could indicate that several causative fungi were involved and the common occurrence of closely associated lumen hyphae with clamp connections and the white fibrous nature of the decayed wood (Section 5.5.4) suggested that WR basidiomycetes were responsible.

Nicole *et al.* (1995) produced TEM images of microhyphae of *P. noxious* in wood cell walls of *B. papyrifera.* Within cell walls they were found to be associated with primary and secondary wall breakdown and alteration of the middle lamellae. Two essentially different types of penetration pattern were observed. The first consisted of penetration perpendicular to the long axis of wood cells and the second consisted of longitudinal penetration intramurally in xylem cells. Nicole *et al.* (1995) suggested that these microhyphae shared the salient features of fine hyphae, with a diameter of 0.3 – 0.5μm (proboscis, penetration and T-branch hyphae) described for SR fungi by Eaton (1985), in Nicole *et al.* (1995). The microhyphae of *P. noxious* did not form bore holes, suggesting they had an essentially different function to the smallest hyphae associated with bore holes (0.5μm; Eaton and Hale, 1993). Nicole *et al.* (1995) cited reports of microhyphae produced by *Fomes annosus* (Peek *et al.*, 1972) and *Ganoderma applanatum* (Murmanis *et al.*, 1984). The fine pentration hyphae described by Eaton and Hale (1993) and by Zabel and Morrell (1992) for sapstain fungi and for plant pathogenic fungi that possess a penetration peg associated with a transpressorium appear essentially different to the microhyphae described by Nicole *et al.* (1995). They were not believed to exert mechanical pressure like proboscis hyphae (Eaton and Hale, 1993) and did not expand like proboscis hyphae do once extension has ceased i.e. there was no evidence of oscillatory growth. These micro-hyphae were a similar diameter to the finer hyphae produced by type T1 but did not branch within the cell wall or expand to accommodate expanding tunnels like type T1 hyphae. Therefore three essentially different types of tunnelling hyphae have been reported:

1. unbranched (except during formation of satellite cavities) classical SR hyphae with an oscillatory growth pattern, that have dramatic variations in width (0.2 – 5 μm) rather like a string of sausages (produced by ascomycetes, deuteromycetes and basidiomycetes);
2. unbranched microhyphae of preferential WR fungi of uniform width (0.3 – 0.5 μm);
3. highly branched WR hyphae as described for type T1 and by Singh and Wong (1996) and Xiao *et al.* (1997), the width of which varies from 0.5-5 μm at the tip, gradually widening up to 2 – 5 μm at the distal end.

Section 4.3.4.2

T2

Some of the features of T2 were similar to a decay pattern in Baltic redwood described by Daniel *et al.* (1992) in their account of "SR and multiple T-branching by the basidiomycete *O. mucida*". Formation of either a single or a collection of closely associated T-branches parallel to the S2 CM of pine tracheids was common to both decay patterns. This feature was also produced by *A. auricularia-judea* in birch (Anagnost *et al.*, 1998). Whilst T-branches were closely associated in many micrographs viewed it was not clear that they were of the multiple type produced by *O. mucida* which produced a "stack" of connected T- and L- branches. It was possible that in some instances several unconnected T-branches were close together and had then coalesced. Multiple T-branching associated with types T3 and C3 were not connected or closely associated with other defining features of type T2 and were considered to be a different decay type.

The BH and hat of the T-branch was typically of a greater diameter and of a greater range of orientations within the wood cell wall (than is typical of previously reported decay types). BH's produced by *A. auricularia-judea* and *O. mucida* were 90^0 to the lumen wall but those produced by type T2 ranged from 90^0 to essentially parallel i.e. the BH became a tangentially (concentrically) orientated tunnel. Alignment of T-branches with the S1 CM was of a similar frequency to alignment with S2 CM, a feature absent in wood attacked by *A. auricularia-judea* and *O. mucida.* T-branching was always an early feature of the decay sequence produced by type T2 but L-branches, although not referred to by Daniel *et al.* (1992), appeared to be very common in LM micrographs of wood decay by *O. mucida.* Another feature not referred to directly was the orientation of the multiple branching pattern which in some cases consisted of a parent hypha orientated tangentially with side branches (T- and L- branches) parallel to the S2 CM. The parent tangential hypha could be considered as essentially the same feature as a tangential BH/tunnel formed by type T2, except that the diameter was much greater and the branching was a single T-branch for type T2.

Daniel *et al.* (1992) described later stages of decay by *O. mucida* as possessing features typical of those produced by basidiomycete fungi. The micrographs referred to showed coalesced T-branches and merged BHs which it could be argued look similar to merged erosion troughs and BHs produced by simultaneous WR fungi. However the micrographs referred to also showed cavities parallel to the S2 CM with apical proboscis hyphae, which appear continuous with BHs and zones of merged cavities, as was demonstrated for type T2 i.e. it was apparent that the cavities and associated BHs were the cause of wall removal in merged zones. This decay pattern has a very different mechanism of wood cell wall removal compared to simultaneous WR or any other widely accepted decay type caused by basidiomycetes. However Daniel *et al.* (1992) inadvertently make a very useful point in that if the early defining stages of decay by *O. mucida* or type T2 are not visible, it would be possible to mistake later stages of decay for simultaneous WR which could partly explain the lack of reports that define it as a distinctive type. The LM micrographs reported by Daniel *et al.* (1992) were of a very high quality and are probably not representative of the majority of LM micrographs that have been studied within the field of wood decay research and therefore it is likely that important diagnostic features associated with early decay would have been missed. Alternatively it would be possible to misinterpret the decay pattern produced by *O. mucida* and type T2 as a mixture of decay micromorphology caused by a combined attack by a SR fungus and WR fungus. This must have been almost inevitable in wood from the field that had been attacked by fungi with a decay type possessing a mixture of micromorphology similar to that produced by *O. mucida* including serrated SR cavities, multiple T-branching patterns, BHs and cavities with long proboscis hyphae.

An important difference between classical SR cavitation and decay caused by type T2, *A. auricularia-judea* and *O. mucida*, is that for SR the oscillatory growth pattern is a much more prominent feature i.e. a multiple chain of discrete cavities is a defining event in the decay sequence that is repeated many times until decay ceases. For some unknown reason the T-branch stage of T2, *A. auricularia-judea* and *O. mucida* is the defining decay event, not the subsequent oscillatory cavitation sequence. For type T2, apical proboscis hyphae were produced by the hat of the initiatory T-branch and this also appeared to be the case for *O. mucida*. Therefore it is

reasonable to assume that *O. mucida* and the fungus or fungi responsible for type T2 possess the genes that encode the biochemical events associated with the triggers of oscillatory growth. The possibility that WR fungi possess genes encoding a cavitation capability not normally expressed was discussed by Worrall (1997) as part of his arguments for suggesting an evolution of WR fungi from SR fungi and, BR fungi from WR fungi. If Worrall's (1997) hypothesis is correct it is to be expected that a more powerful stimulus would be required to trigger cavitation in basidiomycete WR fungi compared to deuteromycete and ascomycete SR fungi. The decay patterns produced by type T2 and *O. mucida* were observed in pine whereas *A. auricularia-judea* produced a similar pattern in birch. It is significant that similar patterns have been observed in both a softwood and a hardwood since this suggests that differences in lignin content and type do not have a definitive influence on presence or absence as is sometimes the case for fungi known to have dual type 1 and 2 SR capabilities and for fungi known to have dual preferential and simultaneous WR capabilities.

The common occurrence of closely associated early stages of type T2 and stepped tunnels similar to SR cavities suggested that sometimes oscillatory extension was a feature of type T2. These tunnels contained hyphae with clamp connections or were continuous with lumen hyphae with clamp connections. Lumen hyphae with clamp connections were also typically closely associated with early decay features of T2. It therefore seems highly likely that all features of type T2 were produced by basidiomycete fungi. Wood decayed by type T2 was typically bleached and fibrous (section 5.5.4) but was sometimes present in wood of a less fibrous and less bleached nature (type 2) that was also typically associated with classical SR micromorphology. Bleached fibrous wood is usually attributed to decay by basidiomycete WR fungi which supports the microscopic evidence that type T2 is caused by basidiomycete WR fungi.

It is not known why SR cavities often remain as discrete chains and do not form a conical tube or tunnel by longitudinal coalescence of individual cavities. This feature is probably the consequence of a combination of the oscillatory growth pattern plus an unknown feature of the chemistry of wood cell wall degradation. Preston (1979) developed a mathematical model to account for the shape of SR cavities. It was

proposed that enzymes released by cavity hyphae diffuse at different rates across and along the cellulose microfibrils, and that these rates are so regulated that a diamond or conical shaped cavity is produced. Daniel and Nilsson (1998) suggested that absence of enzyme secretion from hyphal segments involved or incorrect stereochemistry of cell wall polymer groups hindering enzyme activity could also be involved. The continuous nature of tunnelling formed by some WR basidiomycetes suggests that whatever factor prevents coalesce of SR cavities is absent. Possibly the more comprehensive lignin degrading capability of basidiomycetes alters the diffusion dynamics of the model proposed by Preston (1979). Anagnost (1998) suggested that a SR mechanism may be different to a WR mechanism by production of an enzyme system that is sensitive to the spatial arrangement of particular cell wall components, such as the nature of the crystalline lattice structure and the arrangement of the CM.

Tunnels produced by type T2 that were not stepped and were essentially SR-like resembled cavities seen in libriform fibres of oak (*Q. robur*) that had been naturally infected by the BR basidiomycete *F. hepatica* (Schwarze *et al.* 2000). *F. hepatica* caused SR-like cavitation in libriform fibres high in syringyl lignin but caused BR in fibre-tracheids high in guaiacyl lignin. Schwarze *et al.* postulated that this was related to the tendancy of BR fungi to attack softwoods high in guaiacyl lignin. They also suggested that the dual decay modes of *F. hepatica* were a legacy of adaptation during evolution alongside evolution of advanced libriform fibres from primitive fibre-tracheids. This suggestion is particularly relevant to the suggestion that some types of cavitation referred to as diffuse cavitation by Daniel and Nilsson (1998) and Anagnost *et al.* (1994) had features suggestive of a BR decay mechanism. This suggests that some fungi with a dual BR-SR capability express cavitation and a BR decay simultaneously whilst others express each in isolation in response to differences in wood cell wall chemistry.

T2 decay removed large volumes of wood with a relatively small number of T-branching events compared to decay by *A. auricularia-judea* and *O. mucida* that contained a higher density of finer T-branches. Therefore whilst all three decay types shared a decay initiation event (T-branching) and were the result of attack by a basidiomycete, it appeared that the stimulus controlling multiple T-branching was

suppressed in type T2. This may have been a reflection of fungal species differences, environmental factors or their interaction. The lower frequency of hyphal branching and the larger dimensions of BHs and T-branches of type T2 suggests that this decay pattern was more closely related to simultaneous WR than the decay caused by *A. auricularia-judea* and *O. mucida.* In contrast, the decay pattern reported by Schwarze *et al.* (1995) entitled "Soft rot and multiple T-branching by the basidiomycete *Inonotus hispidus* in ash and London plane" appeared more closely related to SR than either *A. auricularia-judea* and *O. mucida* since chains of cavities with angular apices (steep, straight sided conical shape in 3D) were present. Furthermore there was a large gap (2 - 4μm) separating cavities i.e. the proboscis hypha in this region did not take part in lateral cavity expansion which is a feature typical of SR. The absence of this feature and the presence of stepped tunnels might be typical features of some types of WR tunnelling that involve oscillatory growth. By extension of the same rationale it could be argued that absence of oscillatory growth in pine decayed by type T1 tunnelling and the similar types reported by Singh and Wong (1996) and Xiao *et al.* (1997) in Belian and pine respectively, might be more closely related to WR decay than type T2.

Section 4.3.4.3

Types T3 and C3

The most detailed descriptions of multiple branching patterns caused by fungi are those already referred to in the previous section by Daniel *et al.* (1992), Schwarze *et al.* (1995) and Anagnost *et al.* (1998), although identical and similar patterns in naturally infected wood, attributed to decay by actinomycetes, were also described in detail by Nilsson and Daniel (1990). The suggestion that actinomycetes might be involved was based on the small size of hyphae contained in tunnels and previous absence of their description in the literature. For those patterns that were identical to those later attributed to basidiomycete WR fungi it is almost certain that actinomycetes were not involved and that WR basidiomycetes were responsible. Some of the other patterns involved very fine reticular patterns, including those that appeared to involve anastomosing hyphae, and observations reported for types T3 and C3 suggested that these are also caused either by WR basidiomycetes possessing

tunnelling capabilities or SR fungi respectively (anastomosing hyphae are produced by basidiomycetes and ascomycetes).

Micrographs produced in this study demonstrated that the multiple branching patterns of types T3 and C3 were sub-types of the parent types T1 and SR respectively, although it is not certain that multiple branching is always caused by fungi possessing dual decay capabilities (parent and sub-type). The hypothesis that multiple branching patterns arise when decay fungi respond to stimuli that interrupt the normal decay sequence, causing hyphal branching to repeat many times before the appearance of later features typical of the normal sequence, has not been suggested previously. It is however widely accepted that environmental and wood species factors have a profound effect on expression of decay type (Zabel and Morrell, 1992) and that branching by SR proboscis hyphae is probably affected by wood cell wall chemistry and anatomy (Sulaiman and Murphy, 1995; Daniel and Nilsson, 1998). The reason why types T3 and C3 were so common in this study and were usually linked to parent decay types (i.e. continuous micromorphology of the parent and sub-type was present) is not entirely clear. As discussed in section 4.3, possibly the combination of the selectivity of many unique wood-preservative-site combinations enabled detection of significant decay types that might otherwise be overlooked, in much the same way that SR was overlooked or misinterpreted for many years.

Section 4.3.4.4

Type T4

The mechanism of formation of concentric tunnels in beech fibres by fungal hyphae described for type T4 has not been reported in the literature. The most closely related decay mechanism found in the literature was that produced by *C. globossom* in bamboo (Sulaiman and Murphy, 1995) except that T-branching was not involved in T4 and *C. globossum* did not decay the ML and lumen wall. Whilst removal of the ML suggests a lignin degrading capability beyond that typical of SR fungi, it did not occur until late in decay. Uptake of aniline blue dye by the ML close to hyphae seen penetrating it suggested it was modified before being completely removed. Furthermore the BH through the ML did not enlarge around the penetrating hypha suggesting that the mode of decay was different to classical simultaneous WR. These

observations suggest that the fungus responsible may have been a fungus with a preferential WR decay capability that was largely repressed.

Type T4 was similar to a decay pattern seen in European beech fibres caused by *M. giganteus* (Schwarze and Fink, 1998). Whilst the decay mechanism suggested was difficult to interpret the micrographs suggested that a hyphal branch from a lumen hyphae penetrated the fibre wall, turned abruptly through 90^0 forming a tangential concentric cavity in the process. Of further interest was the report of two other types of decay pattern, one of which involved preferential delignification of fibre walls by lumen hyphae prior to formation of cavities. This supports the suggestion that apparent modification of the ML might be the result of a preferential WR type of decay. The third decay pattern produced by *M. giganteus* had tunnelling features (described as SR) intermediate between types T1 and T2. The lack of other reports of the decay mechanism of T4 is probably because this decay type looked very similar to wood containing large irregular BHs caused by classical simultaneous WR.

Section 4.3.4.5

Tunnelling bacteria

The only significant departure of TB micromorphology found in this study from that reported in the literature was the high frequency of alignment of tunnelling with the CM of both the S2 and S1 layers, as discussed in Section 4.3.3. The other significant aspect of TB decay micromorphology was the hypothesis put forward concerning a putative mechanism for bacterial penetration suggested by micrographs described in section 4.2.5.5. In essence, these observations suggested a different function for cross walls and to some extent encapsulating slime compared to the functions generally accepted in the literature.

As suggested by the review of Singh and Butcher (1991), slime may play an important role in bacterial mobility. It was suggested that slime enables a bacterial gliding mechanism to operate. Bacteria were believed to be surrounded by slime which forms a tunnel wall and that the cross walls (described as bands by Singh and Butcher (1991)) were left behind as a consequence of build up of slime during a period when the bacterium is relatively inactive. Whilst Singh and Butcher (1991)

give the most detailed analysis of the mechanism of TB movement during wood decay, they stated that the mechanism of deposition of discrete bands of slime is not clear. Since cross wall formation is such a unique feature amongst wood decay microorganisms and leaves a unique imprint of tunnel orientation it is worth considering in more detail. A clue that could contribute to an increased understanding of the mechanism of cross wall formation and its possible significance to bacterial mobility is the frequency of cross wall occurrence within tunnels.

Singh and Butcher (1991) compared micrographs and suggested that cross wall frequency is related to wood species, with a greater frequency between wood species than within a species. However examination of micrographs presented by Singh and Butcher (1991) did not appear to consistently substantiate this suggestion. A more consistent feature that was not reported was the apparent increased cross wall frequency that occurred when tunnels were in close proximity. This was possibly because the putative avoidance mechanism forced bacteria in adjacent tunnels to change orientation within a tunnel as a prelude to, or simultaneous with, a change in tunnel direction away from the adjacent tunnel. In situations where a tunnel is surrounded by many other tunnels the frequency of reorientation would increase. These observations are consistent with the hypothesis that the cross walls are an imprint of the movement mechanism itself rather than an imprint of an immobile period. It is to be expected that the wood cell wall surrounding the bacterium provides a resistant surface required for gliding motion. It is also to be expected that the tunnel void behind the bacterium offers less resistance, and it is therefore logical that this is where a slime build up would occur as a result of the pushing motion associated with gliding. Slime would build up in the void as a result of it having been squeezed from between the surface of the bacterium and the wood cell wall and would preferentially build up at the distal end of the bacterium.

Alternatively the cross wall may provide a cohesive surface that assists with forward motion. The absence of apical growth by a tubular hypha in TB, may be compensated for by production of a cross wall. The cross wall, in combination with the tunnel wall, would be expected to physically restrain a flexible bacterium, achieving an effect similar to the containment of fungal cytoplasm by the rigid hyphal wall. Apical

pressure exerted by cytoplasmic flow or localised apical pressure via exocytosis or other cross-wall and cross-membrane transport mechanisms, cannot be ruled out.

Daniel and Nilsson (1998) described TB as globose. Whilst they also mentioned the considerable plasticity of the bacterial cell wall it is perhaps misleading to describe these bacteria as globose. This is because micrographs presented by Singh and Butcher (1991) and those produced in this study showed the enormous capacity for shape transformation and conformation of the bacterium to accommodate a narrow and expanding cavity during wood cell wall penetration. The following description of this penetration process was given. "Prior to its entry, the bacterium becomes pear-shaped, the protruding apical region being involved in boring a small hole into the wall. This obviously involves localised enzyme dissoluion of the S3 layer and part of the underlying S2 layer. When the bacterium is about half way into the wall the apical region of the bacterium flattens out creating a larger hole. During bacterial penetration of the wood cell wall, a close correspondence between the shape of the bacterium and contours of the wall around the hole is always seen. When the bacterium is nearly three-quarters of the way into the cell wall it abruptly changes direction of movement, usually turning at right angles to the initial direction. The bacterium then enters completely and continues to advance."

During the "three-quarters" entry stage described by Singh and Butcher (1991) the bacterium appeared to possess a flying saucer shape, not unlike a fat T-branch. Such a shape fits with the observation that at this stage the bacterium turns through 90^0 since this might be a response to a plane of wood cell wall weakness or contrast at the S3-S2 interface. It is perhaps surprising that the remarkable plasticity exhibited by a unicellular bacterium has not been suggested to play a key role in ongoing tunnel formation by TB. Current belief that an essentially globose bacterium (Daniel and Nilsson, 1998), "sinks" into the wood cell wall (Singh and Butcher, 1991), and then undergoes a start-stop penetration process does not fit well with the observations of Singh and Butcher (1991) or those of this study. In view of this enormous capacity for plasticity it seems likely that it would be exploited during ongoing tunnelling and not just during penetration.

Perhaps the term plasticity inadequately describes the apparent fluidity of these remarkable bacteria. In this regard, an observation of Daniel and Nilsson (1998) may be of particular significance. They described bacteria from a tentative pure culture of TB, as being surrounded by a slime capsule or tube. It is possible that this capsule might function as a highly flexible "cell wall". It might explain how the extreme contortions are achieved during the close conformation of the bacterium surface with the wood cell wall during cell wall penetration. The slime capsule might prevent cell lysis that would otherwise occur in the absence of a fully functional cell wall, a state that might be required to achieve the contortions observed by Singh and Butcher (1991). A high magnification (x 50,000) micrograph of tunnelling attack in *P. sylvestris* pulped fibre was interesting in this regard (Singh and Butcher, 1991). A double membranous structure surrounding the bacteria was identified. The dimensions of the gap between the double membrane of TB were similar to those seen in micrographs of the double membrane of *Escherichia coli* (Alberts *et al.*, 1983). This gap, seen as an electron opague region was identified as peptidoglycan for *E. coli,* a layer believed to provide rigidity. Since TB appear to possess a double membrane-peptidoglycan conformation typical of a Gram negative bacteria, their unusual plasticity might require its modification during the distortion associated with wood cell wall penetration. Absence of a cell wall, was suggested by Hale and Eaton (1985) for SR penetration hyphae and it was inferred that the extent of close association between the hypha and the surrounding hyphal protoplast negated the need for the physical constraint normally provided by a cell wall. Perhaps a similar phenomenon occurs for TB. The physical constraint normally provided by the cell wall is provided by the slime capsule during penetration of TB and a combination of the wood cell wall and distal slime (cross wall) performs this function during tunnelling.

An extension of the putative gliding motion imprint theory is suggested by TEM micrographs of bacterial tunnelling in Belian fibres (Singh and Butcher, 1991) and the observations of this study. Where bacteria were bisected parallel to their long axis they appear pear-shaped (a shape commonly observed in this study), as described for a bacterium penetrating the S3 from the lumen (Singh and Butcher, 1991). This suggests the following sequence of tunnelling events: 1) the posterior end of the

bacterium exposed to the tunnel void is embedded in slime and remains in this position for a defined period of time; 2) the narrow apex of the bacterium extends as wood cell wall degradation creates new space; 3) forward extension that occurs in the absence of movement of the posterior bacterium surface results in elongation and narrowing of the bacterium; 4) forward extension continues for a defined period, possibly limited by a finite bacterial cytoplasmic volume after which; 5) the posterior bacterium surface breaks away from the encasing slime and leaves a cross wall behind either before, during or after lateral expansion of the elongated bacterium; 6) forward extension may or may not be continuous but in either case the bacterium undergoes rhythmic elongation and shortening, similar to the process described for penetration of the S3 layer by Singh and Butcher (1991).

This putative tunnelling mechanism purports that the cross wall is not a consequence of a period of immobility of the whole bacterium as suggested by Singh and Butcher (1991) and others, but is formed by a period of immobility of that portion of the bacterium surface that is exposed to the lumen during active degradation and extension at the anterior surface. This putative mechanism is consistent with the observations of Singh and Butcher (1991) presented in support of degradation only occurring around the advancing front of the tunnel: 1) the diameter of tunnels is similar to that of the TB which produce them, 2) the wood cell wall in the immediate vicinity of the tunnel appears intact, 3) there is a clear direction to TB movement as suggested by the concavity of the cross walls. Eaton and Hale (1993) suggested that degradation of the tunnel wall was not restricted to the anterior region of the bacterium on the basis of variable tunnel diameter. However if bacteria alter their orientation during tunnelling as suggested by the frequency and relative positioning of cross walls, particularly when tunnels are in close association, this would result in variability of tunnel diameter through repositioning of the long axis of bacterium.

The putative tunnelling mechanism is further supported by another observation from TEM micrographs presented by Singh and Butcher (1991). The distal slime (cross-wall) was consistently more electron dense than the surrounding wood cell wall margins of tunnels in bracken fern (*Pteridium aquilinum*) stem. If the slime formed a wall of essentially the same thickness in all tunnels during tunnelling, as suggested by

Singh and Butcher (1991), the wood cell wall margins would be expected to have a layer of electron dense slime. The electron dense nature of the slime is probably a consequence of a concentration of lignin breakdown residues within the slime. A slime deposit beyond the cross wall only appears to occur where tunnels are in close proximity, where adjacent tunnels are not separated by wood cell wall. A complete encapsulating slime layer, as observed for non-attached bacteria from a tentative pure culture of TB (Daniel and Nilsson, 1998), is probably less important for a bacterium closely associated with the tunnel margins. This is because the tunnel margins provide an effective barrier to possible adverse environmental factors and reduces a requirement for a degradative product delivery system and physical restraint. However when tunnels within the wood cell wall are too dense for complete avoidance, lysis of the intervening wood cell wall occurs, at which point there is a requirement for a more complete encapsulating slime layer of a similar thickness to the cross walls. An interesting observation in this regard was seen in high magnification micrographs of EB (Daniel and Nilsson, 1998) showing bacteria encapsulated in a thick dark layer. This appeared to be an encapsulating glycocalyx as described by Eaton and Hale (1993). The glycocalyx surrounding EB appeared considerably thicker than for TB. This observation is consistent with the location of EB on the lumen surface where exposure to environmental stress is greater than occurs for a tunnelling bacterium located at the apex of an encapsulating tunnel.

The maximum separation between cross walls was a distance similar to the longest dimension seen for TB which was 1.2 μm for tunnels in *H. foetidium* fibres (Eaton and Hale, 1993). This distance was only seen for bacteria that appeared constricted at one end by the surrounding wood cell wall which is consistent with the elongation phase of the putative cross wall formation mechanism described above. In this study bacteria separated from the wood cell wall by a void on all sides were typically shorter (0.6 – 0.9 μm). Whilst shrinkage may contribute to occurrence of such a void, a consistently narrower, often undetectable void around the anterior region of pear-shaped bacteria suggests that this feature is not an artifact but is related to forward motion. The variable diameter of tunnels in this study (0.6 – 0.9 μm) was probably a consequence of sectioning through bacteria in varying orientations and at varying stages of oscillatory elongation and contraction. Observations of bacteria constricted

around the leading anterior end, such as pear-shaped bacteria, are considerably less common in micrographs of Singh and Butcher (1991) which may have contributed to a lack of attention to their possible significance. However lower frequency is possibly an inevitable consequence of the lower probability of bisecting a bacterium so as to reveal it's pear shape.

Butcher and Singh (1991) reported that long straight sections of tunnel often occurred within the S1 layer and suggested that this was due to physical constriction within a layer of similar dimensions to TB. However this phenomenon was observed in micrographs of decayed radiata pine for which the S3 layer is known to be approximately 0.1 – 0.2 μm thick (unpublished data of Donaldson), considerably narrower than commonly observed for bacteria. Since the review of Singh and Butcher (1991) there has been an increased awareness of the significance of concentric lamellae within the S2 layer of wood cell walls during penetration by SR hyphae and it appears probable that such a phenomenon might also be involved in orientation during forward motion of TB. This is strongly suggested by micrographs of Singh and Butcher (1991) showing various stages of bacterium penetration of the wood cell wall from the lumen and which illustrate the alignment of a bacterium with the S3-S2 interface. This alignment is possibly triggered by a plane of weakness or contrast between the two layers of different lignin composition.

Section 4.3.5

Cavitation decay

Section 4.3.5.1

Soft rot

Probably the most significant finding in terms of new information concerning SR was the very high frequency of occurrence of connected cavities of different shape, dimensions and orientation within the S1 and S2 layers of pine tracheids. This suggests that many decay fungi are capable of moving between these layers, and in response to the different CM orientation and possibly wall chemistry, the form and orientation of the cavity undergoes significant changes. An incorrect but natural conclusion to reach in situations where cavity chains in different layers were not

clearly connected, would be that such widely different cavity forms must have been caused by different species of decay fungi. Possibly of even greater significance was the observation that the cavity form within the S1 layer had similarities with simultaneous WR and those in the S2 layer were essentially of a classical SR form. This is further evidence that decay fungi undergo substantial changes in decay mechanisms in response to changes in cell wall chemistry, although it is likely that preservative-wood interaction effects would also be involved because wall chemistry has a significant effect on wood preservative uptake. The smooth wavy edges and rounded apices of cavities in the S1 had similarities to wall remnants of wood attacked by simultaneous WR fungi. Some of the cavity forms seen were intermediate between tunnelling of type T2 and classical SR, suggesting that this was caused either by a WR fungus producing a tunnelling type of decay or a SR fungus producing a decay type with attributes of WR erosion. If the evolutionary hypothesis of Gilbertson (1980) and Worrall *et al.* (1997) is correct, it is possible that a large number of fungi may possess dual decay capabilities of WR and SR and that these are usually more frequently encountered under the highly varied conditions typical of field exposure.

Leightley (1980) observed cavities in tracheids of *P. eliottii* and Baltic redwood and in fibres of CCA treated *Eucalyptus maculata* that were perpendicular to the long axis of cellulose microfibrils. It was difficult to determine which wall layers the cavities were in but it was suggested that they could be located within the S3 layer. Perpendicular cavities were not associated with the S3 layer in this study. Leightley's (1980) LM micrographs showed that the perpendicular cavities were typically wider (up to 8 μm) than those of the S2 layer (2 - 3μm) as was seen in this study. A typical oscillatory growth pattern could not be seen for perpendicular cavities, another feature in common with observations of this study. Cavities associated with T-branches within the S2 layer appeared to be connected to perpendicular cavities suggesting a common causative fungus. The interconnectedness of the cavities of different orientation resulted in a criss-cross pattern referred to by Leightley (1980) as reticular.

Leightley (1980) discounted the possibility that the perpendicular cavities were the

result of unusual cellulose microfibril orienation within the wood cell wall on the basis that the same pattern was seen in three wood species and because TEM micrographs suggested no link between cavity formation and particular cell wall layers. Khalili *et al.* (2000) has linked a similar reticular cavity pattern in kapok fibre cell walls to different cellulose microfibril orientation in different cell wall layers. Khalili *et al.* (2000) cited work of Fengel and Wenzkowski (1986) which suggested that kapok fibre cell walls consisted of three main layers where the outer layer consisted of cellulose microfibrils orientated perpendicularly to the long axis of the fibre. This cellulose arrangement was consistent with the observation of Khalili *et al.* (2000) that perpendicular cavities formed by *Humicola alopallonella* in the outer kapok cell wall layer, were aligned with this perpendicular cellulose microfibril orientation.

Greeves and Nilsson (1982) reported perpendicular cavities in CCA treated birch (*B. verrucosa*), CCA treated European beech (*F. sylvatica*), and cuprammonium caprylate treated European beech. On the basis of observations from SEM micrographs it was suggested that these cavities were located in the S1 layer, however examination of the micrograph included in the publication suggested that the S2 layer was also involved. The S1 layer in radiata pine is approximately 0.1 – 0.2 μm thick and the transition zone between the S1 and S2 is in the order of 0.05 μm (unpublished data of Donaldson, *Forest Research*). Soft rot penetration hyphae are 0.2 – 0.4 μm diameter (Hale and Eaton, 1985) and subsequent lateral cavity expansion typically results in cylindrical cavities 0.5 – 1.5 μm diameter. In an SEM micrograph in Greeves and Nilsson (1982) showing two adjacent fibres, one contained only perpendicular cavities, many of which were coalesced and the second adjacent fibre contained only longitudinal cavities. The apparent topography of the micrograph coupled with the known dimensions of the S1 layer and those of SR hyphae and surrounding cavities strongly suggests that the cavities seen were primarily in the S2 layer. Therefore, the S1 probably initiates hyphal alignment, possibly at the T- or L-branching phase of decay, perhaps at the S1-ML interface, and then subsequent lateral cavity expansion into the S2 occurs.

The expression of different growth patterns within the S1 and S2 layers by SR fungi is likely to be a response to differences in wall structure and, or chemistry. It is particularly interesting that cavities in the S1 layer do not appear to produce an oscillatory growth pattern typical of growth within the S2 layer, or do so with a lower frequency. Khalili *et al.* (2000) suggested that the minimum width of cell wall of kapok fibre necessary for proboscis hypha formation was >0.3 μm on the basis that proboscis hyphae are 0.2 – 0.4 μm diameter. However proboscis hyphae were commonly observed at the apex of perpendicular cavities and therefore if they were initiated by hyphal branches aligned with the S1 this suggests that S1 thickness was not the critical factor for proboscis formation within this layer. Sulaiman and Murphy (1995) reported initiation of tangential T-branch hyphae at narrow lamella of the parenchyma cell wall of bamboo. Bamboo parenchyma cell walls have alternating narrow (0.04μm) and broad (0.2μm) lamellae, thought to possess different physical and chemical composition (e.g. lignin type and content or cellulose microfibril orientation). TEM micrographs showed that initiation of T-branch formation occurred at the thin lamellae. Subsequent extension and expansion of hyphae within the broad lamella at the boundary of the narrow lamellae resulted in formation of a crescent-shaped cavity. TEM micrographs (Nilsson and Daniel, 1983) showed concentrically arranged half-moon shaped cavities in the fibre cell walls of *H. foetidium* and their argument that the lateral expansion of cavities was restricted by the high lignin content of concentric narrow lamellae was very convincing. A similar restriction would be imposed on perpendicular cavity hyphae by the ML. The ML may also trigger T- or L- branching at the S1 – ML interface and if so it is then logical that orientation would be in response to the CM alignment of the S1 layer, not the S2 layer.

Therefore, it is suggested that perpendicular cavities are a response to the high lignin ML coupled with the perpendicular alignment of the S1 CM. Such a model would only apply to hyphae penetrating towards the ML. A corollary to this putative model involves an extension of the suggestion of Khalili *et al.* (2000) concerning the minimum thickness of a micro-fibril containing wall required for proboscis formation. It is suggested that the thickness of the S1 was insufficient for an oscillatory growth pattern as occurred in the S2 layer. The greater width of

perpendicular cavities could be related to the non-homogeneity of wall structure between the ML and the S1. Unlike cavities within the S2 that are surrounded by helical CM, a cavity initiated at the ML – S1 interface would probably encounter less resistance between layers in the tangential plane resulting in greater cavity expansion in this plane compared to the radial plane. The ML – S1 interface is likely to a more abrupt region of non-homogeneity than the S1 – S2 interface because the transition between these two layers is known to occur gradually over a distance of 0.05μm in radiata pine (unpublished data of Donaldson) (the contrast of lignin content is more abrupt at the S1-ML interface). A similar mechanism would explain the close association of perpendicular tunnels of type T1 with the ML.

Cavities within the S1 layer that crossed the ML of pine tracheids were more common in this study than cavities within the S2 layer that crossed the ML. Whilst it is known that SR fungi cross the ML by means of proboscis hyphae, in this study cavities within the S1 typically did not change diameter upon crossing the ML. This suggested that the fungus involved possessed a lignin degrading capability beyond that typically exhibited by SR fungi which is further evidence of a decay mode related to WR. Sulaiman and Murphy (1995) reported concentric tangential cavities in bamboo caused by *C. globossom*, a fungus traditionally considered a reference SR fungus. This fungus is now known to cause several cavitation types not previously considered typical of SR (Table 1.3). Whilst these decay types all result in highly efficient removal of the S2 layer of softwoods, efficient removal of either the S1 of softwoods or the ML (both high in lignin and containing resistant guaiacyl-rich lignin) by *C. globossom* have not been reported. Perhaps therefore it is more likely that wood with connected cavity chains of widely divergent form between the S2 and S1 layers is more likely to have been caused by WR basidiomycetes undergoing a SR-like decay mode.

Section 4.3.5.2

C1

SR cavities that are wider in the tangential plane than the longitudinal plane are not widely reported in the literature. The expansive tangential dimension coupled with an irregular erosion of the cavity wall suggests that the causative fungus may possess a

different degradation mechanism to classical biconical and cylindrical SR. The great majority of references that cite production of lateral branches from the parent cavity hypha were reported to form satellite cavities alongside the parent cavity after T- or L- branching. This is essentially different to type C1 where lateral branches do not form cavities but are associated with the lateral expansion of the parent cavity. A key point of interest was the difficulty of identifying individual cavities once decay had advanced beyond early stages and the subsequent resemblance of radial tracheid wall sections to remnants of wall decayed by simultaneous WR erosion. Clearly this has important diagnostic implications similar to those highlighted by Anagnost (1998) in her account of the possibility of confusing serrated edges of cavities caused by SR type 1 with serrated erosion caused by type 2 SR. Preferential expansion of cavities in the tangential plane was probably a response to constriction by radial concentric layers i.e. expansion within a homogenous tangential plane. This was also likely for types C2 and C6.

Type C1 appears to be an intermediate form between classical SR, where cavities are formed by a single parent hypha, and types C5 and C6 that involve multiple branching within a "super-cavity" resulting in complete removal of the S2 layer.

Section 4.3.5.3

C2

The defining feature of type C2 was cavities with diffuse edges containing aniline blue dye in pine tracheids. The only other decay type that produced this feature was C6, also in pine tracheids. The depth of aniline blue uptake was approximately 2 - 5μm. Since no aniline blue uptake could be seen by cavity walls of other cavitation types and bearing in mind the limits of LM resolution are in the order of 0.2μm, this represents a very different staining response to that observed for cavity and tunnel walls of other decay types. It suggests that the causative fungus of type T2 produces degradative products that diffuse into the wall causing its modification so that aniline blue dye is taken up. The significance of this diffuse attack is also relevant to type C6 and is discussed in Section 4.3.5.6.

Section 4.3.5.4

C4

Cavitation of the outer S2, and S1 layers, commencing near the ML and subsequently advancing towards the lumen has not been widely reported but a similar decay pattern was described by Courtois (1963) as type 10 SR. The term cavitation was used to describe type C2 because it was sandwiched between the S2 and ML, however the serrated edge of the S2 seen in radial sections was essentially the same as occurs for erosion of the lumen wall of hardwood fibres decayed by fungi causing type 2 SR (Daniel and Nilsson, 1998). The initiation of cavitation close to the ML could be due to the ML offering a plane of resistance to radial penetration hyphae that triggers T-branching prior to cavitation.

It is not clear why a serrated type 2 SR attack, that is commonly reported in the literature was not seen in this study, whereas type C4 that is not reported was very common. Lumen hyphae of similar dimensions to those within cavities were typically present suggesting that the causative fungus did not possess a type 2 SR capability or that it was suppressed. This is surprising in view of the widely held belief that SR fungi capable of causing cavitation also cause erosion in hardwood fibres (Daniel and Nilsson, 1998).

Anagnost (1998) discussed the possibility of confusing serrated edges of cavities caused by SR type 1 with serrated erosion caused by SR type 2 and it is also possible that type C4 could be similarly mistaken for SR Type 2. Worrall *et al.* (1997) produced an LM micrograph showing decayed birch fibres with serrated edges, caused by *Eutypella parasitica*, the features of which suggest that type C4 decay was involved. Worrall *et al.*'s (1997) description of the pattern was "erosion channels with serrated edges" however the serrations referred to appear to be on the inside of cavities, not erosion of the lumen wall. Anagnost (1998) described "angular channels" in birch caused by *Daldinia concentrica* and referred to this decay as type 2 SR, however this is also believed to be type C4.

Type C4 was similar to type T4 because in both, an apparently intact ML remained sandwiched between cavities within the S2 of adjacent fibres. Therefore it seems

likely that the suggested affiliations with a WR mode of attack discussed for type T4 may also apply to type C4. However, Daniel and Nilsson (1998) suggested that extensive ML breakdown is diagnostic for WR when attempting to distinguish it from type 2 SR. Therefore the intact nature of the ML in fibres attacked by type C4 could suggest a closer affiliation to SR than WR.

Section 4.3.5.5

C5 (beech only)

The defining features of type C5 were complete removal of the S2 layer of beech fibres by small branched hyphae underneath an intact lumen wall. A similar decay pattern was seen in southern yellow pine tracheids decayed by the SR fungi *A. cuboidea* (Anagnost, 1994) and by *Chaetomella raphigera* (Anagnost *et al.*, 1998). Anagnost (1994) proposed the new term diffuse type 1 SR to describe this decay type. Initially Anagnost considered the possibility that this decay was caused by lumen hyphae, as is believed to occur for some Type 2 SR in softwoods (Daniel and Nilsson, 1998), since hyphae could not be seen in cavities. However it was suggested that their absence was due either to rapid autolysis or was a function of their small size preventing detection using LM. However hyphae were subsequently observed in TEM micrographs and in slide cultures of early decay examined under LM. Anagnost (1994) suggested that it was likely that diffuse type 1 was previously misinterpreted as type 2 SR in Douglas-fir (Nilsson 1973; Zabel and Morrell 1992). Anagnost also suggested that diffuse cavities at a late stage of decay may resemble 'BR' caused by *Hypoxylon mediterraneum* previously described by Francis and Leightley (1984). Hyphae were described as growing longitudinally within the S2 and were purported to release enzymes and/or other substances which were able to diffuse away from the hyphae and degrade the S2. Anagnost pointed out that the gap between sound wall and the hyphae observed in TEM micrographs was up to 2.5μm compared to 1μm for type 1 SR cavities. Anagnost (1994) and Anagnost *et al.* (1998) did not refer to any other details of the mechanism of association between the cavity hyphae and the wood cell wall or whether hyphae were branched.

LM micrographs produced in this study showed that branched cavity hyphae were intimately associated with indentations or V-shaped troughs within the cavity on the

ML side of the wall. It was also clear that cavity expansion was concentric, around the perimeter of the fibre and that almost complete removal of the S2 typically occurred prior to collapse of the residual overlying lumen wall. In later stages the V-shaped troughs coalesced and cavities became oblong in radial section. Corbett (1965) referred to erosion attack by SR as type 2 attack and is considered to be the result of enzyme secretion from hyphae in the wood cell lumen. Corbett (1965), as cited in Eaton and Hale (1993) observed V-shaped notches in the wood cell wall which occurred contiguous to the hyphae growing along the lumen wall surface. Fine hyphae were also seen penetrating the cell wall, surrounded by a notch. It is likely that such a decay pattern was the same as the V-shaped troughs formed in cavities of type C5 once the lumen wall had collapsed or was sloughed off during sectioning or was not visible because of occlusion and, or transparency.

Unlike Anagnost's (1994) description of longitudinal hyphae, hyphae were of an essentially random orientation. The close association of hyphae with the bottom of V-shaped troughs within the cavity suggests a degradative mechanism similar to simultaneous WR. Anagnost *et al.* (1998) did not elaborate on Daniel and Nilsson's (1998) suggestion that diffuse cavity attack may involve release of diffusible degradative products similar to those produced by BR fungi. Interestingly the descriptions of diffuse cavity attack by Anagnost (1994) and that of Daniel and Nilsson (1998) are different, even though the later publication refers to the first as providing a more detailed description. Daniel and Nilsson (1998) described a typical type 1 cavity having well defined edges comprising what appeared to be sound wood cell wall. Their description of a diffuse cavity attack was as follows: "diffuse degradation sometimes occurred that extended beyond the cavity edge". They go on to say: "observations using polarised light suggested that the cellulose was degraded in a way reminiscent of BR attack". Daniel and Nilsson (1998) point out that this diffuse zone extended as far as the middle lamella from cavities within the S2 layer. Therefore what this essentially describes is a SR cavity hypha surrounded by a lytic zone, or void, which is surrounded by a diffuse zone of degraded or modified cell wall within which loss of cellulose birefringence suggests loss of cellulose. This description of diffuse cavity walls shared a resemblance with the diffuse edge of cavities of type C2 and those of type C6 but not type C5 where cavity edges were

similar to those described for type 1 SR. Anagnost *et al.* (1994) reported that *S. lignicola, C. funicola and C. globossum* also produced diffuse cavity decay in southern yellow pine, although it was not clear from LM micrographs that micromorphology comparable to that produced by *A. cuboidea, C. raphigera* and type C5 was present.

TEM micrographs (Anagnost *et al.*, 1994) of wood attacked by *A. cuboidea* suggested that cell wall breakdown within the proximity of the diffuse cavity is different to that illustrated by TEM micrographs of wood with BR (Highley and Murmanis, 1985). Cavity cell wall consisted of a heterogeneous, open matrix of electron-opaque cell wall degradation products randomly interspersed with voids of various shapes and sizes. Brown rotted cell wall (Highley and Murmanis, 1985) was homogenous, finely grained, more electron-dense and contained no voids in wood with moderate decay and in wood with advanced decay, a layered lamellate structure prevailed that suggested concentrically arranged voids had formed during cell wall shrinkage. The S2 layer within the diffuse zone was more electron dense and more grainy than sound wood seen in another micrograph showing the S2 layer adjacent to a type 1 cavity formed by *P. dimorphosphora* (uranyl acetate - lead citrate fixation technique was used in both studies). These TEM observations of wood with BR and diffuse SR cavitation suggested that different mechanisms are involved.

Micrographs showing advanced decay of beech fibres by type C5 showed remnants of birefringent wall that looked like angular cavity apices. This observation coupled with the observation that the intact overlying lumen wall was typically birefringent even in advanced decay suggested that the bulk of birefringence loss was the result of highly efficient S2 removal and not the result of diffusible degradative products modifying wood cell wall beyond the cavity wall. Therefore diffusible degradative products produced by diffusible type 1 SR probably have a much shorter range (2.5 μm) than BR (approximately 1 wood cell thickness (20 – 50 μm)).

Type C5 always occurred in beech fibres in contrast to type 1 diffuse SR caused by *A. cuboidea* (Anagnost, 1994) and *C. raphigera* (Anagnost *et al.*, 1998) that was observed in southern yellow pine tracheids. Furthermore *A. cuboidea* caused types 1

and 2 SR in birch and *C. raphigera* caused type 1 SR in birch, but no diffuse type 1. The following fungi were also reported by Anagnost (1994) to have caused diffuse type 1 in pine and either type 1 only or types 1 and 2 only in birch: *Bispora betulina*, *Phialophora melinii*, *Alternaria alternata*, *Bipolaris sorokiniana*, *C. aureum* and *Phoma fimeti*. In this study type C6, that was also characterized by multi-branched hyphae which caused complete removal of the S2, only occurred in pine tracheids, but the micromorphology was nevertheless distinctive from both C5 and diffuse type 1.

TEM micrographs showing diffuse cavities in wood attacked by *A. cuboidea* also showed an interesting feature not discussed by Anagnost *et al* (1994) which challenges the basic concept of a typical SR cavity with a single longitudinal hypha surrounded by a cavity. One TEM micrograph of a longitudinal wood section shows 14 hyphae, each approximately 1 μm diameter, clustered together in the centre of a cavity with a lateral width of 3.5 μm and a tangential width of at least 10 μm (cavity extended beyond micrograph margins). Whilst SR cavities can contain lateral and radial hyphal branches from the parent longitudinal hypha it is unusual to find a large number of essentially parallel hyphae positioned as observed for *A. cuboidea*. If these hyphae had originated from individual coalesced cavities it would be expected that they would occupy the cavity space evenly. Instead they are clustered together in the centre of the cavity with very little (>0.15 μm) or no space between them, or their imprint (seen as a void in the residual cell wall breakdown material). Perhaps this feature is an important clue as to the mechanism of diffuse cavity attack. It suggests that coalescence of cavities is not the primary mechanism of S2 degradation and removal since the bulk of residual cell wall material was not associated with individual hyphae but instead surrounded a group of hyphae in the centre of a single large cavity. This further suggested that at some point soon after T-branching (possibly closely associated multiple branching) and early cavity formation, these small cavities coalesce and from that point on the hyphae acted collectively to form a single cavity. This could account for the lack of discrete cavities either side of heavily degraded cell wall as described for wood decayed by *A. cuboidea*. If lateral and tangential degradation of the S2 by a collective of hyphae becomes the dominant feature of this pattern of attack, as opposed to oscillatory longitudinal extension, then

this might be expected to result in formation of an abrupt transverse decayed - sound wood boundary as seen. If lateral and tangential degradation becomes very efficient as might be the case if a rapid diffusible mechanism, similar to BR, is involved, this could result in the loss of the ordered wood cell wall stoichiometry required for formation of longitudinal proboscis hyphae.

If a diffusible BR type degradative mechanism is initiated by a group of hyphae in a diffuse cavity, it can be expected that such a mechanism would be very efficient. This is suggested, partly on the basis that the cavity might initially concentrate the degradative agents within the confines of the cavity. It is also possible that the short lived nature of the cavity hyphae, as noted by Anagnost *et al.* (1994) might be the consequence of a highly efficient and rapid degradative mechanism in which the products of hyphal autolysis "fuel" an oxidative diffusible degradative process. A similar phenomena is a well established mechanism of plant immune responses, in which plant cells autolyse thereby providing a substrate for reactive oxygen species (ROS) that subsequently kill invading pathogens (Fellbrich *et al.*, 2002; Bolwell *et al.*, 2002). ROS reactions are known to be important for both white rot and brown rot fungi during wood decay (Leonowicz *et al.*, 1999; Hofrichter *et al.*, 2002) and it has been suggested that diffuse SR may share some features of brown rot decay biochemistry (Daniel and Nilsson, 1998). Therefore, what is proposed, is that some of the hyphae become sacrificial (hence their evanescence) providing substrate for an oxidative, partially diffusible, degradative system. This degradative system then rapidly degrades surrounding wood cell wall in a highly efficient manner such that within the confines of degraded patches, almost complete degradation occurs (as shown by micrographs in Anagnost *et al.* (1994)). Towards the end of the degradation sequence substrate (lysed hyphae) for ROS reactions deplete. A possible consequence of such a process is that in some instances the autolytic ROS-fueled reactions might destroy all hyphae within a cavity. Such an event, coupled with destruction of the wood cell wall stoichiometry (limits or prevents formation of longitudinal proboscis hyphae) would result in contained patches of heavily degraded wood cell wall, as observed by Anagnost *et al.* (1994) and in LM micrographs of advanced type C5 in this study.

The close proximity of hyphae within cavities as described for TEM micographs in Anagnost *et al.* (1994) may corroborate with such a putative degradative process. The closeness of hyphae might be expected to negate any protective effect from diffusible degradative products afforded by extracellular sheath. If cavity hyphae are touching, as suggested by TEM micrographs, it is difficult to predict a protective effect similar to that suggested for BR fungi surrounded by a mucilagenous sheath (Zabel and Morrell, 1992). In type 1 SR close association of many hyphae does not occur, each hyphae being surrounded by cell wall and therefore the putative protection effects of mucilage might become important in cavities containing multi-branched hyphae. Therefore the closeness of hyphae might increase the likelihood of autolysis caused by hyphal degradative products concentrated within the cavity.

Section 4.3.5.6

C6

Micromorphology suggestive of a decay pattern closely related to type C6 could not be found in the literature. Type C6 and C5 both caused S2 removal through the collective actions of branched hyphae. However, the hyphae of type C6 were typically aligned with the CM of the S2 layer in pine tracheids and hyphae were of a diameter typical for SR cavity hyphae and were less evanescent. Another key difference was that hyphae of type C6 typically penetrated the wall close to the lumen wall, perhaps at the S3-S2 interface. It appeared that the degradative products and, or mucilage stained by aniline blue was expansive in the longitudinal and tangential planes between penetrating hyphae and that cavity edges were sometimes diffuse as described for type C2. In contrast, the cavity wall seen in radial sections was typical of type 1 SR, where a clearly defined abrupt boundary occurred between the lytic zone that was stained blue and the unstained, apparently sound cavity wall. This suggested that wall degradation was more efficient in the longitudinal and tangential plane, possibly at a concentric radial interface, compared to degradation in the radial plane i.e. across the wall lamellae. These observations suggest that a "sheet-like" degradation spreads rapidly in the longitudinal and tangential planes but more slowly in the radial plane. Expansion in the cavity in the longitudinal and tangential planes is caused primarily by the collective actions of degradative products released from lateral regions of the cavity hyphae, and by apical extension of proboscis hyphae.

Preferential expansion at the interface of concentrically arranged planes of strength and weakness fits with the convincing arguments of Nilsson and Daniel (1983) and Sulaiman and Murphy (1995) who suggested that crescent-shaped SR cavities were the result of preferential expansion within concentric thick lamellae between narrow lamellae of wood cell walls. The major difference between type C6 and the crescent-shaped SR cavitation was that the former resulted in formation of a "super-cavity" containing multiple branched hyphae. The reason that branches did not form satellite cavities was believed to have been caused by partial suppression of the mechanism controlling oscillatory growth (as discussed in section 4.3.5.5 for the putative ROS-fuelled degradative mechanism of type 1 diffuse cavitation). That is, removal of cell wall immediately behind the extending hyphal apex was very efficient thereby preventing significant extension of the proboscis hypha before cavity expansion. Micrographs showing radial sections suggested that very short "stubby" proboscis hyphae were present.

If causative fungi of type C6 possess dual type 1 SR and C6 decay capabilities then it is likely that an unknown stimulus results in suppression of the oscillatory growth response and increased degradative efficiency within the tangential plane thereby shifted the decay pattern from production of chains of cavities to concentric removal of the S2 by a "collective" of cavity hyphae. The decay strategy of type C6 appears to combine some of the advantageous attributes of SR, WR and BR.

1. The resistant S3 was bypassed (essentially a stress-tolerant strategic decay response) enabling an efficient erosion of the less resistant S2 layer.
2. The encapsulating effects of the overlying S3 / lumen wall concentrates the degradative products produced, perhaps without the need for mucilage and other membranous extensions that have been suggested to assist in delivery of degradative products produced by lumen hyphae of WR and BR fungi.
3. It is possible that type C6 produces degradative products that are more diffusible than those produced by SR and perhaps possess some of the attributes of the low molecular weight oxidative products produced by BR fungi. Alternatively they may be more efficient at crossing CM bundles as suggested by Anagnost (1998).

Worrall *et al.* (1997) argued that BR fungi were more highly evolved than WR and SR fungi because the biochemistry of decay by-passed the protective actions of lignin by production of a diffusible (low molecular weight / non-enzymatic) oxidative decay mechanism. This was considered a more efficient and more evolved decay mechanism because it was simpler (fewer complex lignin degrading enzymes), faster (early high weight loss) and more energy efficient (lignin breakdown is considered to be a low efficiency process in terms of contribution to oxidative phosphorylation).

A consequence of a BR strategy is that whilst it probably confers a competitive advantage in softwoods that are more resistant to WR and SR because of high total lignin and high guaiacyl:syringyl lignin ratio, the concurrent loss of lignin degrading capability reduces competitiveness in hardwoods and in situations were tunnelling away from unfavourable conditions in the lumen prevail (e.g. high moisture, extractives, allopathic chemicals, blockages and preservatives etc.). Fungi possessing a type C6 decay capability may have evolved to become adaptable to a wide range of conditions by retaining SR and WR features but also by acquiring some BR capability. To argue that BR is more highly evolved because of the efficiency of its decay mechanism within a relatively narrow spectrum of field conditions (as suggested in chapter 5) may be a mute point if fungi such as those producing type C4 achieve greater competitiveness by becoming more adaptable through acquisition of more evolved decay capabilities and persistence of less evolved capabilities. Efficiency and adaptability are both important components of the successfulness of decay strategies, the importance of each varying according to the prevailing conditions.

Type C6 was more common in pine tracheids than beech fibres. In beech the putative "sheet-like' mechanism of the expanding cavity was less apparent and extending hyphae were orientated randomly i.e. they did not align with the S2 CM as occurred in pine. The cavity edges were also straighter, more angular and were non-diffuse. It is widely reported that decayed wood cell wall edges are more angular in hardwoods than softwoods for SR decay (Zabel and Morrell, 1992; Eaton and Hale, 1993). Where hyphae were in close contact with the S2 layer, erosion troughs very similar to those caused by simultaneous WR formed so that once the overlying lumen wall

collapsed or was sloughed off during sectioning, the underlying S2 would in all likelihood be diagnosed as WR erosion.

Section 4.3.5.7

C7 (pine only)

The defining feature was expansive lateral serrations of cavity walls. Unlike type 1 SR, lateral (tangential) extensions were often longer than longitudinal extensions and these were typically associated with lateral branches of the parent cavity hypha whereas shallower extensions were not. Presence or absence of lateral hyphal branches could suggest two fundamentally different decay mechanisms were involved but these were grouped under type C7 because the limits of LM resolution sometimes prevented reliable separation i.e. later hyphal branches may have been present but were not visible. The most important aspect of type C7 was that it was a very common type that might easily be mistaken for type 2 SR in hardwoods. Of particular interest was the finding that cavities were often very close to the lumen wall, resulting in its collapse. Furthermore wood attacked by type C7 often contained many closely spaced cavities that merged early on in the decay sequence. These factors resulted in a decay pattern almost indistinguishable from type 2 SR. Anagnost *et al.* (1998) referred to the difficulty of distinguishing type 1 SR that had a serrated edges from type 2 erosion of the serrated type described by Daniel and Nilsson (1998). Typically, wood with merged cavities was situated in close proximity to discrete cavities, enabling elucidation of the cavitation mechanism. After initiation of type C7 followed by collapse of the lumen wall decay may continue as erosion. The presence of proboscis extensions are believed to be a diagnostic feature of a cavitiation mode of decay. In some micrographs of type C7 and C6, proboscis extensions were present at the end of what appeared to be a trough. Possibly the causative fungus is more efficient at removal of the S3 than is typical for type 1 SR i.e. lignin degrading capability was more efficient, thereby causing early collapse of the overlying lumen wall.

Type C7 was only observed in pine tracheids. This has important implications for diagnosis of wood decay type. Anagnost *et al.* (1998) compiled a key for this purpose based on observations of decay produced in birch and southern yellow pine by known

decay fungi. One of the assumptions made was that the propensity for V-shaped indentations in cell walls was diagnostic of SR in hardwoods, especially type 2, and U-shaped erosion was diagnostic for basidiomycete simultaneous WR. Clearly this key would not be useful for decay types found in radiata pine and European beech since V-shaped indentations were more common in softwoods in this study. Furthermore, several types of cavitation produced U-shaped erosion of the cavity wall (C2 and C6) that could be mistaken for WR once the overlying lumen wall had collapsed. V-shaped indentations were common in beech for erosion type E1 but this type typically had distinctive concentric indentations that were different to those described by Anagnost *et al.*, (1998). The lack of versatility of this key is probably a reflection of both the laboratory conditions employed and the confusion arising from similarities between decay types once initiatory cavitation micromorphology was obscured by later decay features, or coalescence of early features, or early collapse of the lumen wall.

Section 4.3.6

Erosion decay

Section 4.3.6.1

White rot

Cell wall thinning caused by longitudinal lumen WR hyphae (Highley and Murmanis, 1987; Messner and Stachelberger, 1984; Cowling, 1961) and close association between hyphae and U-shaped erosion troughs (Blanchette, 1994) are the most widely reported features of simultaneous WR but were extremely rare in preservative treated wood in this study. Erosion of fibres of *Phoenix canariensis* by *Wolfiporia cocos*, intermediate between the U-shaped erosion troughs and even erosion, reported by Adaskaveg *et al.* (1991) was similar to some of the erosion patterns seen in this study. Here the epicenter of deepest erosion was clearly linked to the position of individual hyphae in the approximate centre of the fibre lumen but the erosion trough was much wider than the U-shaped troughs typically described in the literature. However the observations of this study suggested that some WR fungi cause the bulk of erosion during a somewhat random penetration of wood. A clear distinction between lumen hyphae parallel to the wood cells and perpendicular BH hyphae could

not be made. Typically hyphae crossed the cell wall at an oblique angle forming an erosion feature intermediate between a classical erosion trough and a BH.

Serrated bore holes were seen with a similar frequency to rounded BHs. This was in conflict with the literature in general because rounded smooth erosion is considered diagnostic for WR, enabling distinction from type 2 SR. Striations of the lumen wall seen in this study are also not widely reported as features of WR erosion. However TEM studies of Schmidt *et al.* (1997) showed very small erosion troughs (0.3 – 0.5 μm accross) produced by the white pocket rot *Physisporinus vitreus* in *Pinus sylvestris* tracheids and referred to similar erosion troughs cited by Eriksson *et al.* (1990) caused by *Phellinus pini* and *Phlebia tremellosa*. These troughs were a similar diameter to the striations seen in this study and may be related features.

The distance between WR hyphae and the extremities of erosion was typically within the range 5-10μm which is considerably greater than has often been considered as typical for WR (Ruel *et al.,* 1984; Eaton and Hale, 1993) although reports of thinning associated with hyphae located centrally in wood cell lumenae suggested greater distances are involved (Adaskaveg *et al.*, 1991). It is possible that enzymes arrive at the wood cell surface via extracellular membranous structures (EMS) or extracellular slime (Foisner *et al.*, 1985) or fungal sheath (Benhamou *et al.*, 1987; Murmanis *et al.* 1984 as cited in Nicole *et al.*, 1995). These extracellular materials are believed to act as a matrix for channeling and concentrating both extracellular fungal enzymes and wood cell wall breakdown products. Immunolocalisation studies of wood degrading enzymes have provided evidence that fungal sheaths are directly involved in wood degradation (Nicole *et al.*, 1995). It is interesting that aniline blue typically stained degradative products between hyphae and cavities or tunnels but similar staining was not seen around WR hyphae in cell lumenae.

Section 4.3.6.2

Erosion type E1

In beech fibres the defining feature of type E1 was concentric V-shaped troughs formed by side branches of a parent hypha aligned longitudinally within the lumen. These troughs were V-shaped in both radial and tangential planes. The intriguing

aspect of this type was its concentric symmetry, a feature difficult to explain for a decay pattern initiated as erosion. There are convincing arguments to account for concentric cavitation patterns (Nilsson and Daniel, 1983; Sulaiman and Murphy, 1995) that are based on the presumption that concentric radial lamellae act as planes of resistance that trigger T- and L-branching of penetrating proboscis hyphae and which also determine the pattern of subsequent cavity expansion. However it is more difficult to account for concentrically arranged erosion troughs. This was a dilemma in this study and a convincing argument for their mechanism of formation could not be derived. It was tempting to suggest that these troughs were V-shaped notches within the wall of type C5 cavities that appeared to be troughs because of early collapse of the overlying lumen wall. However in wood with type C5 it was always possible to locate overlying lumen wall in some areas, and the longitudinally aligned lumen hyphae were not seen. It was believed that type E1 and C5 may under some circumstances share a causative decay fungus since the curved shape of the V-notches and their concentric orientation were highly distinctive and were present for both types, although they were less symmetrical for type C5. V-shaped notches have been widely reported as diagnostic for type 2 SR, especially in hardwoods (Nilsson and Daniel, 1983; Zabel and Morrell, 1993).

Section 4.3.6.3

Erosion type E2

Formation of irregular troughs and BH's are typically diagnostic for simultaneous WR. However specific reference to wide troughs (up to 30μm) with sharp serrated edges and BH's formed by large (dia. 7 - 15μm) anastomosing (fused) hyphae as occurred for type E2 could not be found. Hyphae had thin walls, were short lived and formed sheet-like hyphae with narrow (0.5μm) protuberances that appeared to initiate penetration of tracheid walls. Lumen hyphae had well defined walls but when penetrating the cell wall the wall appeared to merge with a diffuse degraded wall boundary. This was different to the pattern described for C2 where cavities contained a well defined hyphae and then a lytic zone with a diffuse wall edge stained by aniline blue because for E2 their was no visible gap between the hypha and the diffuse wall. Sheet-like hyphae were different to the mycelium mats compromised of many hyphae sometimes seen associated with wood with BR decay.

Whilst the most common range for diameter of fungal hyphae is 2 – 5μm, some of the largest hyphae, other than vessel hyphae associated with linear organs, are up to 10 – 15 μm in diameter and are produced by certain holocoenocytic forms such as occur in the genera *Stereum*, *Phanaerochaete* and *Coniophora* (Raynor and Boddy, 1988). Whilst hyphal fusion (anastomoses) are a fundamental process in both the vegetative and reproductive development of ascomycetes and basidiomycetes they were only observed in this study when associated with type E2 decay (they may also have be present in some of the very fine reticular decay patterns reported as type T3 but the limits of resolution of LM prevented confirmation). In culture they typically form behind the mature colony margin where it is normal for hyphae to direct their growth towards each other and then fuse, either tip to tip or tip to side, although the nature of the homing mechanism involved is poorly understood (Raynor and Body, 1988).

Section 4.3.6.4

Erosion bacteria

All EB micromorphology and its interpretation reported in the review of Singh and Butcher (1991) was essentially the same as described in this study.

Section 4.4

Conclusions

1. The decay types identified (based on micromorphology) in this study were in three categories.

a) Decay adequately described by one of the accepted decay types of current decay classification nomenclature.
b) Decay possessing mixtures of micromorphological features representative of more than one of the accepted decay types.
c) Decay micromorphology not previously reported.

The finding that many decay types belonging to category b) had a similar or greater frequency of occurrence compared to those of category a) challenged some of the currently held views on decay classification nomenclature and the relative

importance of different types of decay fungi in preservative treated wood. New insight into the micromorphology of the early stages of several decay types enabled new and more detailed interpretation of the mechanism of their formation.

2. Several of the fungal decay types defined in this study did not fit neatly within the decay types BR, SR (Types 1, 2 and diffuse) and WR (simultaneous and preferential). This was attributed to a high frequency of occurrence of "intermediate decay fungi" that possess diverse decay capabilities, a finding that is concurrent with increasing evidence within the literature that a significant number of fungi possess multi decay capabilities. It is suggested that possession of more than one decay capability is much more common than previously considered and that this has been largely overlooked because few studies have tested the significance of diverse combinations of site, wood species and preservative. Overly rigid decay classification boundaries that place emphasis on taxonomic affinity and biochemical reactions under laboratory conditions, fail to accommodate the diversity of decay types that occur under field conditions.

3. Decay fungi that bypass the decay resistant S3 layer, producing multi-branched hyphae that collectively erode the S2 layer in a fashion similar to simultaneous WR, forming a "super-cavity" under an overlying lumen wall, were very important in preservative treated beech fibres and pine tracheids. Whilst some of the micromorphology associated with this type of decay has been reported as diffuse type 1 SR, its significance has been largely overlooked.

4. Results suggested that several important decay types have been overlooked or misinterpreted because their early defining features are easily masked by later features. This is particularly important for early cavitation decay that forms near or at the S3-S2 interface, and where the overlying lumen wall quickly disintegrates, has been sloughed off during sectioning or has been overlooked in low resolution LM micrographs. It is suggested that this has resulted in misinterpretation of cavitation decay types as erosion (Corbett's type 2 erosion).

5. The greatest diversity of decay micromorphology occurred in wood with

macroscopic features similar to WR or intermediate between WR and SR. Such wood contained mainly cavitation and tunnelling micromorphology, much of which was attributed to basidiomycete WR fungi. Classical simultaneous WR and type 2 SR were almost completely absent from preservative treated pine and beech suggesting that fungal erosion decay is of minor significance in preservative treated wood and that decay fungi that produce it in other situations (e.g. in untreated wood) adopt a tunnelling or cavitation mode in preservative treated woods (i.e. they adopt a stress tolerant strategy in response to the high lignin content and/or preservative content of the lumen wall.

6. Cellulose microfibril orientation in both the S2 and S1 layers determined orientation and positioning of the great majority of decay types, including those currently thought to be essentially random such as TB decay. Contrary to the literature, orientation parallel to or affected by the S1 CM, was of similar significance as alignment with the S2 CM. Concentric radial wood cell wall structure plays an important role in determining the positioning, orientation and limits of expansion of several decay types in both pine and beech, consolidating the suggestions of several recent papers.

7. The frequency of occurrence of decayed wood with a homogenous mixture of macroscopic and microscopic features of BR and WR suggested that some decay fungi produce an intermediate decay type.

8. A putative model was derived to account for the high frequency and morphology of SR cavities and cavities and tunnels of other decay types that were orientated perpendicular to the longitudinal wood cell wall axis. The S1-ML interface triggered formation of tangential hyphal branches followed by non-oscillatory growth and preferential expansion in the tangential plane. Whilst the S1 layer cellulose microfibrils cause orientation of hyphae within the tangential (concentric) plane it is too narrow to trigger normal SR oscillatory growth.

11. Tunnelling bacteria decay micromorphology challenged several currently held views on the mechanism of decay. In particular, a putative bacterial penetration

mechanism purported that a bacterium undergoes rhythmic elongation and shortening. Apical extension, elongation and narrowing of the bacterium occurs until the finite volume of the bacterium causes the distal region to break away from its temporarily fixed location, leaving behind a cross wall.

CHAPTER FIVE

Field test site, preservative and wood species effects on decay type and its impact on wood preservative performance

Section 5.1

Experimental

The objective was to determine the effects of site, preservative and wood species on decay type and its impact on wood preservative performance.

The rationale for selection of a diverse range of field test site conditions was explained in chapter 4. Decay type is currently defined by the species of the causative decay microorganism and by changes to wood structure and chemistry. These three factors provide complementary information about the nature of decay type and it could be argued that an ideal experimental design for studying decay type should undertake to measure all three. However, the number of samples generated by large field studies dictates that a rapid method of assessment be employed to measure decay type. Of equal importance, this traditional approach to studying wood decay is not suited to studying decay type in preservative treated wood in soil contact.

Decay in field test stakes is represented by the formula

wood[1] x preservative[2] x environment[3] x decay microorganism(s)[4] = decay type[5].

In the majority of prior ecological studies of wood decay in ground-contact, reviewed by Rayner and Boddy (1988), the emphasis has been on measuring 4 which is notoriously difficult and of questionable reliability in terms of proving a link between fungal species identified and decay type present in wood exposed in soil (i.e. application of Koch's Postulates is not possible because field conditions cannot be reproduced in a laboratory). Possibly this emphasis has resulted from the preconception that a clear delineation exists between most decay fungi in terms of decay type caused. For example, if a fungus known to cause simultaneous WR under laboratory conditions is isolated from wood in soil contact there is likely to be an assumption that its primary significance in that situation was as a simultaneous WR

fungus as currently defined by laboratory studies. However, as pointed out by Rayner and Boddy (1988) "there has been a tendency to regard wood decay as though the resource and the organism were two simple reagents off a laboratory shelf whose interaction results in a consistent and predictable outcome." They went on to say "this is, of course, far from the truth: not only is wood itself a variable and heterogenous resource but as with all other eukaryotes the decay fungi cannot be regarded as fixed entities. Rather they vary, and this variation occurs not only between different individuals, but also because of the indeterminate nature if the mycelial body form, within an individual. Causes of variation include genotypic differences, direct effects of environment on metabolic functioning, changes to differences in gene regulation and random effects. The implications of variation, both between individuals and during the development of single individuals, are considerable, not only with respect to taxonomy and diagnosis but also in terms of the ways in which populations of decay fungi establish in and interact with woody resources."

The complexity of the determinate factors described by Rayner and Boddy (1988) is such that attempts to link a single phenomenon such as taxonomic affinity or successional sequences of arrival of microorganisms on wood, with the final outcome i.e. decay type, is fraught with the possibility of incorrect assumption or at best is overly simplistic. The idea that fungal species is only one determining factor of decay type in nature, perhaps not the most important one, has perhaps been overlooked because of the prior emphasis on laboratory studies using single isolates tested over a narrow range of exposure conditions. It is therefore not surprising that the emphasis in ecological studies has been on identification of fungal species. If a significant number of decay fungi are able to express several decay types, as a steadily increasing number of publications have reported (chapter 4) then the relevance of identifying fungal species in ecological studies is further diminished. Alternatively, if decay type is determined by complex microbial communities, as suggested in the review by Zabel and Morrell (1992), that are difficult to delineate and identify using current isolation techniques, then this is another reason why placing emphasis on identification of microorganism species is unlikely to be rewarding. Perhaps once a sufficiently comprehensive library of molecular probes is available, identification of complex microbial communities in wood in the field may become a more realistic

target. However, even if fungal species present in communities are known, determination of their relative contributions to the final outcome of decay type is still likely to be difficult task since expression of decay capability is likely to be dependant on exogenous factors.

The concept of adaptation of decay microorganisms to wood as it changes after initial soil contact is widely accepted (Eaton and Hale, 1993), as is the concept of selectivity of particular wood species - preservative combinations caused by preservative tolerance and wood lignin type (Zabel and Morrell, 1992). However, the emphasis has been on selection of the cause of decay as defined by the decay microorganism species and its taxonomic affinity rather than on the whole equation i.e. the product of the 4 factors:

wood[1] x preservative[2] x environment[3] x decay microorganism(s)[4] = decay type[5].

In other words the currently accepted equation is

wood[1] x preservative[2] x environment[3] = decay microorganism(s)[4] = decay type[5].

It is suggested that this places emphasis in the wrong place. For example if the decay mechanism of the decay microorganism or microorganism community alters in response to a combination of 1, 2 and 3, as is likely, then the primary product is the decay type, not the decay microorganism.

In prior ecological studies, the range of wood, preservative and environmental variables was typically small and did not enable comparison of their effects systematically as was possible in this study. Furthermore, the 5.5 – 6.5 year exposure of wood, particularly preservative treated wood, ensured establishment and advancement of decay, a feature absent from most previous studies that focussed on colonisation prior to significant advancement of decay. Most diagnostic features of currently defined decay types are detectable using light microscopy (LM) which is sufficiently rapid to enable examination of many wood samples as are generated by an ecological study of this type.

Section 5.2

Results

Section 5.2.1

Macroscopic decay features and associated micromorphology

Iterative analysis of the micromorphology associated with macroscopic decay types present at all sites resulted in the development of a decay type schematic illustrated in Table 5.1. Photographs of each macroscopic decay type plus details of their recognition are given in Section 5.5.4 (Appendix 5). Data on MSRs and decay types for each site are in Sections 5.5.1 and 5.5.3 and in Section 5.5.2, respectively (Appendix 5).

Table 5.1: Macroscopic features of decay and associated micromorphology

Macroscopic features		Decay type				
Colour		1	2	2a	3	4*
1	White / bleached			✓		
2	Bleached	✓*]				
3	Brown	✓]				✓
4	Slight lightening		✓]			
5	Unchanged		✓			
6	Slight darkening		✓]		✓*]	
7	Grey - brown				✓*	
8	Grey - black				✓]	
Texture						
1	Powdery	✓ (dry)				
2	Fibrous		✓]	✓]		
3	Spongy		✓	✓*	✓	✓
4	Cheesy	✓ (wet)	✓]			
Decay type based on micromorphology		BR (BR-WR)†	Highly Variable$	Highly Variable$	Soft rot	Soft rot

* = only present at Glenbervie and Glenbervie Forest; * = secondary feature, only present at some sites;] = features were often merged; † = decay type only present at some sites; (wet) = BR had a cheesy texture when wet but became powdery and brittle when dry; $ = types 2 and 2a contained diverse micromorphology (Table 5.9).

Decay type 1 contained brown rot (BR) micromorphology alone, except at a few sites where a type intermediate between white rot (WR) and BR (BR-WR) existed. Similarly, types 3 and 4 always contained soft rot (SR) micromorphology alone. However, wood with decay types 2 and 2a contained a great diversity of decay

micromorphology at all sites i.e. many different decay types, as defined by micromorphology, produce wood with a texture and colour of these types. For example ACQ treated pine at Whakarewarewa (WKA) site had types 2 and 2a, both of which were found to contain micromorphology typical of tunnelling bacteria (TB), erosion bacteria (EB), white rot (WR) and 4 other decay types tentatively defined as cavitation type 3 (C3) and 10 (C6) and tunnelling types T2 and T3 (Section 4.2). Other types of micromorphology with macroscopic features of types 2 and 2a were present in other treatments or at other sites.

Subtle differences of colour and texture sometimes existed within a given macroscopic type and this could in some instances be related to site, preservative or wood species. For example, at Hanmer (HAN) there were two distinctive shades of brown for wood with BR, only one of which was associated with a black mycelium, suggesting that different fungi were responsible. Similarly, at Tapanui (TAP) the shade of brown was different again, and this type also occurred at Esk Valley (ESK). Since these subtle differences of colour were believed to be attributable to attack by different species of fungi, and identification of species was beyond the scope of this study, it was decided not to complicate the macroscopic decay type descriptions by recording subtle differences of colour and texture. Whilst it was sometimes possible to distinguish several macroscopic sub-types within a macroscopic type, they could only be reliably distinguished in certain situations, not across all sites for all treatments. For example TB attack produced a distinctive, slightly bleached cheesy type of decay at Tomblesons mandarin orchard (TOM) and at the tropical site at Canal Creek (CC), but when mixed with other micromorphology associated with type 2 or 2a, as occurred at other sites, it was impossible to identify macroscopically across all sites.

The extent of separation between pockets of different decay type on a stake was highly variable and was to a degree dependent on the decay types involved. Where a given decay type occurred in a separate pocket a decay rating was readily assigned to that type. Whilst many stakes contained more than one macroscopic decay type they were typically distributed within overlapping pockets and it was usually possible to estimate the relative proportions of each type and the depth of decay associated with

each type. A minority of stakes had macroscopic decay types that were thoroughly mixed. However when this occurred it was usually possible to determine which macroscopic types were involved after microscopic examination had enabled their identification. All stakes with such a mixture of macroscopic types were then given the same decay rating for each type involved. Proportionately more stakes within this more difficult category were examined microscopically to characterise decay type.

The selectivity of preservative and wood species for particular decay types was a common phenomenon at all sites and was indispensable for linking macroscopic features with micromorphology and subsequent separation of the various decay types, especially for types 2a and 2 that contained such diverse micromorphology. Typically, each macroscopic type and in some cases a single group of micromorphological features that defined a decay type, was present in isolation on at least some of the stakes of at least one treatment group, a phenomenon that was highly site specific. For example, at TOM, CAZ and ACQ treated pine stakes typically contained decay pockets of TB attack only. It was therefore possible to clearly establish colour and texture of this decay type. Once similar correlations had been established between macroscopic features and micromorphology for all treatment-site combinations it was then possible to identify decay type with a greater degree of confidence at the next assessment. Iterative correlative analysis across all sites was therefore important and by the fourth years assessment micromorphology of most decay types had been thoroughly characterised. Some macroscopic features always contained the same micromorphology at all sites but others did not. Typically, a given set of macroscopic features contained the same micromorphology within a site-treatment group combination and it was therefore possible to predict the decay micromorphology in those stakes not examined microscopically. Therefore, reliable correlation between macroscopic and microscopic features was only feasible if micromorphology was characterised for each macroscopic decay type within each treatment group (treatment group refers to all retentions of a preservative for this purpose). Retention effects on decay type where minor compared to site, preservative type and wood species effects. The most important effect of retention was on the timing of arrival of a given decay type where lower retentions were decayed earlier in the study by the same decay type.

The exercise of identifying mixtures of macroscopic features was often made simpler by the fact that two or more decay types within a pocket were different colours and textures. For example at the TOM site, CCA treated pine often had pockets of decay containing a mixture of SR of a dark grey colour and spongy texture, and TB of a slightly bleached beige colour and cheesy texture. It was therefore possible to identify decay pockets that consisted of a blend of the decay features of the two types at that site. Random microscopic examination of wood with mixtures of decay typically corroborated with predicted decay type.

Section 5.2.2

Significance of decay type occurrence and diversity in preservative treated wood

Section 5.2.2.1

Affect of decay type on preservative performance

The relative importance of different macroscopic decay types at each site is given by significant differences (at a 5% level of probability) between percentage contribution of each decay type to MSR across all treatments at each site (Table 5.2).

Key results (Tables 5.2 and 5.3) are:

1. Site had a profound affect on decay type.

2. There was no obvious link between overall site hazard (site hazard index) to variously preservative treated pine and beech and the importance of single macroscopic decay types, as given by percentage contribution to MSR.

3. The contribution of each macroscopic decay type, typically varied greatly between sites (Table 5.2).

Table 5.2: Percentage contribution of each decay type across all treatments at each site, their significant differences (highest retentions only), and site decay hazard index

Type 1 Brown rot			Type 2 Many decay types*			Type 2a Many decay types*			Type 3 (plus type 4†) Soft rot		
Site[N]	%	Sign.	Site	%	Sign.	Site	%	Sign.	Site	%	Sign.
HAN[12]	27.74	a	HH[5]	31.88	a	CK[1]	65.21	a	TOM[2]	52.82	a
TAP[9]	24.96	ab	MM[11]	30.98	a	OAK[6]	59.67	a	TAP[9]	40.08	b
GBF[4]	20.07	bc	PT[10]	29.10	ab	PT[10]	46.09	b	OAK[6]	37.04	cb
ESK[7]	18.43	cd	TOT[13]	26.90	abc	ESK[7]	43.26	cb	GB[3†]	33.1	cd
WKA[8]	13.85	d	TOM[2]	23.69	bcd	HH[5]	42.84	cb	TOT[13]	32.44	cd
GB[3]	7.59	e	HAN[12]	21.00	cd	WKA[8]	40.90	cb	GBF[4†]	31.24	cde
OAK[6]	3.29	ef	GB[3]	20.40	cd	TOT[13]	40.66	cb	ESK[7]	31.17	cde
MM[11]	0	f	WKA[8]	19.85	cd	GB[3]	38.91	cb	MM[11]	30.32	cde
PT[10]	0	f	GBF[4]	17.52	de	MM[11]	38.70	c	CK[1]	26.66	def
CK[1]	0	f	TAP[9]	10.78	ef	GBF[4]	31.17	d	WKA[8]	25.4	def
TOM[2]	0	f	CK[1]	8.13	f	HAN[12]	28.76	de	HH[5]	25.28	ef
HH[5]	0	f	ESK[7]	7.14	f	TAP[9]	24.18	de	PT[10]	24.81	ef
TOT[13]	0	f	OAK[6]	0.00	g	TOM[2]	23.49	e	HAN[12]	22.5	f

MSR = Mean soundness reduction. † = type 4 only present at GB and GBF. * = decay micromorphology associated with macroscopic types 2 and 2a was diverse. [N] = index of site decay hazard, where 1 is the most severe (Table 3.1). Sign. = significant difference: MSR values with the same letter in a column do not differ significantly at a 5% level of probability.

Table 5.3: Percentage contribution of each decay type to MSR across all treatments at each site (highest retentions only)

Site[n]	Macroscopic decay type			
	T1 (Brown rot)	T2 (Mixed)	T2a (Mixed)	T3 & 4 (Soft rot)
CK[1]	0	8.13	65.21	26.66
TOM[2]	0	23.69	23.49	52.82
GB[3]	7.59	20.4	38.91	33.1
GBF[4]	20.07	17.52	31.17	31.24
HH[5]	0	31.88	42.84	25.28
OAK[6]	3.29	0	59.67	37.04
ESK[7]	18.43	7.14	43.26	31.17
WKA[8]	13.85	19.85	40.9	25.4
TAP[9]	24.96	10.78	24.18	40.08
PT[10]	0	29.1	46.09	24.81
MM[11]	0	30.98	38.7	30.32
HAN[12]	27.74	21	28.76	22.5
TOT[13]	0	26.9	40.66	32.44

[N] = index of site decay hazard where 1 is the most severe

Whilst it was clear that site had a profound affect on decay type occurrence in variously preservative treated pine and beech, the significance of decay type on preservative performance was not straightforward. In terms of relevance to prediction of wood preservative performance, the existence of a severe individual decay type hazard at a site with an overall low hazard is important. For example HAN had the

second least severe decay hazard (hazard index 12) in terms of overall MSR (Table 3.1) but was the most aggressive site in terms of BR decay hazard. In contrast, Glenbervie Forest (GBF) had the second most severe BR hazard but was the fourth most severe site overall (equal second at 5% level of probability), and CC with the second highest overall hazard (not significantly different to the site with the highest decay hazard, Glenbervie (GB)) had no BR hazard. ANOVAR showed that there was a significant site-preservative interaction affect so it was to be expected that the performance of some treatments was not consistent with overall performance.

Table 5.4: Percentage contribution of each decay type to MSR for each treatment across all sites and their significant differences (highest retentions only)

Type 1 Brown rot			Type 2 Many decay types*			Type 2a Many decay types*			Type 3 (plus type 4†) Soft rot		
Treat-ment[N]	%	Sign.	Treat-ment	%	Sign.	Treat-ment	%	Sign.	Treat-ment	%	Sign.
PACQ4[4]	31.58	a	PC4[3]	64.01	a	Pine[13]	82.19	a	BCC2[7]	61.08	a
POIL2[8]	14.17	b	PCAZ4[3]	38.77	b	Solv[11]	62.46	b	BOIL[10]	52.63	b
PCCA5[5]	12.63	bc	PACQ4[4]	31.29	c	Beech[12]	46.59	c	BCAZ2[6]	52.44	b
PC4[2]	11.73	bcd	PCC4[1]	23.43	d	POIL2[8]	41.36	cd	BCCA3[9]	51.86	b
Solv[11]	11.73	bcd	Beech[12]	23.33	d	BCCA3[9]	34.74	d	PCCA5[5]	41.11	c
PCC4[1]	10.87	bcd	PCAA5[5]	19.45	d	BOIL[10]	33.93	de	PCC4[1]	38.68	c
BCAZ[6]	9.43	bcde	BCAZ2[6]	11.15	e	PCC4[1]	27.02	ef	POIL2[8]	34.1	cd
PCAZ[3]	9.38	bcde	POIL2[8]	10.37	e	BCAZ2[6]	26.98	ef	PCAZ4[3]	30.36	de
BCC2[7]	7.26	cde	BCCA3[9]	9.28	e	PCCA5[5]	26.81	ef	Beech[12]	25.48	de
BOIL[10]	6.40	de	Solv[11]	8.66	e	BCC2[7]	23.66	fg	PACQ4[4]	19.83	ef
Beech[12]	4.60	e	BCC2[7]	8.00	e	PCAZ4[3]	21.49	fg	Solv[11]	17.15	f
Pine[13]	4.14	e	BOIL[10]	7.04	e	PACQ4[4]	17.30	gh	PC4[2]	13.55	fg
BCCA3[9]	4.12	e	Pine[13]	4.55	e	PC4[2]	10.71	h	Pine[13]	9.12	g

MSR = mean soundness reduction. [N] = index of overall treatment performance across all sites where 1 is the best (Table 3.1). Sign., * and † = as for Table 5.2.

Table 5.5: Percentage contribution of each decay type to MSR for each treatment across all sites (highest retentions only)

Site	Macroscopic Decay Type			
	T1 (Brown rot)	T2 (Mixed)	T2a (Mixed)	T3 & 4 (Soft rot)
PCC4[1]	10.87	23.43	27.02	38.68
PC4[2]	11.73	64.01	10.71	13.55
PCAZ4[3]	9.38	38.77	21.49	30.36
PACQ4[4]	31.58	31.29	17.3	19.83
PCCA5[5]	12.63	19.45	26.81	41.11
BCAZ2[6]	9.43	11.15	26.98	52.44
BCC2[7]	7.26	8	23.66	61.08
POIL2[8]	14.17	10.37	41.36	34.1
BCCA3[9]	4.12	9.28	34.74	51.86
BOIL[10]	6.4	7.04	33.93	52.63
Solv[11]	11.73	8.66	62.46	17.15
Beech[12]	4.6	23.33	46.59	25.48
Pine[13]	4.14	4.55	82.19	9.12

[N] = index of treatment performance where 1 is the best

Key Results (Tables 5.4 and 5.5) are:

1. The overall performance of most treatments across all sites (performance index) was determined by a complex mixture of decay types.

2. Collectively, decay types 2 and 2a accounted for between 32 and 74% (mean = 50%) of the MSR reduction for preservative treated pine and beech (excluding solvent, oil and untreated) across all sites. Therefore, in terms of importance for durability of preservative treated pine and beech they were highly significance. These decay types are made up of a complex mixture of decay micromorphology (largely cavitation and tunnelling) much of which does not fit neatly within current decay classification nomenclature or has not been fully characterised in terms of mechanism of formation within the wood cell wall and taxonomic affinity (chapter 4).

3. All treatments had significantly different susceptibility for at least one decay type.

4. Preservative treated beech had significantly more SR than all pine treatments and untreated beech.

5. Preservative treated pine was significantly more susceptible to BR than treated beech. CAZ treated wood was an exception (CAZ treated pine had the lowest susceptibility of pine treatments whereas CAZ treated beech had the highest susceptibility of beech treatments).

6. ACQ treated pine was significantly more susceptible to BR than all other treatments. The contribution to overall MSR across all sites was 31%, more than double the next highest value (14%).

7. Untreated wood and wood treated with oil or solvent, particularly pine, had significantly more type 2a decay than the majority of other treatments.

Whilst single macroscopic decay types did not appear to be linked to overall decay hazard or overall treatment performance it was clear that treatments had significantly different decay type susceptibilities (Table 5.4). This is important because it means that in some situations overall site decay hazard given by site MSR or overall performance of a treatment given by treatment MSR across all sites is likely to be misleading. In view of this it could be argued that the only way to predict performance reliably is to have knowledge of the characteristics of the intended exposure site. However, Table 5.4 suggested that discrepancies between overall hazard and individual site hazards for a given treatment (site-interaction effect) are only profound at sites with a severe BR hazard. It is of course possible that more complex site-interaction effects involving mixtures of decay types might also be significant but were more difficult to detect, particularly within a 5.5 – 6.5 year exposure period that is probably not adequate for prediction of longer term durability in all situations.

This issue is particularly significant at sites of a low overall hazard but which pose a high hazard to some treatments because of their decay type susceptibility as explained for HAN. It is further illustrated by the observation that of the 8 highest site-MSR values for PACQ4, 7 occurred at the 7 sites with a BR hazard (Table 3.2). Therefore failure to expose ACQ treated pine and other similar treatments to a severe BR hazard prior to entering service could result in use in unsuitable situations. As discussed in chapter 3, the retention of 1.56% m/m a.i. ACQ for radiata pine stakes (PACQ4) is higher than the NZS 3640 minimum requirement of 1.02% m/m a.i

The rationale for examining the significance of decay type MSR across all sites for the highest preservative retention only (in-ground retention recommended by supplier), was that this would facilitate observation of decay type effects associated with performance of preservative treated wood in ground contact in service situations i.e. for wood treated with preservative at retentions recommended for in-ground use. However, a possible disadvantage of this approach is that the 5.5 - 6.5 years exposure may be an inadequate duration to fully test the effect of decay type for higher preservative retentions. On the basis that preservative depletion will occur with time it might be possible to predict longer term effects of decay type on wood treated with

higher retentions by examining decay type effects in wood across all retentions tested (Tables 5.6 and 5.7).

Table 5.6: Percentage contribution of each decay type to MSR across all treatments for each site and their significant differences (all retentions analysed collectively)

Type 1 Brown rot			Type 2 Many decay types[$]			Type 2a Many decay types[$]			Type 3 (plus type 4[†]) Soft rot		
Site	%	Sign.	Site	%	Sign.	Site	%	Sign.	Site	%	Sign.
HAN[12]	45.38	a	MM[11]	45.11	a	CK[1]	61.42	a	TOM[2]	49.43	a
ESK[7]	30.12	ab	PT[10]	36.98	b	OAK[6]	44.86	b	OAK[6]	40.87	b
GBF[4]	30.05	bc	HH[5]	35.64	b	PT[10]	41.87	b	TAP[9]	39.42	b
TAP[9]	27.65	cd	TOM[2]	34.18	b	TOT[13]	40.85	b	HH[5]	35.29	c
WKA[8]	19.47	d	TOT[13]	27.24	c	WKA[8]	34.33	c	TOT[13]	32.40	cd
GB[3]	16.32	e	GB[3]	23.20	cd	HH[5]	31.96	cd	ESK[7]	31.91	cde
OAK[6]	13.71	ef	WKA[8]	20.87	de	MM[11]	29.82	cde	CK[1]	30.07	def
MM[11]	0	f	GBF[4]	16.68	ef	ESK[7]	28.58	def	GBF[3]	27.71	ef
PT[10]	0	f	TAP[9]	14.97	fg	GBF[4]	26.00	ef	WKA[8]	27.27	fg
CK[1]	0	f	ESK[7]	11.23	gh	GB[2]	25.19	gf	MM[11]	25.33	fg
TOM[2]	0	f	CK[1]	10.87	gh	HAN[12]	21.38	gh	HAN[12]	25.07	fg
HH[5]	0	f	HAN[12]	9.24	h	TAP[9]	17.96	ih	PT[10]	24	gh
TOT[13]	0	f	OAK[6]	0.56	i	TOM[2]	16.39	i	GB[2]	21.15	h

Sign., [$] and [†] = as for Table 5.1.

Table 5.7: Percentage contribution of each decay type to MSR for each treatment across all sites and their significant differences (all retentions analysed collectively)

Type 1 Brown rot			Type 2 Many decay types[$]			Type 2a Many decay types[$]			Type 3 (plus type 4[†]) Soft rot		
Treat-ment	%	Sign.	Treat-ment	%	Sign.	Treat-ment	%	Sign.	Treat-ment	%	Sign.
PACQ	31.81	a	PC	51.99	a	Pine	82.19	a	BCC	54.53	a
PCAZ	20.33	b	PCAZ	30.10	b	Solv	62.46	b	BOIL	52.63	a
PCCA	15.85	c	PACQ	25.51	bc	Beech	46.59	c	BCAZ	50.5	a
PCC	15.20	c	Beech	23.33	cd	BCC A	42.41	cd	BCC A	41.4	b
PC	14.48	c	PCCA	19.21	de	POIL	39.07	de	POIL	37.46	bcd
POIL	14.15	c	PCC	15.65	e	PCC	35.68	e	PCCA	34.99	cd
Solv	11.73	cd	POIL	9.32	f	BOIL	33.93	ef	PCC	33.47	cd
BCAZ	11.49	cd	BCC A	9.19	f	BCAZ	29.98	fg	PCAZ	30.53	de
BCC	8.80	de	Solv	8.66	f	PCCA	29.95	fg	PACQ	26.01	e
BCC A	7.00	ef	BCC	8.52	f	BCC	28.15	g	Beech	25.48	e
BOIL	6.40	ef	BCAZ	8.03	f	PCAZ	19.04	g	Solv	17.15	f
Beech	4.60	ef	BOIL	7.04	f	PC	17.90	g	PC	15.63	f
Pine	4.12	f	Pine	4.55	f	PACQ	16.67	g	Pine	9.14	g

Sign., [$] and [†] = as for Table 5.1.

Inclusion of lower retentions in the statistical analysis, with a few exceptions, did not greatly alter the outcome of significant differences between sites or treatments or suggest any new effects of decay type on performance of preservative treated wood. Where the % decay type contribution to overall MSR value changed greatly, typically there was no obvious explanation. A possible exception occurred for PCAZ which had 20% BR across all sites when all retentions were analysed compared to 9% for the highest retention only. This is in contrast to PACQ which remained unchanged at 32%. This is probably due to the higher BR resistance of the highest PCAZ retention compared with the lower retentions of PCAZ and the highest PACQ retention.

Section 5.2.2.2

Affect of preservative, wood species and site on decay type diversity

Fifty percent of the overall MSR of preservative treated pine and beech was caused by decay types that did not fit neatly within current decay classification nomenclature and/or has not been fully characterised, and it is therefore useful to examine its distribution in more detail. An example of inter-site variability of decay type percentage contribution to MSR for each treatment is illustrated by comparison of TOM (Figure 5.1) and WKA (Figure 5.2).

Figure 5.1: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Tombleson's mandarin orchard

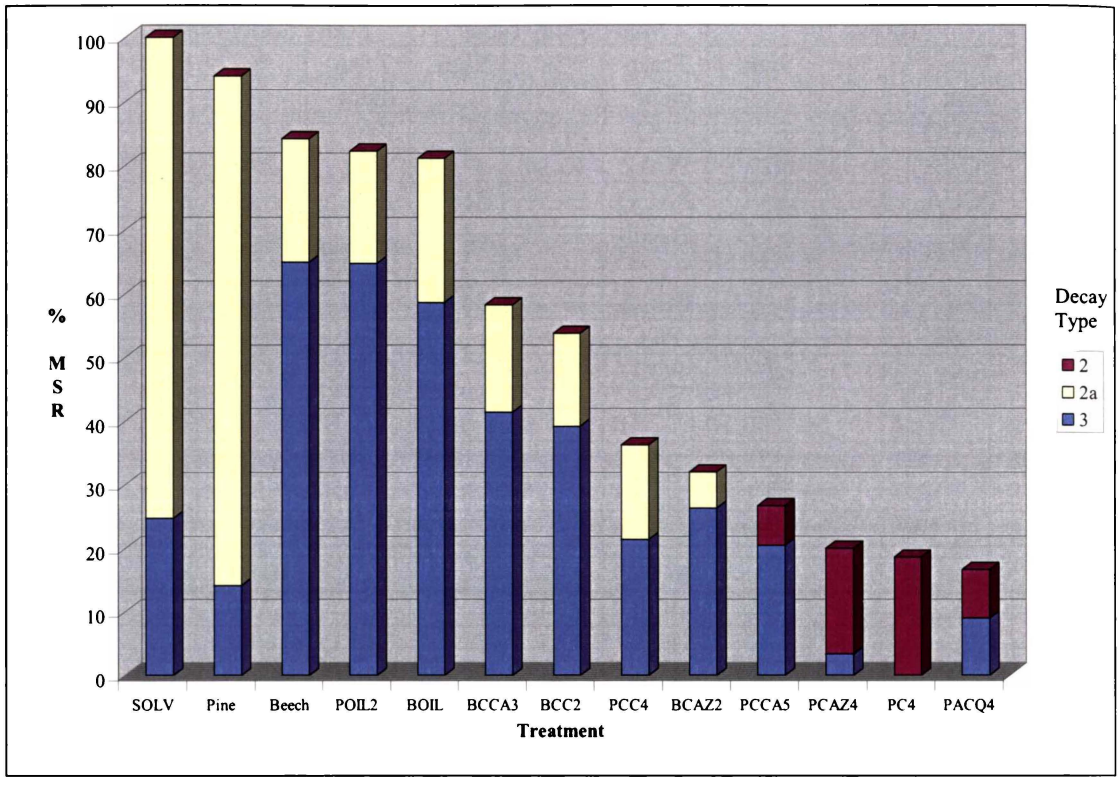
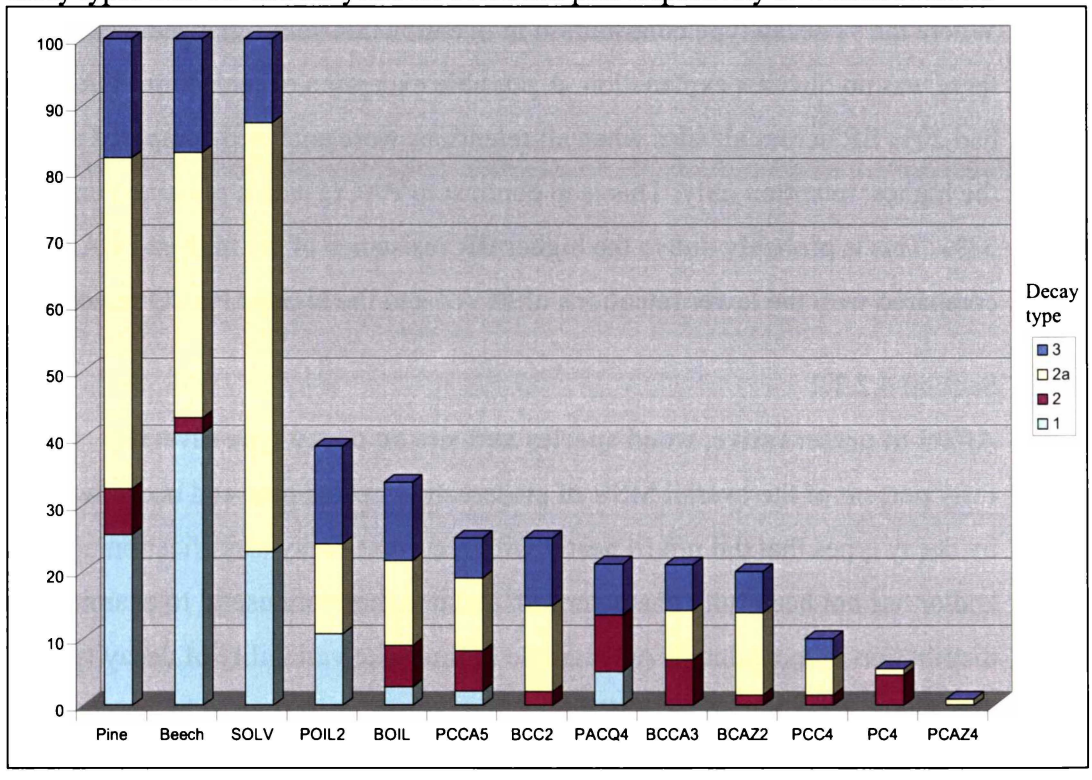


Figure 5.2: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Whakarewarewa



These two sites are not unusual in terms of likely conditions of exposure for preservative treated wood in ground contact. WKA and TOM are pastoral and horticultural sites respectively both typical of situations where preservative treated wood is widely used. Decay caused by types 2 and 2a was 61 and 47% for TOM and WKA respectively. Their complex mixture of decay micromorphology was grouped into the decay types described in chapter 4, and it was difficult to show this degree of complexity graphically but is given in Tables 5.8 and 5.9 for TOM and WKA sites, respectively. Whilst all sites had complex mixtures of decay, because the site-treatment combinations used in this study were highly selective, a given decay type was typically either abundant or absent and therefore illustration of inter-treatment decay type variability was typically straightforward.

Table 5.8: Decay types at Tomblesons Mandarin Orchard (TOM)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro *													
BR	1													
BR/WR	1/2a													
WR	2a	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a						✓							
C2	2, 2a													
C3	2, 2a					✓	✓							
C4	2, 2a		✓								✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a				✓	✓	✓							
C7	2, 2a				✓	✓	✓							
T1	2, 2a	✓		✓	✓	✓	✓	✓		✓				
T2	2, 2a	✓	✓	✓	✓	✓	✓	✓						
T3	2, 2a			✓	✓	✓	✓	✓						
T4	2, 2a													
TB	2, 2a	✓			✓	✓	✓		✓					
EB	2, 2a													
E1	2, 2a										✓	✓	✓	✓
E2	2, 2a													

Micro* = micromorphology, macro* = macromorphology. ✓ = decay present.

Table 5.9: Decay types at Whakarewarewa (WKA)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BR/WR	2a / 1													
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a													
C2	2, 2a				✓	✓	✓							
C3	2, 2a				✓	✓	✓							
C4	2, 2a										✓	✓		
C5	2, 2a										✓	✓		
C6	2, 2a				✓	✓	✓							
C7	2, 2a				✓	✓	✓							
T1	2, 2a	✓		✓		✓	✓	✓	✓					
T2	2, 2a	✓			✓	✓	✓		✓					
T3	2, 2a	✓		✓	✓	✓	✓	✓	✓					
T4	2, 2a		✓								✓	✓	✓	✓
TB	2, 2a				✓	✓								
EB	2, 2a	✓			✓	✓			✓					
E1	2, 2a										✓	✓		
E2	2, 2a													

micro* = micromorphology, macro* = macromorphology. ✓ = decay present.

Key Results (Tables 5.8 and 5.9) are:

1. Only SR was present in all treatments at both sites.

2. The occurrence of all other decay types was dependant on treatment and/or site.

The degree of complexity in terms of distribution of decay type between treatments that occurred at WKA and TOM was similar at the other 11 sites (Tables 5.12 – 5.22 in Appendix 5, Section 5.5.2). The occurrence of decay types at each site across all preservatives and on each treatment across all sites is given in Tables 5.10 and 5.11, respectively.

Table 5.10: Decay type occurrence at each site across all treatments

Type		HH	MM	GB	OAK	TAP	TOT	WK	GBF	CC	HAN	PT	ESK	TOM
Micro*	Macro*													
BR	1			pb	pb	pb		pb	pb		pb		pb	
BR/WR†	2a / 1	p			p	p								
WR	2a	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb
SR	2, 3	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb	pb
C1	2, 2a	p	p	p	p	p	p							
C2	2, 2a	p	p	p	p	p	p	p		p				p
C3	2, 2a	p	p	p	p	p	p							
C4	2, 2a	b	ND	b	b	b	b	b	b	b	b	b	b	b
C5	2, 2a	b	ND	b	b	b	b	b	b	b	b	b	b	b
C6	2, 2a	pb	p	p	p	p	p	p	p	p				p
C7	2, 2a	p		p	p	p			p	p				
T1	2, 2a	p	p	p	p	p	p	p	p	p	p	p	p	p
T2	2, 2a	p	p	p	p	p	pb	p	p	p	p	p	p	P
T3	2, 2a	p	p	p	p	p	p	p	p	p	p	p	p	p
T4	2, 2a	b	b		b	b	b	b						
TB	2, 2a	p	p	p	pb	pb		p		p	p			p
EB	2, 2a	p	p		pb	pb				p				
E1	2, 2a	b	b	b	b	b	b	b	b	b	b	b	b	b
E2	2, 2a	p					p							

b = decay present in beech treatments only; p = decay present in pine treatments only; pb = decay present in pine and beech treatments; ND = no data (treated beech not exposed); micro* = micromorphology; macro* = macromorphology. † = I.D. of BR/WR macroscopically was difficult as explained in section 4.2.3 and therefore was likely to have contributed to MSR values of both types 1 and 2a in some instances. However this decay type was only present at 3 sites so its overall contribution to MSR across all sites was minor.

Key Results (Table 5.10) are:

1. Numbers of decay type across all treatments as defined in chapter 4, ranged from 10 to 18 per site.

2. The following decay types were present at all sites: WR, SR, C4, C5, T1, T2, T3, and E1.

3. All other types were present at a minimum of 5 sites, except type E2.

4. Most decay types were specific to wood species.

    - Types BR/WR, C1, C2, C3, T1, T3 and E2 only occurred in pine.
    - Types C4, C5 and E1 only occurred in beech.
    - BR, WR and SR was common in both wood species.

- TB and EB were present in pine at 9 and 5 sites respectively but occurred at only 2 sites in beech.

Many of the decay types that were common on many of the treatments at most sites did not fit neatly within current decay type nomenclature i.e. WR (simultaneous and preferential), BR, SR (Types 1 and 2), TB and EB.

Table 5.11: Decay type occurrence for each treatment across all sites

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BR/WR	2a / 1	✓			✓	✓	✓		✓					
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a				✓	✓	✓				✓			
C2	2, 2a	✓			✓	✓	✓	✓	✓					
C3	2, 2a	✓		✓	✓	✓	✓	✓		✓				
C4	2, 2a		✓								✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a	✓			✓	✓	✓		✓	✓	✓			
C7	2, 2a				✓	✓	✓							
T1	2, 2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			
T2	2, 2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
T3	2, 2a	✓		✓	✓	✓	✓	✓	✓	✓				
T4	2, 2a	✓	✓	✓							✓	✓	✓	✓
TB	2, 2a	✓		✓	✓	✓	✓		✓		✓	✓	✓	✓
EB	2, 2a	✓			✓	✓	✓		✓		✓	✓		
E1	2, 2a		✓							✓	✓	✓	✓	✓
E2	2, 2a	✓			✓	✓	✓			✓				

micro* = micromorphology, macro* = macromorphology. ✓ = decay present.

Key Results (Table 5.11)

1. Numbers of decay type across all sites per treatment ranged from 8 to 15.
2. PACQ, PCAZ and PCCA had the most varied decay types (15). In contrast, treated beech had fewer decay types.
3. All treatments had SR, WR and BR.
4. BR/WR, C2, C3, T3 and E2 were only present in pine.
5. C7 was only present in treated pine.

Figures showing MSR and associated decay types for stakes of all preservation retentions (section 5.5.3) showed a clear dose response across retentions with increasing MSR as retention increased. In general, preservative retention had little effect on the % contribution of each decay type to overall MSR, suggesting that retention was not an important selective criterion within the retention range studied.

Section 5.3

Discussion

Most of the comparative ecological studies of the establishment of decay microorganisms on untreated and preservative treated wood in ground contact have focused on the timing of their arrival and position on or within the stake in terms of depth and position relative to the ground line. Sampling and culturing techniques have been used in an attempt to select significant decay microorganisms. In many studies a second step has been to screen isolates, usually fungi, for their decay capabilities by measuring weight loss in standard laboratory decay tests, and by using microscopy to determine micromorphology and, or chemical analysis to determine changes in the ratio of key wood components. Many of these studies have been termed successional on the basis that they identify the order of arrival (succession) of different groups of microorganisms. Referring to the successional studies of Corbett and Levy (1963) and Banerjee and Levy (1971), Eaton and Hale (1993) described the following sequence for birch and pine posts:

1. "bacteria, moulds and sugar fungi in the surface regions;
2. staining fungi and SR fungi which penetrated into the wood via the ray parenchyma;
3. SR fungi which penetrated to greater depths;
4. Basidiomycotina isolated by 4-6 months following installation in the soil."

Other studies of fungal succession on untreated wood have produced similar results (Raynor and Boddy, 1988). Typically, moulds such as *Trichoderma* spp., *Fusarium* spp. and *Penicillium* spp. arrive before wood decay fungi, usually within a few weeks (Corbett and Levy, 1963; Käärick, 1968; Butcher, 1968). Arrival of SR decay micromorphology typically occurs before BR. Concurrently, the frequency of

isolation of fungal species with known SR potential is greater than for BR species early on (Merrill and French, 1965; Käärick, 1968). Merrill and French (1965), as cited in Zabel and Morrell (1992), reported that SR arrived after 6 weeks and pockets of BR arrived after 12 weeks. Kaarick (1968) conducted a 4-year study of decay development in pine and spruce poles in Sweden and found that the vertical positioning relative to the ground line and soil type effected fungal isolations. A concept sometimes suggested by successional investigations is that early colonisers prepare wood for later colonisers. The term succession was originally coined to describe a predictable series of changes within a community of higher plants or animals, ending with a steady-state condition (Zabel and Morrell, 1992). Zabel and Morrell (1992) suggested that the use of this term is not entirely appropriate because almost without exception claims of succession in the wood decay process start with sound wood and end with onset of decay. Zabel and Morrell (1992) went on to say that this describes early colonisation only since the end of decay is marked by mineralisation of the wood. Lack of information in the literature for later stages of wood decay ecology is a reflection of the length of time wood takes to decay under field conditions, usually measured in years rather than months.

Some authors have discussed arrival on wood largely within the context of adaptation to changing conditions on and within the wood (Rayner and Boddy, 1988; Eaton and Hale, 1993). Competitive saprophytic ability (CSA) (Garret, 1970), a measure of the ability of a fungus to effect colonisation and establishment on or in a substrate, changes with time in response to changing physical and chemical substrate conditions. For example, many cosmopolitan soil fungi and bacteria typically exhibit a ruderal competitive strategy (Cooke and Rayner, 1984) during colonisation within hours or days of wood contacting moist soil. Their CSA is high at this stage in the successional sequence because of their early arrival due to a cosmopolitan spore distribution, high growth rates and rapid production of large numbers of spores that further facilitates spread. However their CSA diminishes as readily available, non-structural wood nutrients deplete, or upon arrival of other stress factors such as low oxygen or presence of toxic secondary metabolites (e.g. antibiotics). At this time in the sequence fungi adapted to whatever stress prevails assume a greater CSA and have been referred to as stress-tolerant (Cooke and Rayner, 1984). Other fungi that

have the ability to persist for long periods, excluding other potential colonisers termed combative, are adapted to defense of wood and can repel other fungi, either by antibiosis or pathogenesis (Zabel and Morrell, 1992). *Lenzites betulina* parasitises and replaces some *Coriolus* sp. (Rayner *et al.*, 1987; as cited in Zabel and Morrell, 1992). Many fungi possess attributes that favour adaptation to more than one colonisation strategy (Raynor and Boddy, 1988).

Also important within the concept of CSA is population level within the vicinity of the wood. Early on in succession the large numbers of conidiospores produced by moulds and many SR fungi confer an advantage because they increase the probability of early arrival and likelihood of successful establishment through sheer numbers. However, production of large resting spores such as the chlamydospores observed for BR fungi at the dry site HAN, may confer a competitive advantage when it is necessary to overcome draught stress by lying dormant for extended periods. Alternatively, larger spores may be able to overcome a higher preservative retention because of their higher inoculum potential. Similarly, the presence of soil mycelium associated with roots and stumps containing BR at ESK or the mycelium amongst the decomposing litter layer at GBF may have increased inoculum potential of BR fungi, enabling them to overcome preservative retentions that would prevent colonisation by spores. However other sites with similarly high BR hazards such as TAP and GB were typically moist throughout the year and had no apparent elevated inoculum potential associated with either litter or rotting woody material. At the pastoral sites HAN and WKA, elevated soil inoculum potential almost certainly was a factor in facilitating spread of BR between stakes, as discussed in chapter 3. As with the great majority of decay types observed, BR was widely distributed across sites of varying conditions and therefore its presence did not appear to be linked to any single environmental factor.

Succession of microorganisms was not measured in this study and therefore its significance cannot be measured directly, however several observations suggest that it was not of great importance in terms of determining decay type. Many of the wood species-preservative-site combinations were highly selective and appeared to determine decay type. Whilst ruderal microorganisms almost certainly colonise stakes

early on, utilising readily available non-structural wood nutrients, this probably does not affect decay type because of the overwhelming selectivity of the wood-preservative-site combination that subsequently selects specific decay fungi. Furthermore, this selective pressure is probably in a state of flux as environmental conditions fluctuate. Arrival of basidiomycetes after SR, as observed in several successional studies may also occur but is unlikely to have had a primary role in decay type determination in this study. Such observations in successional studies are more likely to be a reflection of the fact that many soil fungi have a SR potential and exhibit a ruderal strategy and therefore the chances of their isolation early on is high.

The same attributes that confer a high CSA to these fungi during expression of ruderal competitive strategies also make them much easier to isolate, thereby further distorting their significance. Butcher (1968) and Eaton and Hale (1993) pointed out that succession on preservative treated wood in soil contact results in similar patterns of succession to those that occur on untreated wood but that the rates are slower. On the basis of fungi isolated from untreated and CCA treated Baltic redwood and birch, Clubbe (1982) suggested that succession on CCA treated wood was fundamentally different. On untreated wood SR fungi were replaced by basidiomycetes but on CCA treated wood SR remained the dominant decay type. Several authors have referred to such an alteration of succession by a preservative as being a response to the selection of preservative tolerant decay microorganisms (Butcher, 1968; Clubbe, 1982; Blanchette *et al.* 1990).

According to the literature, successional sequences do not appear to vary greatly across different wood species and between untreated and preservative treated wood prior to arrival of the causative decay microorganism. Bearing in mind that it is well known that wood species (hardwood verses softwood) and preservative have a profound affect on decay type it seems likely that succession prior to arrival of the causative agent of decay is largely coincidental. Compared to the overwhelming significance of site on decay type, and the selectivity of wood species, preservative type and their combinations, the importance of early sequences of succession on decay type is likely to be minor. Microorganism community structure during decay is likely to be of greater significance but very little is known for preservative treated

wood in ground contact (Zabel and Morrell, 1992). It seems likely that decay microorganisms possessing stress tolerant strategies adapted to wood-preservative factors are more important than microorganisms possessing either ruderal or combative strategies. Most of what is known about combative strategy amongst basidiomycete decay fungi is related to colonisation of untreated wood, especially forest floor material and laboratory experiments involving replacement and stalemate responses of pure cultures of paired fungi (Cooke and Rayner, 1984; Rayner and Boddy, 1988) and its relevance to preservative treated stakes in ground contact is questionable and beyond the scope of this discussion.

At the time when most ecological studies of wood decay in ground contact were conducted, the diversity of decay type and the taxonomic affinities of different decay fungi was not fully appreciated. In particular the possible significance of basidiomycetes causing cavitation similar to SR was not taken into account. In a review of decay types and their implications for tree hazard assessment Schwarze *et al.* (1997) suggested that production of SR cavities by basidiomycetes could be a response to pathogenic tree reactions such as production of phenolic substances and physical obstructions of the lumen (e.g. gums and resins). As discussed in chapter 4, the observations of this study coupled with recent demonstrations of SR type cavitation formed by basidiomycetes (Worrall *et al.*, 1997; Anagnost, 1998) in dead wood, suggested that cavitation and tunnelling caused by basidiomycete fungi is much more common than previously recognised. If the suggestion of Schwarze *et al.* (1997) is correct then it may also apply to preservative treated wood, in which case basidiomycetes that cause classical WR micromorphology in untreated wood or wood unstressed in other ways (e.g. optimal moisture and aeration conditions), may cause cavitation and tunnelling in treated wood or in wood stressed in other ways such as high moisture and low oxygen. Successional and related ecology studies my have drawn inappropriate conclusions for the following reasons.

1. Basidiomycetes isolated would have been assumed to be either classical WR or BR fungi whereas they may have been of greater significance as causative agents of cavitation or tunnelling decay types.

2. Deuteromycetes and ascomycetes isolated would have been assumed to have caused all cavitation observed. Alternatively isolation of such fungi with known SR potential is likely to have been considered indicative of incipient SR i.e. the SR fungi were present but they had not yet caused significant wood cell wall damage, whereas there presence might be coincidental. Basidiomycetes, often harder to isolate, may have been responsible for what was diagnosed as SR, and the more abundant and easier to isolate soil fungi possessing a SR potential may have been assumed to have been the cause. As discussed in chapter 4, not all types of basidiomycete cavitation are readily distinguishable from SR caused by deuteromycetes and ascomycetes. Prior to the relatively recent findings that basidiomycetes produce SR type cavitation, all types of cavitation associated with fungi would almost certainly have been diagnosed as SR caused by deuteromycetes and ascomycetes.

3. Decay type observations in the laboratory by single isolates may have been misleading since field conditions may have caused expression of a different decay type by the same decay microorganism. The assumption that application of Koch's Postulates using single isolates in laboratory decay tests would reveal the type of decay caused in the field is not necessarily valid because field conditions cannot be reproduced in a laboratory.

As previously mentioned, Rayner and Boddy (1988) have pointed out that "there has been a tendency to regard wood decay as though the resource and the organism were two simple reagents off a laboratory shelf whose interaction results in a consistent and predictable outcome." They went on to say "this is, of course, far from the truth: not only is wood itself a variable and heterogenous resource but as with all other eukaryotes the decay fungi cannot be regarded as fixed entities. Rather they vary, and this variation occurs not only between different individuals, but also, because of the indeterminate nature if the mycelial body form, within an individual. Causes of variation include genotypic differences, direct effects of environment on metabolic functioning, changes to differences in gene regulation and random effects. The implications of variation, both between individuals and during the development of single individuals, are considerable, not only with respect to taxonomy and diagnosis

but also in terms of the ways in which populations of decay fungi establish in and interact with woody resources." The complexity of the determinate factors described by Rayner and Boddy (1988) is such that attempts to link a single phenomenon such as taxonomic affinity or succession with the final outcome i.e. decay type, is fraught with the possibility of incorrect assumption or at best is overly simplistic.

Assumptions made in this study may also have caused misinterpretation. For example the majority of wood sections examined using LM and CLSM were from the sound-decayed wood interface since this is were the defining features of decay micromorphology are most easily observed and interpreted. It was assumed that early features of decay were the most important in determining the mechanism of formation. However this method could have resulted in missing important features associated with later decay. Whilst many sections of heavily decayed wood taken as close as possible to wood with early decay were also examined, LM lacks the resolution required to elucidate many closely associated micromorphological features that occur in wood with advanced decay.

However, in this study the nature of the preservative-wood species combination typically predetermined decay type, sometimes irrespective of site conditions, and this was a powerful selective tool for separation of a complex mixture of decay types that might otherwise have been impossible to elucidate. However such an observation was only possible because of the large number of sites tested. Had a smaller number of sites been tested, incorrect conclusions might have been reached regarding apparent links between decay type occurrence and environmental factors, as has probably occurred in the literature. For example presence of EB at the water logged site Hari Hari (HH), presence of BR at the dry site HAN and presence of TB at the horticultural site TOM would have fitted neatly with the great majority of previous reports regarding prevalence of each of these decay types (Blanchette *et a*l. 1990; Kim and Singh, 2000). However a key result of this study was the common occurrence of the majority of decay types across many different sites.

It was difficult to establish consistent correlation between single environmental factors and decay type in this study. This was of particular interest with regards

moisture availability which is likely to be one of most important environmental factors that influence decay fungi. In a recent review by Kim and Singh (2000) it was pointed out that SR, EB and TB are more tolerant of high moisture and reduced oxygen compared to basidiomycete WR and BR fungi. This was suggested as the main reason why they have frequently been observed in wood in very wet, often submerged or waterlogged, environments.

A review by Blanchette *et al.* (1990) reported that SR has been described by several authors as occurring other a wide range of moisture conditions including very wet and close to fibre saturation point. They also pointed out that deep SR was more common in moderately wet wood whereas surface erosion, suggestive of slower decay, occurs under very wet conditions. In this study presence of preservative treated beech was the most important selective factor for SR and pine treated with copper containing preservatives was the most important selective factor for EB and TB. Amongst the many sites where bacterial decay was important, moisture conditions were highly variable and included two of the driest sites, OAK and Mt. Mee (MM) but did not include two of the wettest sites, HH and Peat (PT). This means that factors other than high moisture are more important selective criteria in many ground contact situations.

High moisture and associated low oxygen probably favours bacteria and SR indirectly i.e. other microorganisms such as basidiomycetes are excluded (Eaton and Hale, 1993). The widely accepted view that the minimum moisture requirements for decay by basidiomycetes is close to the fibre saturation point (approximately 0.3g of water g^{-1} of oven dry wood; Griffin, 1977) was questioned by Griffin (1977) on the basis that complete decomposition of 1 gram of cellulose by microbial action liberates 0.555g of water. Production of metabolic water by microbial communities in wood in the field might assume greater significance than occurs under laboratory conditions and therefore currently available data may be misleading. Butcher (1975) pointed out that whilst soil with a moisture content close to 100% of its water holding capacity was optimal for promoting SR in wood, decay of pine and beech occurred at soil moisture contents between 30 and 105%. It is unlikely that wood degrading bacteria would decay wood more effectively in water logged conditions compared to moderate moisture conditions where oxygen is not restricted.

Blanchette *et al.* (1990) reported that bacteria cannot compete successfully with fungi if the substrate and the environmental conditions are suitable for fungal decay. However TEM micrographs clearly showed the close coexistence of TB and SR in cooling tower timbers (Singh *et al.* 1992; Singh *et al.* 1994; Singh and Wakeling, 1997) and in wood from CCA treated posts (Clausen, 1995). Within the macroscopic decay type groups 2 and 2a, in treated pine, erosion and TB decay often occurred in similar positions on the same stake, as the majority of other decay types reported (e.g. T1, T2 and T3). Some of the decay types associated with 2 and 2a were likely to have been caused by WR basidiomycetes, suggesting that they are able to cause decay under similar conditions to SR. The fungi and bacteria associated with these decay types, reported in micrographs of chapter 3, strongly suggested that they were living at the time of examination. Therefore fluctuating conditions over the exposure period was unlikely to have caused such close coexistence suggesting that conditions suitable for different causative fungi and bacteria occurred simultaneously at several sites tested.

Several authors have reported TB decay in CCA treated pine posts exposed in horticultural soils (Butcher, 1984; Hedley and Drysdale, 1984; Drysdale and Hedley, 1984) where moisture conditions were most likely to have been moderate. The absence of TB from ESK, an irrigated horticultural site may have been due to the loose well-drained soil conditions that created low moisture stress or stress brought about by moisture level fluctuation. Some sites (TOM, TAP, OAK and CC) where TB was important had heavy clay soils that typically have a high water holding capacity and therefore encourage maintenance of moist conditions in stakes. The OAK site was of particular interest because of its very low rainfall. Possibly the high clay soil, litter layer and tree cover maintained adequate moisture for the important erosion and TB presence. Therefore maintenance of moist conditions coupled with a powerful wood-preservative selective criterion is possibly more important than high moisture in isolation. Basidiomycetes produce tunnelling and cavitation under similar conditions to those reported for SR occurrence both in this study and in others (Worrall et al. 1997; Anagnost, 1998).

Therefore it seems likely that many fungi of widely divergent taxonomic affinities, and bacteria, are capable of causing decay over a wide range of moisture conditions. This study showed that factors other than moisture, such as wood species and preservative type, or their combinations are more important selective criteria than moisture. If, as argued in chapter 4 and as suggested by many of the publications listed in Table 1.4, basidiomycetes cause several types of tunnelling and cavitation decay that is common in preservative treated wood it is possible that some of the reports concerning types of decay and their reported correlation with particular moisture contents, may have been misleading. It has been reported that some heart-rot basidiomycete decay fungi are tolerant of low oxygen (Rayner and Boddy, 1988) and occur in very wet wood in cooling tower timbers (Schmidt *et al.* 1997). Blanchette *et al.* (1990) reported that brown and WR fungi may tolerate very low oxygen concentrations. As discussed in chapter 4, decay types thought to have been caused by basidiomycete WR fungi were common at the water-logged sites HH and PT. Some of the previous reports of SR cavitation in wood exposed to wet environments may have been caused by basidiomycetes.

During storage of stakes at 4^0C in plastic bags it was noted that after prolonged storage (2 - 3 years) stakes that had been recorded as containing decay types T1, T2 and T3 sometimes produced mycelial strands that were associated with bleached, decayed wood. This bleaching was much more intense than previously recorded. This prompted further microscopical examination and it was discovered that classical WR wall thinning was present, as compared with the earlier observation of tunnelling only. Whilst this phenomenon was not investigated systematically, the limited observations made suggested that bundles of stakes in a plastic bag kept at 4^0C induced expression of a different decay type. White rot fungi that produce classical WR micromorphology may have been present but not active previously, or the fungi responsible for the tunnelling decay types may have reverted to a different decay type in response to new conditions. Cooke and Rayner (1984) reported that psychrophilic fungi exhibit a stress-tolerant competitive strategy and it is possible that 4^0C constituted a more significant stress factor than the original wood-preservative combination that selected tunnelling decay types.

The increased degree of bleaching in decayed wood that changed type 2 to 2a was observed on a number of occasions after prolonged storage. Possibly the redistribution of moisture within the stake bundle altered the selective criteria. This suggestion was supported by another observation made at the PT site where the above ground portions of stakes had more bleaching than wetter, below ground portions. In some instances there was also classical WR above ground and tunnelling decay below, although the latter was also common above ground and was often associated with bleached wood. A corollary to the hypothesis that cool-store storage conditions changed decay type expression was as follows. Once mycelial strands form within stake bundles, the inoculum potential of the fungus at the preservative treated wood interface increased to a point where it could overcome the preservative present whilst producing an erosion type of decay as opposed to the prior tunnelling form. The layered nature of stake bundles coupled with moisture redistribution may have been conducive to formation of mycelial strands, whereas in soil contact, in the absence of forest litter, the appropriate stimulus was absent. It is also possible that within a bundle of stakes, an associated increase in preservative retention diversity allowed formation of mycelial strands on a stake of lower retention and that having assumed a mycelial growth pattern, the fungus was then able to overcome higher preservative retentions in adjacent stakes (stakes were typically stored as groups of different retentions of the same preservative).

Schwarze et al. (1997) used the term facultative or transient SR to describe cavitation caused by basidiomycetes that was similar to that produced by deutermycetes and ascomycetes. Furthermore, Worrall *et al.* (1997) discussed the likelihood that decay fungi recognised for production of a particular decay type may possess latent genes for other decay capabilities as also inferred by Boddy and Rayner (1988). However the terminology of Schwarze *et al.* (1997) is possibly misleading since it makes the assumption that less commonly observed types of decay caused by basidiomycetes are less typical or "normal" expressions of the decay capability of the fungus. It may fail to take adequate account of Rayner and Boddys' (1988) assertion that "decay fungi cannot be regarded as fixed entities", but are highly variable due to "genotypic differences, direct effects of environment on metabolic functioning, changes to differences in gene regulation and random effects". What is considered "normal"

might have been overly influenced by laboratory observations that do not reflect field situations. It therefore seems appropriate to place emphasis on decay type expression being the product of a complex mixture of environmental factors, substrate factors, inherent fungal physiology and genetics and their interactions. Terminology that places undue emphasis on any one of these factors is unlikely to be robust enough to stand scrutiny across the diverse exposure conditions under which decay occurs. Therefore the terms facultative and transient to describe one form of decay that is probably produced by many taxonomically divergent basidiomycetes and which this study suggested occurred over a wide range of environmental conditions, is misleading. Within the context of wood exposed in the field, it is useful to consider decay type is a continuum. Position of decay microorganisms within the continuum varies in response to the product of many factors and wide overlap probably occurs between widely taxonomically divergent species.

A number of reviews have discussed the significance of environmental factors on different decay microorganisms (Butcher, 1975; Raynor and Boddy, 1988; Blanchette *et a*l. 1990; Zabel and Morrell, 1992; Eaton and Hale, 1993). In addition to moisture and oxygen availability, pH, nitrogen availability, light, minerals and vitamins have also been discussed. The overall impression is that most decay fungi are able to tolerate a wide range of all the factors discussed, which fits with the finding of diverse decay occurrence at all sites tested. Whilst much of the information was based on field observations, most of the specific data concerning the effects of moisture, pH, nitrogen etc. was based on laboratory studies and its relevance to field situations is questionable. Blanchette *et al.* (1990) reported that BR fungi tolerate more acidic conditions than WR fungi, and have a poor tolerance of high pH. The latter report does not however fit with reports that the dry rot fungus *Serpula lacrymans* is more common in wood associated with alkaline building materials (Bech-Andersen 1987 and 1991).

Ph was not measured at test sites but it is known that *Nothofagus* leaf litter (Ross and Tate, 1993) as occurred at Totara flat TOT, and PT (Brady, 1984) at the Peat site are considerably more acidic (within the pH range 2.8 – 4) than most forest soils that are in turn known to be more acid than most mineral loam soils (Brady, 1984). Brown rot

had one of the narrowest site distributions, present at 7 out of 13 sites. Interestingly all the non-BR sites (TOM, PT, CC, TOT, MM, HH) had extremes of one or more of the following factors: high temperature (CC and MM), high (TOM) and low pH (PT and TOT) and high moisture availability (high rainfall and, or poor drainage) (CC, HH and PT). Presence of many of the tunnelling and cavitation decay types believed to have been caused by WR basidiomycetes at these sites and at sites with BR, may be an expression of their greater tolerance of a wide range of environmental conditions.

Tolerance of copper containing preservatives has been reported for some SR fungi (e.g. *Lecythophora hofmannii*; Nilsson and Henningsson, 1978. *Phialophora* spp.; Daniel and Nilsson, 1988), some BRs (e.g. *Antrodia vaillantii* and *Poria spp.:* Eaton and Hale, 1993) and for wood degrading bacteria (Singh and Butcher, 1991; Eaton, 1994). Some BR fungi produce copious amounts of oxalic acid, believed to be an important degradative product (Bech-Anderson, 1987; Green *et al.*, 1991; Shimada *et al.*, 1991: as cited in Larsen *et al.* 1995; Green and Clausen, 2003; Clausen and Green, 2003) and it has been suggested that oxalic acid reacts with copper to form insoluble copper oxalate, thereby detoxifying copper containing preservatives (Eaton and Hale, 1993). A number of studies have shown that presence of wood preservatives in wood alters microbial colonisation, favouring those fungi possessing wood preservative tolerance (Greaves and Savory, 1965; Henningsson and Nilsson, 1976; Nilsson and Henningsson, 1978; Sorkhoh and Dickinson, 1975: as cited in Zabel and Morrell, 1992).

The importance of TB and BR on ACQ and CAZ treated pine may in part have been related to the high pH of ammoniacal ACQ and ethanolamine based CAZ. With a few notable exceptions, bacteria are generally considered to thrive at higher pH values compared to fungi (Stanier *et al.*, 1987). Interestingly the only two sites were ACQ and CAZ treated pine was almost exclusively attacked by TB only was at CC and TOM. The owner of TOM site reported that the soil was slightly alkaline and CC was a highly leached tropical soil and severe leaching has been linked to soil alkalinity (Brady, 1984). Perhaps the combined alkalinity of the ACQ and CAZ preservative and the higher pH of soil at these two sites was especially selective of TB.

The absence or rarity of TB from ACQ and CAZ treated beech was probably due to the powerful selective nature of the lignin composition of beech (low total lignin and low guaiacyl:syringyl lignin ratio) for SR fungi and those fungi responsible for other types of cavitation (C4 and C5) prevalent in beech. Possibly the low pH (4.5) associated with the high resin acid content of radiata pine (Kininmonth and Whitehouse, 1991) would normally constitute an unfavourable pH for TB and the powerful alkalinity of ACQ and CAZ raises the pH to levels that favour TB. Soft rot fungi, *Phialophora* spp. in particular have been reported as tolerant of copper (Daniel and Nilsson, 1988) and SR was common in CCA treated pine, which could suggest that it was not the copper which was inhibiting SR in ACQ and CAZ. However, the copper retentions in ACQ (0.69% m/m) and CAZ (0.57% m/m) treated pine were higher than in CCA (0.18% m/m) treated pine. It has also been reported that ammoniacal preservative systems achieve better wood cell wall copper absorption (Eaton and Hale, 1993). This would be expected to improve performance against type 1 SR and other cavitation decay types. The wood-preservative factors that could affect decay type occurrence are complex and difficult to elucidate.

Poor performance of CCA treated hardwoods, Australian *Eucalyptus* sp. in particular, has been attributed to a number of factors (Eaton and Hale, 1993) including the following.

1. Poor macrodistribution of preservative solution that results in low uptake by fibres.
2. Poor microdistribution across the cell wall, and poor absorption by the S2 layer in particular.
3. Close association of lignin, guaiacyl lignin in particular, with cellulose microfibrils in the S2 layer of softwoods is believed to confer resistance to SR. It has been suggested that the absence of a similar protective affect in hardwoods is the main reason why much higher copper retentions are required.
4. High tannin content of hardwoods is thought to compete with cellulose and lignin for CCA components resulting in precipitation reactions away from the S2 layer (e.g in cell luminae).

In this study the most important observation regarding hardwood verses softwood SR decay susceptibility was that beech was much more susceptible to SR than radiata pine, irrespective of preservative type. The wood species effect appeared to be far more potent as a selective criterion compared to the effects of the preservatives CCA, CAZ and CC since beech treated with all three was more susceptible to SR than any other decay type. However decay types C4, C5 and E1 were also important in beech regardless of preservative treatment. However it may be significant that untreated beech was significantly less susceptible to SR compared to several other decay types associated with macroscopic types 2 and 2a. This suggested that the selective affect of beech (low lignin and a low guaiacyl:syringyl) became a more important selective factor once these other decay types had been excluded by preservative. This further suggested that in this study the third factor listed above was the most significant i.e. a preservative-wood type interaction effect was important for SR selection. It is likely that tunnelling decay types produced by WR basidiomycetes are subject to the same effects of preservative as described for SR. The main difference was that SR was more common in beech and tunnelling decay types were more common in pine.

Tunnelling perpendicular to the wood cells long axis, thought to have been initiated by T- and L- branching at the S1 - ML interface, was more common in pine treated with the oil based preservatives creosote (C) and chlorothalonil plus chlorpyriphos (CC). In water based preservative treated pine tunnelling was also initiated at the S1 – ML interface but branching was also common in the S2 layer. In pine treated with C and CC the tunnelling expanded laterally across the S2 but frequency of branching was much lower i.e. the initial point of tunnelling initiation (S1-ML interface) determined the course of tunnelling for a much longer duration. This suggested that preservative distribution across the cell wall affected tunnelling patterns. Perhaps the oil based preservatives penetrated across the S2 layer more efficiently than water based treatments. Alternatively, copper depletion (chapter 6) may have resulted in the preservative retention within the S2 layer dropping below the toxic threshold.

Decay fungi that bypass the decay resistant S3 layer, producing multi-branched hyphae that collectively erode the S2 layer in a fashion subsequently similar to simultaneous WR, forming a "super-cavity" under an overlying lumen wall (C5 and

C6), were very important in preservative treated beech fibres and pine tracheids. Whilst some of the micromorphology associated with this type of decay has been reported as diffuse type 1 SR (Anagnost, 1994) its significance has been largely overlooked. It could be argued that the expansive or diffuse removal of the S2 layer by these fungi, thought to be WR basidiomycetes, is essentially more efficient than either tunnelling and cavitation patterns that leave intact intervening walls until late in the decay sequence. As discussed in chapter 5, several authors have described an evolutionary sequence of WR from SR. Therefore it is suggested that decay types C5 and C6 are more "evolved" stress-tolerant decay responses, more efficient at overcoming preservative and/or high lignin concentration within the lumen wall compared to the cavitation response of deuteromycetes and ascomycetes (Blanchette *et al.* 1990) and the tunnelling response produced by basidiomycetes (Schwarze *et al.*, 1997).

The finding that preservative retention did not affect decay type greatly suggested that the threshold retention required to exert a selective effect was achieved at the lowest retention used. Whilst data from assessments earlier than 5.5 years exposure for beech and 6.5 for pine were not reported, lower retentions were attacked by the same decay type as higher retentions were at a later date. Once preservative depletion mechanisms had occurred and possibly the inoculum potential of the decay microorganism had reached a high enough level (as discussed for BR at Hanmer and Whakarewarewa in chapter 3), decay in higher retentions then occurred.

At dry sites like HAN the increased importance of BR was probably partly a response to the greater tolerance of BR basidiomycetes to low moisture conditions compared to SR fungi. However at wetter sites such as TAP, ESK and GB, high BR and SR hazards occurred together, although typically not on the same stake. Brown rot tended to occur in isolation and resulted in "sudden" (within 12 months) failure as opposed to progressive failure, (particularly for all but the highest preservative concentration), suggesting that once it arrived, rapid decay ensued and also that arrival was a rarer phenomena than arrival of SR. Reports of fungi in the monokaryon condition suggest single basidiospores (haploid) are able to initiate decay (Zabel and Morrell, 1992). The grouping of stakes with BR at TAP and HAN in the absence of basidiocarps on

stakes suggested that soil mycelium may be involved in spread of BR between stakes. Possibly the production of chlamydospores by BR at HAN may have facilitated spread, perhaps by insects and other arthropods or in soil water (especially during sudden heavy rainfall). Also of interest in this regard was the absence or low incidence of BR at sites with a thick litter layer (OAK and TOT) that had been expected to give a competitive advantage to cord forming forest floor basidiomycetes some of which are known to cause BR (Raynor and Boddy, 1988). Possibly the importance of BR fungi at GBF coupled with absence at TOT and low incidence at OAK was largely due to the generally recognised greater incidence of BR and WR on softwoods and hardwoods respectively (Eaton and Hale, 1993; Zabel and Morrell, 1992). At non-forest sites including HAN, WKA and TAP, there was a high incidence of BR in beech treatments. That BR fungi were able to decay beech at these sites suggested that in some situations lack of competition from WR and SR fungi rather than lack of hardwood decay capability by BR fungi was the dominant selective criteria.

Unlike BR that could be linked to an elevated decay hazard caused by preservative tolerance at otherwise low hazard sites, such clear examples of linkage between decay type and preservative performance was not obvious for other decay types. Treatment susceptibility clearly affected decay type occurrence but this did not appear to have an obvious affect on preservative performance. The influence of temperature and water availability discussed in chapter 3 appeared to be the main contributing factor to preservative performance, irrespective of decay type occurrence. Very little is known about competitive strategy of decay microorganisms in preservative treated wood (Zabel and Morrell, 1992). At warm, moist temperate and tropical sites, and where other important physico-chemical parameters such as pH are not extreme, decay microorganisms possessing a stress-tolerant strategy, as was suggested for BR fungi at HAN (combined low moisture / high preservative retention tolerance), are less competitive. At these sites, conditions suitable for sustaining a greater diversity of decay microorganisms are likely to prevail. Whilst decay microorganisms that occur at these sites appeared to display preservative tolerance by causing decay in stakes with high preservative retentions, this may be largely a reflection of indirect (non-physiological) tolerance mechanisms rather than the physiological mechanism

reported for copper detoxification by oxalic acid produced by BR fungi. Localised surface depletion may be exacerbated at these sites due to elevated activity of preservative depletion mechanisms such as ion exchange with clay micelles and humus molecules (Brady, 1984), greater diffusion of preservative out of wood in soil with high moisture, and enhanced biodegradation by secondary (non-decay) microorganisms.

Section 5.4
Conclusions

1. The overall performance of preservative treated radiata pine and European beech field test stakes accross 13 test sites of widely varying soil type, vegetation and climate was determined by a complex mixture of decay types.

2. The only detectable profound site – (wood species x preservative) interaction effect occurred at sites with a low overall decay hazard but which harboured aggressive BR fungi. ACQ treated pine was the most severely affected treatment, suggesting that it has a high susceptibility to failure in ground-contact situations where BR is likely to occur.

3. Fifty percent of the mean soundness reduction across all sites was caused by decay types poorly characterised by current wood decay classification nomenclature, several of which were believed to have been caused by WR basidiomycete fungi.

4. The determinate factors of decay type in preservative treated pine and beech stakes was highly complex and was defined by the formula

wood[1] x preservative[2] x environment[3] x decay microorganism(s)[4] = decay type[5].

- One, 2 and 3 all had significant effects (5% level of probability) on decay type, and there were examples of significant site – (wood x preservative) interaction effects.

- Specific combinations of 1, 2 and 3 were highly selective of decay type and it was possible to predict decay type on the basis of detailed knowledge of all three but on the basis of knowledge of either 1 and 2 only or 3 only, it was more difficult to predict decay type. However wood species was highly selective of decay type across all sites and in some cases preservative type introduced another layer of selectivity.

5. Results of this study suggest that previous attempts to link a single phenomenon to decay type occurrence have been overly simplistic. For example, successional sequences of microorganisms are probably of little consequence to decay type because of the overriding significance of wood species x preservative x site. Similarly, assumptions concerning prevalence of certain decay types under extreme environmental conditions only (e.g. bacteria in wet situations) have also have been misleading.

6. All decay types recognised by current classification nomenclature were important across diverse in-ground exposure conditions, as were many poorly characterised types and therefore site conditions alone rarely determined decay type.

7. The results of this study coupled with the observation of an increasing number of publications that suggest decay fungi possess multiple decay capabilities, indicated that the significance of 4 in the above formula was not as important as 1 x 2 x 3. Therefore in some in-ground exposure situations the decay capability encoded by the genotype of a single species is not fixed but changes in response to the product of 1 x 2 x 3. It may in part explain the common occurrence of decay micromorphology that possessed a mixture of

features that in the past have been associated with more than one recognised decay type but which were believed to have been caused by an individual fungal species in this study. These mixtures where reproduced in response to specific combinations of 1 and 2 across many sites in the same consistent and reproducible way that features of recognised decay types, caused by individual fungal species, occur together. This observation, coupled with the homogenous nature of associated macromorphology (a feature not typical of wood decayed by mixtures of basidiomycete fungi) suggested they were produced by individual fungi of a single species.

8. In a significant number of the examples of novel mixtures of decay micormorphology, hyphae with clamp connections were the causative agent and the conclusion was that basidiomycetes are more important agents of decay in preservative treated wood than is currently recognised, causing several decay types that are easily mistaken for SR. On the basis that basidiomycetes are generally considered to be more efficient wood degraders than ascomycetes and deuteromycetes it is perhaps not surprising to find that basidiomycetes are of greater importance in decay of preservative treated wood in ground contact than was previously recognised. If basidiomycetes have evolved more efficient solutions to degrading the inherently refractory lignocellulosic complex in wood and therefore carry a greater diversity of latent genes encoding different decay capabilities, it is to be expected that they would be more adept at overcoming the additional obstacle provided by fungicides contained in wood preservatives.

9. Publications that refer to basidiomycetes "causing SR" confuse decay classification nomenclature, as have suggestions that certain broad taxonomic affiliations dictate decay type (e.g. all decay caused by ascomycetes and deuteromycetes is defined as SR decay). It is necessary to recognise that decay in preservative treated stakes in ground contact is defined by a complexity of factors beyond those achieved in laboratory studies. Therefore information derived from laboratory studies which has contributed to the current decay classification nomenclature is too simplistic and lacks the

versatility to describe decay type diversity in preservative treated pine and beech exposed in soil in the field. This probably applies to preservative treated softwoods and hardwoods in general.

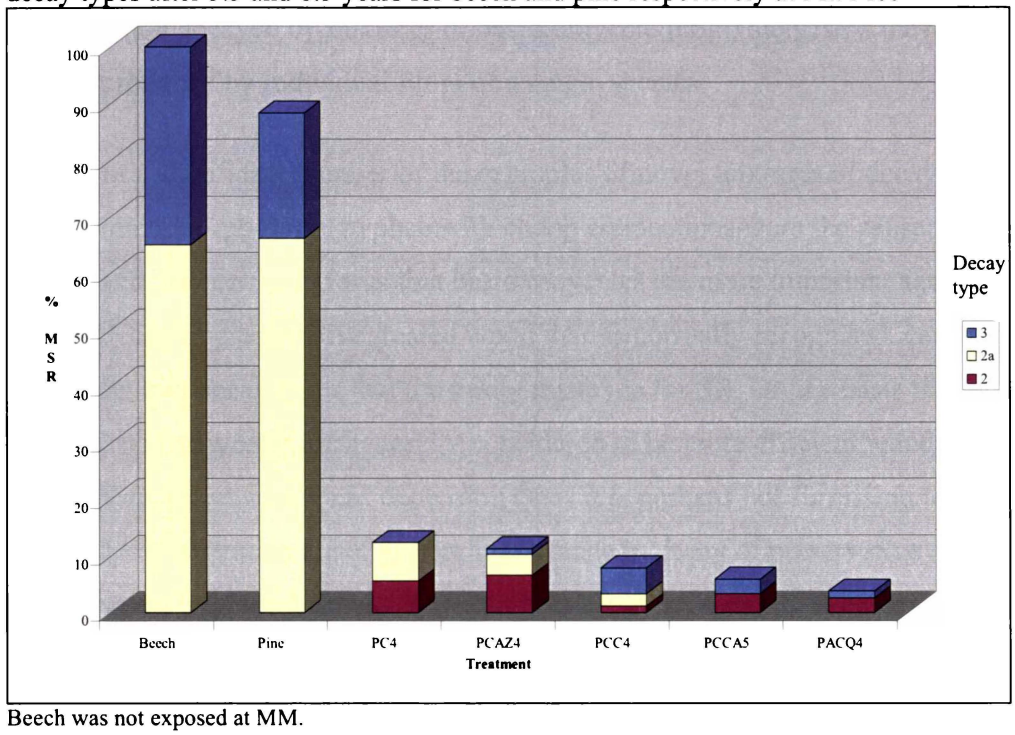
Section 5.5

Appendix 5

Section 5.5.1

Site mean soundness reduction (MSR) and associated macroscopic decay types.

Figure 5.3: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Mt. Mee



Beech was not exposed at MM.

Figure 5.4: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Totara Flat

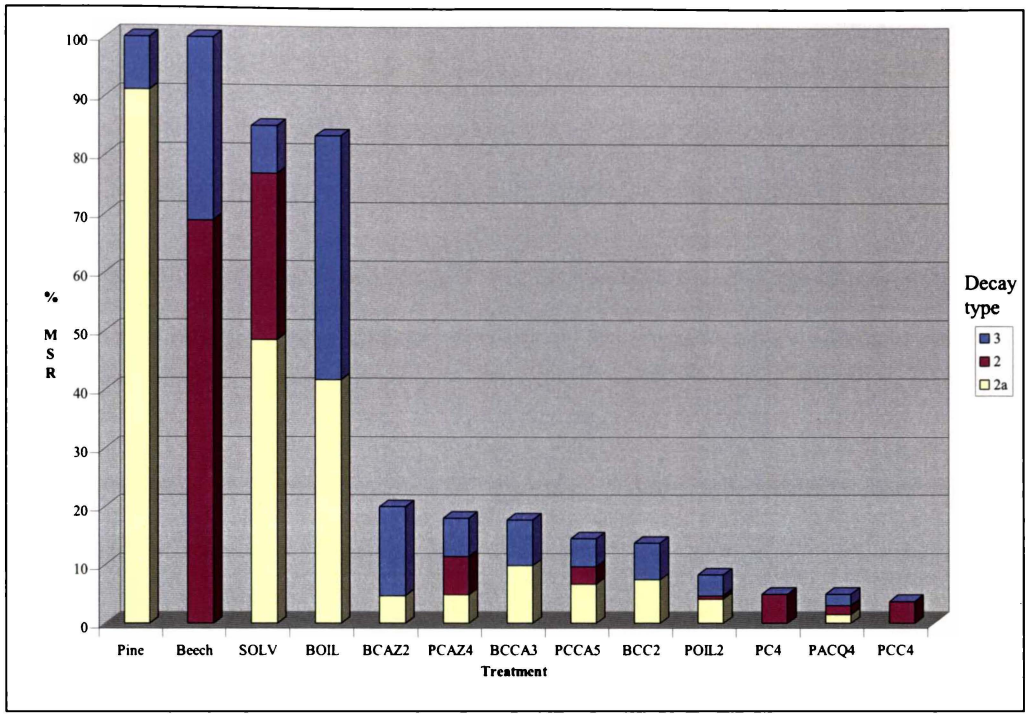


Figure 5.5: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Hari Hari

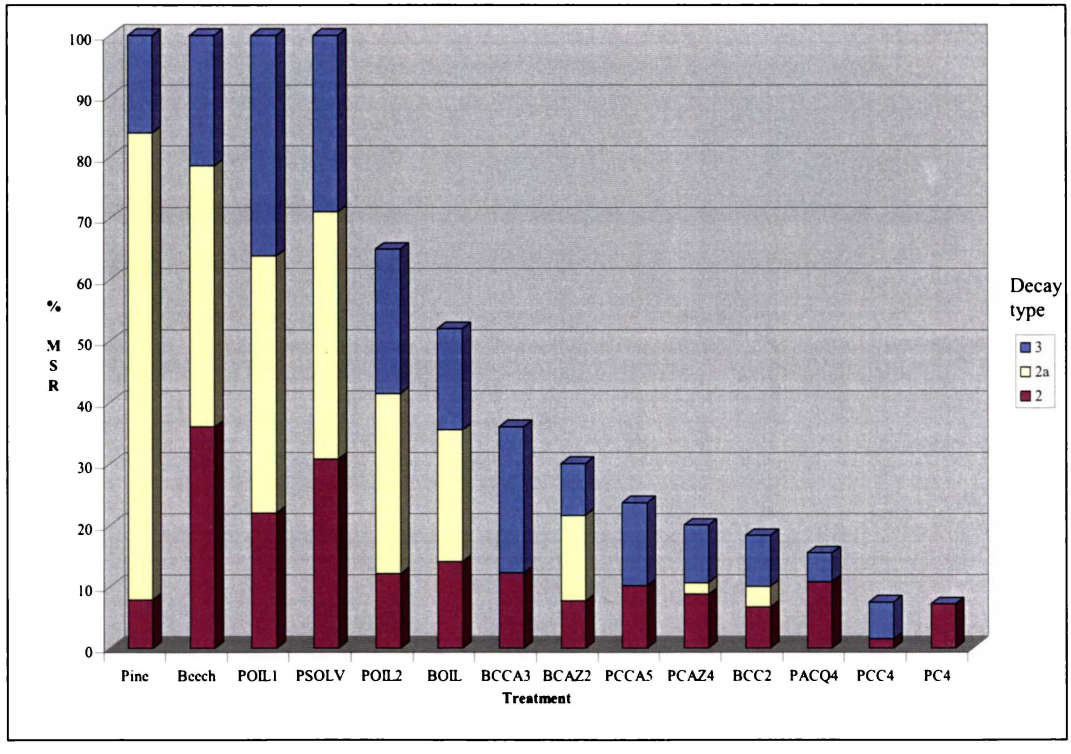


Figure 5.6: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Tapanui

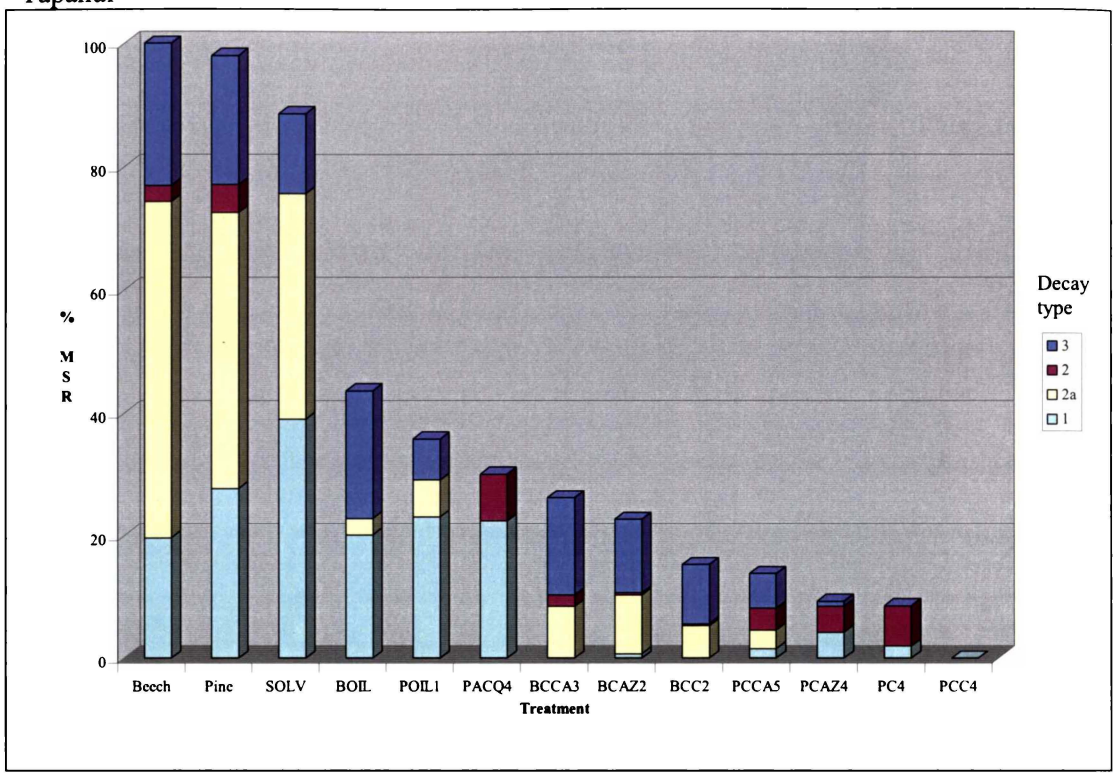


Figure 5.7: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Oak Forest

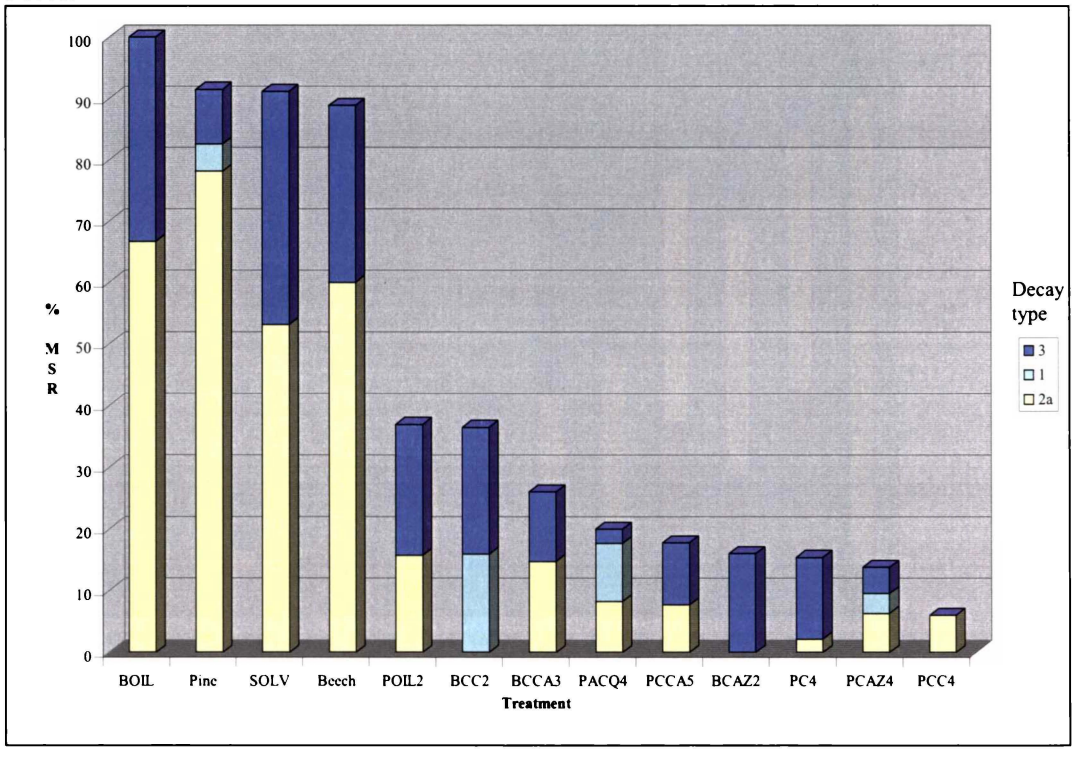


Figure 5.8: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Glenbervie Forest

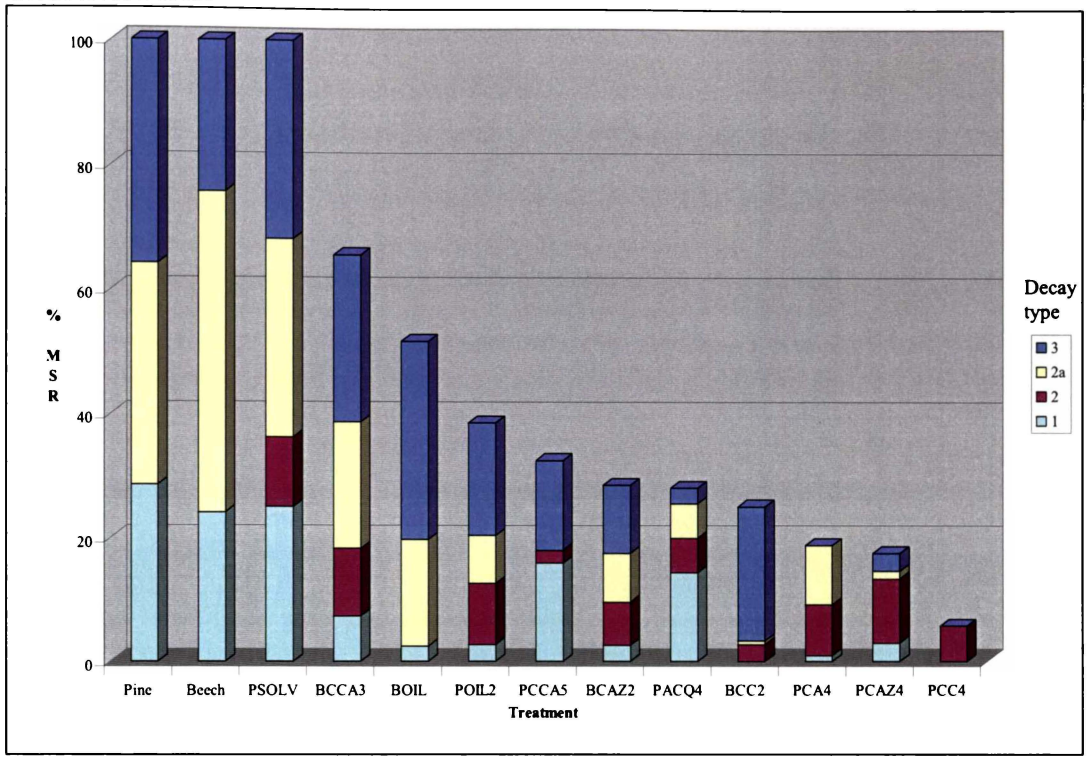


Figure 5.9: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Peat Site

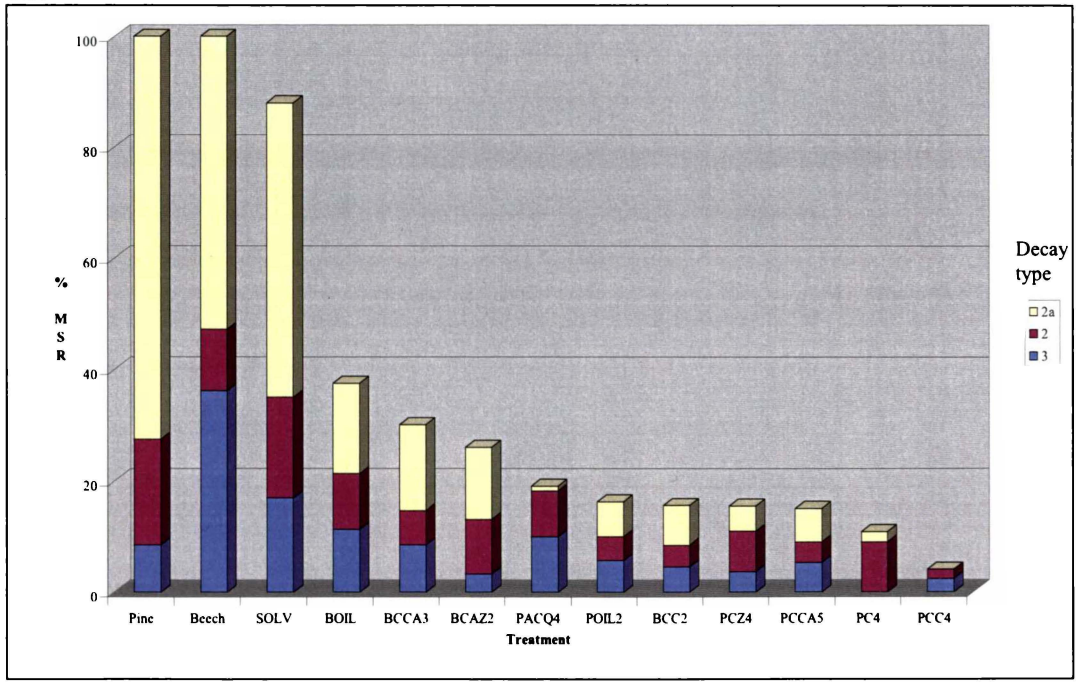


Figure 5.10: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Hanmer

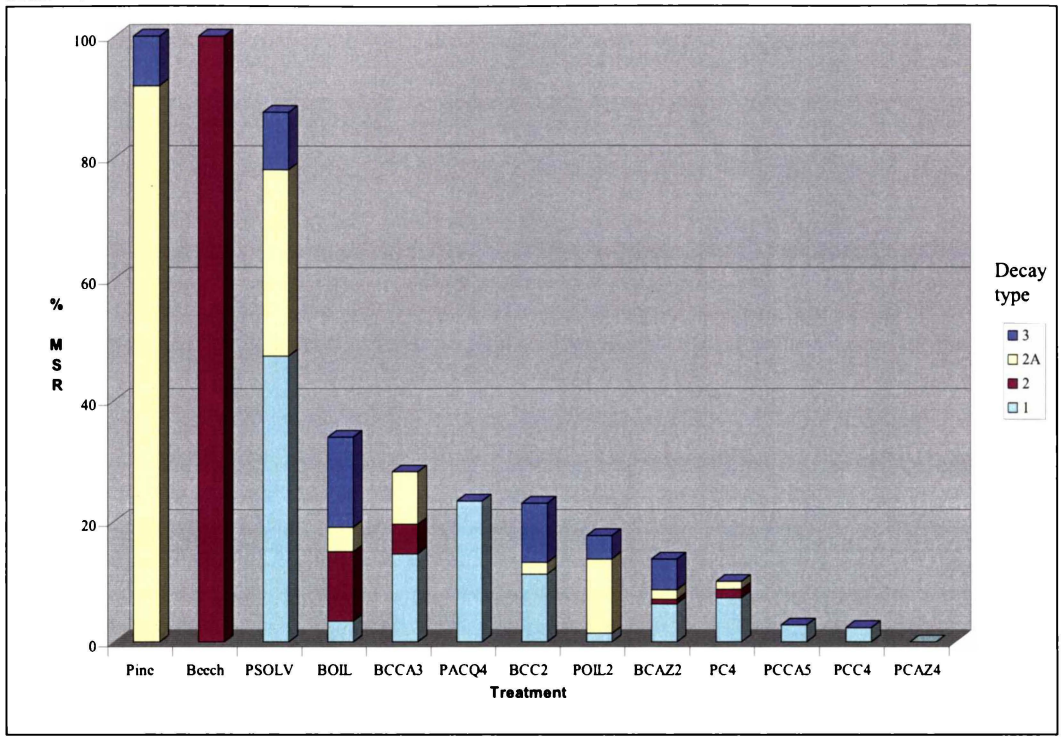


Figure 5.11: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Canal Creek

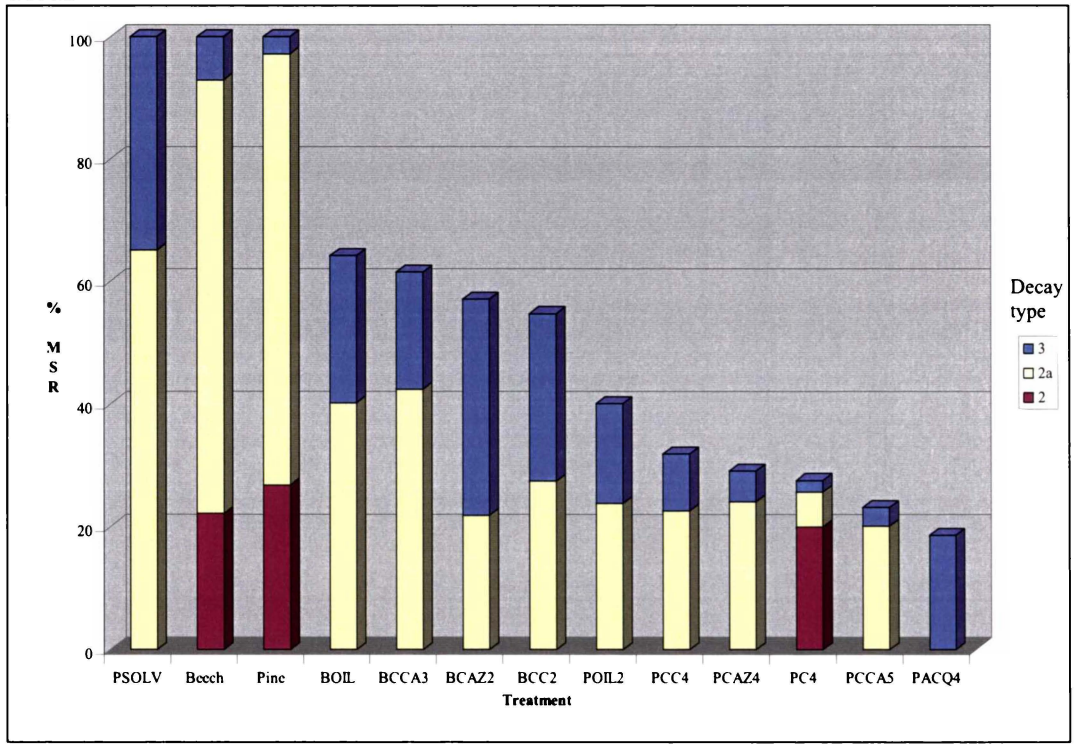


Figure 5.12: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Esk Valley

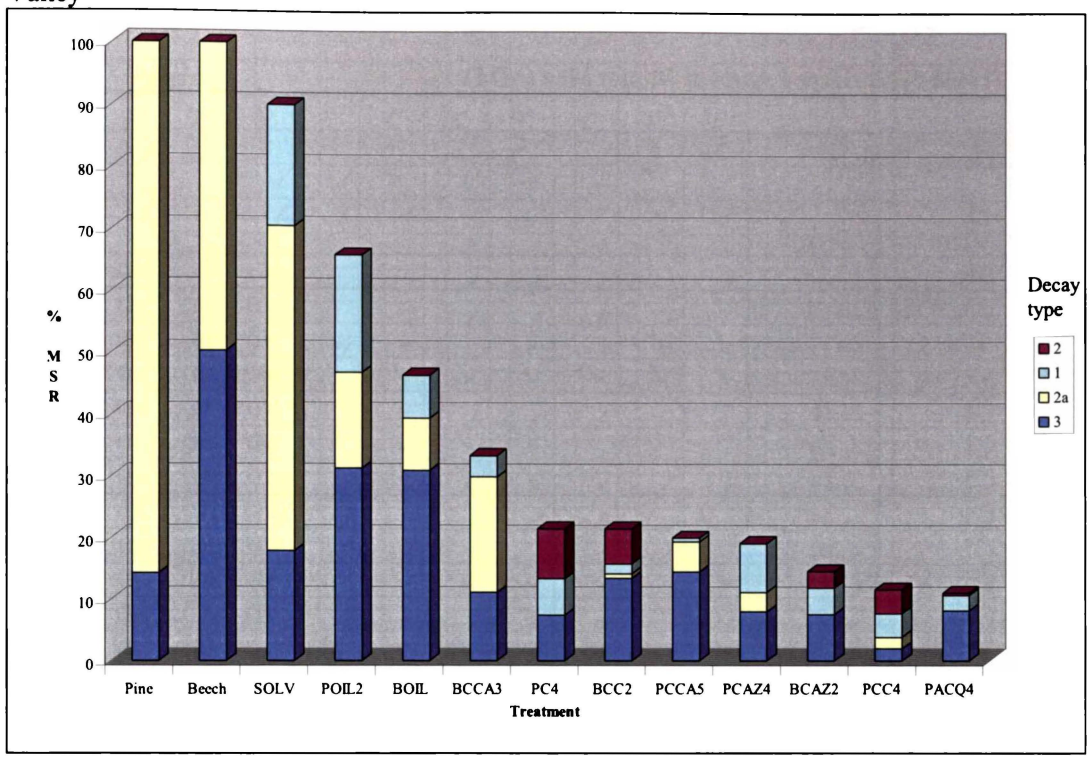
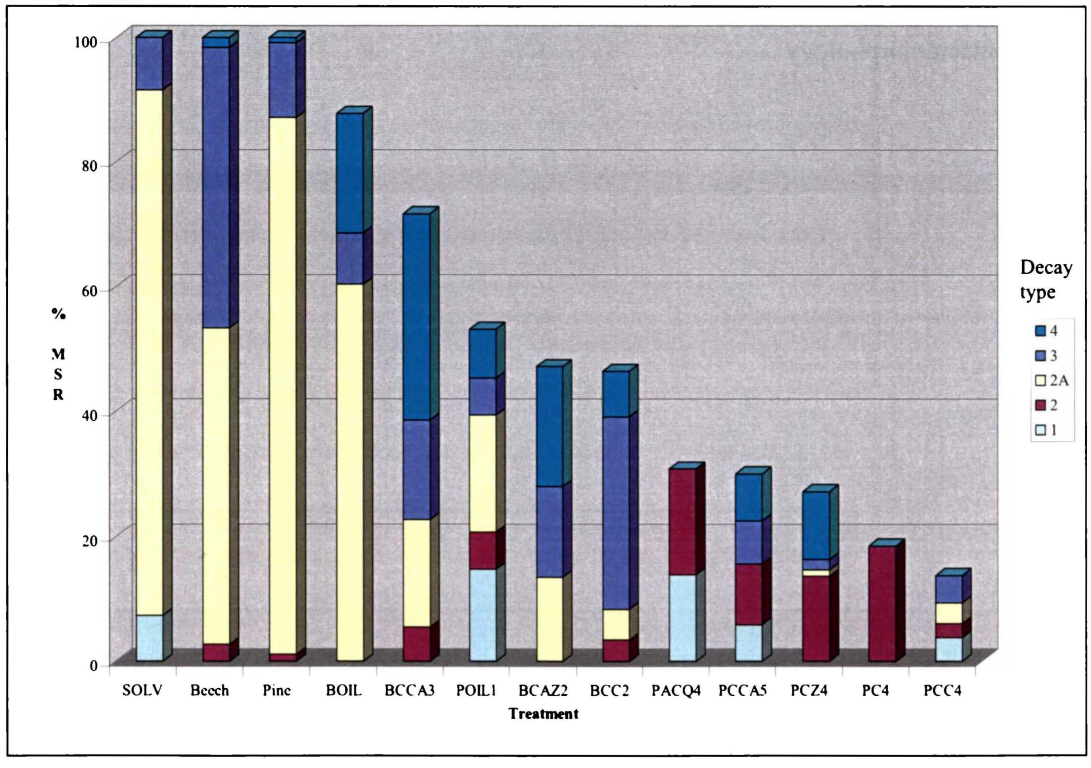


Figure 5.13: MSR for stakes treated with the highest preservative retention and associated decay types after 5.5 and 6.5 years for beech and pine respectively at Glenbervie



Section 5.5.2

Microscopic decay types for each treatment at each site

Table 5.12: Decay types at Mount Mee (MM)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1								ND	ND	ND	ND	ND	ND
BR/WR	2a / 1								ND	ND	ND	ND	ND	ND
WR	2a	✓	✓		✓		✓	✓	ND	ND	ND	ND	ND	ND
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	ND	ND	ND	ND	ND	ND
C1	2, 2a						✓		ND	ND	ND	ND	ND	ND
C2	2, 2a				✓	✓	✓		ND	ND	ND	ND	ND	ND
C3	2, 2a				✓	✓	✓		ND	ND	ND	ND	ND	ND
C4	2, 2a								ND	ND	ND	ND	ND	ND
C5	2, 2a								ND	ND	ND	ND	ND	ND
C6	2, 2a	✓			✓	✓	✓		ND	ND	ND	ND	ND	ND
C7	2, 2a								ND	ND	ND	ND	ND	ND
T1	2, 2a	✓		✓		✓		✓	ND	ND	ND	ND	ND	ND
T2	2, 2a	✓	✓						ND	ND	ND	ND	ND	ND
T3	2, 2a	✓		✓	✓	✓		✓	ND	ND	ND	ND	ND	ND
T4	2, 2a		✓						ND	ND	ND	ND	ND	ND
TB	2, 2a				✓	✓			ND	ND	ND	ND	ND	ND
EB	2, 2a				✓	✓			ND	ND	ND	ND	ND	ND
E1	2, 2a		✓						ND	ND	ND	ND	ND	ND
E2	2, 2a								ND	ND	ND	ND	ND	ND

ND = no data (treatments not exposed), micro* = micromorphology, macro* = macromorphology

Table 5.13: Decay types at Totara Flat (Tot)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1													
BR/WR	2a / 1													
WR	2a	✓	✓		✓	✓	✓		✓		✓			
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a				✓		✓							
C2	2, 2a	✓			✓	✓	✓		✓					
C3	2, 2a	✓			✓	✓	✓							
C4	2, 2a		✓								✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a				✓	✓	✓							
C7	2, 2a													
T1	2, 2a	✓	✓	✓	✓	✓	✓	✓	✓	✓				
T2	2, 2a	✓	✓		✓	✓	✓			✓				
T3	2, 2a				✓	✓		✓						
T4	2, 2a										✓	✓	✓	✓
TB	2, 2a													
EB	2, 2a													
E1	2, 2a		✓								✓	✓	✓	✓
E2	2, 2a	✓			✓	✓	✓							

micro* = micromorphology, macro* = macromorphology

Table 5.14: Decay types at Hari Hari (HH)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1									ND				
BR/WR	2a / 1	✓			✓	✓	✓		✓	ND				
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	ND	✓	✓	✓	✓
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	ND	✓	✓	✓	✓
C1	2, 2a				✓	✓	✓			ND				
C2	2, 2a				✓	✓	✓			ND				
C3	2, 2a				✓	✓	✓			ND				
C4	2, 2a		✓							ND	✓	✓	✓	✓
C5	2, 2a									ND	✓			
C6	2, 2a				✓	✓	✓			ND	✓	✓		
C7	2, 2a				✓	✓	✓			ND				
T1	2, 2a			✓	✓	✓	✓	✓		ND				
T2	2, 2a				✓					ND		✓		
T3	2, 2a									ND				
T4	2, 2a									ND	✓		✓	
TB	2, 2a						✓			ND				
EB	2, 2a				✓	✓	✓			ND				
E1	2, 2a									ND	✓			
E2	2, 2a	✓			✓	✓	✓		✓	ND				

P (T1) = predominantly perpendicular tunnels, micro* = micromorphology, macro* = macromorphology

Table 5.15: Decay types at Tapanui (TAP)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
BR/WR	2a / 1					✓	✓							
WR	2a	✓	✓			✓	✓		✓	✓	✓			
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a						✓				✓			
C2	2, 2a				✓	✓	✓							
C3	2, 2a				✓	✓	✓							
C4	2, 2a										✓		✓	
C5	2, 2a									✓	✓	✓	✓	
C6	2, 2a				✓	✓	✓							
C7	2, 2a				✓	✓	✓							
T1	2, 2a	✓		✓	✓	✓	✓	✓	✓	✓				
T2	2, 2a	✓					✓	✓	✓		✓	✓	✓	
T3	2, 2a	✓		✓	✓	✓	✓	✓	✓	✓				
T4	2, 2a		✓								✓	✓	✓	✓
TB	2, 2a	✓			✓	✓	✓		✓		✓	✓	✓	✓
EB	2, 2a	✓			✓	✓	✓		✓		✓	✓		
E1	2, 2a									✓	✓	✓	✓	✓
E2	2, 2a													

micro* = micromorphology, macro* = macromorphology

Table 5.16: Decay types at Oak (OAK)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1	✓			✓	✓	✓	✓	ND	ND			✓	
BR/WR	2a / 1				✓	✓	✓		ND	ND				
WR	2a	✓	✓						ND	ND				
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	ND	ND	✓	✓	✓	✓
C1	2, 2a						✓		ND	ND	✓			
C2	2, 2a				✓	✓	✓		ND	ND				
C3	2, 2a				✓	✓	✓		ND	ND				
C4	2, 2a								ND	ND	✓	✓	✓	✓
C5	2, 2a								ND	ND	✓	✓	✓	✓
C6	2, 2a				✓	✓	✓		ND	ND				
C7	2, 2a						✓		ND	ND				
T1	2, 2a	✓		✓	✓	✓	✓	✓	ND	ND				
T2	2, 2a	✓					✓	✓	ND	ND	✓	✓	✓	
T3	2, 2a	✓		✓	✓	✓	✓	✓	ND	ND				
T4	2, 2a		✓						ND	ND	✓	✓	✓	✓
TB	2, 2a	✓			✓	✓	✓		ND	ND	✓	✓	✓	✓
EB	2, 2a	✓			✓	✓	✓		ND	ND	✓	✓		
E1	2, 2a								ND	ND	✓	✓	✓	✓
E2	2, 2a								ND	ND				

Micro* = micromorphology, macro* = macromorphology

Table 5.17: Decay types at Glenbervie forest (GBF)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BR/WR	2a / 1													
WR	2a	✓	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a													
C2	2, 2a				✓	✓	✓							
C3	2, 2a				✓	✓	✓							
C4	2, 2a										✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a	✓			✓	✓	✓							
C7	2, 2a				✓	✓	✓							
T1	2, 2a			✓	✓	✓	✓	✓		✓				
T2	2, 2a		✓	✓	✓	✓	✓	✓						
T3	2, 2a			✓	✓	✓	✓	✓						
T4	2, 2a													
TB	2, 2a													
EB	2, 2a													
E1	2, 2a										✓	✓	✓	✓
E2	2, 2a													

Micro* = micromorphology, macro* = macromorphology

Table 5.18: Decay types at Peat (PT)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1													
BR/WR	2a / 1													
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a				✓	✓	✓							
C2	2, 2a				✓	✓	✓							
C3	2, 2a						✓							
C4	2, 2a										✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a													
C7	2, 2a													
T1	2, 2a	✓		✓	✓	✓	✓	✓	✓	✓				
T2	2, 2a	✓	✓	✓	✓	✓	✓	✓	✓	✓				
T3	2, 2a			✓	✓	✓	✓	✓	✓	✓				
T4	2, 2a													
TB	2, 2a													
EB	2, 2a													
E1	2, 2a										✓	✓	✓	✓
E2	2, 2a													

Micro* = micromorphology, macro* = macromorphology

Table 5.19: Decay types at Hanmer (HAN)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BR/WR	2a / 1													
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3, 2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a													
C2	2, 2a				✓	✓	✓							
C3	2, 2a					✓	✓							
C4	2, 2a										✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a													
C7	2, 2a													
T1	2, 2a			✓				✓		✓				
T2	2, 2a													
T3	2, 2a			✓				✓						
T4	2, 2a													
TB	2, 2a													
EB	2, 2a													
E1	2, 2a										✓	✓	✓	✓
E2	2, 2a													

Micro* = micromorphology, macro* = macromorphology

Table 5.20: Decay types at Canal Creek (CK)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	Absent													
BR/WR	Absent													
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3, 2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a													
C2	2, 2a				✓	✓	✓	✓						
C3	2, 2a			✓	✓	✓	✓	✓		✓				
C4	2, 2a										✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a				✓	✓	✓		✓					
C7	2, 2a				✓	✓	✓							
T1	2, 2a			✓			✓	✓		✓				
T2	2, 2a	✓	✓	✓			✓	✓		✓				
T3	2, 2a			✓			✓	✓						
T4	2, 2a	✓									✓	✓	✓	✓
TB	2, 2a			✓	✓	✓	✓							
EB	2, 2a													
E1	2, 2a									✓	✓	✓	✓	✓
E2	2, 2a													

Micro* = micromorphology, macro* = macromorphology

Table 5.21: Decay types at Esk Valley (ESK)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BR/WR	2a / 1													
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3, 2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a													
C2	2, 2a													
C3	2, 2a													
C4	2, 2a		✓								✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a													
C7	2, 2a													
T1	2, 2a			✓				✓		✓				
T2	2, 2a	✓												
T3	2, 2a													
T4	2, 2a													
TB	2, 2a				✓	✓	✓							
EB	2, 2a													
E1	2, 2a										✓	✓	✓	✓
E2	2, 2a													

Micro* = micromorphology, macro* = macromorphology

Table 5.22: Decay types at Glenbervie (GB)

Decay Type		Pine	Beech	PC	PACQ	PCAZ	PCCA	PCC	Solv	POIL	BCCA	BCAZ	BCC	BOIL
Micro*	Macro*													
BR				✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
BR/WR					✓	✓	✓							
WR	2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SR	2, 3, 4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C1	2, 2a						✓							
C2	2, 2a				✓	✓	✓							
C3	2, 2a				✓	✓	✓							
C4	2, 2a		✓								✓	✓	✓	✓
C5	2, 2a										✓	✓	✓	✓
C6	2, 2a	✓			✓	✓	✓							
C7	2, 2a				✓	✓	✓							
T1	2, 2a	✓		✓	✓	✓	✓	✓		✓				
T2	2, 2a	✓	✓		✓	✓	✓	✓						
T3	2, 2a	✓		✓	✓	✓	✓	✓						
T4	2, 2a	✓									✓	✓	✓	✓
TB	2, 2a	✓			✓	✓	✓		✓					
EB	2, 2a	✓			✓	✓	✓							
E1	2, 2a										✓	✓	✓	✓
E2	2, 2a													

Micro* = micromorphology, macro* = macromorphology

Section 5.5.3

Mean Soundness reduction and associated macroscopic decay types for each treatment at each site

Figure 5.14: MSR for all treatments and associated decay types at Glenbervie

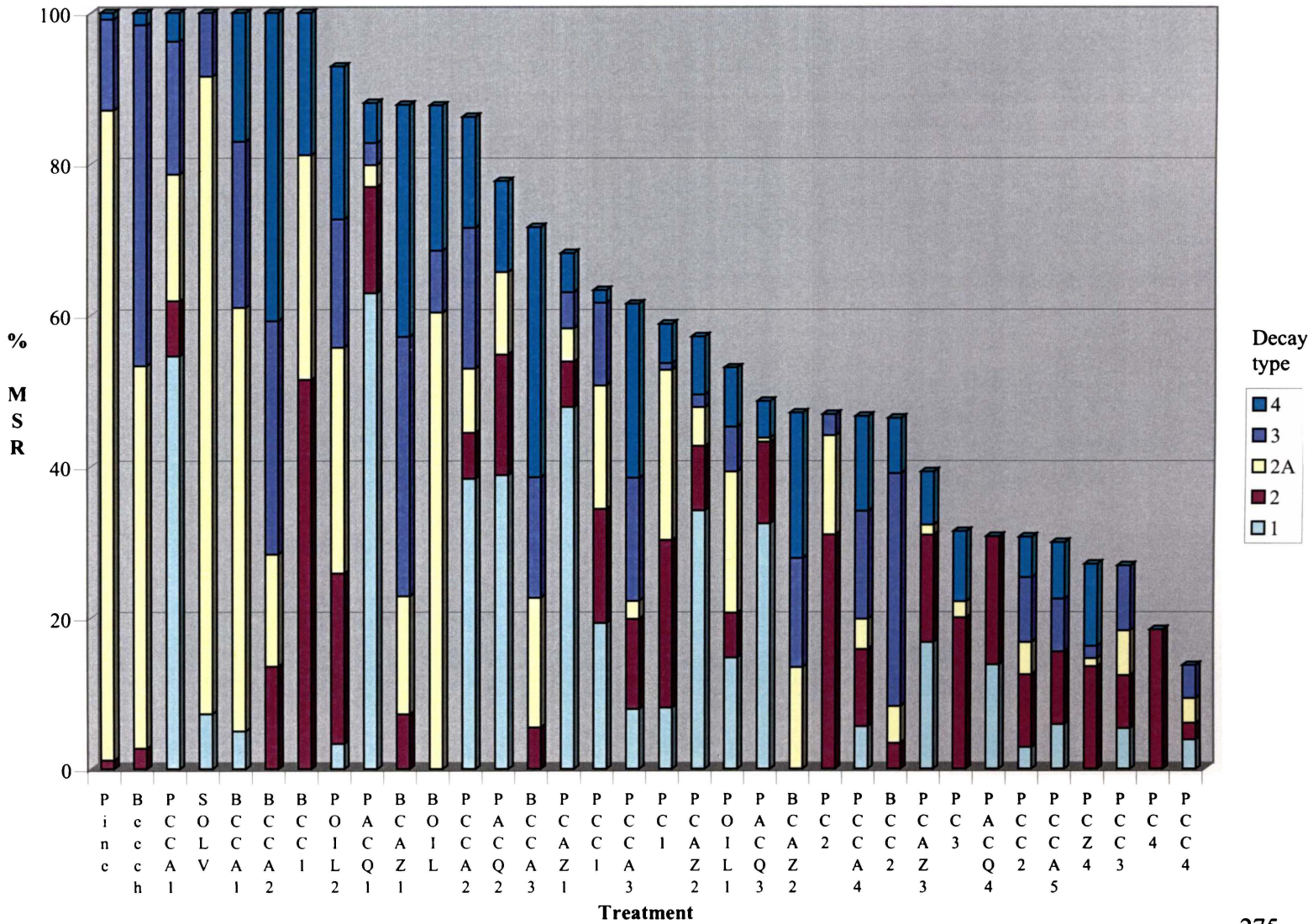


Figure 5.15: MSR for all treatments and associated decay types at Glenbervie Forest

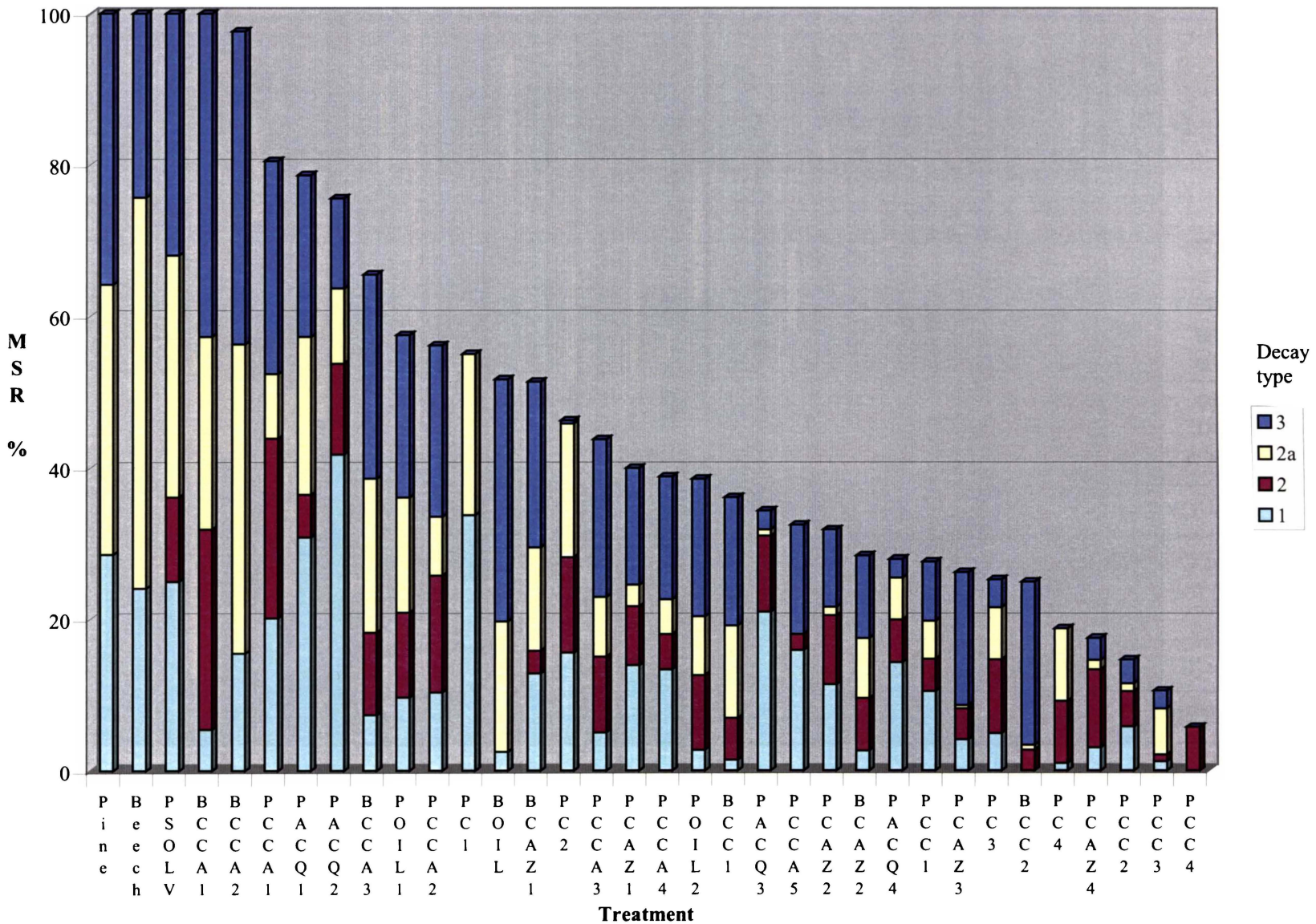


Figure 5.16: MSR for all treatments and associated decay types at Hanmer

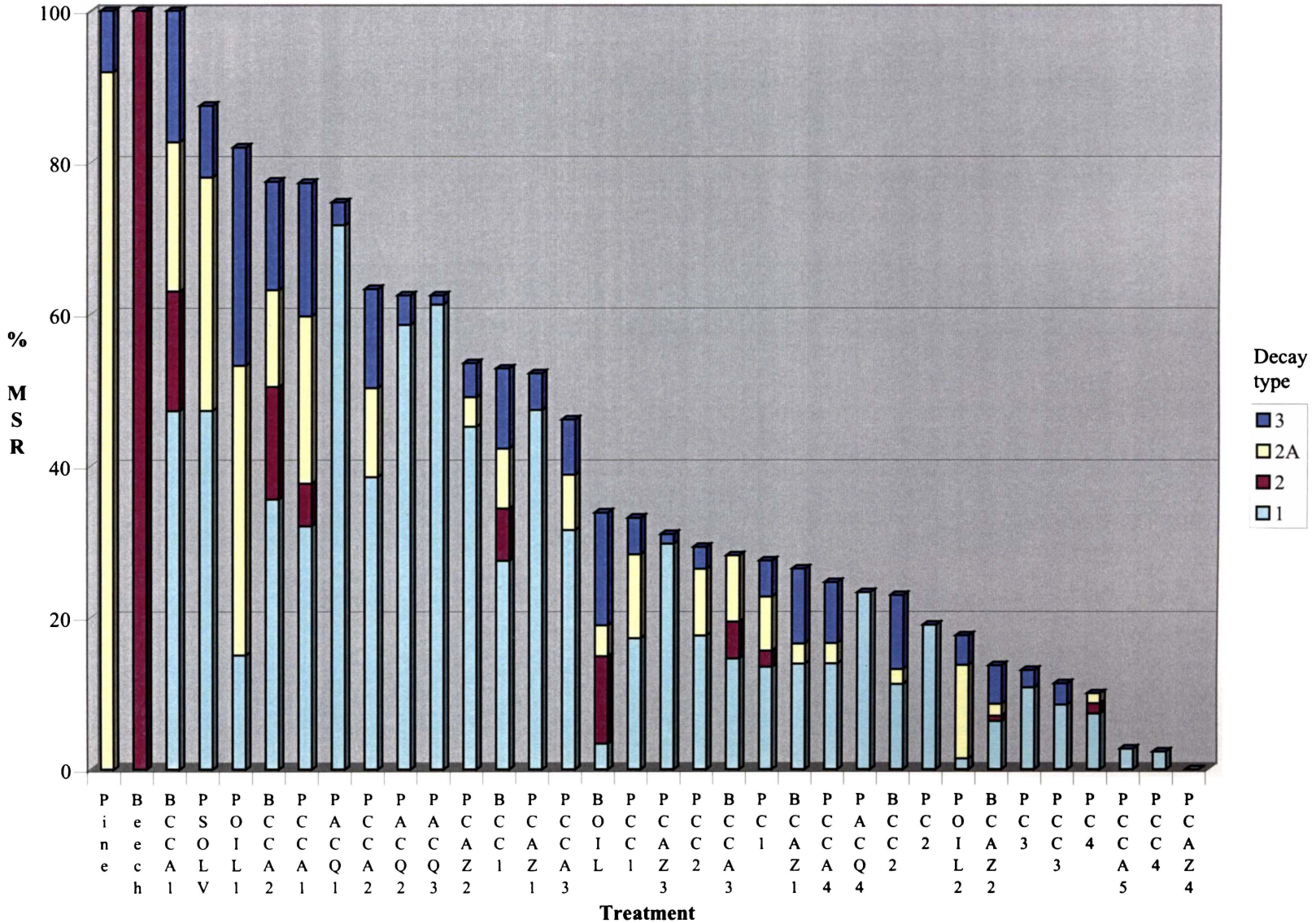


Figure 5.17: MSR for all treatments and associated decay types at Hari Hari

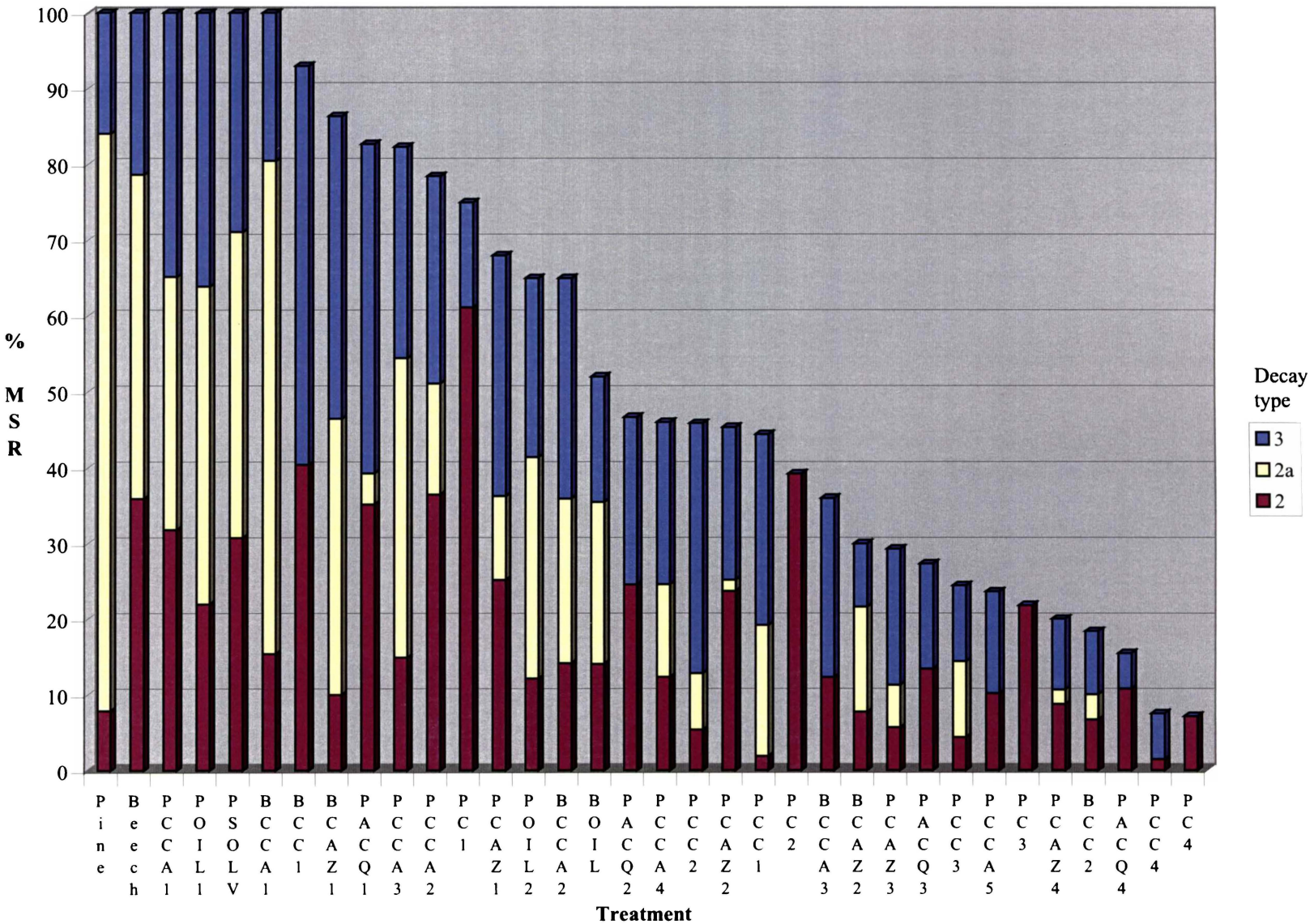


Figure 5.18: MSR for all treatments and associated decay types at Canal Creek

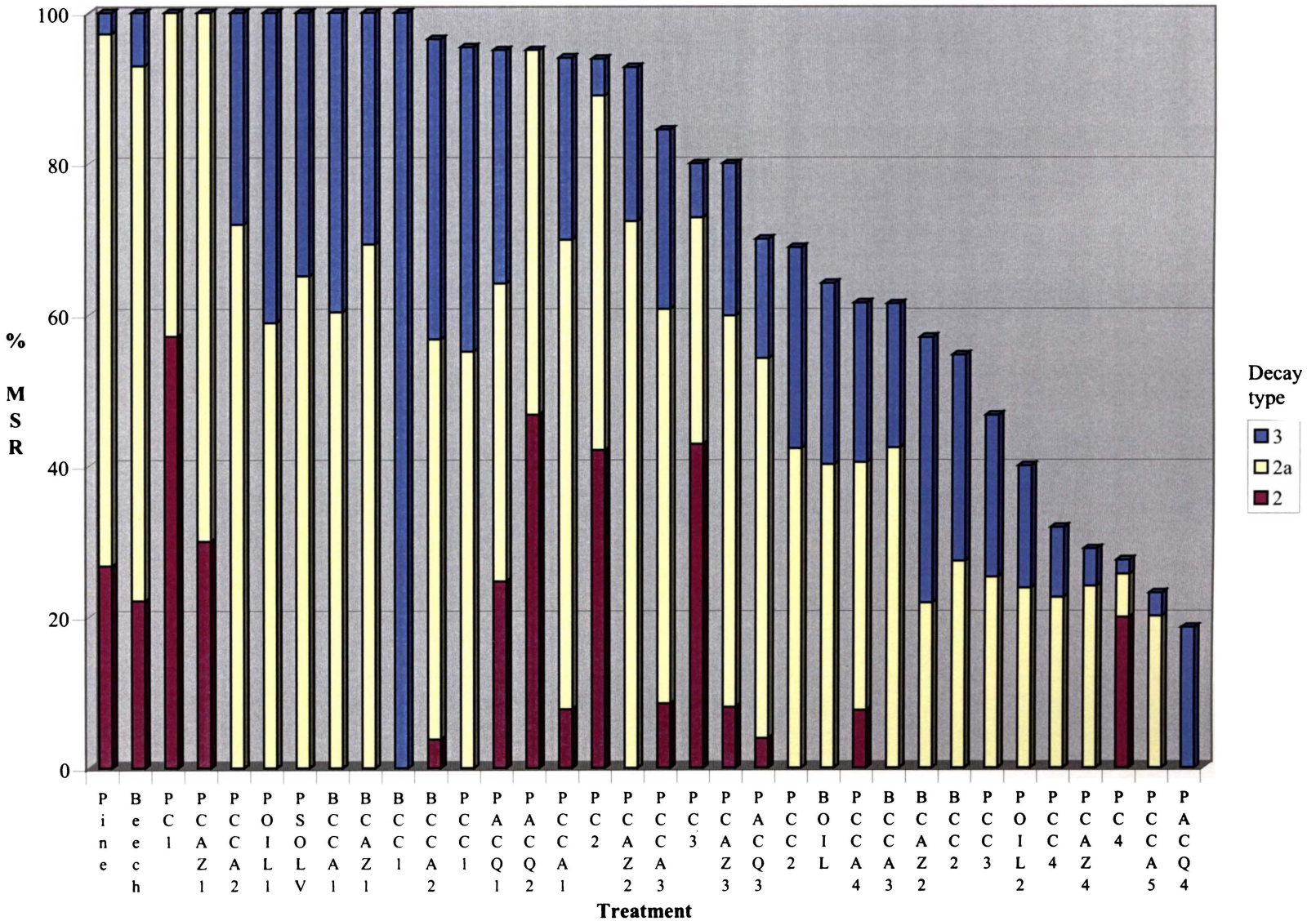


Figure 5.19: MSR for all treatments and associated decay types at Tapanui

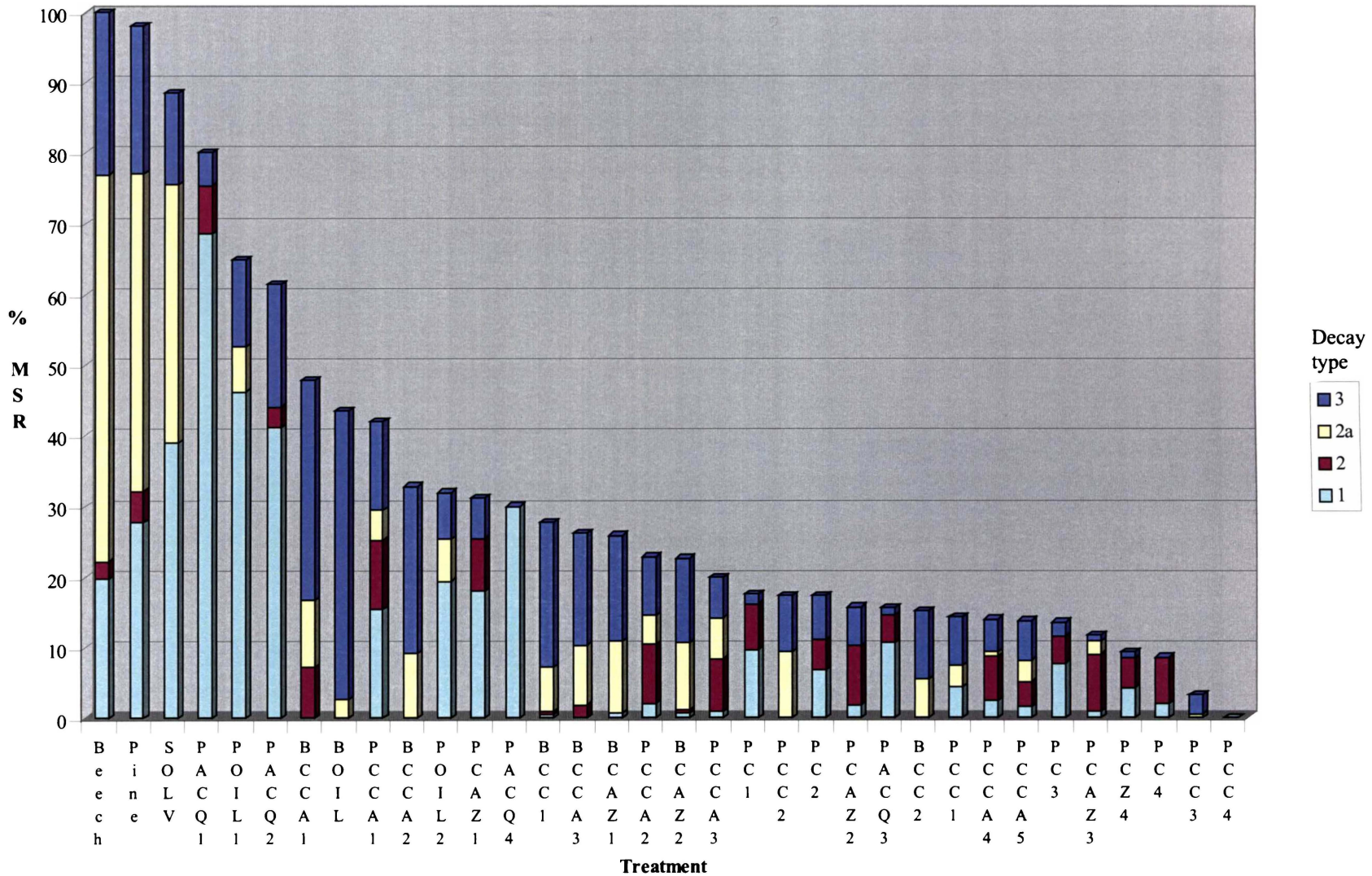


Figure 5.20: MSR for all treatments and associated decay types at Mt Mee

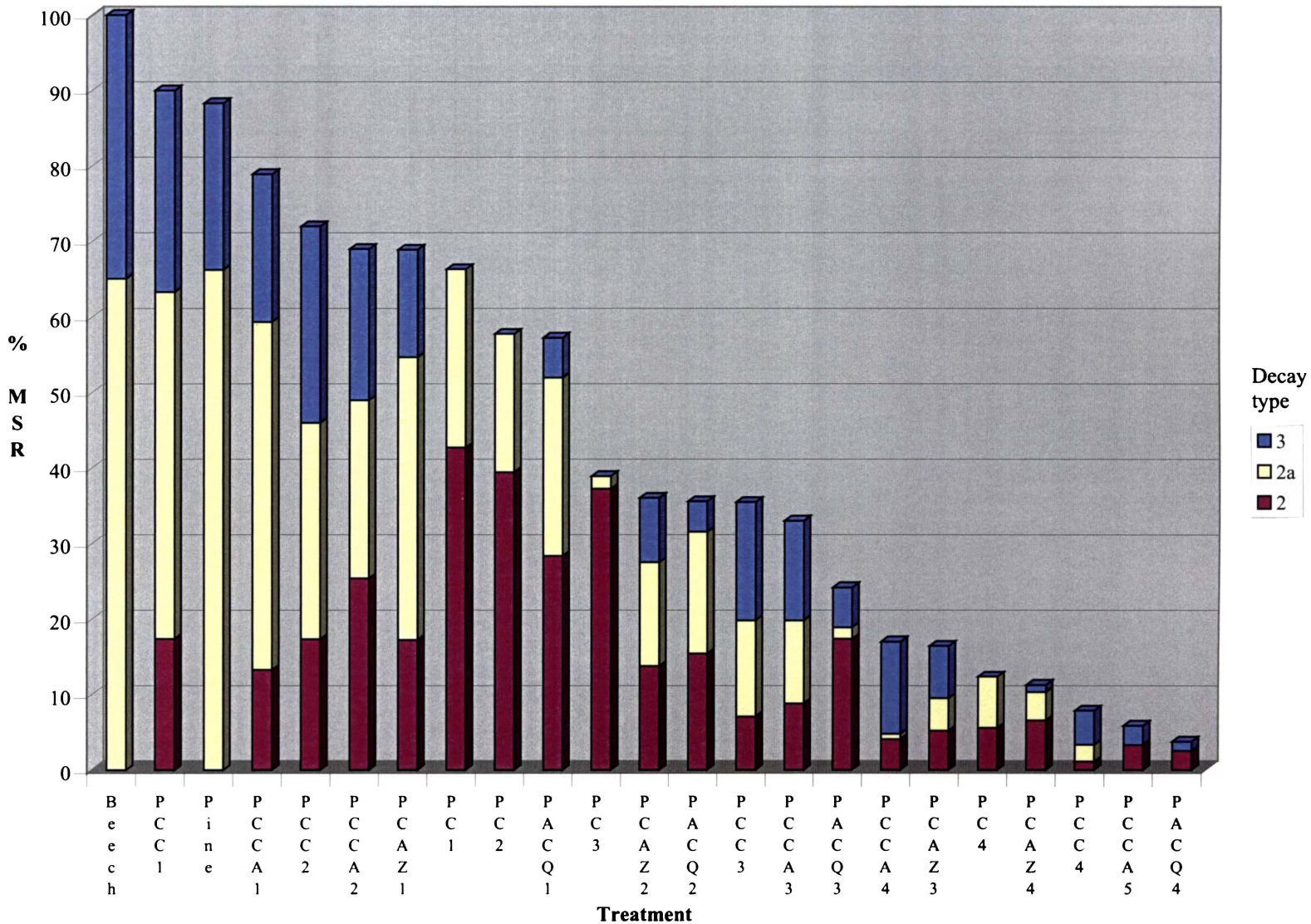


Figure 5.21: MSR for all treatments and associated decay types at Tomblesons Mandarin Orchard

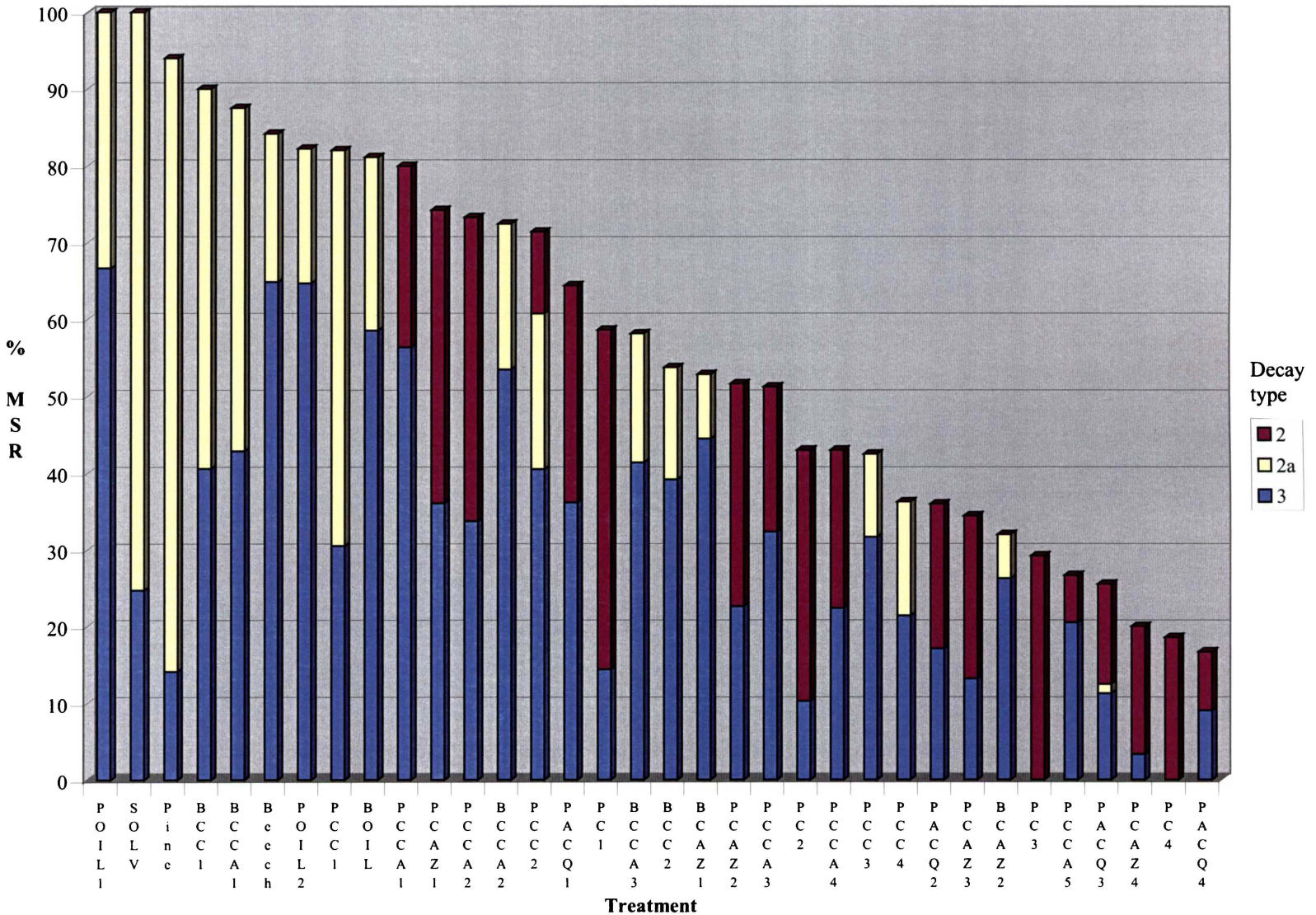


Figure 5.22: MSR for all treatments and associated decay types at Oak Forest

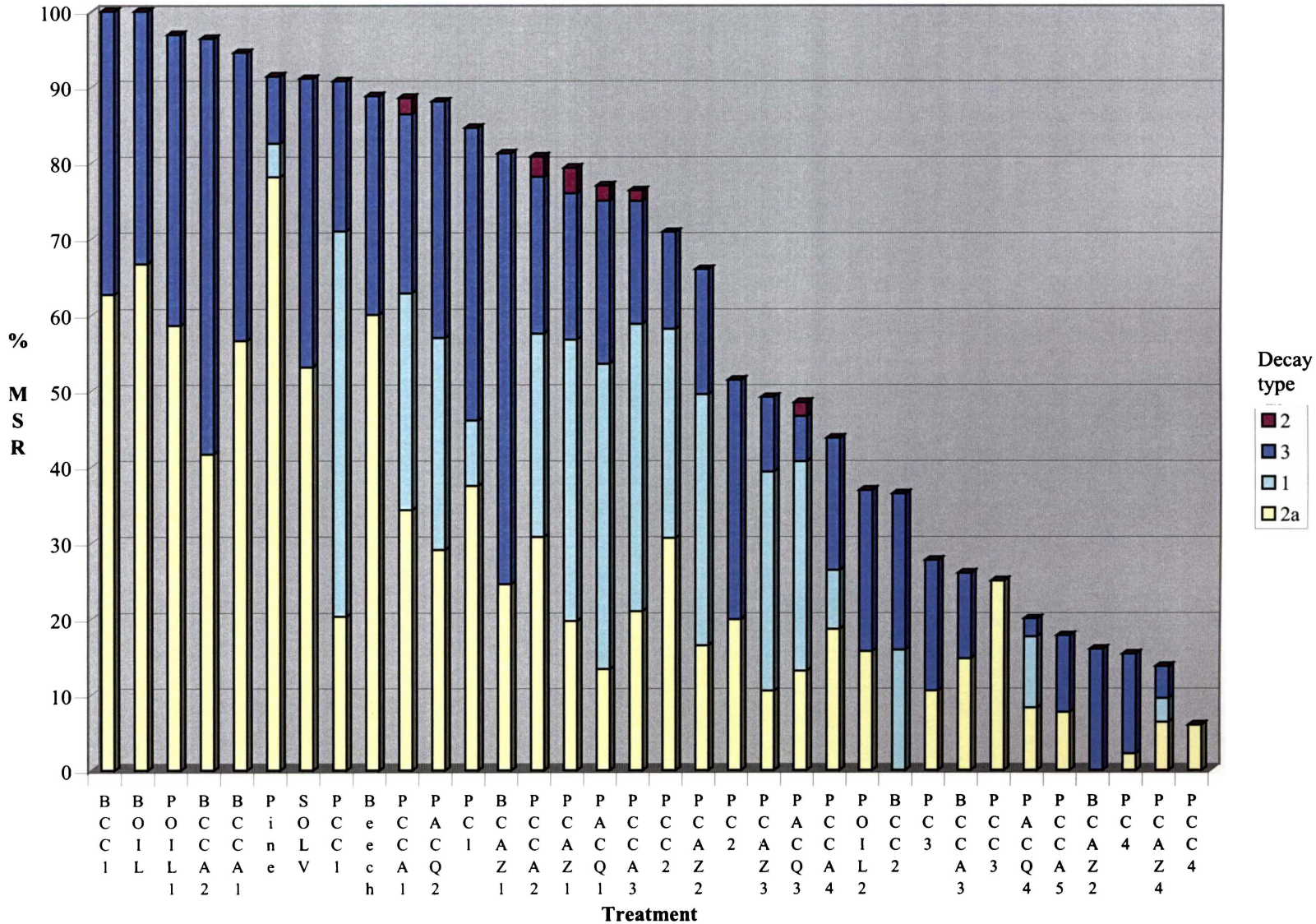


Figure 5.23: MSR for all treatments and associated decay types at Peat Site

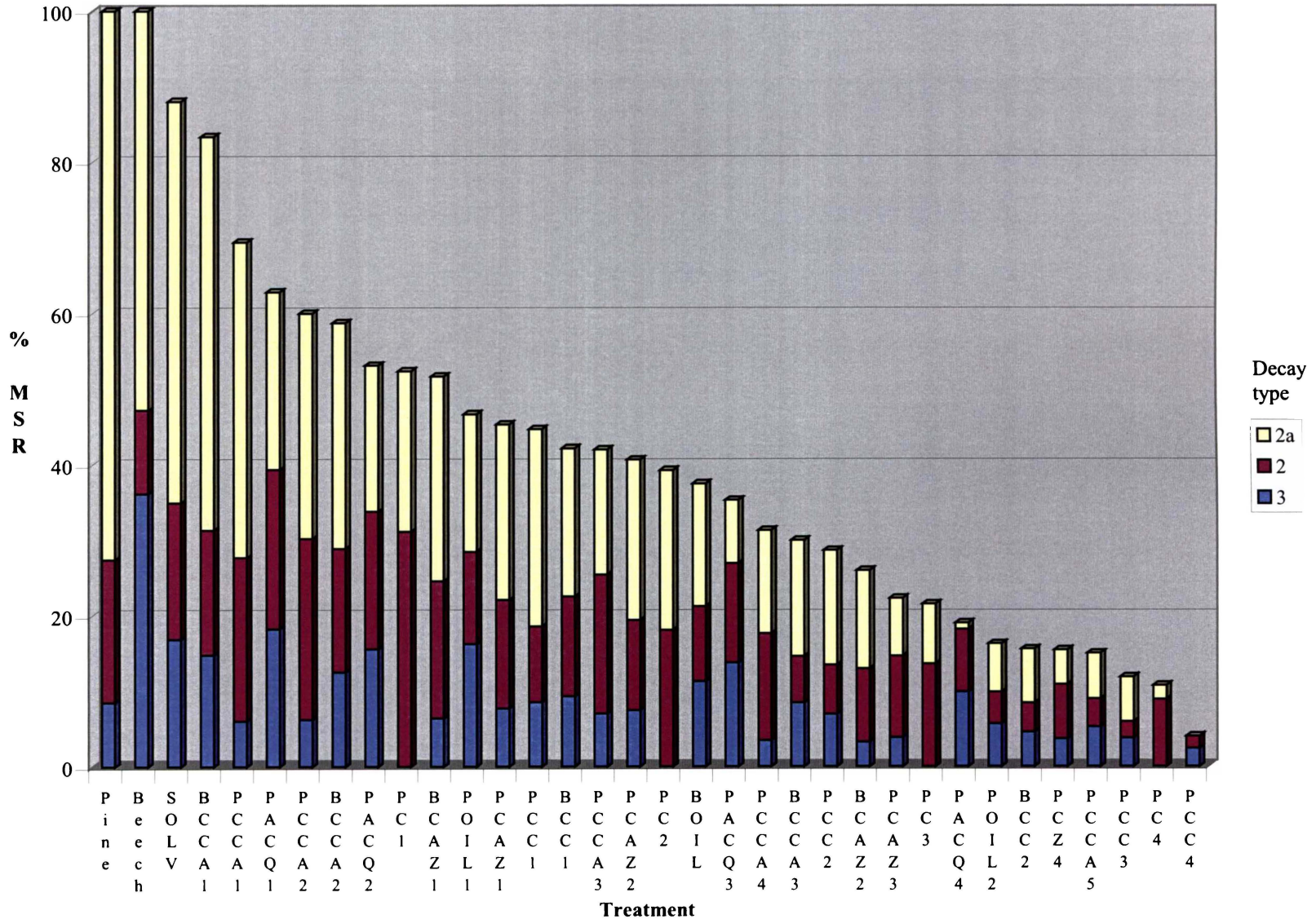


Figure 5.24: MSR for all treatments and associated decay types at Whakarewarewa

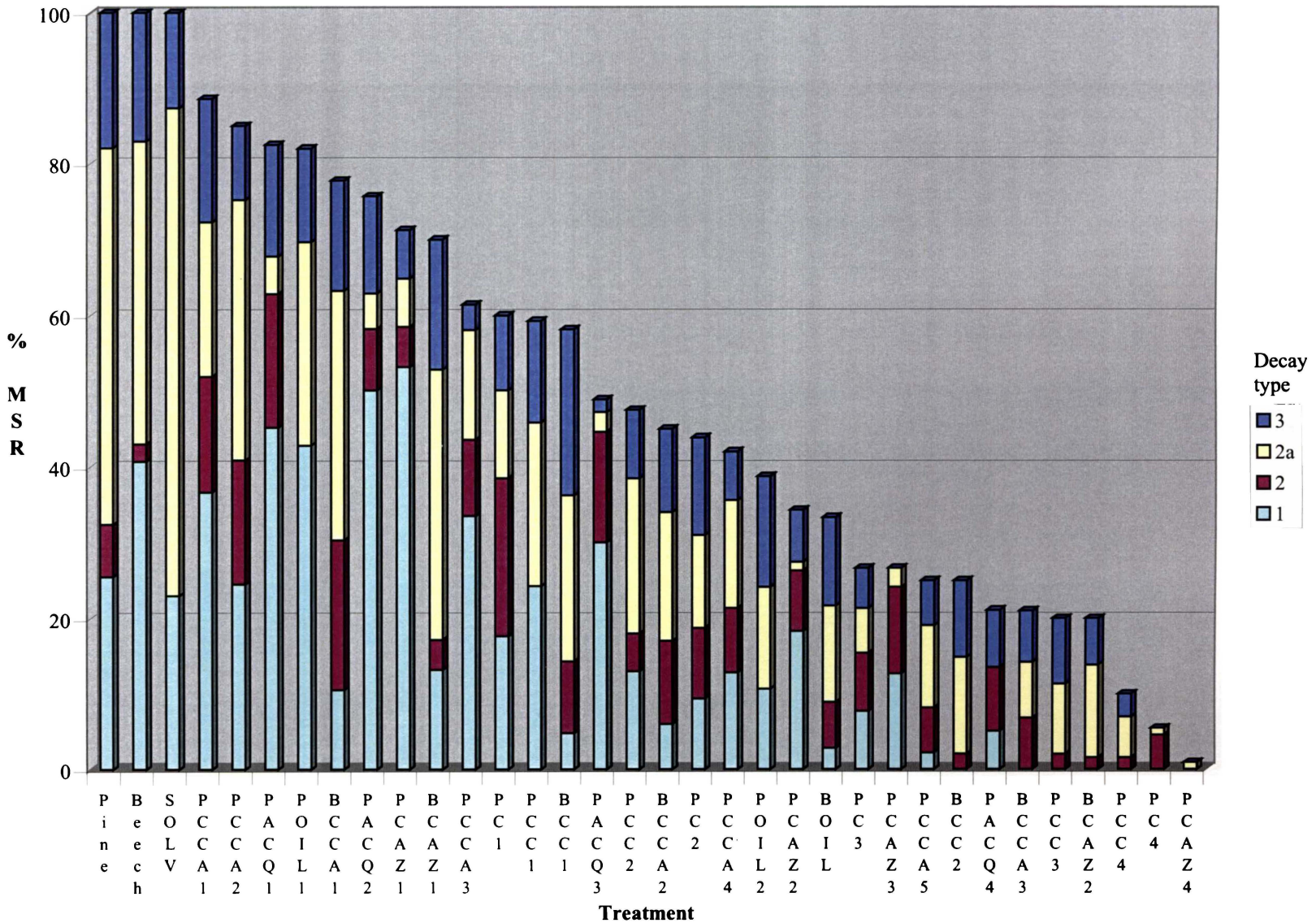


Figure 5.25: MSR for all treatments and associated decay types at Esk Valley

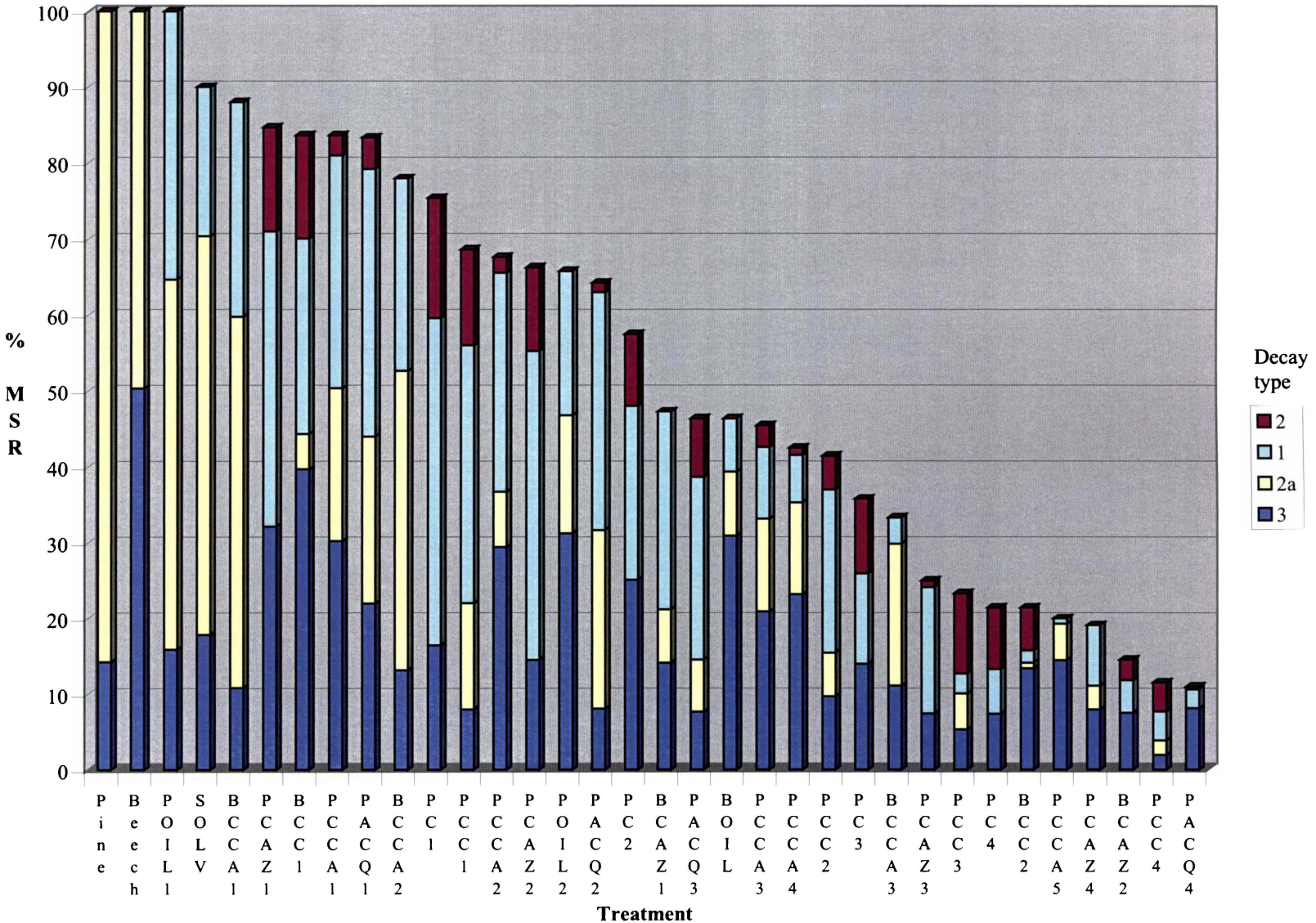
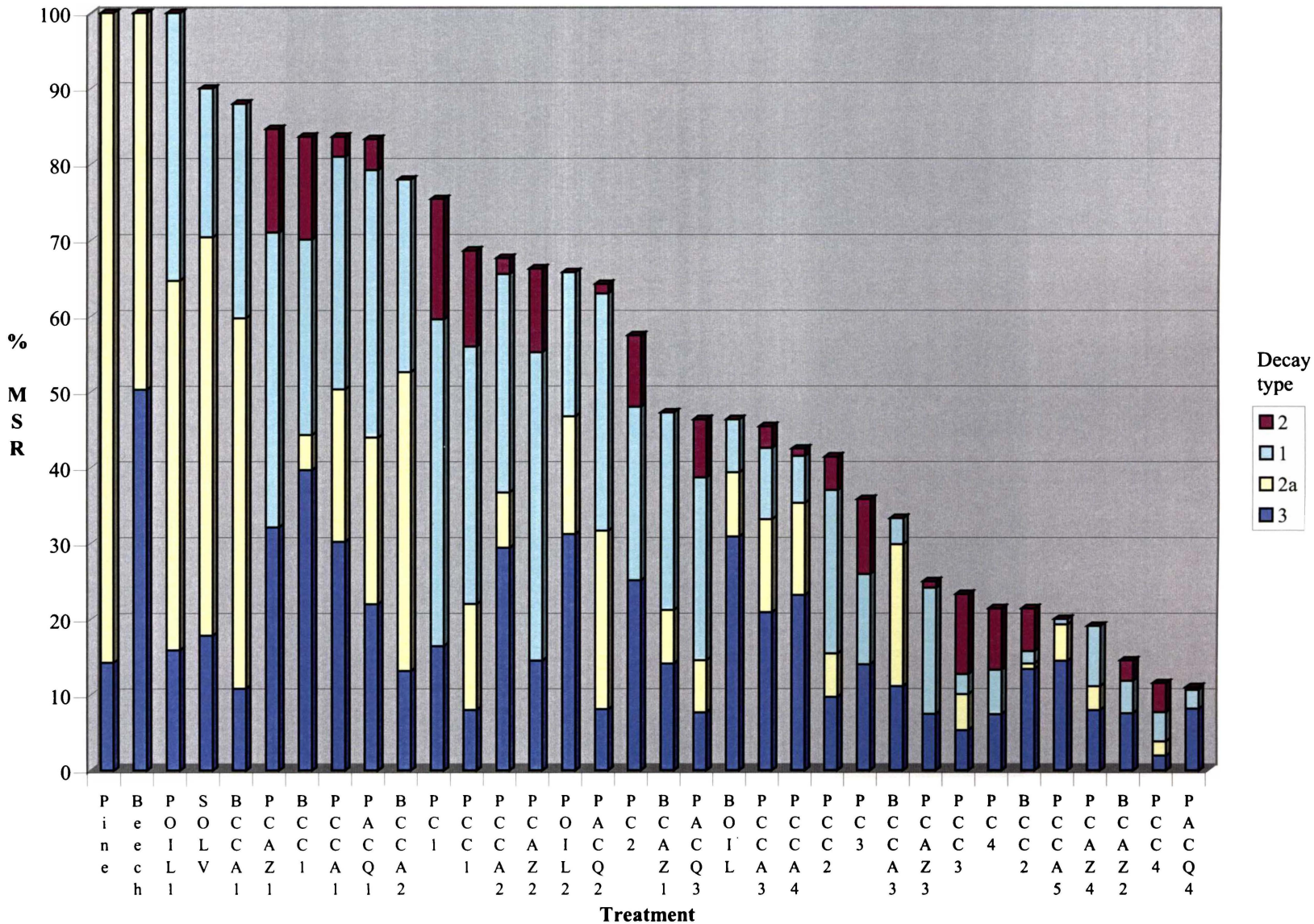


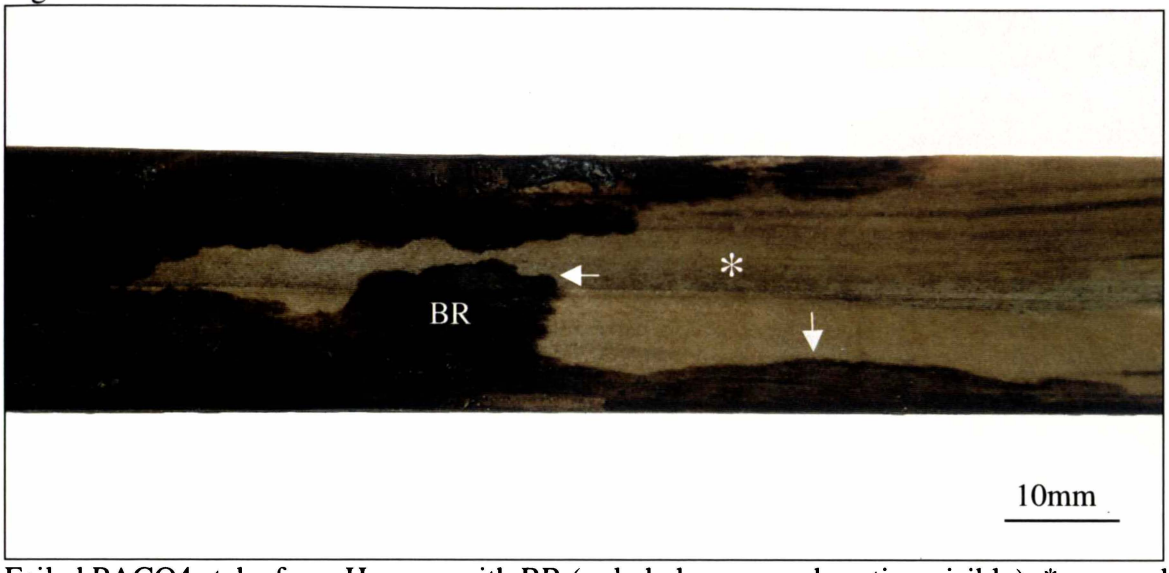
Figure 5.26: MSR for all treatments and associated decay types at Oak Forest



Section 5.5.4
Macroscopic decay type photographs

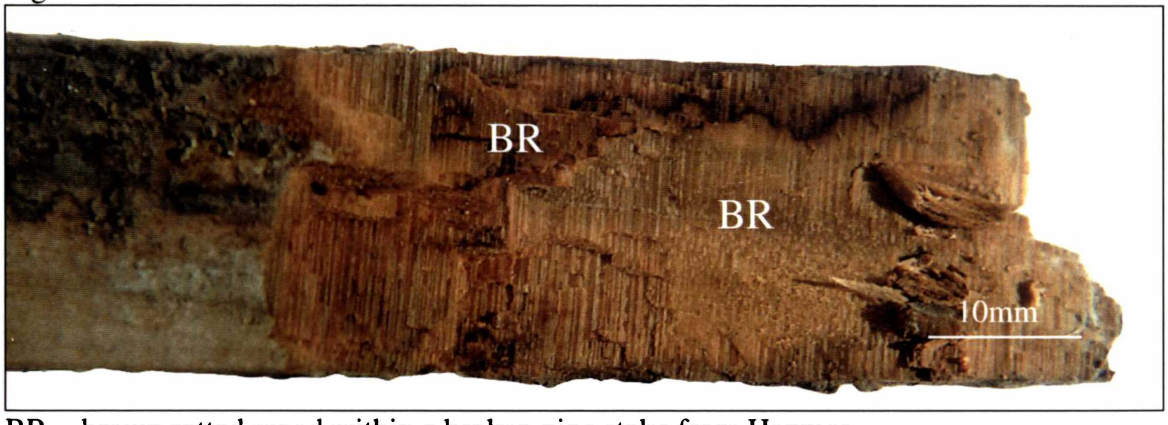
Type 1: brown rot (BR)

Figure 5.27



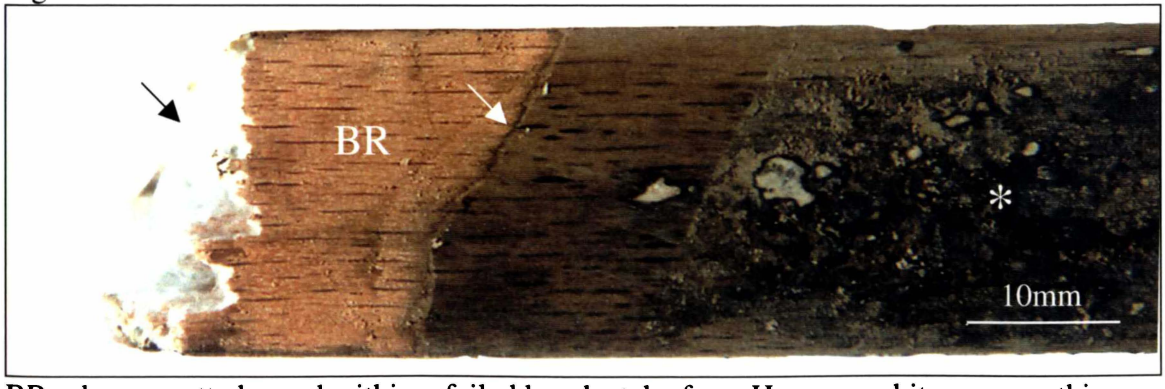
Failed PACQ4 stake from Hanmer with BR (only below ground portion visible); * = sound wood; arrow = border of decay and sound wood.

Figure 5.28



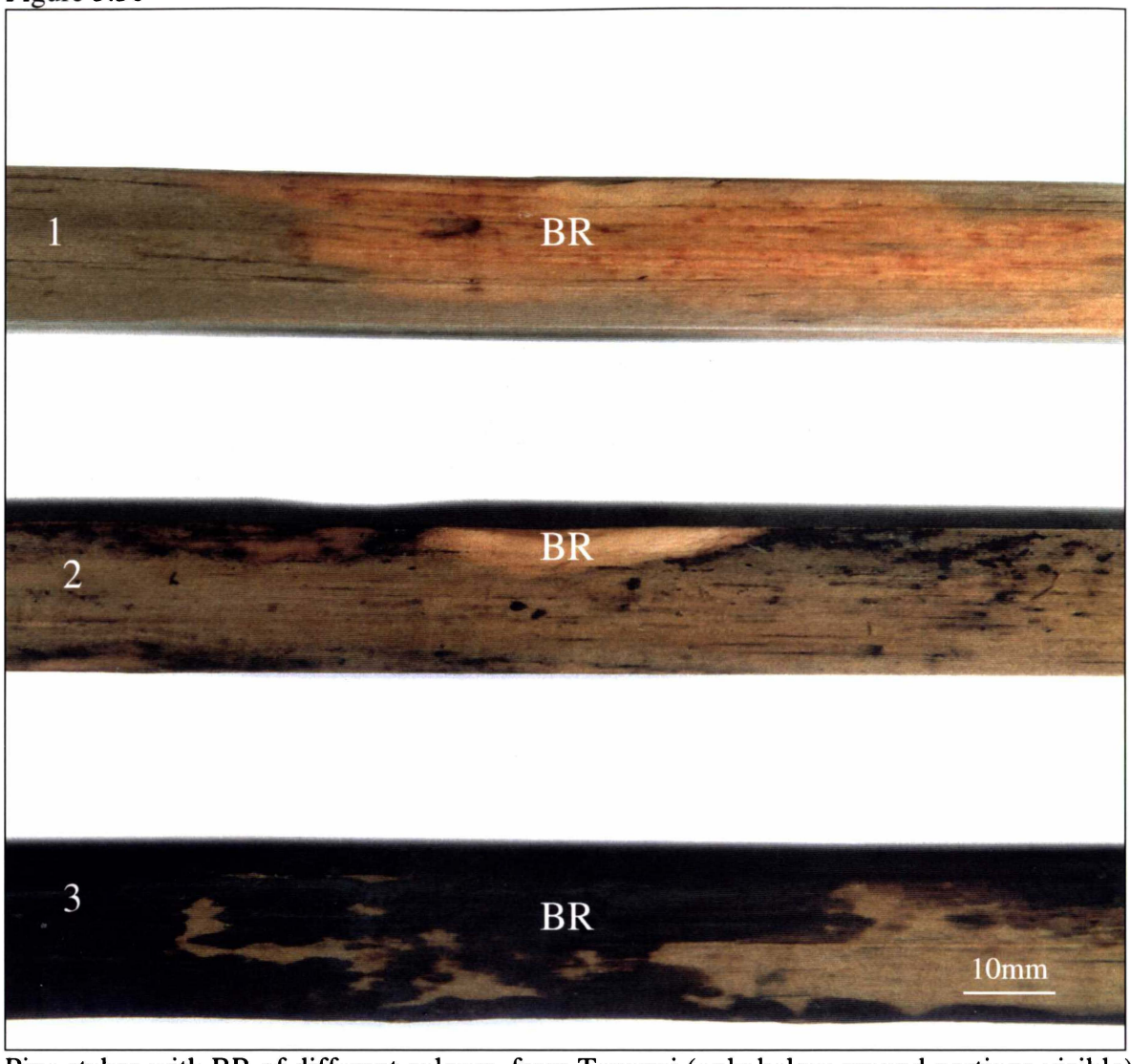
BR = brown rotted wood within a broken pine stake from Hanmer

Figure 5.29



BR = brown rotted wood within a failed beech stake from Hanmer; white arrow = thin (<1mm) shell of wood with SR overlying wood with BR; black arrow = mycelium that appeared several days after stake had broken; * = black mycelium.

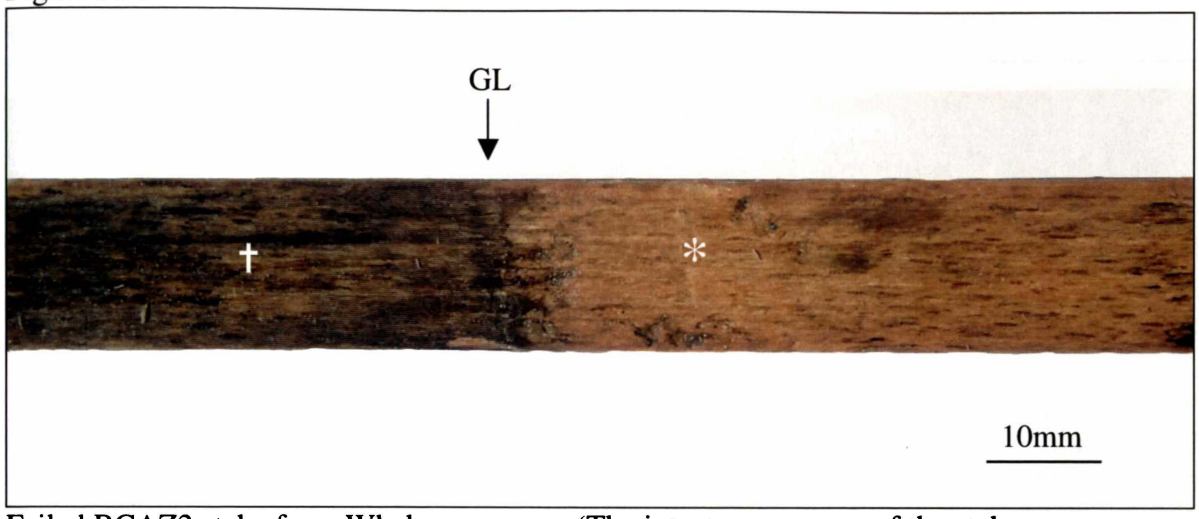
Figure 5.30



Pine stakes with BR of different colours, from Tapanui (only below ground portions visible). 1 and 2 are slightly bleached. 1 is a PACQ4 stake showing decolourisation of the green copper colour that occurred for all copper treated stakes with early brown rot at Tapanui. Stakes 1, 2 and 3 had soundness reduction values of 30%, 40% and 100% respectively.

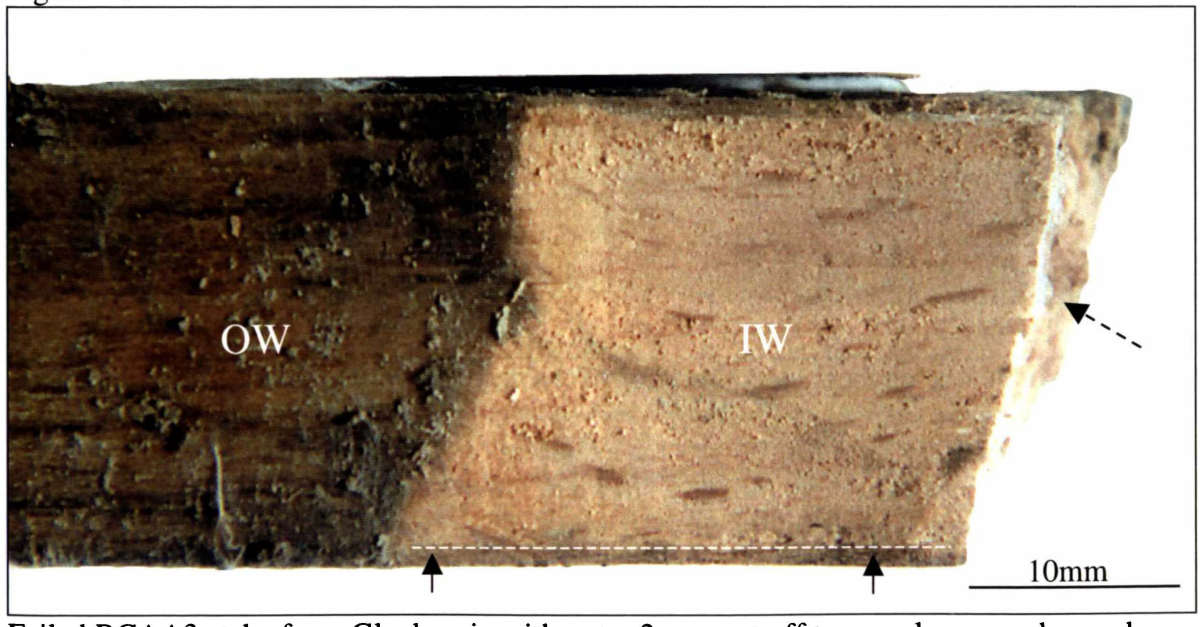
Types 2 and 2a

Figure 5.31



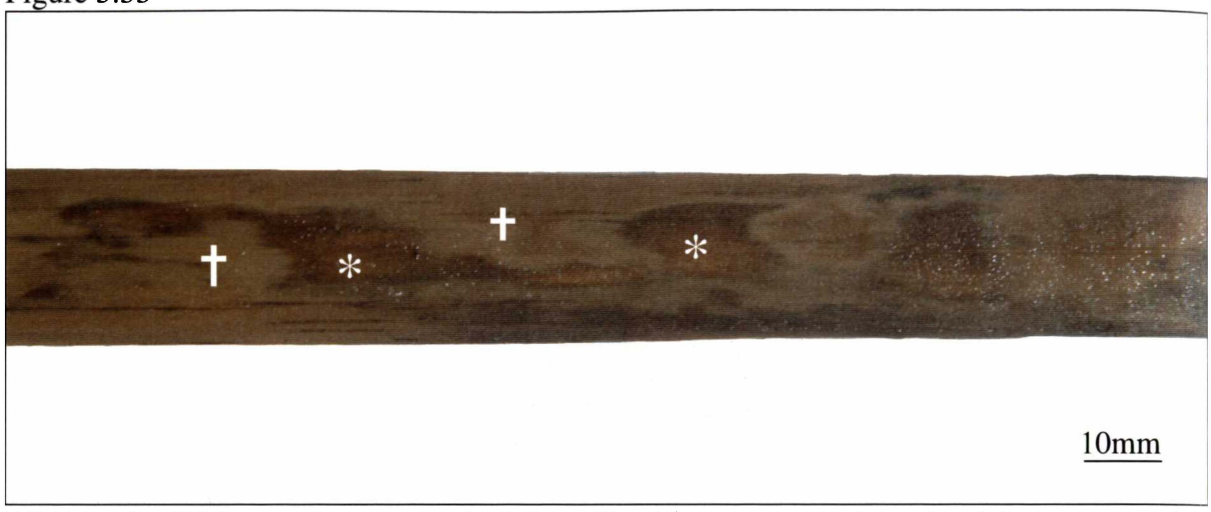
Failed BCAZ2 stake from Whakarewarewa. (The intact appearance of the stake was deceptive. Stakes of this appearance snapped to produce a carrot like fracture when tapped on a boot toe as this stake did after this photograph had been taken (see Figure 5.31). * = Decayed wood (below ground) was essentially the same colour before and after decay. Some areas were slightly lighter and some slighly darker but micromorphology was similar throughout all areas. GL = ground line; † = above ground portion had an outer shell (<1mm) of darkly stained wood associated with secondary moulds and soft rot. This overall appearance was very common at all sites for all treated beech stakes, especially the water borne preservatives, CCA and CAZ. A common variation to overall appearance occurred when the thin shell of stained wood extended to the below ground portions as was the case for the stake shown in Figure 5.31. However the internal wood remained a colour typical of types 2 and 2a.

Figure 5.32



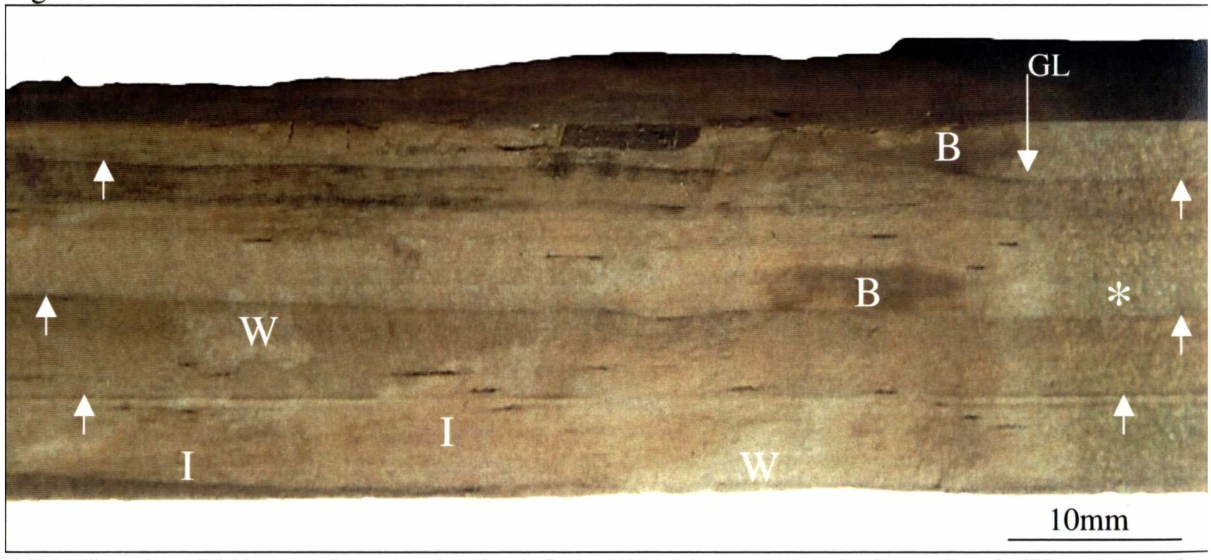
Failed BCAA3 stake from Glenbervie with outer 2 mm cut off to reveal spongy decayed wood, the colour of which was essentially unaltered by decay (below ground portion only). Dashed arrow = carrot fracture with white mycelium growing out of end grain. IW = inner wood; OW = outer wood showing surface darkening caused by secondary moulds and SR. Arrows and associated dashed line give position of outer shell of gray coloured wood with soft rot (1.5 mm thick).

Figure 5.33



PCAZ stake (20% soundness reduction) from Tomblesons mandarin orchard (below ground portion only). * = sound wood; † = decayed wood was slightly lighter in colour than sound wood. Apart from sporadic soft rot cavities, this stake was exclusively attacked by tunnelling bacteria (TB). The light brown colour, cheesy texture and distribution relative to sound wood was highly distinctive. However this pattern only occurred on PACQ and PCAZ stakes at a few sites where TB was very common. When other micromorphology associated with types 2 and 2a was present the patchy distribution of attack was lost, becoming more homogenous over the woods surface, and the colour was more variable (within the colour definition of types 2 and 2a).

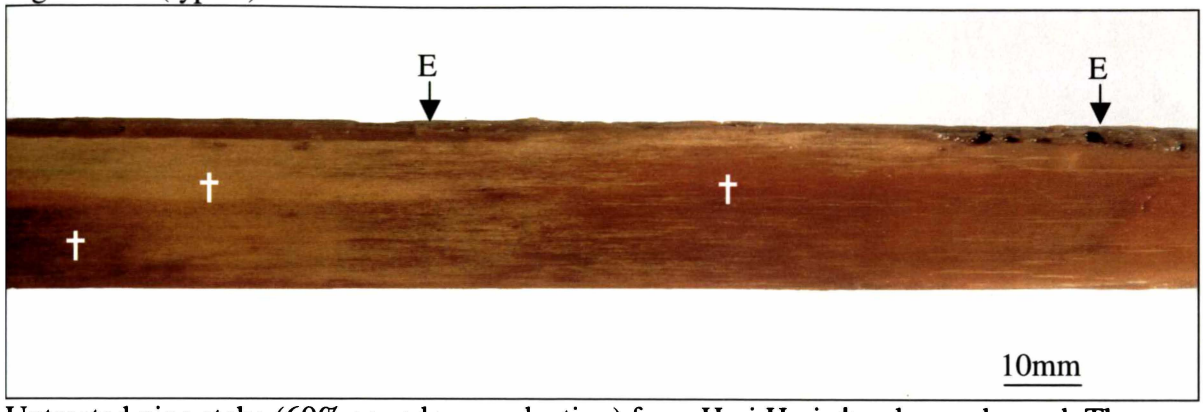
Figure 5.34



PCAZ3 stake (30% soundness reduction) from Tomblesons mandarin orchard. GL = ground line; * = sound wood; white arrow = boundary between early and late wood (wood grain). Apart from sporadic SR cavities, TB was the exclusive type of decay on this stake. This stake had been a similar appearance to the stake shown in Figure 5.32 prior to surface drying. Whilst colour change is common for decayed wood of most types after drying, the greatly increased heterogeneity of colour variation across the wood surface was an unusual feature of TB attack. Decayed wood changed from light brown to either dark brown (B), silvery white (W) or an intermediate colour (I). The silvery white decay pockets were typically shallower (<0.5mm) than the brown pockets. No difference in decay micromorphology (TB attack only), either before or after drying, was detected between pockets of different colour. Possibly the colour of decayed wood in shallow pockets was dominated by early decay characteristics

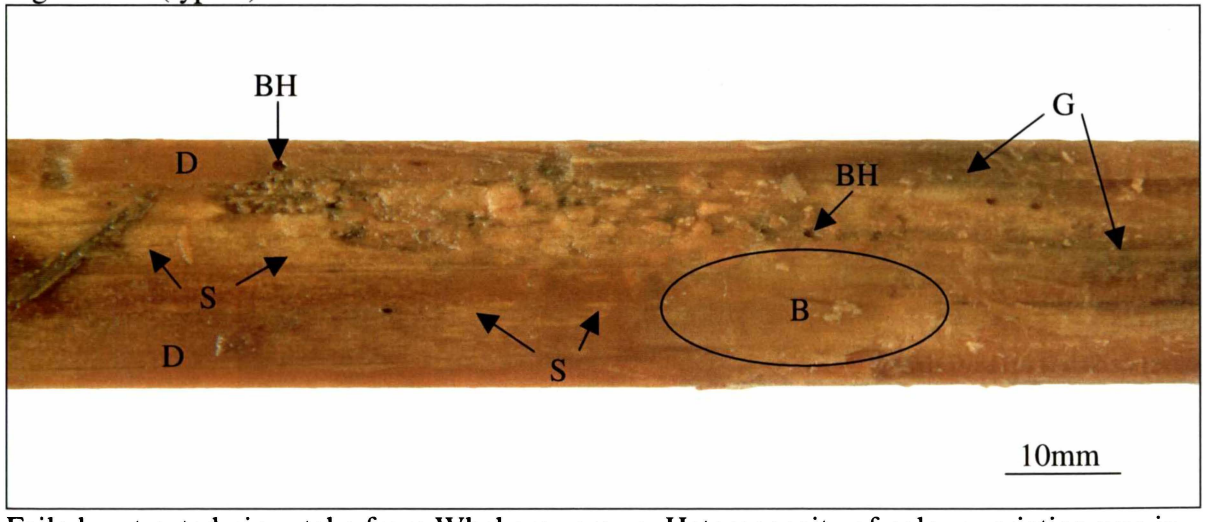
that may have involved bleaching, as was also suggested by decayed wood with some types of early brown rot attack which was also brown in more severely decayed wood (Figure 5.29).

Figure 5.35 (type 2)



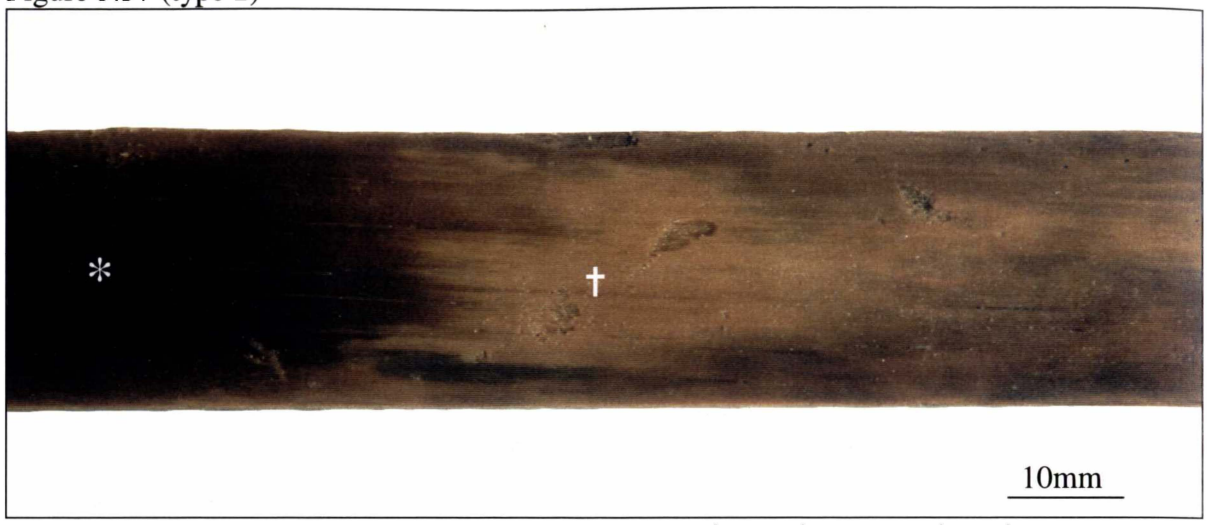
Untreated pine stake (60% soundness reduction) from Hari Hari. † = decayed wood. The heterogeneity of colour exhibited by this stake was deceptive in terms of decay recognition since all areas had similar micromorphology. The lighter coloured areas contained air bubbles and the darker (orange-brown) areas were saturated with water. The darker wood changed to the lighter colour if surface water was squeezed out with a fingernail. The intact appearance of the stake was also deceptive since the entire surface was soft. Only the edges in some areas were showing signs of erosion, and also contained evidence of borer (E). (This stake was not an original untreated pine stake installed during the first year but had been installed in year 5 to mark the position of a stake that had been removed earlier for microscopy and not returned. Most untreated stakes had failed after 2 years and therefore it was necessary to use reinstalled stakes to determine micromorphology during the last 2 years of the study.)

Figure 5.36 (type 2)



Failed, untreated pine stake from Whakarewarewa. Heterogeneity of colour variation was in part due to water content of decayed wood, as explained for Figure 5.34, and partly due to slight bleaching. D = water saturated wood; S = surface water partly removed; B = lightly bleached wood within ring. Decay micomorphology, whilst highly varied overall and representative of several decay types, was similar in all areas irrespective of colour variations. BH = borer hole. A slight gray stain was present in some areas (G). Stained wood contained brown hyphae, possibly belonging to a sapstain fungus.

Figure 5.37 (type 2)



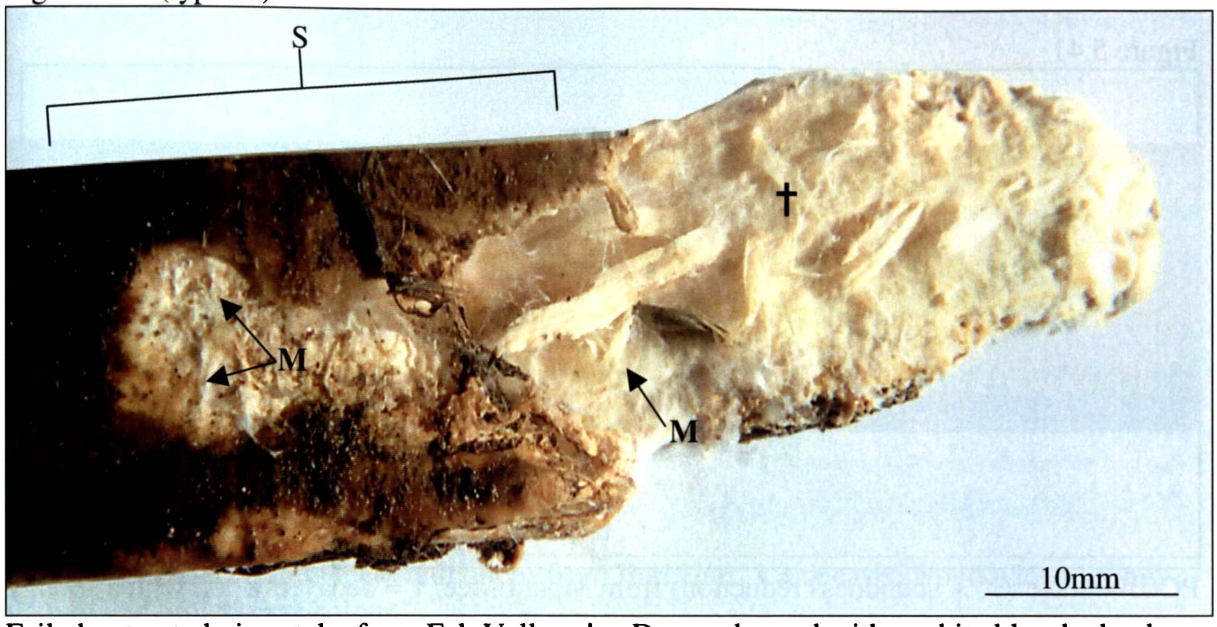
PACQ4 stake (30% soundness reduction) from Totara flat (below ground portion). * = sound wood (dark colour was caused by preservative treatment of late wood). † = decayed wood with a cheesy-spongy texture and lighter colour than sound wood.

Figure 5.38 (Type 2a)



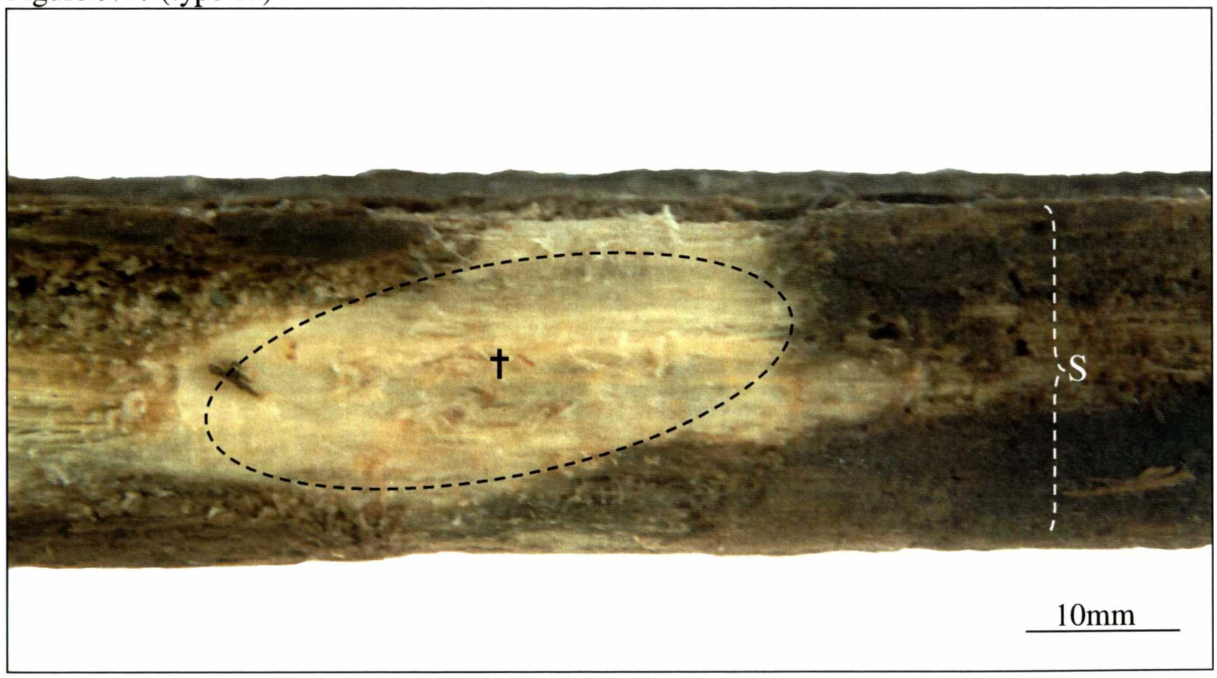
PACQ3 stake (40% soundness reduction) from Esk valley. *1 = sound wood. *2 = sound wood with surface layer cut away to reveal colour of internal wood; L = late wood; E = early wood; white arrow = late wood-early wood boundary; black arrow = fungal mycelium; † = decayed wood with a fibrous texture and bleached colour.

Figure 5.39 (type 2a)



Failed untreated pine stake from Esk Valley. † = Decayed wood with a white bleached colour and fibrous texture; M = fungal mycelium; S = shell of sound wood (<0.5mm thick) overlying decayed wood).

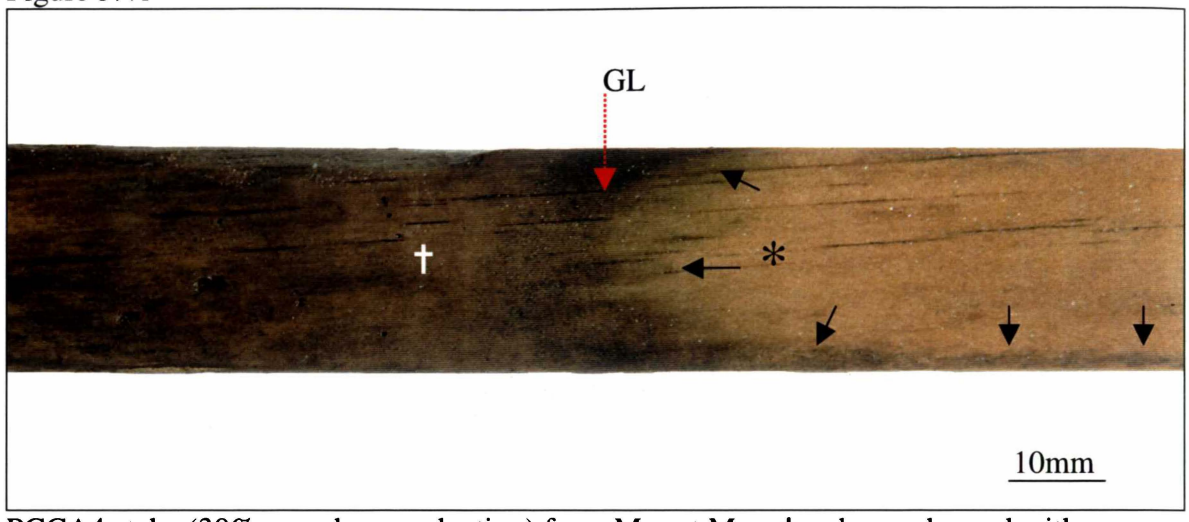
Figure 5.40 (type 2a)



BCCA3 stake (40% soundness reduction) from Cannal Creek (below ground portion only). † = decayed type 2a wood with a fibrous texture and bleached white colour. S = shell (2mm thick) of gray soft rot (type 3) overlying type 2a decay.

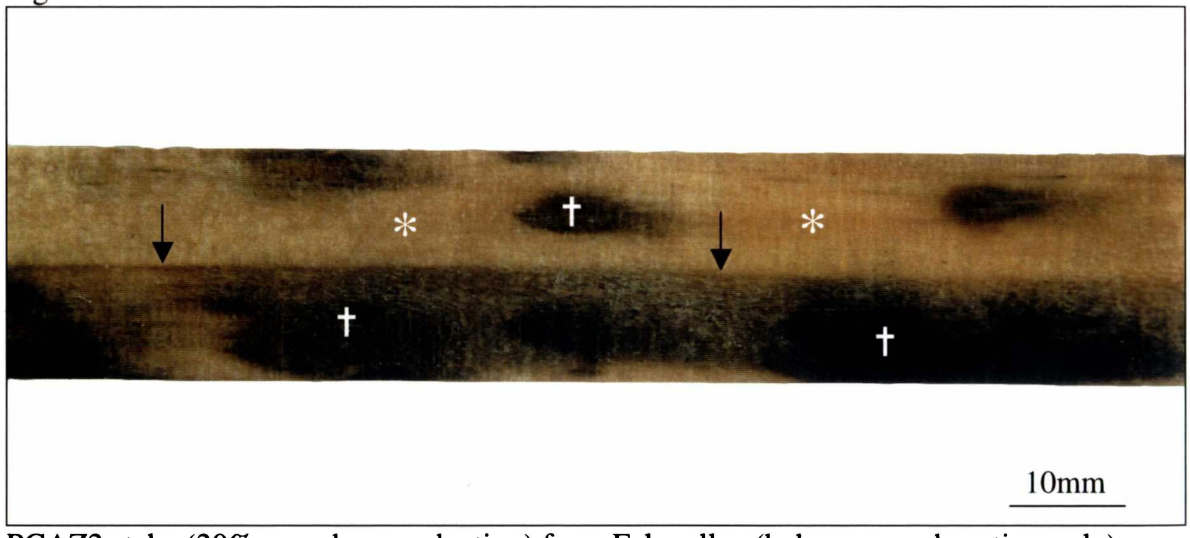
Type 3 (soft rot)

Figure 5.41



PCCA4 stake (30% soundness reduction) from Mount Mee. † = decayed wood with a spongy texture and gray-black colour. * = sound wood. Black arrows mark decayed wood boundary. GL = ground line.

Figure 5.42



PCAZ2 stake (20% soundness reduction) from Esk valley (below ground portion only). † = pockets of decayed wood with a spongy texture and black colour. * = sound wood. Arrow = late wood-early wood boundary.

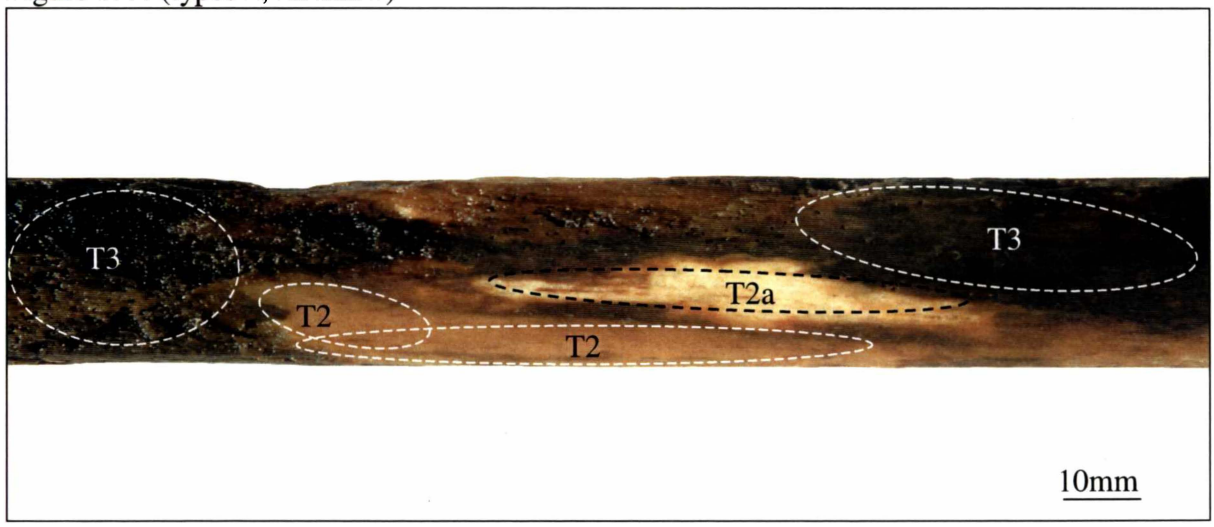
Figure 5.43



PACQ3 stake (30% soundness reduction) from Hari Hari. † = decayed wood with a spongy texture and brown-gray colour.

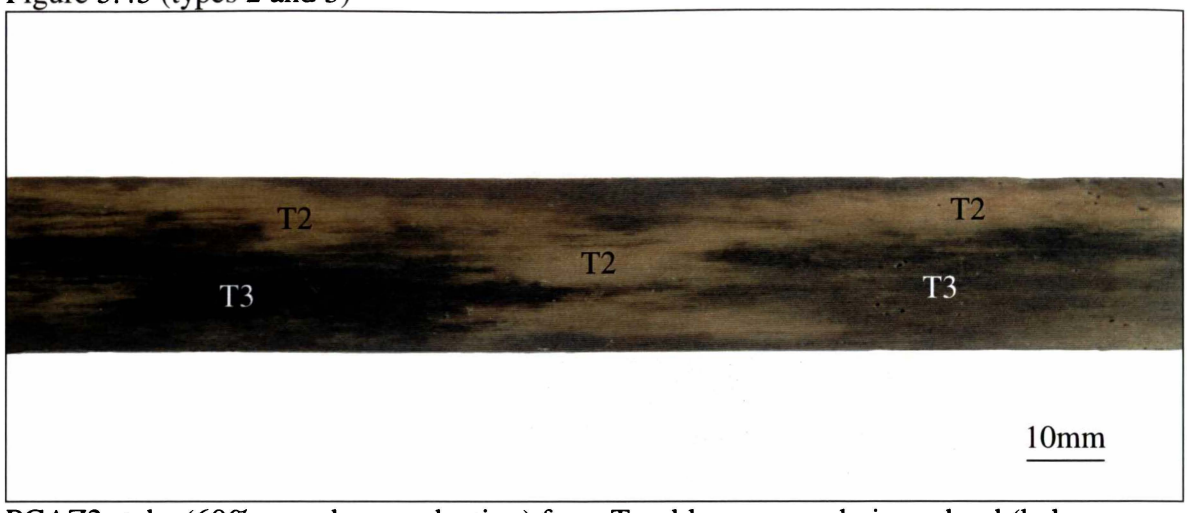
Stakes with mixtures of different decay types

Figure 5.44 (types 2, 2a and 3)



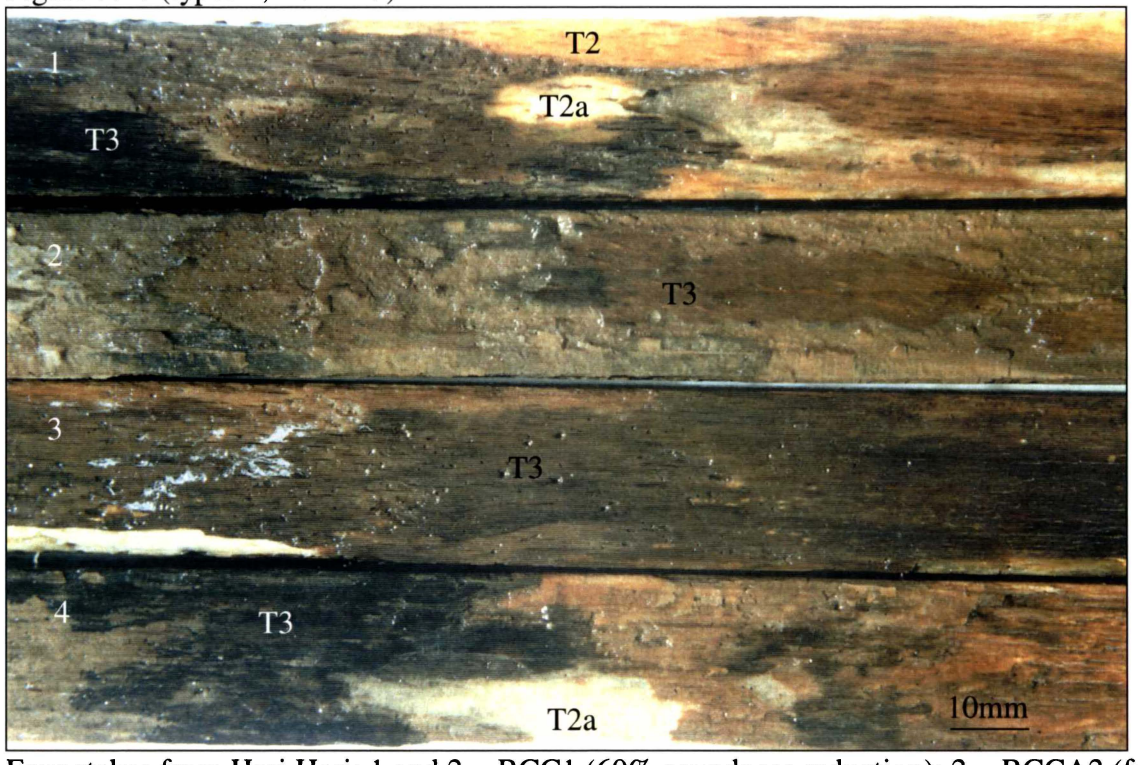
BCCA3 stake (60% soundness reduction) from Cannal Creek. T2 = type 2; T2a = type 2a; T3 = type 3. Dotted lines mark approximate position of decay type boundaries. Areas between boundaries contained a mixture of micromorphologies of respective types within boundaries. Pockets of T2 and T2a were typically deeper and often occurred underneath an overlying shell of T3. T3 often had an overlying layer of mould that may also have been the causative SR fungus, as was suggested for this stake.

Figure 5.45 (types 2 and 3)



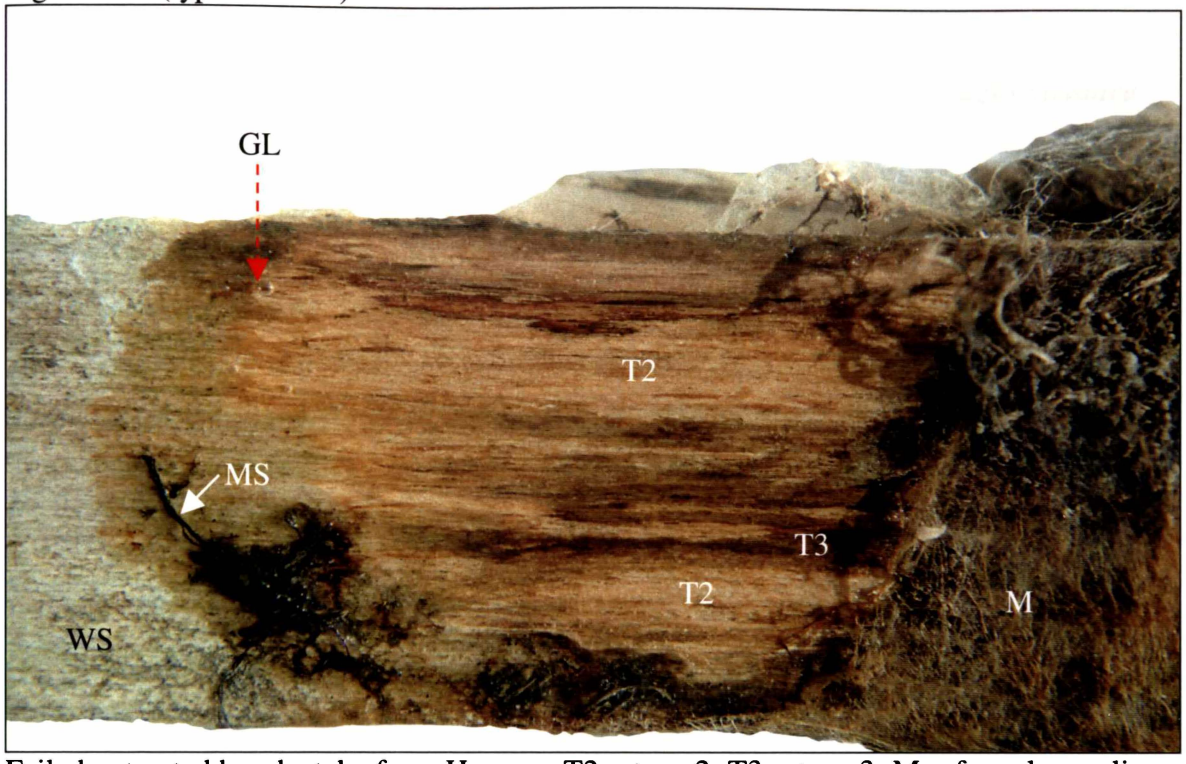
PCAZ2 stake (60% soundness reduction) from Tomblesons mandarin orchard (below ground portion only). T2 = type 2; T3 = type 3.

Figure 5.46 (types 2, 2a and 3)



Four stakes from Hari Hari: 1 and 2 = BCC1 (60% soundness reduction); 2 = BCCA2 (failed) and 4 = untreated beech (failed). T2 = type 2; T2a = type 2a; T3 = type 3. Whilst the micromorphology of these stakes was not determined, stakes with such complex mixtures of decay types have large areas of decayed wood between pockets of a single type, containing mixtures of micromorphological features representative of neighbouring individual pockets.

Figure 5.47 (types 2 and 3)



Failed untreated beech stake from Hanmer. T2 = type 2; T3 = type 3; M = fungal mycelium; Ms = mycelial strand; GL = approximate position of groundline; WS = weathered surface wood. The complex mixture of decay pockets which merge into one another for this stake was unusual and only occurred for untreated beech at a few sites, of which Hanmer was the most complex. The precise nature of micromorphology in untreated beech at Hanmer was not elucidated since all stakes failed in the first year and the severity of decay prevented examination of diagnostic early decay.

CHAPTER SIX

Effect of site on preservative depletion from *Pinus radiata* and *Fagus sylvatica*

Section 6.1

Introduction

The objective was to determine the significance of site and wood species on preservative depletion for a traditional preservative (CCA) and a candidate replacement preservative (CuAz). The rationale for selection of sites likely to possess diverse preservative depletion hazards is explained in chapter 3. Only stakes treated with the highest retentions were studied:

- 0.72% m/m a.i. CCA (Tanalith C) treated radiata pine,
- 0.42% m/m a.i. CCA (Tanalith C) treated European beech,
- 0.59% m/m a.i. copper plus tebuconazole (25:1) (Tanalith E) treated radiata pine,
- 0.56% m/m a.i. copper plus tebuconazole (25:1) (Tanalith E) treated European beech.

Section 6.2

Results

Predicted CCA, Cu and tebuconazole in stakes before exposure and actual retentions determined by chemical analysis after 5.5 and 4.5 years exposure for pine and beech respectively, and the residual percentage, for all sites are given in Section 6.5 (Appendix 6) in Tables 6.3 – 6.6. A close correspondence of predicted and actual retention was demonstrated for unexposed stakes of PCCA5 (5% deviation), PCAZ4 (0.7% deviation) and BCAZ2 (1.8% deviation) (Tables 2.2, 2.5 and 2.8 rspectively). This established a high degree of confidence that predicted retentions for exposed stakes chosen for depletion analysis were close to the actual retentions at the commencement of exposure. As explained in Section 2.1.2, the quantity of Tanalith C added to the treatment solution used for BCCA was unknown and the discrepancy between predicted and actual suggested a mistake had been made during preparation of the treatment solution. On the basis that the actual mean retention determined by

chemical analysis was 0.43 instead of the predicted 0.75 for the stakes analysed (see Table 2.6), an adjusted predicted retention was calculated for exposed stakes as 0.72 x 0.43/0.75 = 0.42% m/m a.i. where 0.72 was the predicted retention for exposed stakes.

Mean residual preservative active ingredient after exposure for each site and standard deviations are given in Table 6.1 for CCA and in Table 6.2 for CuAz treated pine and beech. ANOVAR and LSD statistical analysis were performed to test the significance of wood species (Table 6.7) and site (Tables 6.8 and 6.9) and are given in Appendix 6 (Section 6.5). Effects of above and below ground exposure, wood species and site were profound and were illustrated graphically (Figures 6.1 – 6.12).

Figure 6.1: Mean percentage residual CCA in above and below ground portions of pine and beech stakes after 5.5 and 4.5 years respectively across all sites (59 pine and 51 beech stakes)

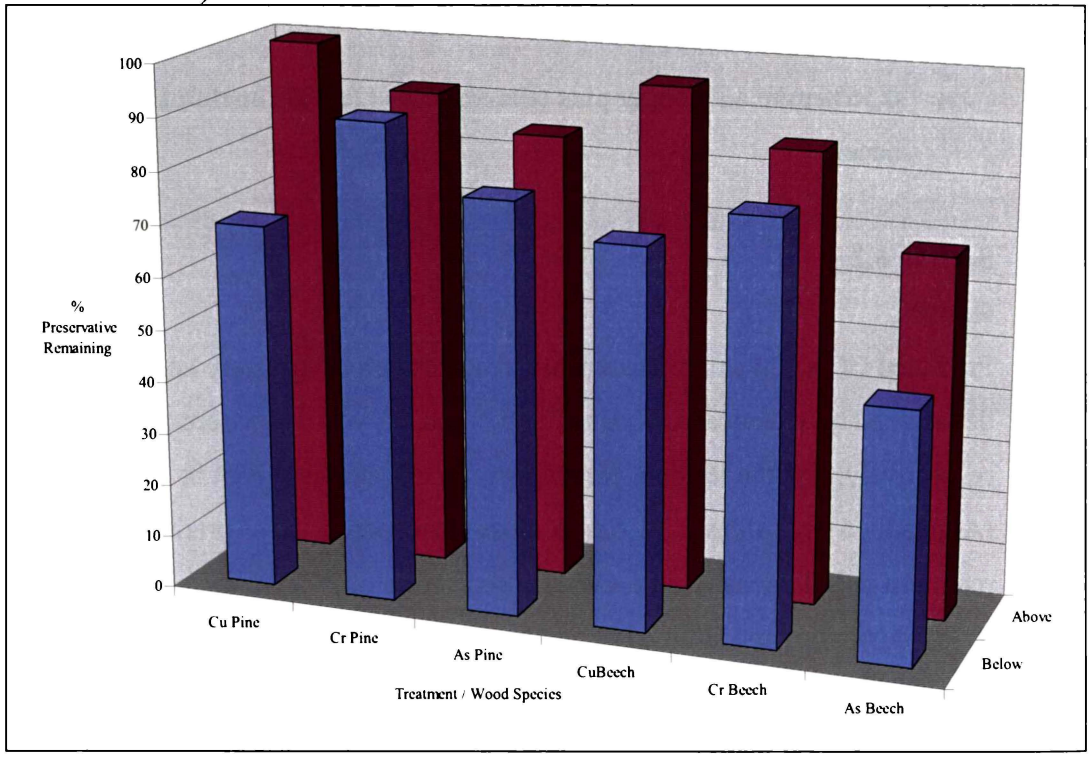


Table 6.1: Mean percentage residual CCA in pine and beech stakes after 5.5 and 4.5 years respectively

Site	Pine					
	Above Ground			Below Ground		
	Cu	Cr	AS	Cu	Cr	AS
MM	123.26*	114.03*	104.88*	103.83*	106.26*	97.08
OAK	107.29*	98.46	91.58	82.40	97.46	88.19
TOM	106.24*	98.64	93.78	94.08	99.76	86.90
GBF	110.59*	90.94	89.52	85.31	85.97	76.91
CC	92.86	81.73	70.54	45.16	76.67	57.97
HH	80.02	90.97	82.49	38.72	85.09	67.62
HAN	101.53*	87.57	78.53	81.41	89.55	81.31
TOT	73.70	78.33	69.27	26.93	77.68	59.22
GB	90.36	87.90	81.41	47.72	88.03	70.75
ESK	106.49*	83.77	81.04	96.58	87.85	80.39
TAP	100.70*	85.68	81.87	63.14	87.11	82.76
WKA	90.17	96.37	88.59	81.62	100.95*	86.92
PT	114.59*	98.22	93.07	57.30	96.21	78.97
Mean	**99.83**	**91.74**	**85.12**	**69.55**	**90.66**	**78.08**
SD	**18.87**	**11.67**	**12.39**	**29.29**	**12.42**	**15.57**
n	**59**	**59**	**59**	**59**	**59**	**59**
	Beech					
OAK	93.46	75.51	66.46	80.47	72.94	53.10
TOM	98.89	88.13	79.23	100.55	96.25	65.87
GBF	100.71*	92.05	72.55	81.95	88.85	56.71
HH	85.48	80.56	56.17	63.38	73.63	40.52
HAN	89.40	76.65	62.05	84.52	76.52	53.07
TOT	80.60	69.10	54.40	58.54	67.05	32.41
GB	95.14	87.94	64.78	76.16	83.96	47.18
TAP	105.00*	93.90	85.52	74.85	85.78	60.13
WKA	105.54*	99.30	64.12	70.82	74.23	33.63
PT	104.73*	95.09	78.55	27.54	71.08	28.64
MM	95.90	85.82	68.38	71.88	79.03	47.13
Mean	**95.90**	**85.82**	**68.38**	**71.88**	**79.03**	**47.13**
SD	**16.13**	**14.90**	**16.57**	**22.54**	**13.41**	**16.70**
n	**51**	**51**	**51**	**51**	**51**	**51**

SD = standard deviation across all stakes (SDs for predicted and actual retentions for all stakes is given in Table 6.3); n = number of stakes analysed. *% preservative remaining was in some cases greater than 100% because predicted retention before exposure was less than actual retention after exposure. This may have been due to loss of wood density during exposure. Most stakes selected for chemical analysis were rated as 100% sound but slight decay might have been undetected or wood may have been weathered, causing loss of wood density and a corresponding increase in retention (grams of preservative/100g wood). Therefore above ground losses were probably greater than the data suggested. Photodegradation may have reduced outer wood density thereby elevating residual values.

Key results of (Figure 6.1) are:

1. The above ground portion of CCA treated pine had lost less than 1% Cu compared to 8% for Cr and 15% for As.

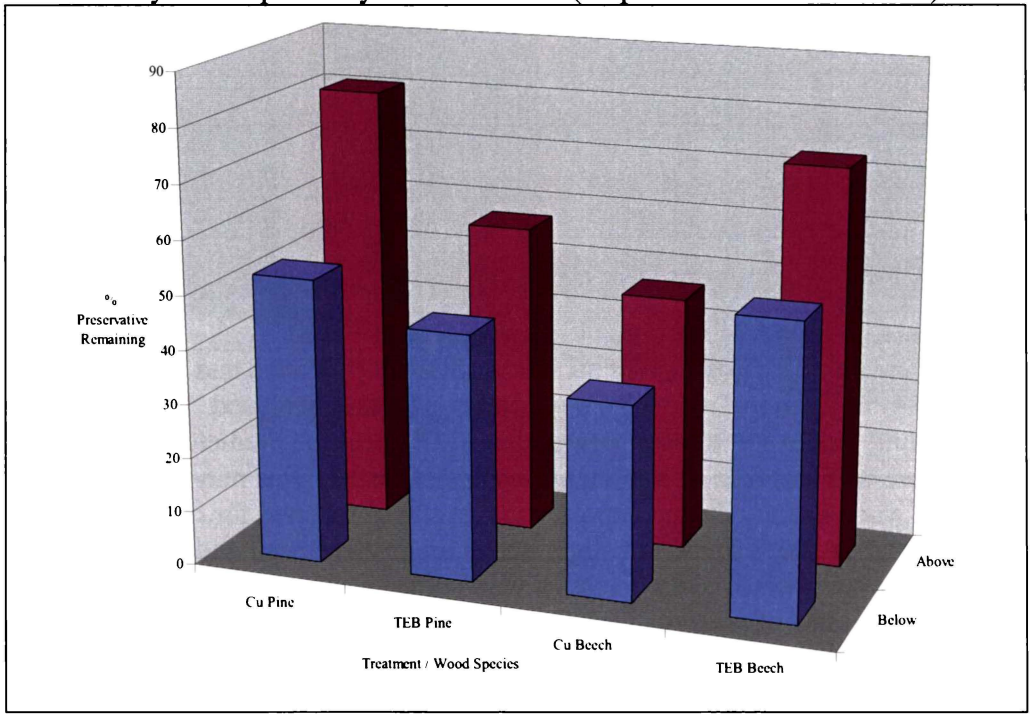
2. Increased Cu (30%) loss for the below ground portion compared to above ground portion (<1%), was much greater than for Cr (8 versus 9%) and As (15 versus 22%).

3. Cr loss was the least affected by below ground exposure for pine and beech.

4. Whereas Cu loss was most affected by below ground exposure for pine, for beech As was most affected.

5. All three elements were more susceptible to loss from beech than pine for both above and below ground exposure, except below ground Cu (30% loss for pine and 28% for beech). Arsenic in particular was more susceptible to loss from beech for both above ground (32% for beech versus 15% for pine) and below ground (53% for beech versus 22% for pine).

Figure 6.2: Mean residual tebuconazole (TEB) and Cu in pine and beech stakes after 5.5 and 4.5 years respectively across all sites (56 pine and 58 beech stakes)



Key results (Figures 6.1 and 6.2)

1. Copper loss from CuAz treated pine was greater than for CCA treated pine for both above ground exposure (19% for CuAz versus <1% for CCA) and below ground exposure (47% versus 30%).

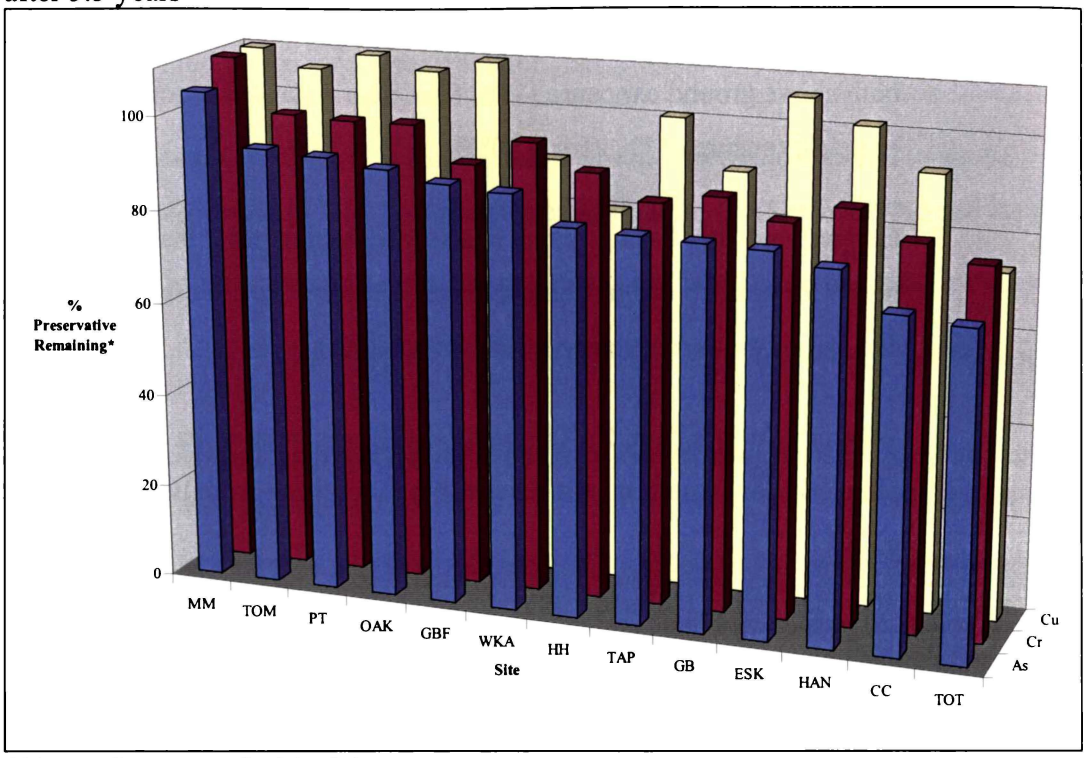
2. Copper loss from CuAz treated beech was much greater than for CCA treated beech for both above ground exposure (53% for CuAz versus 4% for CCA) and below ground exposure (64% versus 28%).

3. Whereas Cu loss from CuAz treated beech was greater than for CuAz treated pine, tebuconazole was less susceptible to leaching from beech compared to pine.

Table 6.2: Mean percentage residual copper and tebuconazole in pine and beech stakes after 5.5 years and 4.5 years respectively

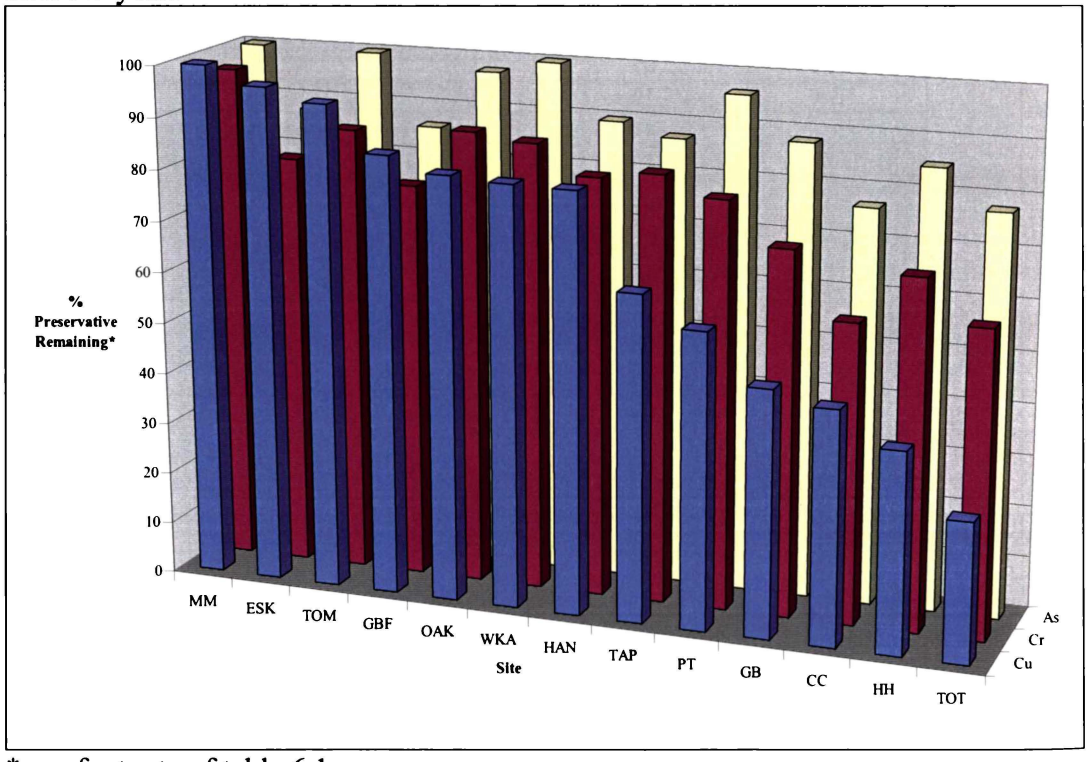
Site	**Pine**			
	Above Ground		**Below Ground**	
	Copper	**tebuconazole**	**Copper**	**tebuconazole**
PT	75.25	62.67	13.31	40.99
WKA	86.53	69.25	70.39	48.06
TAP	74.35	65.39	51.58	50.85
ESK	91.40	45.61	71.69	39.29
GB	78.20	45.00	42.23	39.69
TOT	73.77	46.81	30.68	38.04
HAN	82.01	47.01	67.75	33.65
HH	75.10	47.39	36.60	38.47
CC	76.44	31.12	53.98	29.40
GBF	86.34	49.27	64.78	46.36
TOM	81.81	85.83	65.09	72.54
OAK	89.98	94.28	63.23	67.28
Mean	**80.93**	**57.46**	**52.60**	**45.38**
SD	**9.56**	**22.05**	**19.73**	**15.05**
n	**56**	**56**	**56**	**56**
	Beech			
OAK	37.95	66.48	33.53	52.55
TOM	56.86	76.04	35.66	63.80
GBF	58.45	79.78	48.04	57.95
HH	33.78	69.34	21.02	44.84
HAN	31.42	73.94	28.38	63.09
TOT	34.31	70.32	26.51	39.13
GB	56.38	73.93	37.39	49.16
ESK	33.24	57.48	31.33	50.32
TAP	58.72	77.02	45.68	55.93
WKA	58.76	78.84	49.29	61.17
PT	55.90	82.15	37.42	50.24
Mean	**46.89**	**73.21**	**35.84**	**53.47**
SD	**10.58**	**13.98**	**19.34**	**11.78**
n	**58**	**58**	**58**	**58**

Figure 6.3: Mean percentage residual CCA in the above ground portion of pine stakes after 5.5 years



*%: see footnote of table 6.1.

Figure 6.4: Mean percentage residual CCA in the below ground portion of pine stakes after 5.5 years



* see footnote of table 6.1.

Key results (Figures 6.3 and 6.4) are:

1. Below ground CCA loss was much more variable between sites than above ground loss indicating that inter-site above ground effects, such as rainfall on loss were of less significance than below ground effects such as rainfall plus soil conditions.

2. The sites with the greatest CCA depletion hazard were either wet (Canal Creek (CK), Hari Hari (HH) and Glenbervie (GB)) or acid (Peat (PT)) or both (Totara flat (TOT)).

3. The effects of site moisture and acidity were considerable for Cu loss but minor for Cr and intermediate for As.

The key inter-site effects on CCA loss for pine (Figures 6.3 and 6.4) were also observed for beech (see Figures 6.9 and 6.10 in Appendix 6, Section 6.5).

Figure 6.5: Mean percentage residual copper and tebuconazole in the above ground portion of pine stakes after 5.5 years

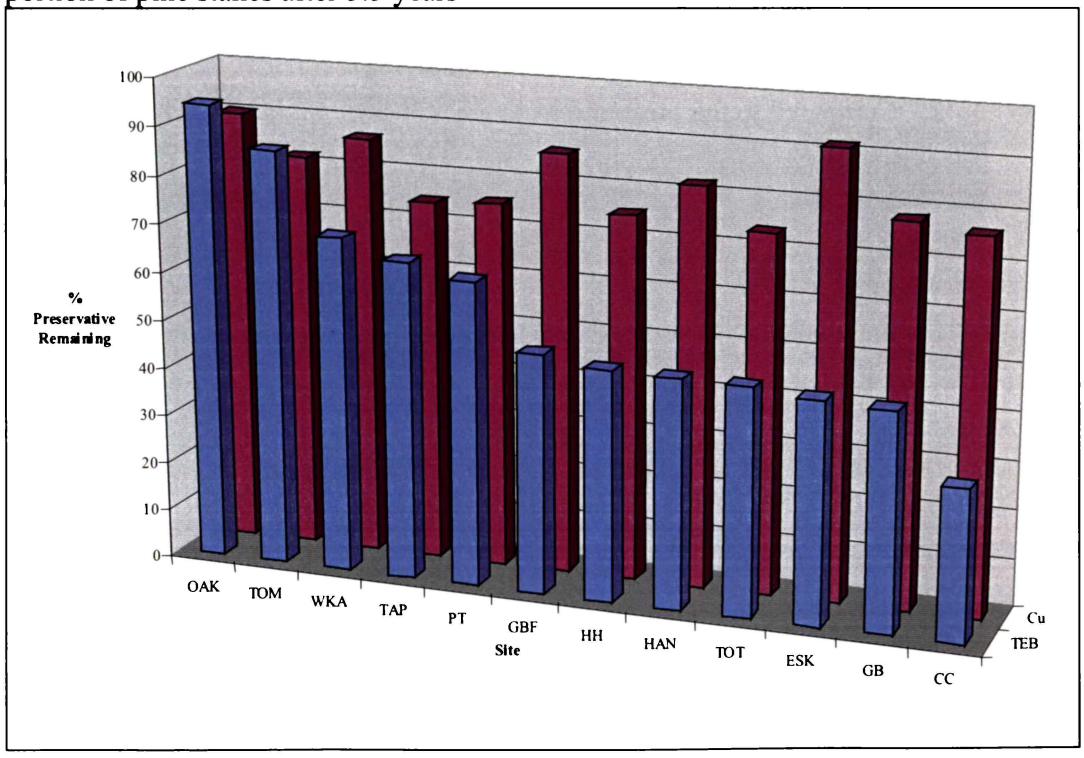
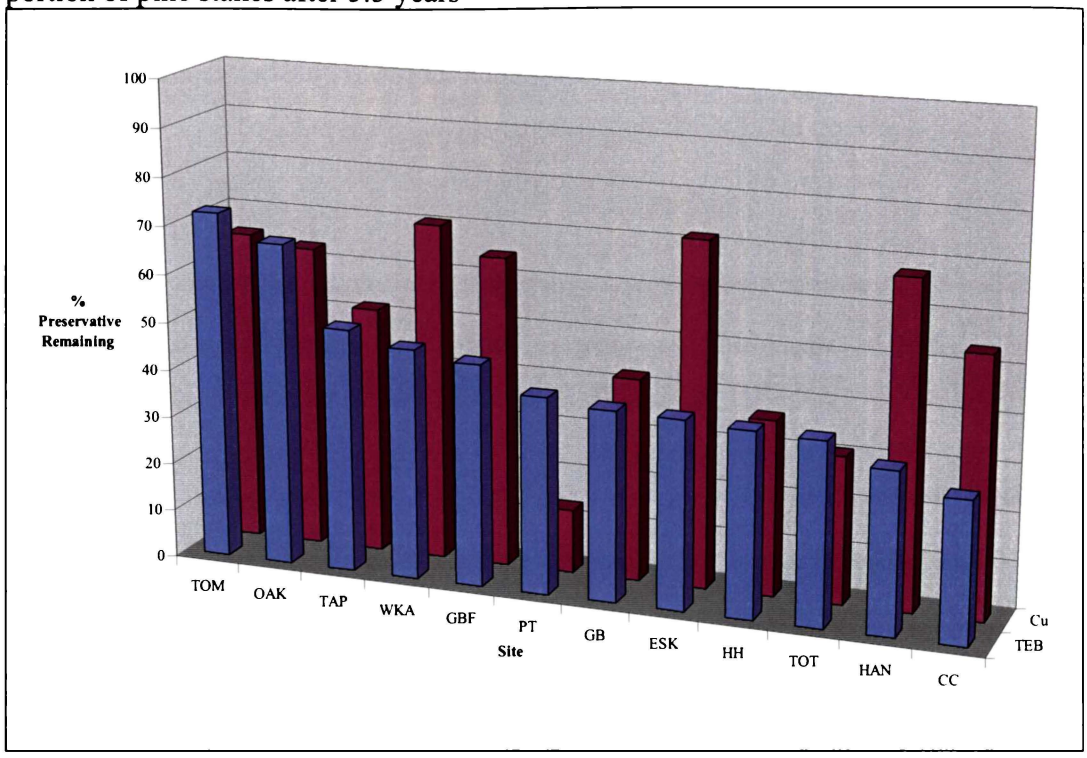


Figure 6.6: Mean percentage residual copper and tebuconazole in the below ground portion of pine stakes after 5.5 years



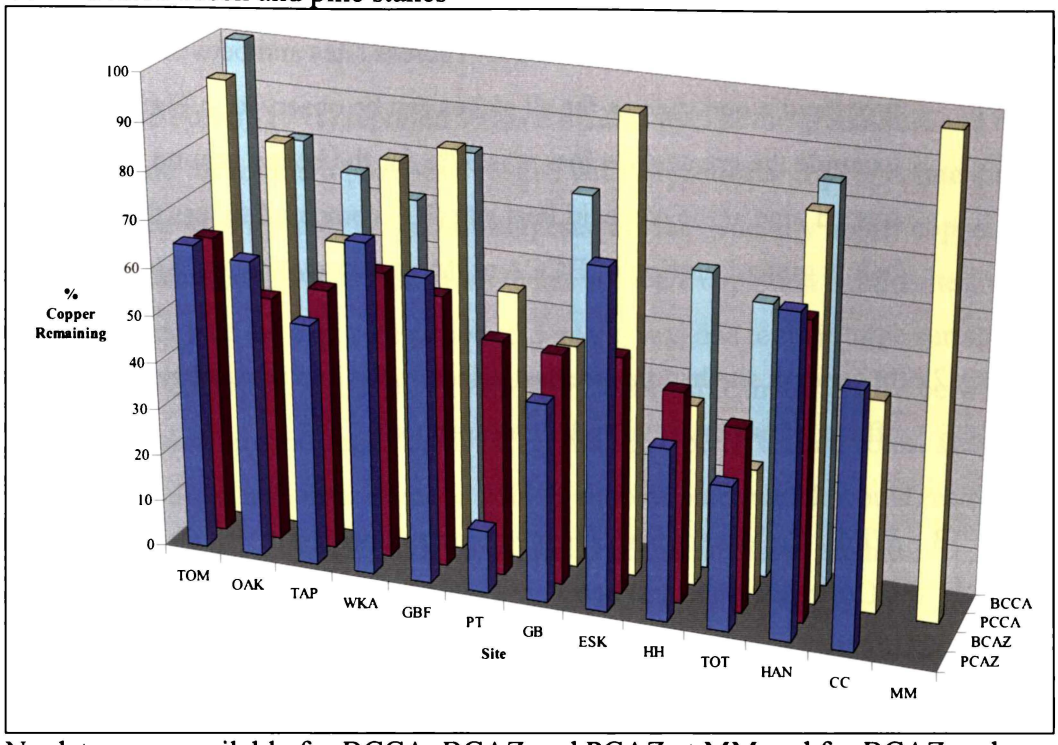
Key results (Figures 6.5 and 6.6) are:

1. The key inter-site effects listed for Cu loss from CCA treated pine (Figures 6.3 and 6.4) also applied to Cu loss from CuAz treated pine.

2. Compared to the profound increase in loss of Cu from the below ground portion compared to the above ground portion, this effect was less pronounced for tebuconazole, suggesting that the factors affecting loss below ground have proportionately less affect on tebuconazole than Cu. This was partly due to the greater effect of below ground effects on Cu loss but also because tebuconazole suffered relatively high loss above ground. This suggested that the above ground affects on preservative loss were more significant for tebuconazole than they were for Cu.

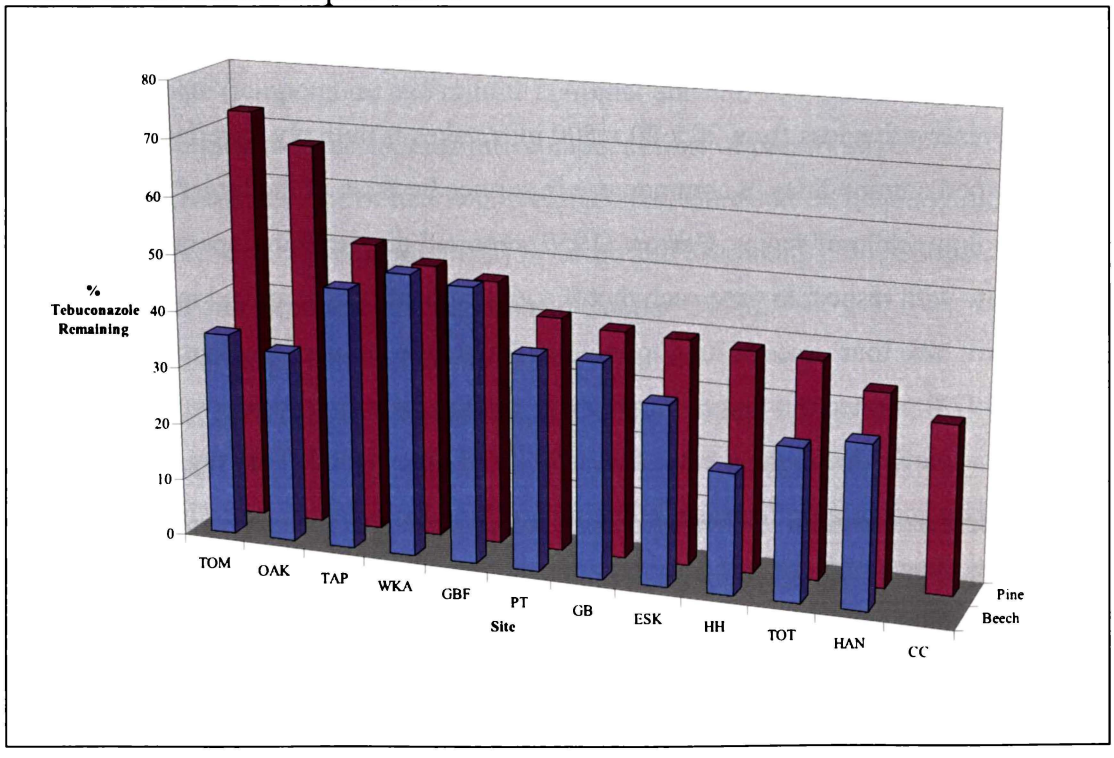
The key inter-site effects on tebuconazole loss for pine (Figures 6.5 and 6.6) were also observed for beech (see Figures 6.11 and 6.12 in Appendix 6, Section 6.5) except that the intersite differences were smaller for tebuconazole loss from beech were less than for pine.

Figure 6.7: Mean percentage residual copper in the below ground portion of CCA and CuAz treated beech and pine stakes



No data were available for BCCA, BCAZ and PCAZ at MM and for BCAZ and BCCA at CC.

Figure 6.8: Mean percentage residual tebuconazole in the below ground portion of CuAz treated beech and pine stakes



Key Results (Figures 6.7 and 6.8) are:

1. The profound variability of Cu loss across sites and between preservative types and wood species for all stakes can be observed in Figure 6.8. For example the greatest Cu loss was 87% for the below ground portion of CuAz treated pine at the wet acid Peat site (PT) compared to less than 1% for the below ground portion of BCCA at the arid stony site Mt. Mee (MM).

2. In general, site had the greatest effect on Cu loss variability (compared to the effects of preservative and wood species).

3. Whilst Cu loss for CuAz treated pine and beech was much greater than for CCA treated pine and beech, the size of the difference between them was highly dependent on site.

4. For CuAz treated wood the effects of all variables (site, preservative and wood species) on tebuconazole loss were less than for Cu loss, except the affect of **above**-ground exposure which caused greater tebuconazole loss.

Section 6.3

Discussion

Preservative loss from 20 x 20 x 500 mm stakes is unlikely to reflect loss from larger wood commodities in common use in service because of the larger surface area to volume ratio of stakes. Harrow (1959) reported that leaching operates by the square law with respect to time such that if a given quantity is removed in a certain time it will take four times as long to remove double the amount. Cockcroft and Laidlaw (1978) pointed out that earlier higher leaching rates from preservative treated timber was partly due to the greater accessibility of outer wood. This suggests that the outer wood acts as a buffer zone, reducing leaching from deeper wood by isolating it from the effects of close association with soil. Seventy five percent of the volume of a 20 x 20 x 500 mm stake is in the outer 5 mm zone compared to 10% for a 200 x 200 mm house pile. This is likely to result in a large proportion of the wood in a stake being

exposed to the primary mechanisms of preservative loss associated with close contact between wood cell walls and soil.

Johnson and Foster (1991) reported 79% residual copper at the groundline and 87% above ground for southern pine posts treated with ammoniacal copper borate that had been exposed for 11 years. This result suggests that the generally higher copper depletion rates from 20 x 20 x 500 mm stakes at severe depletion hazard sites, that occurred after half the exposure period (5.5 years) was due to their large surface area to volume ratio. Osborne and Fox (1995) found no significant difference between above and below ground depletion in CCA (AWPA type C) treated poles. This could be related to the small surface area to volume ratio of poles which would increase the effects of the putative "buffer zone" on reduced depletion below ground.

Wood preservative depletion rate is likely to be a complex phenomenon controlled by many factors including the following.

1. Soil chemistry (particularly acidity) and physical structure (Plackett, 1984; Archer and Jin, 1994; Wang *et al.*, 1998; Edlund and Nilsson, 1999; Cooper *et al.*, 2000; Schultz et al., 2000).
2. Climate (temperature and rainfall) (Archer and Jin, 1994; Le Bow, 1996).
3. Wood factors (species, moisture content at time of preservative treatment,presence of heartwood/extractives, latewood/earlywood ratio, compression wood, acidity etc.) (Warren and Solomon, 1990; Cooper, 1991; Kartel and Lebow, 2001; Kaldas and Cooper, 1996).
4. Wood drying method (Cooper *et al.* 1997).
5. Rate of preservative fixation (Ruddick *et al.*, 1994).

The main mechanisms of preservative loss probably involve, mass transfer (leaching) (Schultz *et al.*, 2000), cationic exchange with mineral ions (mainly those bound to clay micelles and humic molecules), complexation reactions and biodegradation. The rate of flux of preservative compounds and elements probably depends on the gradient, not only of the actual active preservative components themselves but other

constituents in and around the wood. For example, copper leaching may be affected by total soil content of heavy metals since this affects the overall gradient (Wang *et al.* 1998). Factors affecting preservative loss are likely to be highly interrelated. For example availability of cations for cationic exchange reactions is highly dependant on soil pH and therefore it is likely to be difficult to elucidate the individual contributions of soil cationic exchange capacity and pH to preservative loss. Further complexity is likely to arise from the precise chemistry of individual constituents of mass transfer, cationic exchange and complexation reactions. For example, organic acids have been shown to cause greater leaching than inorganic acids (Warren and Solomon, 1990; Cooper, 1991; Lebow, 1996) suggesting that factors beyond pH are important. Humic acid is known to undergo complexation reactions with many soil minerals and therefore the difference between organic and inorganic acids may be linked to a dual pH-complexation reaction mechanism occurring for organic acids that is absent for inorganic acids.

Unpublished data of Robert Franich (*Forest Research*) showed that radiata pine could be impregnated with silicate particles with a 9 nanometer diameter using a wood preservation pressure cylinder. Silicates of this size form colloids in solution, the micelles of which are typically larger than the individual particles. Silicate micelles in soil have a diameter as small as 10 nanometers and humus micelles are believed to be at least as small (Brady, 1984). Humic molecules and clay micelles have a high cationic exchange capacity (Brady, 1984) and this has been suggested as a mechanism of depletion of cationic preservative elements such as copper. At wet sites it is possible that colloidal clay and humus molecules will impregnate wood cell luminae. It is known that field test site stakes "wick" materials above the ground line (Eaton and Hale, 1993) and this may be an important mechanism of impregnation by colloidal minerals (e.g. clay) and organic substances (e.g. humus). The wet sites had a much higher loss hazard and it would be expected that high soil moisture would facilitate a wick action.

At very wet sites, wick action may be similar for small and large wood commodities but at the majority of sites where more moderate moisture conditions are present, the effect of wood commodity diameter is likely to be significant. The base of stakes is

typically associated with top soil, whereas for posts and poles it is usually placed within the subsoil layer. The capacity for water and associated colloid uptake is likely to be reduced at the base of a post seated in compacted subsoil if soil moisture is restricted. Therefore it is possible that colloids taking part in preservative active element depletion by cationic exchange reactions may act within cells beyond those in immediate soil contact and that the impregnation effects of a "wick" action may be more pronounced for small stakes compared to larger commodities in service.

It is generally recognised that preservative treated wood immersed in fresh water is susceptible to leaching of preservative because of the increased potential for preservative constituents to diffuse from wood and, or take part in chemical exchange reactions (Hochman, 1967; Irvine *et al.*, 1972; Lebow, 1996; Cooper *et al.*, 2000). It is also widely accepted that immersion in saline water (marine and estuarine) is a greater hazard than fresh water because solutes can take part in chemical exchange reactions with ionic wood preservative constituents (Richardson, 1993). It is known that use of saline estuarine water rather than water with a low or less reactive solute concentration can greatly reduce the service life of CCA treated radiata pine in cooling towers (Irvine *et al.*, 1972). Schultz *et al.* (2000) cited several publications in support of the statement that preservative depletion is greater in soil contact compared to water (Nicholas, 1988; Cooper *et al.*, 1997; Wang et al., 1998). However it is not clear from the literature that this is entirely correct since whilst above ground exposure is almost certainly a lower depletion hazard compared to below ground in the majority of situations, there are likely to be immersion situations which do not involve soil that are as severe as in-ground exposure. For example wood in water cooling towers is potentially a greater hazard than exposure to soil, if pH and solute concentration and type is not carefully controlled.

Plackett (1984) conducted laboratory leaching studies of ground CCA (Tanalith C) treated radiata pine and found that aqueous solutions of calcium chloride ($CaCl_2$), magnesium nitrate ($MgNO_3$) or calcium nitrate ($CaN0_3$) (0.03M-1.0M) increased copper loss compared to distilled water. For example % copper losses of 3, 13, 17, 23, 30, 33 and 36% were obtained for $CaCl_2$ at molar concentrations of zero, 0.19, 0.38, 0.75, 1.5, 2.25 and 3.00 respectively. Whilst losses from sawdust are likely to

be much higher than losses from stakes in the ground, Plackett (1984) concluded that the higher calcium and magnesium levels typical of horticultural soils was likely to increase loss of copper from CCA treated wood in service. However perhaps the method used by Plackett (1984) was misleading because the importance of clay and humus / ion interaction effects were not tested. In soil, cations are typically associated with the charged surfaces of colloidal clay and humus and free ions in solution are a small fraction of the total. Results of this study suggested that low pH of soil was a more important factor affecting loss, particularly copper loss, than presence in horticultural soils. For example loss of copper and arsenic from CCA treated wood at the horticultural sites Tombleson's mandarin orchard and Esk Valley vineyard were lower than occurred from wood at the majority of other sites, especially the waterlogged and or acidic sites (Peat and Hari Hari). Several authors have reported that low acid conditions caused by inorganic acids increase copper loss from CCA treated wood (Rennie et al., 1987; Cooper, 1988; Murphy and Dickinson, 1990; Eatvelde *et al.*, 1995). Eatvelde *et al.* (1995) simulated aquatic exposure conditions in laboratory leaching studies and showed that copper leaching was greatest under acid conditions and chromium and arsenic loss was greatest under neutral pH.

Murphy and Dickinson (1990) used a modification of the laboratory leaching method EN84 (1980) to investigate the effects of acid rain (sulphuric acid / ammonium nitrate based buffer solution) on CCA (American Wood Preservers Association type B and C and British Standard 4072 (1974) type 1) loss from pine blocks buried in soil. Up to 40% copper was lost from blocks after leaching in "rainfall" at pH 3.0 but no copper was lost at pH 5.6. Arsenic (20%) was lost from Type B CCA treated pine at pH's of 3.0 and 5.6 and to a lesser extent (12%) from BS 4072 Type 1 CCA treated wood but only at pH 5.6. Chromium was not leached from any CCA treated wood at pH 5.6, with only the "possible" loss of 6% chromium at pH 3.0 from wood treated with the BS 4072 Type 1 CCA. These results may not reflect loss from stakes in the field because the buffering capacity of soils used by Murphy and Dickinson (1990) would likely have had a significant impact on loss. Possibly the "permanently low" pH associated with a peat bog (Peat site) would have posed a greater leaching hazard than occurred under the conditions employed in the simulated leaching study.

As pointed out by Murphy and Dickinson (1990) the buffering capacity of the 3 soils would have raised pH of the acid "rainfall" at the woods surface. Sulphuric acid introduced into CCA treatment solutions has been shown to reduce durability (Fox *et al.*, 1987) due to increased copper leaching. The mechanism discussed involved absence of normal copper fixation reactions because low pH prevented reduction of chromium (VI) to chromium (III). These results and those of Dickinson and Murphy (1990) suggested that the fixation reactions that normally greatly reduce leaching of CCA can be inhibited and/or reversed under conditions of low pH. The majority of reports of the effects of low pH on CCA leaching from wood refer to inorganic acid induced low pH. However Clausen and Smith (1998) showed that 81% of copper oxide (CuO), 62% chromium trioxide (CrO_3) and 89% of arsenic pentoxide (As_2O_5) was removed from ground CCA treated (6.4kg/m^3 salt) decking after refluxing with oxalic acid (pH 2.0) for 24 hours at room temperature. Peat soils have been reported to have a pH as low as 2.8 (Brady, 1984) and therefore pH was almost certainly the major factor causing large losses of copper from CCA and CuAz treated pine and beech exposed at the Peat site. Kamden et al. (1996) reported that typically arsenic is the most susceptible to leaching from CCA treated wood and chromium is the least susceptible. However the studies of Eatvelde *et al.* (1995) , Clausen and Smith (1998) and Dickinson and Murphy (1990) suggested that under very acid conditions of below pH 3 copper is more susceptible to leaching than arsenic.

The results of this study showed that copper was more susceptible to leaching than arsenic at most sites tested. Possibly this is a reflection of the severity of leaching conditions that occur for small field test stakes, that do not occur for most preservative treated wood commodities which have a lower surface area to volume ratio. Of the three elements in CCA, the greatest difference between above and below ground loss occurred for copper. This suggested that the below ground factors that influence loss were of greater significance for copper. Conversely, chromium was more affected by above ground influences compared to arsenic and copper. The finding that chromium loss above ground was greater than copper loss for both pine and beech was surprising but its consistency across all sites may be indicative of a differential above ground effect. Possibly weathering depletion factors are important. A major affect of ultraviolet light on wood is preferential lignin degradation (i.e.

ahead of cellulose and hemicellulose). Lignin plays an important role during CCA fixation reactions and therefore the suggestion that chromium is affected more by above ground depletion mechanisms could be related to reversal of chromium-lignin fixation to a greater degree than occurs for copper-lignin fixation reversal, although an interaction effect may also be important.

The mechanism of CCA fixation described by Pizzi (1982) involved early adsorption and reduction of chromium at cellulose sites and subsequent complexation reactions with guaiacyl units of lignin ($CuCrO_4$, $CrAsO_4$, $Cr_2O_7^{2-}$, $HCrO_4^-$, $CrO4_2^-$). Whilst complexation of Cu^{2+} with lignin guaiacyl units also occurred, as did precipitation of $CrAsO_4$ onto cellulose, Pizzi's model suggested that the overall importance of lignin for complexation reactions of chromium appeared to be greater than for cellulose. This is probably an overly simplistic view of what is a complex series of reactions but if lignin is degraded ahead of cellulose, it is possible that this "releases" proportionately more chromium than copper. One of the potential problems with Pizzi's model is that it was derived using cellulose and lignin substrates under laboratory conditions. The accessibility and reactivity of hydrogen bonded crystalline cellulose, encapsulated in lignin, as occurs in wood cell walls is likely to be considerably less than occurred under the experimental conditions employed by Pizzi (1982). Therefore the lignin and hemicellulose may be of greater significance than implied by Pizzi's model in terms of reaction sites for CCA. Accordingly it might be expected that photodegradation of lignin could have important effects on CCA leachability although it is unclear why chromium loss was proportionately higher than arsenic and copper.

It is recognised that solution concentration of CCA treatment solutions during impregnation can affect subsequent susceptibility to leaching (Cockcroft and Laidlaw, 1978). The CCA retentions of wood employed in the leaching studies of Plackett (1984) and Murphy and Dickinson (1990) were typical of those used for ground contact and those used by Clausen and Smith (1998) were typical of above ground exposed situations such as occurs for decking. At these retentions the significance of treatment solution on subsequent susceptibility to leaching is unlikely to be of practical significance since wood treated with these retentions have an

established performance track record in the respective exposure situations described. Whilst this is also likely to have been the case for the highest retention of CCA treated pine used in this study, the retention achieved for beech was below that aimed for and below the retention that would typically be expected to perform under ground contact conditions (0.42% m/m a.i.). European beech is not usually used in ground contact, and therefore recommended retentions are not available but the NZTPA specifications (1986) require a minimum of 30kg/m^3 of CCA salt in the sapwood of Australian Eucalypt poles. Therefore the low retentions achieved for beech may have contributed to much greater CCA loss than is typical of hardwoods treated with higher CCA retentions.

Kamden *et al.* (1996) reported that wood species influences fixation of CCA and subsequent susceptibility to leaching. In particular, reduction of Cr(VI) to CR(III) was reported to occur at a faster rate in high extractive containing wood species and that there was a general relationship between the rate of fixation and subsequent susceptibility to leaching across all wood species i.e. the faster the fixation reactions moved to completion the greater the subsequent susceptibility to leaching. High tannin containing timbers have been reported to cause premature fixation of CCA in wood cell luminea thereby reducing effective treatment of the cell wall itself (Eaton and Hale, 1993). Possibly the macrodistribution of CCA treatment solution was poorer in beech due to the difficulty associated with penetration of fibre bundles (Eaton and Hale, 1993).

The findings of this study showed that at most field test sites copper loss from pine and beech treated with CuAz preservative was greater than copper loss from CCA treated pine and beech. Sundman (1984) showed that leaching of copper from pine (sawdust) treated with CCA and ammoniacal copper (described as a copper tetrammine system) was the same over the pH range 5-7. It was also reported that unlike ammoniacal copper, CCA was strongly affected by ionic concentration of the leaching solution used. Tanalith E used in this study is formulated by dissolving a copper salt (e.g. copper carbonate) in ethanolamine which yields complexes of copper amine. There appears to be a lack of readily available information concerning copper

amine fixation reactions in the literature, which may be a reflection of commercial sensitivity rather than actual lack of information.

The results of this study demonstrated the profound affect of site on depletion. Since laboratory studies have shown the profound affects of low pH on increasing depletion of CCA from wood, it was not a great surprise to find that the sites likely to have a low pH caused the greatest depletion. A study of Schultz *et al.*, (2002) showed that two loam soils of widely different acidity, texture, base saturation, metal content and cation exchange capacity both caused similar CCA depletion from 19 x 19 x 559 mm southern yellow pine stakes (6.4 kg/m^3 oxide, of AWPA type C CCA). Mean (out of 10-12 stakes) Cu, Cr, and As depletions were 39.7, 13.3 and 31.9% respectively at one site and 33.3, 18 and 25.5% respectively at the other site after 5.5 years. The mean losses of Cu, Cr and As across all sites for radiata pine stakes tested in this study were 30.45, 9.44 and 21.22%. For the most severe site (TOT) Cu, Cr and As loss was 73.07, 22.31, and 40.78% respectively and the least severe sites had close to zero loss. The 2 sites of Schultz *et al.*, (2002) were selected on the basis of possessing very different soil characteristics and the authors were surprised that site had no significant effect on depletion. Both soils were described as highly acid (ph 4.81 and 5.12) and the site with the lower pH had 258% greater exchangeable acidity. This suggests that pH was not in isolation the most important factor affecting depletion, a suggestion that fits with the concept of soil properties being the product of a complex interaction of chemical and physical parameters.

Results suggested that high water availability (high rainfall plus poor drainage) and acidity had important affects on depletion in the current study and therefore the lack of site affect found by Schultz *et al.*, (2002) is probably related to greater variability of the following factors:

- actual water availability at the stake/soil interface,
- chemistry of the soil constituents related to pH,
- pH values were more diverse than those measured by Schultz *et al.*, (2002).

One site of Schultz *et al.*, (2002) had 1.4 meters annual rainfall and was described as having moderately poor drainage and the other had 1.7 meters and was described as well drained. Compared to the severe water logging known to occur at HH, TOT, PT and GB, the sites of Schultz *et al.*, (2002) probably had lower water availability compared to MM and HAN probably had lower water availability. The peat site (PT) would have contained a high humic acid concentration and the *Nothofagus* litter at TOT may also have contained a high concentration of organic acids and this may have significantly increased the depletion hazard as has been suggested previously (Cooper et al., 2000). This suggests that the two most important factors affecting preservative depletion in soil contact is water logging and/or availability of organic acids.

Possibly soil pH is not a reliable indicator of pH at the wood/soil-water interface. Possibly the hydrogen ion concentration associated with the soil water matrix does not reflect hydrogen ion concentration within test stakes whereas organic soil acids are effectively impregnated into the wood structure. Both sites of Schultz *et al.*, (2002) had low organic matter content (2.4 and 2.06%) suggesting that organic acid content was also low compared to many of the sites included in the current study. Sites with a thick litter layer (TOT, GBF, OAK) and the peat site (PT) would inevitably have very high organic matter content at the ground line. Forest litter is known to contain humic acid (Brady, 1984), malonic and lactic acids (Howard et al., 1998) and fulvic acid (Silva *et al.*, 1998). As well at the direct effects of low pH on preservative depletion, low pH increases soil cation exchange capacity and increases soil water metal cation concentration (Brady, 1984) both of which might be expected to increase depletion of cationic preservative constituents. The presence of woody herbaceous material at some of the non-grazed and non-mown sites may also have harboured higher organic matter (HAN, HH, GB, CK) than occurs at mown sites. At HH stakes were encased in a dense reticulum of roots and root hairs of grasses and other plants. Since plant roots are likely to change soil properties at the soil/root hair interface during mineral ion exchange, soil root density may be an important phenomenon at some sites.

Another finding of Schultz *et al.* (2002) was that stakes analysed 11 months earlier (4.6 years exposure) had similar depletion (actually higher depletion but this was probably due to wood variability). This supports the arguments made regarding the importance of a "buffer zone" of outer wood protecting inner wood from depletion mechanisms, as discussed above i.e. high initial loss from the buffer zone occurs, after which loss from deeper wood occurs more slowly according to Harrow's (1959) square law. Whilst it is difficult to separate the impact of the effect of a putative "buffer zone", from the effect of high initial loss arising from early removal of incompletely fixed CCA, it is reasonable to purport that the loss values recorded by Schultz *et al.* (2002) and those of this study cannot be accounted for only by loss of incompletely fixed CCA.

Copper amine solubility in the treatment solution is dependant on high pH and therefore the initial drop in pH in the wood shortly after treatment is likely to decrease solubility. Ammonia causes swelling of the wood cell wall and this is known to aid copper adsorption across the wall (Eaton and Hale, 1993). Probably the significance of copper adsorption is greater for copper amine systems compared to CCA for which precipitation and complexation reactions are also important. The high loss of copper from CCA treated pine and beech that occurred at the acidic sites also occurred for CuAz treated pine suggesting that both fixation systems are susceptible to loss under conditions of low pH. However, it is difficult to explain the greater loss of copper from CuAz treated pine and beech, other than in terms of the overall copper fixation that occurs in CCA treated wood, softwoods in particular, being more efficient than the copper amine adsorption system.

Tebuconazole has low solubility in water and is formulated as a microemulsion in Tanalith E. Information concerning its susceptibility to leaching from preservative treated wood could not be found. Wakeling *et al.*, (1993) reported that loss of a microemulsion formulation of another triazole fungicide, hexaconazole, applied to green radiata pine by dipping and then subjected to a simulated rainfall regime, could not be detected by HPLC analysis of the run-off solution. This suggested that hexaconazole "fixed" to the wood once the emulsion had broken shortly after contact with wood. Radiata pine has a pH of approximately 4.5 (Kinimonth and Whitehouse,

1991) and this could suggest that reversal of fixation due to pH is unlikely to occur in soils of moderate acidity (pH 4.5-6.5: Brady, 1984). Triazole fungicides used as fungicides in horticulture have been reported to breakdown rapidly in soil due to microbial activity (Neera and Dureja, 2000; Munier-Lamy and Borde, 2000). In recognition of this, dual preservative systems are considered important when triazole fungicides are used, particularly for wood in ground contact, because the partner fungicide is purported to prevent microbial breakdown. The finding in this study that tebuconazole loss was not substantially greater than loss of copper from CuAz treated pine and beech below ground suggested that the copper was performing such a protective role. Possibly the primary mechanism of loss of tebuconazole was the same as occurred for copper, although once copper depletion had occurred and its protective effect was diminished, biodegradation could have occurred. Interesting in this regard was the finding that at the Peat site, loss of tebuconazole was substantially less than copper loss, indicating that copper is more susceptible to loss induced by low pH compared to tebuconazole. This also suggested that at this site biodegradation of tebuconazole did not result from loss of the putative protective effects of copper.

The greater loss of tebuconazole from above ground portions of stakes compared to copper for CuAz treated pine and beech is difficult to account for. Failure of above ground portions of CCA treated stakes, not part of this study, to brown rot before below ground failure at Whakarewarewa test site (unpublished data) suggested that in some situations a reversal of the usually higher decay hazard below ground can occur. In this particular example the possibility that above ground preservative depletion caused by local sulphurous emissions from geothermal activity remains unproven. However, such examples suggest that above ground depletion may in some situations be more severe than occurs below ground. Possibly the increased above ground tebuconazole loss was caused by enzyme induced breakdown by mould and, or sapstain fungi which may have been more prevalent in the above ground portions of stakes. The incidence of sapstain was high in above ground portions of stakes at most sites and mould growth was an issue for lower retentions during post treatment fixation storage in plastic bags. These observations suggested that CuAz treated wood was susceptible to some sapstain and mould fungi. Breakdown of quaternary

ammonium compounds by moulds has been reported previously (Burgel *et al.*, 1996; Dubois and Ruddick, 1998).

There is currently considerable interest in the impact of preservative treated wood on the environment (IRGWP 98-50101). Whilst much of the interest revolves around remediation of spillage at old treatment sites (Rodsand *et al.*, 1998), there is also interest in leaching of preservatives from wood commodities in service (Wegan and Lucks, 1998). Possibly the greater susceptibility of copper loss from wood treated with alternatives to CCA, such as CuAz has not been adequately addressed. Focus has been largely on the benefits associated with a move away from chromium and arsenic, both of which face increasing scrutiny because of concerns over adverse affects to health and the environment. However, in use situations where aquatic toxicity of copper is likely to be important such as decking around waterways, the potential for adverse impact from CuAz treated wood may be greater than for CCA treated wood.

Section 6.4

Conclusions

1. Site, wood species and preservative type had a major effect on copper, chromium and arsenic loss from CCA treated pine and beech and loss of copper and tebuconzole from CuAz treated pine and beech. The importance of wood species and preservative type was dependant on site.

2. Above-ground depletions for all preservatives were minor compared to below-ground depletions, except tebuconazole depletion which was considerably higher than above ground depletion of other preservatives which may have been related to degradation by mould and sapstain fungi.

3. The finding that waterlogged sites, and/or sites with low pH caused greatest loss to all treatments irrespective of wood species and preservative type, in the light of low loss at horticultural sites suggested that the influence of extremes of water availability and of low pH was more important than other mechanisms such as cationic exchange reactions with soil. Particularly high

loss at sites where soil was likely to have contained a high organic acid concentration, in the light of the findings of others of no apparent effect of large pH differences between soils of low organic matter content suggested that organic acids (e.g. humic, lactic, malonic and fulvic acids, known to be associated with forest litter) are important agents of depletion.

4. Beech was more susceptible than pine to loss of copper for both preservatives and this may have been attributable to less efficient fixation reactions, perhaps caused by higher extractive content of beech and poor preservative distribution (macro- and micro-) in beech.

5. Contrary to most reports of the relative susceptibility of elemental loss from CCA treated wood, copper was more susceptible to loss from below ground portions of stakes compared to arsenic. Whilst this fits with known effects of low pH (<3.0) this mechanism is unlikely to have accounted for greater mean copper loss across all sites.

6. Chromium was more susceptible than copper to loss from above ground portions of stakes and this may have been related to lignin degradation by ultraviolet light and an associated preferential chromium loss mechanism.

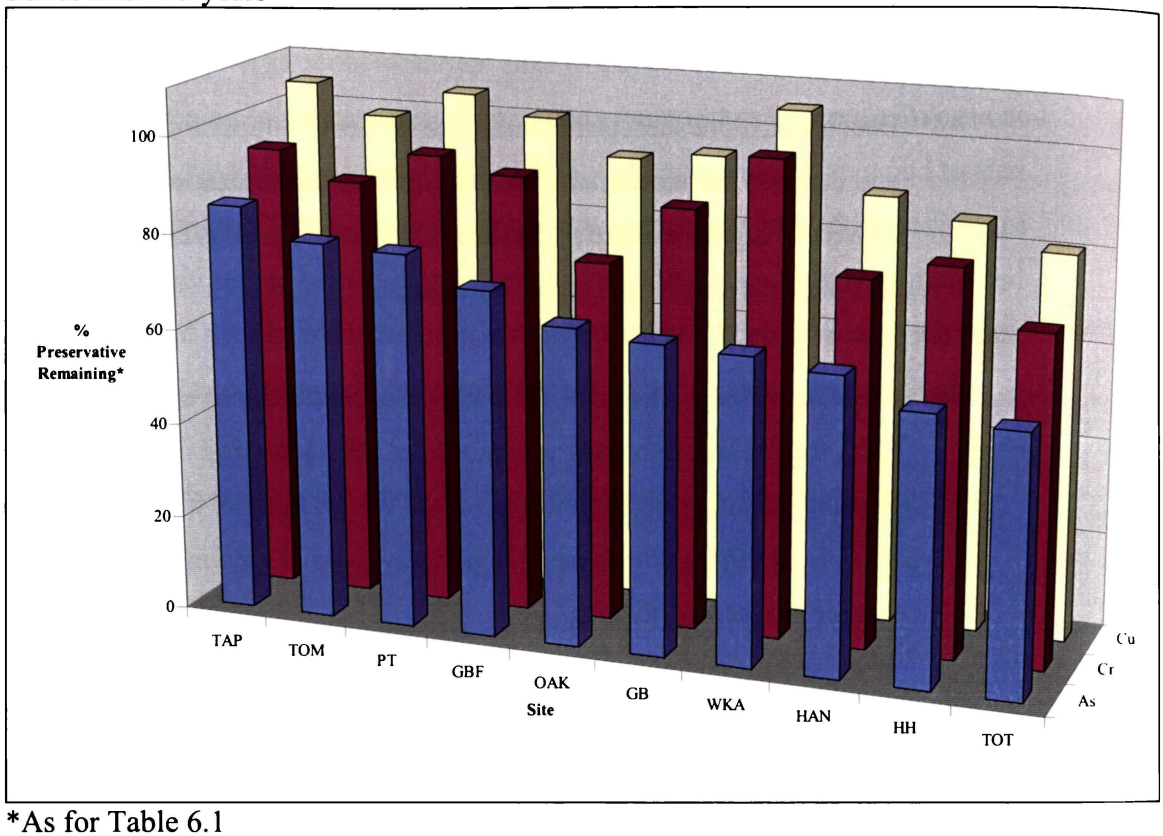
7. Substantially greater loss of copper from CuAz treated wood compared to CCA treated wood across all sites, particularly above ground, may become significant in usage situations where aquatic toxicity of copper is important.

8. The similar degree of tebuconazole and copper loss from CuAz treated pine and beech suggested that copper performed a protective effect, preventing microbial breakdown as would normally occur for triazole compounds in soil contact.

Section 6.5

Appendix 6

Figure 6.9: Mean percentage residual CCA in the above ground portion of beech stakes after 4.5 years



*As for Table 6.1

Figure 6.10: Mean percentage residual CCA in the below ground portion of beech stakes after 4.5 years

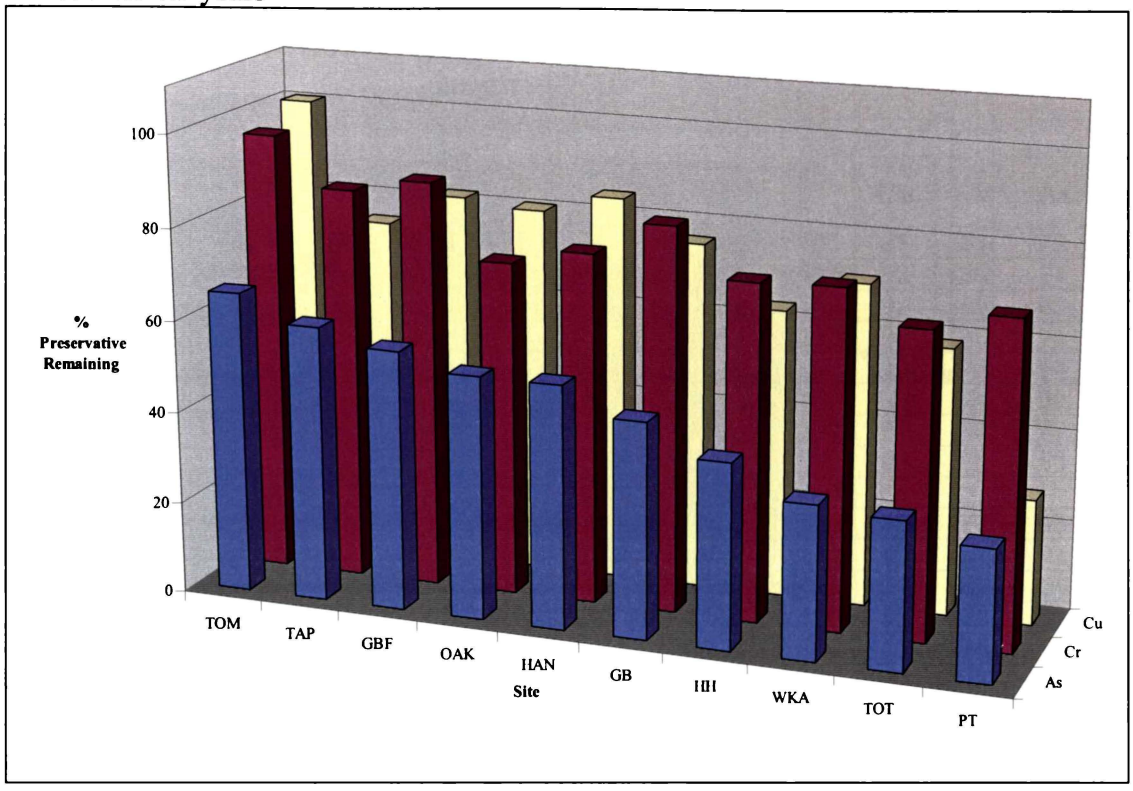


Figure 6.11: Mean percentage residual copper and tebuconazole remaining in the above ground portion of beech stakes after 4.5 years

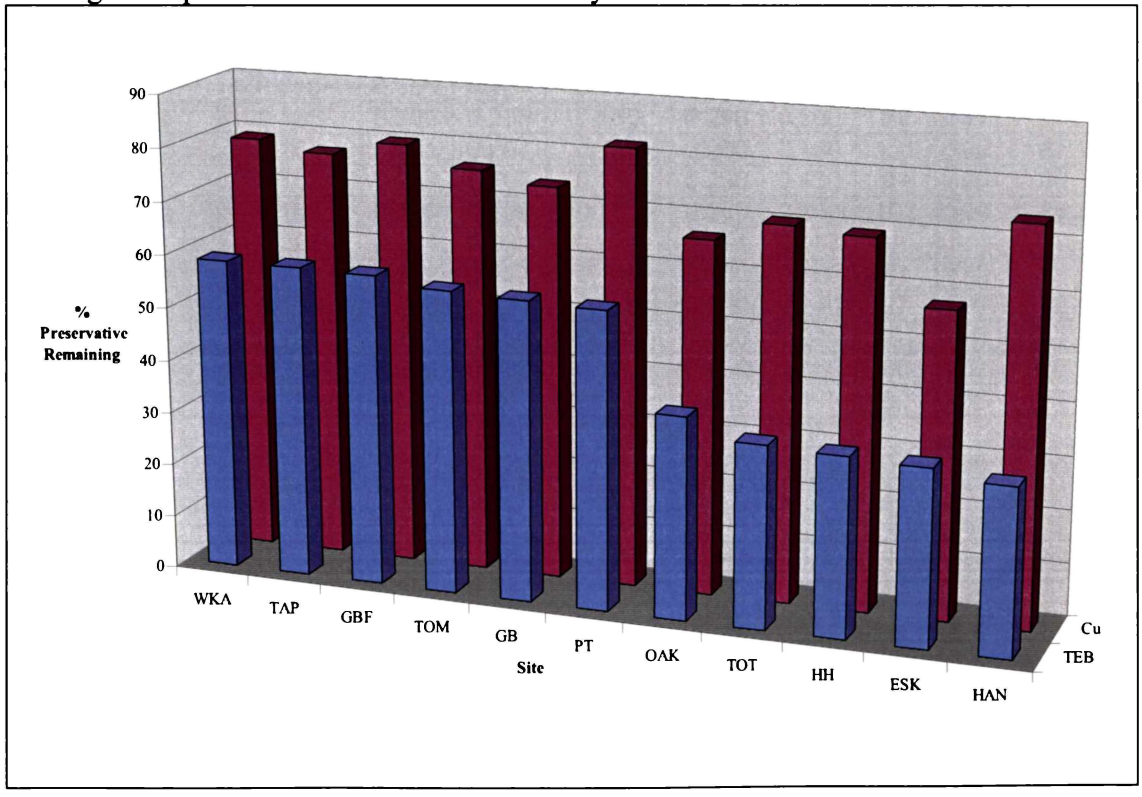


Figure 6.12: Mean percentage copper and tebuconazole remaining in the below ground portion of beech stakes after 4.5 years

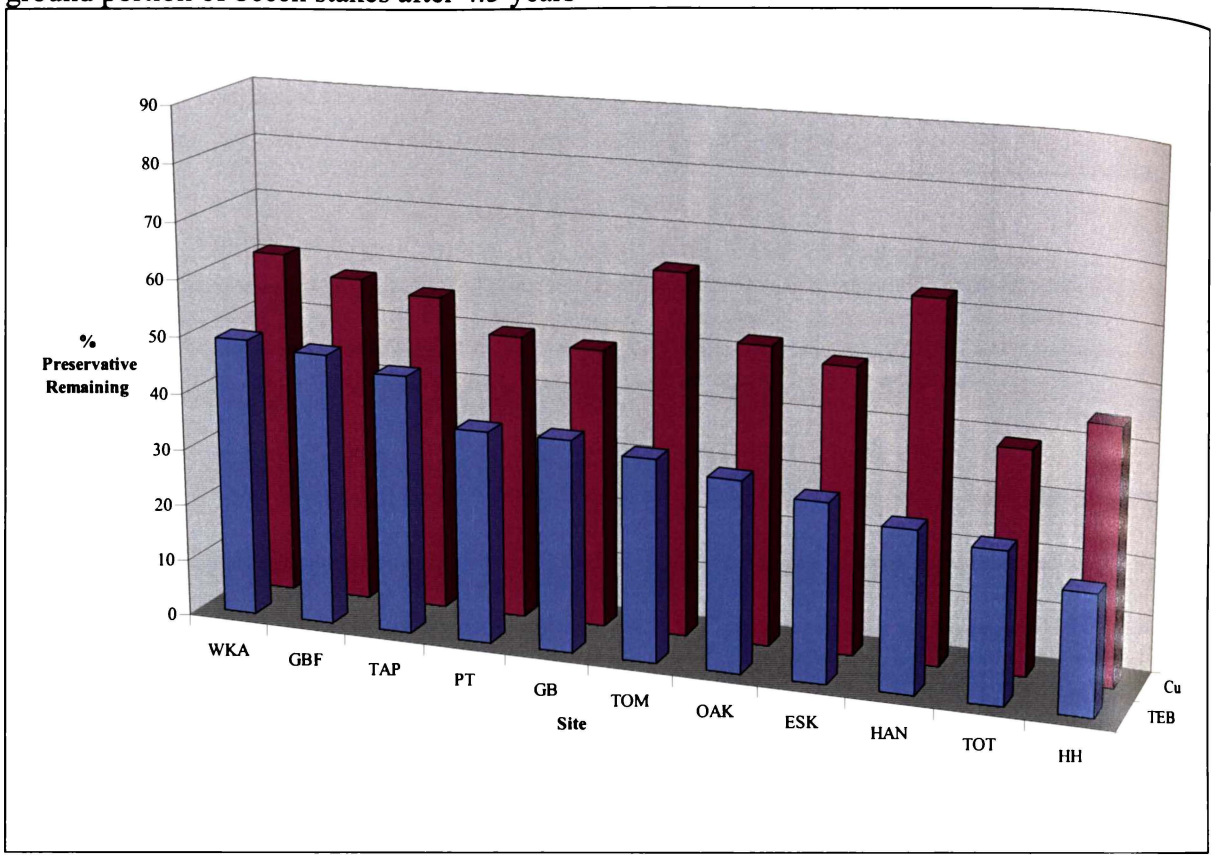


Table 6.3: Predicted CCA retention in pine stakes before exposure, after 5.5 years and percentage remaining

Site	Stake - Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood						% Residual Preservative		
		Predicted			After exposure					
		Cu	Cr	As	Cu	Cr	As	Cu	Cr	As
CK	1a	0.175	0.313	0.222	0.170	0.260	0.160	97	83	72
CK	2a	0.182	0.325	0.231	0.170	0.280	0.170	93	86	74
CK	3a	0.185	0.331	0.235	0.180	0.280	0.160	97	85	68
CK	4a	0.168	0.300	0.213	0.160	0.240	0.160	95	80	75
CK	5a	0.172	0.307	0.218	0.140	0.230	0.140	82	75	64
CK	6a	0.140	0.250	0.178	0.160	0.250	0.160	114	100	90
CK	7a	0.195	0.349	0.248	0.190	0.290	0.190	97	83	77
CK	8a	0.183	0.327	0.232	0.170	0.260	0.160	93	80	69
	SD	0.016	0.030	0.021	0.015	0.021	0.014			
CK	1b	0.175	0.313	0.222	0.070	0.240	0.100	40	77	45
CK	2b	0.182	0.325	0.231	0.090	0.250	0.140	49	77	61
CK	3b	0.185	0.331	0.235	0.100	0.270	0.150	54	82	64
CK	4b	0.168	0.300	0.213	0.070	0.220	0.130	42	73	61
CK	5b	0.172	0.307	0.218	0.070	0.230	0.130	41	75	60
CK	6b	0.140	0.250	0.178	0.120	0.250	0.160	86	100	90
CK	7b	0.195	0.349	0.248	0.160	0.300	0.200	82	86	81
CK	8b	0.183	0.327	0.232	0.140	0.270	0.170	76	83	73
	SD	0.016	0.030	0.021	0.035	0.026	0.030			
ESK	9a	0.216	0.386	0.274	0.230	0.320	0.230	106	83	84
ESK	10a	0.186	0.332	0.236	0.200	0.280	0.190	108	84	81
ESK	11a	0.163	0.291	0.207	0.180	0.250	0.180	111	86	87
ESK	12a	0.162	0.289	0.205	0.160	0.230	0.150	99	80	73
ESK	13a	0.156	0.279	0.199	0.170	0.240	0.160	109	86	81
	SD	0.025	0.044	0.031	0.028	0.036	0.031			
ESK	9b	0.216	0.386	0.274	0.210	0.360	0.250	97	93	91
ESK	10b	0.186	0.332	0.236	0.160	0.290	0.200	86	87	85
ESK	11b	0.163	0.291	0.207	0.180	0.260	0.180	111	89	87
ESK	12b	0.162	0.289	0.205	0.140	0.230	0.130	87	80	63
ESK	13b	0.156	0.279	0.199	0.160	0.250	0.150	102	89	76
	SD	0.025	0.044	0.031	0.026	0.051	0.047			
GB	14a	0.175	0.312	0.222	0.160	0.300	0.180	91	96	81
GB	15a	0.134	0.240	0.171	0.130	0.220	0.140	97	92	82
GB	16a	0.198	0.354	0.251	0.200	0.330	0.220	101	93	87
GB	17a	0.148	0.264	0.188	0.130	0.230	0.150	88	87	80
GB	18a	0.195	0.348	0.247	0.160	0.290	0.190	82	83	77
	SD	0.028	0.050	0.035	0.029	0.047	0.032			
GB	14b	0.175	0.312	0.222	0.100	0.270	0.150	57	86	68
GB	15b	0.134	0.240	0.171	0.050	0.200	0.110	37	83	64
GB	16b	0.198	0.354	0.251	0.080	0.310	0.180	40	88	72
GB	17b	0.148	0.264	0.188	0.070	0.240	0.130	47	91	69
GB	18b	0.195	0.348	0.247	0.110	0.320	0.200	56	92	81
	SD	0.028	0.050	0.035	0.024	0.050	0.036			
GBF	19a	0.222	0.397	0.282	0.270	0.370	0.270	122	93	96
GBF	20a	0.163	0.292	0.207	0.180	0.260	0.180	110	89	87
GBF	21a	0.206	0.369	0.262	0.210	0.320	0.220	102	87	84
GBF	22a	0.184	0.329	0.233	0.210	0.300	0.210	114	91	90
GBF	23a	0.190	0.340	0.241	0.200	0.320	0.220	105	94	91

Table 6.3 Continued

Site	Stake - Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood						% Residual Preservative		
		Predicted			After exposure					
		Cu	Cr	As	Cu	Cr	As	Cu	Cr	As
	SD	0.022	0.040	0.029	0.034	0.040	0.032			
GBF	19b	0.222	0.397	0.282	0.160	0.310	0.200	72	78	71
GBF	20b	0.163	0.292	0.207	0.150	0.260	0.170	92	89	82
GBF	21b	0.206	0.369	0.262	0.200	0.350	0.220	97	95	84
GBF	22b	0.184	0.329	0.233	0.150	0.280	0.190	82	85	81
GBF	23b	0.190	0.340	0.241	0.160	0.280	0.160	84	82	66
	SD	0.022	0.040	0.029	0.021	0.035	0.024			
HH	24a	0.173	0.310	0.220	0.150	0.250	0.170	87	81	77
HH	25a	0.182	0.324	0.231	0.160	0.280	0.180	88	86	78
HH	26a	0.190	0.340	0.241	0.200	0.330	0.230	105	97	95
HH	27a	0.189	0.338	0.240	0.030	0.300	0.160	16	89	67
HH	28A	0.187	0.334	0.237	0.160	0.280	0.180	86	84	76
HH	29A	0.160	0.286	0.203	0.130	0.240	0.160	81	84	79
HH	30A	0.164	0.293	0.208	0.160	0.340	0.220	98	116	106
	SD	0.012	0.022	0.016	0.053	0.038	0.028			
HH	24b	0.173	0.310	0.220	0.040	0.230	0.120	23	74	55
HH	25b	0.182	0.324	0.231	0.060	0.310	0.180	33	96	78
HH	26b	0.190	0.340	0.241	0.070	0.300	0.160	37	88	66
HH	27b	0.189	0.338	0.240	0.170	0.270	0.190	90	80	79
HH	28B	0.187	0.334	0.237	0.050	0.250	0.130	27	75	55
HH	29B	0.160	0.286	0.203	0.040	0.220	0.110	25	77	54
HH	30B	0.164	0.293	0.208	0.060	0.310	0.180	37	106	86
	SD	0.012	0.022	0.016	0.045	0.038	0.033			
MM	31A	0.209	0.373	0.265	0.230	0.380	0.250	110	102	94
MM	32A	0.169	0.301	0.214	0.210	0.340	0.220	124	113	103
MM	33A	0.141	0.251	0.178	0.190	0.320	0.210	135	127	118
	SD	0.034	0.061	0.044	0.020	0.031	0.021			
MM	31B	0.209	0.373	0.265	0.190	0.350	0.230	91	94	87
MM	32B	0.169	0.301	0.214	0.180	0.330	0.210	107	109	98
MM	33B	0.141	0.251	0.178	0.160	0.290	0.190	114	115	106
	SD	0.034	0.061	0.044	0.015	0.031	0.020			
OAK	34A	0.188	0.335	0.238	0.200	0.330	0.220	107	98	92
OAK	35A	0.172	0.307	0.218	0.200	0.320	0.210	116	104	96
OAK	36A	0.196	0.351	0.249	0.210	0.340	0.230	107	97	92
OAK	37A	0.143	0.255	0.181	0.150	0.250	0.160	105	98	88
OAK	38A	0.177	0.316	0.225	0.180	0.300	0.200	102	95	89
	SD	0.020	0.037	0.026	0.024	0.036	0.027			
OAK	34B	0.188	0.335	0.238	0.140	0.310	0.200	75	92	84
OAK	35B	0.172	0.307	0.218	0.150	0.310	0.200	87	101	92
OAK	36B	0.196	0.351	0.249	0.160	0.330	0.220	81	94	88
OAK	37B	0.143	0.255	0.181	0.120	0.260	0.160	84	102	88
OAK	38B	0.177	0.316	0.225	0.150	0.310	0.200	85	98	89
	SD	0.020	0.037	0.026	0.015	0.026	0.022			
PT	39a	0.140	0.249	0.177	0.170	0.230	0.160	122	92	90
PT	40A	0.140	0.249	0.177	0.150	0.260	0.170	107	104	96
	SD	0.000	0.000	0.000	0.014	0.021	0.007			
PT	39b	0.140	0.249	0.177	0.150	0.260	0.180	107	104	102
PT	40B	0.140	0.249	0.177	0.010	0.220	0.100	7	88	56

Table 6.3 Continued

Site	Stake - Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood						% Residual Preservative		
		Predicted			After exposure					
		Cu	Cr	As	Cu	Cr	As	Cu	Cr	As
	SD	0.000	0.000	0.000	0.099	0.028	0.057			
TAP	41a	0.181	0.324	0.230	0.180	0.290	0.200	99	89	87
TAP	42a	0.172	0.307	0.218	0.170	0.270	0.170	99	88	78
TAP	43a	0.147	0.262	0.186	0.140	0.210	0.140	95	80	75
TAP	44a	0.191	0.342	0.243	0.190	0.290	0.200	99	85	82
TAP	45a	0.163	0.291	0.207	0.180	0.250	0.180	110	86	87
	SD	0.017	0.031	0.022	0.019	0.033	0.025			
TAP	41b	0.181	0.324	0.230	0.150	0.320	0.230	83	99	100
TAP	42b	0.172	0.307	0.218	0.100	0.260	0.170	58	85	78
TAP	43b	0.147	0.262	0.186	0.100	0.240	0.160	68	92	86
TAP	44b	0.191	0.342	0.243	0.110	0.290	0.200	58	85	82
TAP	45b	0.163	0.291	0.207	0.080	0.220	0.140	49	76	68
	SD	0.017	0.031	0.022	0.026	0.040	0.035			
TOM	46A	0.148	0.265	0.188	0.160	0.260	0.180	108	98	96
TOM	47A	0.166	0.296	0.211	0.160	0.270	0.180	96	91	86
TOM	48A	0.183	0.327	0.232	0.210	0.340	0.230	115	104	99
TOM	49A	0.187	0.334	0.237	0.190	0.320	0.220	102	96	93
TOM	50A	0.172	0.308	0.219	0.190	0.320	0.210	110	104	96
	SD	0.015	0.027	0.019	0.022	0.035	0.023			
TOM	46B	0.148	0.265	0.188	0.150	0.280	0.180	101	106	96
TOM	47B	0.166	0.296	0.211	0.200	0.320	0.200	121	108	95
TOM	48B	0.183	0.327	0.232	0.160	0.320	0.190	87	98	82
TOM	49B	0.187	0.334	0.237	0.160	0.310	0.200	86	93	84
TOM	50B	0.172	0.308	0.219	0.130	0.290	0.170	75	94	78
	SD	0.015	0.027	0.019	0.025	0.018	0.013			
TOT	51a	0.194	0.346	0.246	0.170	0.280	0.180	88	81	73
TOT	52a	0.153	0.273	0.194	0.130	0.250	0.160	85	92	82
TOT	53a	0.175	0.312	0.222	0.160	0.270	0.180	91	86	81
TOT	54a	0.132	0.236	0.167	0.110	0.200	0.120	83	85	72
TOT	55a	0.193	0.344	0.244	0.110	0.280	0.180	57	81	74
TOT	56a	0.187	0.335	0.238	0.070	0.150	0.080	37	45	34
	SD	0.025	0.044	0.032	0.037	0.053	0.041			
TOT	51b	0.194	0.346	0.246	0.080	0.320	0.190	41	92	77
TOT	52b	0.153	0.273	0.194	0.080	0.230	0.130	52	84	67
TOT	53b	0.175	0.312	0.222	0.010	0.250	0.140	6	80	63
TOT	54b	0.132	0.236	0.167	0.020	0.210	0.110	15	89	66
TOT	55b	0.193	0.344	0.244	0.070	0.280	0.150	36	81	61
TOT	56b	0.187	0.335	0.238	0.020	0.130	0.050	11	39	21
	SD	0.025	0.044	0.032	0.033	0.065	0.047			
WKA	57A	0.147	0.263	0.187	0.160	0.290	0.190	109	110	102
WKA	58A	0.190	0.339	0.241	0.150	0.280	0.190	79	83	79
WKA	59A	0.157	0.281	0.199	0.130	0.270	0.170	83	96	85
	SD	0.023	0.040	0.028	0.015	0.010	0.012			
WKA	57B	0.147	0.263	0.187	0.130	0.320	0.190	88	122	102
WKA	58B	0.190	0.339	0.241	0.140	0.300	0.190	74	89	79
WKA	59B	0.157	0.281	0.199	0.130	0.260	0.160	83	93	80
	SD	0.023	0.040	0.028	0.006	0.031	0.017			

SD = standard deviation

Table 6.4: Predicted CCA retention in beech stakes before exposure, after 4.5 years and percentage remaining

Site	Stake - Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood						% Residual preservative		
		Predicted			After exposure					
		Cu	Cr	As	Cu	Cr	As	Cu	Cr	As
GB	1a	0.103	0.184	0.131	0.110	0.190	0.100	107	103	76
GB	2a	0.091	0.163	0.116	0.080	0.120	0.050	88	74	43
GB	3a	0.112	0.201	0.143	0.110	0.170	0.090	98	85	63
GB	4a	0.119	0.213	0.151	0.100	0.180	0.100	84	85	66
GB	5a	0.118	0.211	0.150	0.120	0.200	0.100	102	95	67
GB	6a	0.097	0.173	0.123	0.090	0.150	0.090	93	87	73
	SD	0.012	0.021	0.015	0.015	0.029	0.019			
GB	1b	0.103	0.184	0.131	0.080	0.160	0.070	78	87	54
GB	2b	0.091	0.163	0.116	0.060	0.100	0.020	66	61	17
GB	3b	0.112	0.201	0.143	0.070	0.170	0.070	62	85	49
GB	4b	0.119	0.213	0.151	0.100	0.190	0.070	84	89	46
GB	5b	0.118	0.211	0.150	0.100	0.200	0.090	85	95	60
GB	6b	0.097	0.173	0.123	0.080	0.150	0.070	82	87	57
	SD	0.012	0.021	0.015	0.016	0.035	0.023			
GBF	7a	0.114	0.204	0.145	0.110	0.190	0.110	96	93	76
GBF	8a	0.111	0.198	0.141	0.120	0.190	0.120	108	96	85
GBF	9a	0.090	0.162	0.115	0.080	0.130	0.060	88	80	52
GBF	10a	0.104	0.185	0.132	0.110	0.180	0.110	106	97	84
GBF	11a	0.096	0.171	0.121	0.100	0.160	0.080	105	94	66
	SD	0.010	0.018	0.013	0.015	0.025	0.025			
GBF	7b	0.114	0.204	0.145	0.090	0.190	0.080	79	93	55
GBF	8b	0.111	0.198	0.141	0.090	0.180	0.080	81	91	57
GBF	9b	0.090	0.162	0.115	0.090	0.140	0.080	99	87	70
GBF	10b	0.104	0.185	0.132	0.080	0.170	0.080	77	92	61
GBF	11b	0.096	0.171	0.121	0.070	0.140	0.050	73	82	41
	SD	0.010	0.018	0.013	0.009	0.023	0.013			
HAN	12a	0.127	0.226	0.161	0.100	0.150	0.100	79	66	62
HAN	13a	0.100	0.179	0.127	0.100	0.140	0.090	100	78	71
HAN	14a	0.128	0.229	0.163	0.120	0.170	0.100	93	74	61
HAN	15a	0.108	0.193	0.137	0.100	0.160	0.090	92	83	66
	SD	0.014	0.025	0.018	0.010	0.013	0.006			
HAN	12b	0.127	0.226	0.161	0.110	0.160	0.080	87	71	50
HAN	13b	0.100	0.179	0.127	0.090	0.140	0.070	90	78	55
HAN	14b	0.128	0.229	0.163	0.100	0.170	0.080	78	74	49
HAN	15b	0.108	0.193	0.137	0.090	0.160	0.080	83	83	58
	SD	0.014	0.025	0.018	0.010	0.013	0.005			
HH	16a	0.113	0.202	0.143	0.120	0.200	0.110	106	99	77
HH	17a	0.107	0.192	0.136	0.110	0.200	0.110	102	104	81
HH	18a	0.100	0.178	0.127	0.070	0.120	0.060	70	67	47
HH	19a	0.132	0.236	0.167	0.050	0.080	0.030	38	34	18
HH	20a	0.114	0.204	0.145	0.130	0.220	0.130	114	108	90
HH	21a	0.101	0.181	0.129	0.050	0.110	0.030	49	61	23
HH	22a	0.120	0.214	0.152	0.120	0.180	0.110	100	84	72
HH	23a	0.113	0.202	0.143	0.100	0.150	0.070	89	74	49
HH	24a	0.115	0.206	0.146	0.120	0.190	0.080	104	92	55
HH	25a	0.110	0.196	0.139	0.090	0.160	0.070	82	82	50
	SD	0.009	0.017	0.011	0.030	0.046	0.035			

Table 6.4: Continued

Site	Stake - Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood						% Residual preservative		
		Predicted			After exposure					
		Cu	Cr	As	Cu	Cr	As	Cu	Cr	As
HH	16b	0.113	0.202	0.143	0.080	0.170	0.060	71	84	42
HH	17b	0.107	0.192	0.136	0.090	0.190	0.100	84	99	73
HH	18b	0.100	0.178	0.127	0.050	0.100	0.040	50	56	32
HH	19b	0.132	0.236	0.167	0.070	0.150	0.070	53	64	42
HH	20b	0.114	0.204	0.145	0.080	0.170	0.070	70	83	48
HH	21b	0.101	0.181	0.129	0.080	0.130	0.050	79	72	39
HH	22b	0.120	0.214	0.152	0.070	0.170	0.080	58	79	53
HH	23b	0.113	0.202	0.143	0.060	0.120	0.030	53	60	21
HH	24b	0.115	0.206	0.146	0.070	0.150	0.040	61	73	27
HH	25b	0.110	0.196	0.139	0.060	0.130	0.040	55	66	29
	SD	0.009	0.017	0.011	0.012	0.028	0.022			
MM	26a	0.113	0.201	0.143	0.080	0.130	0.090	71	65	63
	SD	0.000	0.000	0.000	0.000	0.000	0.000			
MM	26b	0.113	0.201	0.143	0.120	0.180	0.120	106	89	84
	SD	0.000	0.000	0.000	0.000	0.000	0.000			
OAK	27a	0.125	0.223	0.159	0.110	0.150	0.090	88	67	57
OAK	28A	0.102	0.182	0.130	0.110	0.160	0.100	108	88	77
OAK	29A	0.099	0.178	0.126	0.100	0.140	0.080	101	79	63
OAK	30A	0.120	0.215	0.153	0.120	0.170	0.110	100	79	72
	SD	0.051	0.091	0.065	0.050	0.070	0.044			
OAK	27b	0.125	0.223	0.159	0.100	0.140	0.070	80	63	44
OAK	28B	0.102	0.182	0.130	0.090	0.150	0.080	88	82	62
OAK	29B	0.099	0.178	0.126	0.070	0.120	0.060	70	68	48
OAK	30B	0.120	0.215	0.153	0.100	0.170	0.090	83	79	59
	SD	0.029	0.052	0.037	0.022	0.038	0.018			
PT	31A	0.122	0.218	0.155	0.130	0.220	0.140	107	101	90
PT	32A	0.107	0.191	0.136	0.090	0.150	0.070	84	79	52
PT	33A	0.093	0.165	0.117	0.100	0.160	0.090	108	97	77
PT	34A	0.113	0.202	0.144	0.130	0.190	0.120	115	94	83
PT	35A	0.111	0.198	0.141	0.110	0.180	0.120	99	91	85
PT	36A	0.113	0.202	0.143	0.130	0.220	0.120	115	109	84
	SD	0.010	0.018	0.013	0.018	0.029	0.025			
PT	31B	0.122	0.218	0.155	0.030	0.200	0.060	25	92	39
PT	32B	0.107	0.191	0.136	0.070	0.140	0.060	66	73	44
PT	33B	0.093	0.165	0.117	0.020	0.110	0.030	22	67	26
PT	34B	0.113	0.202	0.144	0.020	0.160	0.040	18	79	28
PT	35B	0.111	0.198	0.141	0.020	0.160	0.040	18	81	28
PT	36B	0.113	0.202	0.143	0.020	0.070	0.010	18	35	7
	SD	0.010	0.018	0.013	0.020	0.045	0.019			
TAP	37A	0.125	0.224	0.159	0.130	0.210	0.140	104	94	88
TAP	38A	0.094	0.169	0.120	0.100	0.160	0.100	106	95	83
TAP	39a	0.101	0.180	0.128	0.110	0.190	0.120	109	105	94
TAP	40A	0.128	0.229	0.162	0.130	0.200	0.130	102	88	80
TAP	41a	0.096	0.171	0.122	0.100	0.150	0.100	104	88	82
	SD	0.016	0.029	0.021	0.015	0.026	0.018			
TAP	37B	0.125	0.224	0.159	0.100	0.200	0.110	80	89	69
TAP	38B	0.094	0.169	0.120	0.070	0.140	0.070	74	83	58
TAP	39b	0.101	0.180	0.128	0.080	0.170	0.090	79	94	70

Table 6.4: Continued

Site	Stake - Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood						% Residual preservative		
		Predicted			After exposure					
		Cu	Cr	As	Cu	Cr	As	Cu	Cr	As
TAP	40B	0.128	0.229	0.162	0.100	0.210	0.100	78	92	62
TAP	41b	0.096	0.171	0.122	0.060	0.120	0.050	63	70	41
	SD	0.016	0.029	0.021	0.018	0.038	0.024			
TOM	42a	0.106	0.189	0.134	0.110	0.170	0.110	104	90	82
TOM	43a	0.110	0.197	0.140	0.080	0.130	0.080	73	66	57
TOM	44a	0.117	0.210	0.149	0.120	0.200	0.130	102	95	87
TOM	45a	0.117	0.209	0.149	0.130	0.200	0.130	111	96	87
TOM	46A	0.096	0.171	0.121	0.100	0.160	0.100	105	94	82
	SD	0.009	0.016	0.012	0.019	0.029	0.021			
TOM	42b	0.106	0.189	0.134	0.090	0.160	0.080	85	85	60
TOM	43b	0.110	0.197	0.140	0.110	0.190	0.090	100	97	64
TOM	44b	0.117	0.210	0.149	0.130	0.210	0.120	111	100	80
TOM	45b	0.117	0.209	0.149	0.120	0.210	0.100	102	100	67
TOM	46B	0.096	0.171	0.121	0.100	0.170	0.070	105	100	58
	SD	0.009	0.016	0.012	0.016	0.023	0.019			
TOT	47A	0.124	0.221	0.157	0.110	0.140	0.080	89	63	51
TOT	48A	0.114	0.203	0.144	0.080	0.140	0.080	70	69	55
TOT	49A	0.097	0.173	0.123	0.080	0.130	0.070	82	75	57
	SD	0.014	0.024	0.017	0.017	0.006	0.006			
TOT	47B	0.124	0.221	0.157	0.090	0.150	0.060	73	68	38
TOT	48B	0.114	0.203	0.144	0.070	0.130	0.050	62	64	35
TOT	49B	0.097	0.173	0.123	0.040	0.120	0.030	41	69	24
	SD	0.014	0.024	0.017	0.025	0.015	0.015			
WKA	50A	0.111	0.198	0.141	0.100	0.170	0.080	90	86	57
WKA	51a	0.099	0.177	0.126	0.120	0.200	0.090	121	113	71
	SD	0.008	0.015	0.011	0.014	0.021	0.007			
WKA	50B	0.111	0.198	0.141	0.090	0.160	0.050	81	81	36
WKA	51b	0.099	0.177	0.126	0.060	0.120	0.040	60	68	32
	SD	0.008	0.015	0.011	0.021	0.028	0.007			

SD = standard deviation

Table 6.5: Predicted copper and tebuconazole retentions in pine stakes before exposure, after 5.5 years and percentage remaining

Site	Stake	Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood				% Residual preservative	
			Predicted		After exposure			
			Cu	tebuconazole	Cu	tebuconazole	Cu	tebuconazole
CK	1	A	0.570	0.023	0.410	0.006	72	27
CK	2	A	0.520	0.021	0.420	0.007	81	34
CK	3	A	0.480	0.019	0.390	0.007	82	37
CK	4	A	0.610	0.025	0.430	0.007	70	27
		SD	0.057	0.003	0.017	0.001		
CK	1	B	0.570	0.023	0.290	0.005	51	24
CK	2	B	0.520	0.021	0.410	0.007	79	34
CK	3	B	0.480	0.019	0.220	0.007	46	36
CK	4	B	0.610	0.025	0.240	0.006	39	24
		SD	0.057	0.003	0.085	0.001		
ESK	5	A	0.560	0.022	0.510	0.010	91	46
		SD	0.000	0.000	0.000	0.000		
ESK	5	B	0.560	0.022	0.400	0.009	72	39
		SD	0.000	0.000	0.000	0.000		
GB	6	A	0.530	0.021	0.440	0.008	83	40
GB	7	A	0.620	0.025	0.440	0.009	71	38
GB	8	A	0.560	0.023	0.420	0.009	74	42
GB	9	A	0.460	0.019	0.400	0.008	86	44
GB	10	A	0.570	0.023	0.420	0.008	74	34
		SD	0.059	0.002	0.017	0.001		
GB	6	B	0.530	0.021	0.240	0.008	45	37
GB	7	B	0.620	0.025	0.270	0.009	43	37
GB	8	B	0.560	0.023	0.230	0.008	41	35
GB	9	B	0.460	0.019	0.200	0.008	43	43
GB	10	B	0.570	0.023	0.220	0.007	39	31
		SD	0.059	0.002	0.026	0.001		
GBF	11	A	0.670	0.027	0.610	0.011	91	40
GBF	12	A	0.560	0.022	0.500	0.011	90	51
GBF	13	A	0.550	0.022	0.440	0.011	79	50
GBF	14	A	0.510	0.020	0.490	0.010	96	50
GBF	15	A	0.540	0.021	0.400	0.013	75	59
GBF	16	A	0.490	0.020	0.430	0.009	88	45
		SD	0.063	0.003	0.075	0.001		
GBF	11	B	0.670	0.027	0.390	0.011	58	39
GBF	12	B	0.560	0.022	0.350	0.010	63	47
GBF	13	B	0.550	0.022	0.320	0.011	58	50
GBF	14	B	0.510	0.020	0.340	0.009	66	45
GBF	15	B	0.540	0.021	0.420	0.012	78	57
GBF	16	B	0.490	0.020	0.320	0.008	65	39
		SD	0.031	0.001	0.049	0.001		
HAN	17	A	0.560	0.023	0.490	0.012	87	51
HAN	18	A	0.460	0.018	0.410	0.011	90	58
HAN	19	A	0.610	0.024	0.410	0.009	67	39
HAN	20	A	0.660	0.026	0.550	0.010	84	40
		SD	0.083	0.003	0.061	0.001		
HAN	17	B	0.560	0.023	0.390	0.008	69	34
HAN	18	B	0.460	0.018	0.380	0.008	83	43
HAN	19	B	0.610	0.024	0.350	0.007	58	29

Table 6.5: Continued

Site	Stake	Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood				% Residual preservative	
			Predicted		After exposure			
			Cu	tebuconazole	Cu	tebuconazole	Cu	tebuconazole
HAN	21	B	0.660	0.026	0.400	0.008	61	29
		SD	0.231	0.009	0.144	0.003		
HH	21	A	0.650	0.026	0.460	0.022	71	86
HH	22	A	0.590	0.024	0.480	0.017	81	73
HH	23	A	0.580	0.023	0.410	0.008	71	36
HH	24	A	0.640	0.025	0.440	0.008	69	33
HH	25	A	0.520	0.021	0.460	0.011	88	55
HH	26	A	0.640	0.026	0.450	0.010	70	40
HH	28	A	0.480	0.019	0.390	0.007	81	35
		SD	0.065	0.003	0.031	0.006		
HH	22	B	0.650	0.026	0.220	0.012	34	47
HH	23	B	0.590	0.024	0.250	0.013	42	55
HH	24	B	0.580	0.023	0.180	0.008	31	34
HH	25	B	0.640	0.025	0.180	0.008	28	31
HH	26	B	0.520	0.021	0.200	0.010	38	49
HH	27	B	0.640	0.026	0.270	0.009	42	36
HH	28	B	0.480	0.019	0.220	0.006	46	33
		SD	0.065	0.003	0.034	0.002		
OAK	29	A	0.560	0.022	0.490	0.017	87	76
OAK	30	A	0.540	0.021	0.500	0.019	93	90
OAK	31	A	0.570	0.023	0.530	0.023	93	102
OAK	32	A	0.600	0.024	0.520	0.026	87	109
OAK	33	A	0.610	0.025	0.550	0.023	89	94
		SD	0.029	0.002	0.024	0.004		
OAK	29	B	0.560	0.022	0.310	0.015	55	66
OAK	30	B	0.540	0.021	0.380	0.015	71	72
OAK	31	B	0.570	0.023	0.340	0.017	60	74
OAK	32	B	0.600	0.024	0.430	0.012	72	49
OAK	33	B	0.610	0.025	0.360	0.019	59	76
		SD	0.029	0.002	0.045	0.003		
PT	34	A	0.640	0.025	0.530	0.014	83	56
PT	35	A	0.620	0.025	0.520	0.017	84	67
PT	36	A	0.650	0.026	0.480	0.020	74	75
PT	37	A	0.550	0.022	0.510	0.020	93	89
PT	38	A	0.550	0.022	0.370	0.009	67	43
PT	39	A	0.610	0.024	0.310	0.011	51	46
		SD	0.044	0.002	0.091	0.005		
PT	34	B	0.640	0.025	0.090	0.008	14	31
PT	35	B	0.620	0.025	0.080	0.010	13	42
PT	36	B	0.650	0.026	0.080	0.009	12	34
PT	37	B	0.550	0.022	0.090	0.012	16	53
PT	38	B	0.550	0.022	0.070	0.009	13	42
PT	39	B	0.610	0.024	0.070	0.011	11	44
		SD	0.044	0.002	0.009	0.001		
TAP	40	A	0.610	0.025	0.460	0.017	75	70
TAP	41	A	0.560	0.022	0.410	0.014	74	61
		SD	0.035	0.002	0.035	0.002		
TAP	40	B	0.610	0.025	0.280	0.014	46	59
TAP	41	B	0.560	0.022	0.320	0.010	58	43

Table 6.5: Continued

Site	Stake	Above (a) / Below (b) ground	Retention - grams of preservative per 100 grams of Wood				% Residual preservative	
			Predicted		After exposure			
			Cu	tebuconazole	Cu	tebuconazole	Cu	tebuconazole
		SD	0.035	0.002	0.028	0.003		
TOM	42	A	0.630	0.025	0.540	0.024	86	94
TOM	43	A	0.640	0.026	0.520	0.025	81	96
TOM	44	A	0.620	0.025	0.520	0.020	84	79
TOM	45	A	0.580	0.023	0.470	0.022	80	94
TOM	46	A	0.610	0.024	0.500	0.019	82	80
TOM	47	A	0.600	0.024	0.470	0.018	78	73
		SD	0.022	0.001	0.029	0.003		
TOM	42	B	0.630	0.025	0.390	0.022	62	86
TOM	43	B	0.640	0.026	0.410	0.018	64	68
TOM	44	B	0.620	0.025	0.420	0.018	68	73
TOM	45	B	0.580	0.023	0.400	0.015	68	66
TOM	46	B	0.610	0.024	0.400	0.019	66	79
TOM	47	B	0.600	0.024	0.380	0.015	63	64
		SD	0.022	0.001	0.014	0.003		
TOT	48	A	0.530	0.021	0.390	0.010	73	44
TOT	49	A	0.490	0.019	0.410	0.009	84	45
TOT	50	A	0.460	0.018	0.260	0.008	56	46
TOT	51	A	0.610	0.024	0.440	0.013	73	54
TOT	52	A	0.490	0.019	0.400	0.009	82	45
		SD	0.058	0.002	0.070	0.002		
TOT	48	B	0.530	0.021	0.110	0.007	21	35
TOT	49	B	0.490	0.019	0.160	0.008	33	41
TOT	50	B	0.460	0.018	0.190	0.008	41	44
TOT	51	B	0.610	0.024	0.230	0.010	38	43
TOT	52	B	0.490	0.019	0.100	0.005	21	27
		SD	0.058	0.002	0.054	0.002		
WKA	53	A	0.580	0.023	0.490	0.016	84	68
WKA	54	A	0.560	0.022	0.520	0.014	93	61
WKA	55	A	0.610	0.024	0.480	0.023	79	93
WKA	56	A	0.580	0.023	0.440	0.020	75	86
WKA	57	A	0.470	0.019	0.480	0.007	101	38
		SD	0.053	0.002	0.029	0.006		
WKA	53	B	0.580	0.023	0.430	0.013	74	57
WKA	54	B	0.560	0.022	0.390	0.013	70	59
WKA	55	B	0.610	0.024	0.430	0.012	71	51
WKA	56	B	0.580	0.023	0.410	0.009	70	40
WKA	57	B	0.470	0.019	0.320	0.006	68	33
		SD	0.053	0.002	0.046	0.003		

SD = standard deviation

Table 6.6: Predicted copper and tebuconazole retentions in beech stakes before exposure, after 4.5 years and percentage remaining

Site	Stake	Above (A) / Below (B) ground	Retention - grams of preservative per 100 grams of Wood				% Preservative remaining	
			Predicted		After exposure			
			Cu	tebuconazole	Cu	tebuconazole	Cu	tebuconazole
ESK	6	A	0.530	0.021	0.160	0.007	30	33
ESK	7	A	0.520	0.021	0.340	0.007	66	32
ESK	8	A	0.500	0.020	0.350	0.007	70	34
ESK	9	A	0.550	0.022	0.350	0.007	63	33
		SD	0.021	0.001	0.093	0.000		
ESK	6	B	0.530	0.021	0.130	0.007	25	33
ESK	7	B	0.520	0.021	0.320	0.006	62	31
ESK	8	B	0.500	0.020	0.300	0.006	60	31
ESK	9	B	0.550	0.022	0.300	0.007	54	31
		SD	0.021	0.001	0.089	0.001		
GB	10	A	0.540	0.022	0.420	0.012	77	56
GB	11	A	0.540	0.022	0.410	0.012	75	53
GB	12	A	0.490	0.020	0.340	0.012	69	62
GB	13	A	0.550	0.022	0.400	0.013	73	56
GB	14	A	0.450	0.018	0.340	0.010	75	54
		SD	0.043	0.002	0.039	0.001		
GB	10	B	0.540	0.022	0.290	0.008	53	36
GB	11	B	0.540	0.022	0.290	0.006	53	29
GB	12	B	0.490	0.020	0.260	0.009	53	44
GB	13	B	0.550	0.022	0.280	0.012	51	53
GB	14	B	0.450	0.018	0.240	0.007	53	36
		SD	0.043	0.002	0.022	0.002		
GBF	15	A	0.530	0.021	0.380	0.013	72	60
GBF	16	A	0.560	0.022	0.430	0.013	77	59
GBF	17	A	0.530	0.021	0.480	0.014	91	63
GBF	18	A	0.550	0.022	0.450	0.013	82	57
GBF	19	A	0.480	0.019	0.370	0.010	77	53
		SD	0.031	0.001	0.047	0.002		
GBF	15	B	0.530	0.021	0.290	0.010	55	46
GBF	16	B	0.560	0.022	0.310	0.013	56	56
GBF	17	B	0.530	0.021	0.290	ND	55	ND
GBF	18	B	0.550	0.022	0.310	0.010	57	46
GBF	19	B	0.480	0.019	0.310	0.009	65	44
		SD	0.031	0.001	0.011	0.002		
HAN	1	A	0.520	0.021	0.410	0.007	79	35
HAN	2	A	0.530	0.021	0.370	0.006	70	30
HAN	3	A	0.590	0.024	0.390	0.008	67	32
HAN	4	A	0.550	0.022	0.440	0.007	80	30
HAN	5	A	0.540	0.022	0.400	0.007	74	30
		SD	0.027	0.001	0.026	0.001		
HAN	1	B	0.520	0.021	0.300	0.006	58	29
HAN	2	B	0.530	0.021	0.330	0.006	62	28
HAN	3	B	0.590	0.024	0.370	0.007	63	28
HAN	4	B	0.550	0.022	0.390	0.006	71	29
HAN	5	B	0.540	0.022	0.330	0.006	61	28
		SD	0.027	0.001	0.036	0.000		
HH	20	A	0.470	0.019	0.300	0.007	64	36
HH	21	A	0.600	0.024	0.410	0.009	68	38

Table 6.6: Continued

Site	Stake	Above (A) / Below (B) ground	Retention - grams of preservative per 100 grams of Wood				% Preservative remaining	
			Predicted		After exposure			
			Cu	tebuconazole	Cu	tebuconazole	Cu	tebuconazole
HH	22	A	0.550	0.022	0.440	0.010	80	47
HH	23	A	0.530	0.022	0.400	0.010	75	47
HH	24	A	0.530	0.021	0.340	0.005	64	25
HH	25	A	0.540	0.022	0.360	0.007	67	34
HH	26	A	0.550	0.022	0.340	0.006	62	29
HH	28	A	0.490	0.020	0.300	0.006	61	29
HH	29	A	0.540	0.022	0.430	0.007	80	33
HH	30	A	0.570	0.023	0.420	0.005	73	20
		SD	0.037	0.001	0.053	0.002		
HH	21	B	0.470	0.019	0.190	0.004	40	23
HH	22	B	0.600	0.024	0.290	ND	48	ND
HH	23	B	0.550	0.022	0.260	ND	47	ND
HH	24	B	0.530	0.022	0.260	ND	49	ND
HH	25	B	0.530	0.021	0.260	0.005	49	21
HH	26	B	0.540	0.022	0.270	0.005	50	23
HH	27	B	0.550	0.022	0.250	0.004	46	16
HH	28	B	0.490	0.020	0.240	0.004	49	21
HH	29	B	0.540	0.022	0.150	0.005	28	25
HH	30	B	0.570	0.023	0.300	0.004	52	18
		SD	0.037	0.001	0.045	0.001		
OAK	31	A	0.550	0.022	0.370	0.008	67	37
OAK	32	A	0.500	0.020	0.340	0.007	68	36
OAK	33	A	0.570	0.023	0.350	0.009	62	39
OAK	34	A	0.490	0.020	0.340	0.008	69	40
		SD	0.039	0.001	0.014	0.001		
OAK	31	B	0.550	0.022	0.310	0.008	56	35
OAK	32	B	0.500	0.020	0.260	0.007	52	33
OAK	33	B	0.570	0.023	0.290	0.007	51	30
OAK	34	B	0.490	0.020	0.250	0.007	51	36
		SD	0.039	0.001	0.028	0.001		
PT	35	A	0.520	0.021	0.450	0.013	87	63
PT	36	A	0.550	0.022	0.490	0.017	90	75
PT	37	A	0.550	0.022	0.160	ND	29	ND
PT	38	A	0.520	0.021	0.390	0.008	75	37
PT	39	A	0.490	0.020	0.380	0.010	77	49
		SD	0.025	0.001	0.128	0.004		
PT	35	B	0.520	0.021	0.080	0.009	15	43
PT	36	B	0.550	0.022	0.110	0.008	20	36
PT	37	B	0.550	0.022	0.870	0.006	158	25
PT	38	B	0.520	0.021	0.080	0.006	15	31
PT	39	B	0.490	0.020	0.100	0.006	20	28
		SD	0.025	0.001	0.348	0.001		
TAP	40	A	0.540	0.022	0.430	0.013	79	61
TAP	41	A	0.480	0.020	0.360	0.010	75	49
TAP	42	A	0.540	0.022	0.400	0.016	75	73
TAP	43	A	0.580	0.023	0.430	0.013	74	53
TAP	44	A	0.490	0.020	0.400	0.011	82	58
		SD	0.041	0.001	0.029	0.002		
TAP	40	B	0.540	0.022	0.290	0.013	54	59

Table 6.6: Continued

Site	Stake	Above (A) / Below (B) ground	Retention - grams of preservative per 100 grams of Wood				% Preservative remaining	
			Predicted		After exposure			
			Cu	tebuconazole	Cu	tebuconazole	Cu	tebuconazole
TAP	41	B	0.480	0.020	0.260	0.008	54	43
TAP	42	B	0.540	0.022	0.300	0.014	56	64
TAP	43	B	0.580	0.023	0.340	0.009	59	39
TAP	44	B	0.490	0.020	0.280	0.004	58	23
		SD	0.041	0.001	0.030	0.004		
TOM	45	A	0.500	0.020	0.390	0.009	79	46
TOM	46	A	0.510	0.021	0.370	0.010	72	48
TOM	47	A	0.560	0.023	0.430	0.014	77	62
TOM	48	A	0.490	0.020	0.370	0.011	75	53
TOM	49	A	0.540	0.022	0.390	0.016	72	75
TOM	50	A	0.550	0.022	0.420	0.011	76	49
TOM	51	A	0.540	0.022	0.440	0.014	81	64
		SD	0.027	0.001	0.029	0.003		
TOM	45	B	0.500	0.020	0.350	0.006	71	31
TOM	46	B	0.510	0.021	0.290	0.007	57	35
TOM	47	B	0.560	0.023	0.330	0.014	59	60
TOM	48	B	0.490	0.020	0.320	ND	65	ND
TOM	49	B	0.540	0.022	0.340	0.009	63	40
TOM	50	B	0.550	0.022	0.340	0.008	62	36
TOM	51	B	0.540	0.022	0.390	0.003	72	12
		SD	0.027	0.001	0.030	0.004		
TOT	52	A	0.560	0.023	0.380	0.008	68	33
TOT	53	A	0.550	0.022	0.360	0.009	65	41
TOT	54	A	0.540	0.022	0.420	0.007	78	33
TOT	55	A	0.540	0.022	0.380	0.007	70	31
		SD	0.010	0.001	0.025	0.001		
TOT	52	B	0.560	0.023	0.250	0.005	44	24
TOT	53	B	0.550	0.022	0.250	0.007	45	30
TOT	54	B	0.540	0.022	0.190	0.006	35	26
TOT	55	B	0.540	0.022	0.170	0.006	31	26
		SD	0.010	0.001	0.041	0.001		
WKA	56	A	0.530	0.021	0.430	0.015	82	70
WKA	57	A	0.560	0.023	0.430	0.011	76	48
WKA	58	A	0.470	0.019	0.370	0.011	79	58
		SD	0.046	0.002	0.035	0.002		
WKA	56	B	0.530	0.021	0.320	0.013	61	60
WKA	57	B	0.560	0.023	0.350	ND	62	ND
WKA	58	B	0.470	0.019	0.290	0.007	62	38
		SD	0.046	0.002	0.030	0.004		

SD = standard deviation

Table 6.7: Mean residual % active ingredients in pine and beech stakes treated with CCA and CuAz across 13 sites after 5.5 and 4.5 years respectively, and their significant differences

Treatment	Above ground			Below ground		
	Pine	Beech	Sig. Dif. (5% P)	Pine	Beech	Sig. Dif. (5% P)
Copper (CCA)	91	95	No	89	80	Yes
Chromium (CCA)	84	68	Yes	76	48	Yes
Arsenic (CCA)	80	72	Yes	53	50	Yes
Copper (CuAz)	80	72	Yes	53	50	Yes
tebuconazole	59	46	Yes	46	34	Yes

Table 6.8: Mean % residual copper and tebuconazole across pine and beech stakes treated with Copper-Azole

Site	Above ground				Below ground			
	Cu	Sig. Dif. (5% P)	TEB	Sig. Dif. (5% P)	Cu	Sig. Dif. (5% P)	TEB	Sig. Dif. (5% P)
PT	74	B	60	AB	28	E	37	B
TOT	72	BC	41	DE	34	DE	33	B
HH	72	BC	41	DE	42	CDE	31	B
GB	76	AB	48	CD	47	BCD	38	B
CK	76	AB	31	E	54	ABC	30	B
TAP	76	AB	61	AB	55	ABC	47	A
ESK	64	C	36	E	55	ABC	33	B
OAK	79	AB	69	A	59	AB	52	A
GBF	83	A	53	CB	61	AB	47	A
HAN	78	AB	38	DE	65	A	31	B
TOM	79	AB	70	A	65	A	54	A
WKA	84	A	65	A	67	A	48	A

Sig. Dif. (5% P) = Significant differences at a 5% level of probability. The same letter in a column indicates no significant difference. Sites are listed in order of below ground Cu depletion severity with the most severe site at the top

Table 6.9: Mean % Residual Copper, Chromium and Arsenic Across Pine and Beech Stakes Treated with CCA

Site	Above Ground						Below Ground					
	Cu	Sig. Dif.	Cr	Sig. Dif.	As	Sig. Dif.	Cu	Sig. Dif.	Cr	Sig. Dif.	As	Sig. Dif.
PT	107	AB	98	AB	82	ABC	35	G	77	DE	41	G
TOT	76	E	75	E	64	E	37	GF	74	E	50	FG
HH	83	DE	85	CDE	67	DE	53	EF	78	ED	52	FG
CK	96	ABCD	84	CDE	74	CDE	59	E	82	CDE	67	CD
GB	94	BCD	89	BCD	72	CDE	63	DE	86	CD	60	EDF
TAP	103	ABC	90	BCD	84	ABC	69	CDE	87	CD	72	CB
WKA	96	ABCD	98	AB	79	BCD	77	CDE	91	ABC	66	CDE
OAK	104	ABC	89	BCD	81	BC	81	BC	87	CD	73	CB
GBF	106	ABC	91	ABCD	81	BC	84	BC	87	BCD	67	CD
HAN	91	CDE	75	E	65	E	85	BC	77	ED	53	EFG
ESK	107	ABC	84	DE	81	BC	97	AB	88	BCD	80	B
TOM	103	ABC	93	ABCD	87	AB	97	AB	98	AB	76	CB
MM	110	A	102	AB	94	A	105	A	102	A	94	A

Sig. Dif. = Significant differences at a 5% level of probability. The same letter in a column indicates no significant difference. Sites are listed in order of below ground Cu depletion severity with the most severe site at the top

CHAPTER 7

Use of confocal laser scanning microscopy for examination of wood degrading fungi and bacteria

Section 7.1

Introduction

The objective of this study was to determine the suitability of confocal laser scanning microscopy (CLSM) for examination of wood decay fungi and bacteria in wood. Wood with tunnelling bacteria decay (from Tombleson's mandarin orchard) and a less well known decay type caused by tunnelling hyphae (type T1 as described in chapter 4) (from the Peat site) were chosen for this study on the basis that their defining micromorphology is close to the resolution limits of light microscopy (LM). CLSM used in conjunction with epifluorescence staining techniques may offer improved resolution compared to LM, and at the same time avoid the need for time consuming sample dehydration, fixation and coating procedures that are required to achieve high resolution using well established transmission electron microscopy (TEM) and scanning electron microscopy (SEM). This is an important consideration for ecological studies that require examination of large numbers of wood samples and are not therefore suited to electron microscopy.

Another potential advantage of CLSM over other microscopy techniques can be appreciated by considering a comment of Zabel and Morrell (1992) referring to the difficulty of examining small multidirectional decay features inside an opaque material. Decay micromorphology such as tunnels and the hyphae contained in them sometimes move through wood in a random fashion or across the depth of many focal planes over a short distance. It is therefore difficult to gain a three dimensional perspective of their orientation when thin sections are examined using TEM. Whilst SEM is excellent for examining surface topography of substrates with complex surface relief, such as wood it cannot produce images of internal decay features. Images of multiple sequential sections through the depth of relatively thick sections (5 – 8.5 μm) produced by CLSM, can be reconstructed into an image that has 3D qualities using mathematical interpolation software.

The epifluorescent techniques employed by CLSM are more complicated and time consuming than simple LM staining techniques such as use of aniline blue but this inconvenience may be offset by the capability of multiple non-destructive sectioning. Unlike LM that requires examination of many sections within different planes to gain an appreciation of the 3D interconnectedness of decay features like tunnels, the penetrative nature of laser light produced by CLSM coupled with the unique optical arrangement of the pin-hole aperture (Donaldson and Lausberg, 1998), enables production of a sequential "stack" of images without cutting the sample.

The confocal microscope was patented in 1961 (Lichtman, 1994) but has only seen widespread application since the early 1980's. CLSM has increasingly seen application in medical science (Boon *et al.*, 1994; Mills *et al.*, 1994), biology (Shotton, 1989), fungal cytology (Knight *et al.*, 1993; Kwon *et al.*, 1993), and wood structure (Knebel and Schepf, 1991; Donaldson and Lausberg, 1998). However, at the commencement of this study CLSM had not yet been applied to wood biodegradation studies. To apply CLSM to examination of wood requires use of epifluorescent techniques. One of likely challenges of this procedure is selection of fluorescent stains that enable differentiation of decay microorganisms from wood cell walls which autofluoresce over a wide wavelength band when illuminated by a laser light source. Fluorescent lectins specific to chitin in fungal cell walls have been employed for examining fungi in wood using LM (Morrell *et al.*, 1985; Krahmer *et al.*, 1986) but little information is available for fluorescent staining of bacteria in wood. In this study the feasibility of combining fluorescent stains specific to fungi and bacteria with CLSM was investigated. The fluorescent staining techniques applied to fungi were refined by using wood decayed by pure cultures of the brown rot fungus *Coniophora puteana* and the white rot fungus *Trametes versicolor.* A limited number of wood samples examined using CLSM were also examined using SEM and TEM for comparison (LM micrographs for type T1 and TB are in sections 4.5.3 and 4.5.7 (volume II) respectively).

Section 7.2

Results

Section 7.2.1

CLSM images of wood with white and brown rot

Fungal hyphae that had not been fixed with glutaraldehyde were almost non-fluorescent and therefore glutaraldehyde was required for their resolution (Figure 7.1). Wood cell walls were strongly autofluorescent but glutaradehyde fixed fungal hyphae were clearly visible in both wood luminae and wood cell walls. However glutaraldehyde fixation followed by treatment the WGA stain was more effective than glutaraldehyde alone, giving brighter fluorescence to fungal hyphae (Figure 7.2). Figure 7.3 shows 4 images (a – d), 1.25 μm apart through the depth of a 5 μm section of wood with white rot caused by *Trametes versicolor.* Wood had been embedded in Spurr's resin after treatment with glutaraldehyde and WGA.

Section 7.2.2

CLSM, TEM and SEM images of wood with type T1 (hyphal tunnelling)

The interconnectedness of hyphae of variable diameter (0.2 - 5 μm) in tracheid walls and luminae was established by sequential removal of wood cell wall layers (Figure 7.4). Tunnels were present in all parts of the wood cell wall, including the highly lignified middle lamella (Figure 7.5). The tunnels varied greatly in their orientation and were cut transversely, obliquely, and longitudinally within the plane of a single section, and correspondingly were seen in their circular, oval, and long-channel profiles (Figures 7.5 and 7.6). When present within apical regions of tunnels, hyphae completely filled them (Figure 7.7). Although there may be an initial preference for the secondary wall, as the decay becomes more severe all regions of wood cell walls, including the highly lignified middle lamella, were attacked and degraded. As the decay progressed and the tunnel number increased, adjoining tunnels often coalesced, resulting in the formation of larger irregular voids (Figures 7.8 and 7.9). The tunnels and voids appear empty of any contents except for the occasional presence of hyphae, hyphal residues, or lignin degradation products (Figure 7.8 and 7.10).

Section 7.2.3

CLSM and TEM images of wood with tunnelling bacteria decay

Distortion-free images of transverse sections could only be produced if wood specimens were embedded in Spurr's resin prior to sectioning (Figure 7.11). Figure 12 shows four images (a – d) 2.15 μm apart through a 8.5 μm thick section of a longitudinal section of a tracheid containing tunnels and associated tunneling bacteria (TB). Using 3D reconstruction, bacteria within transverse, longitudinal radial and longitudinal tangential faces of tracheids can be seen in Figure 7.13. The fine detail of tunnelling bacteria decay in pine tracheids is given in Figures 7.14 – 7.22.

Section 7.3

Discussion

Differentiation of fungi and bacteria using epifluorescence CLSM is not straightforward because wood autofluorescence is strong over a wide wavelength band. In the past, cell autofluorescence caused by aldehyde (especially glutaraldehyde) fixation has been regarded as noise (Jones *et al.*, 1994, Haugland 1996). However in the present study glutaraldehyde was found to be a useful means for differentiating fungal hyphae from wood particularly because it was relatively easy to apply and the cost was low. However the degree of differential fluorescence of wood cell wall and fungi or bacteria achieved using glutaraldehyde alone was not consistent, possibly because microbial constituents fixed by glutaraldehyde such as protein had denatured (Strange & Cox, 1976; Welker, 1976). WGA targets chitin, which is present in most fungal cell walls (Wisselingh, 1898; Foster, 1949; Morrell *et al.*, 1985). Therefore the greater reliability of the combined glutaraldehyde-WGA system was probably a reflection of the greater stability of fungal chitin (Foster, 1949; Cochrane, 1958). The intensity of fluorescence of fungal hyphae in samples stained with WGA was generally stronger than that obtained with glutaraldehyde treatment, allowing better differentiation between wood and hyphae. The advantage of using the dual system rather than WGA alone was that glutaraldehyde preserved fungal morphology while imparting some fluorescence which the WGA then increased.

Embedding in Spurr's resin was effective at preventing distortion of fragile decayed wood cell walls, helped retain fluorescence and also enabled direct observation under oil immersion objectives (Kagayama *et al.*, 1996). Embedded samples can also withstand a greater duration and intensity of laser illumination, significantly improving imaging quality, especially when a zoom function is used for imaging at higher magnifications. However, embedding increases sample preparation time and cost and makes this technique largely unsuitable for examination of the large numbers of samples produced in ecological studies.

Phospholipid probes have not been used for examining bacteria in wood before. Phospholipids occur in more than 50% of known species of bacteria, primarily in membranes (Lechevalier, 1977). The combination of glutaraldehyde fixation and phospholipid staining was a reliable method for localising wood-degrading bacteria, which contain a characteristic double membrane envelope containing lipopolysaccharides (Singh and Butcher, 1991). Fluorescent WGA stains are specific for Gram-positive bacteria (Haugland, 1996) and therefore the glutaraldehyde-WGA stain described is useful for distinguishing Gram positive and negative bacteria.

TEM revealed the presence of a "halo" around hyphae in the early stages of tunnelling which was particularly prominent in the S2 layer. The cell wall in the "halo" region appeared more electron lucent, indicating a loss in lignin (Hepler *et al.*, 1970; Maurer and Fengel 1990; Donaldson 1994; Singh 1997). The presence of a "halo" around tunnelling hyphae could suggest local (0.25μm) production of low molecular weight diffusible degradation products small enough to penetrate the intact wood cell wall. This suggestion is consistent with preferential removal of lignin, ahead of cellulose, as occurs for wood decayed by preferential white rot fungi (Eriksson *et al.*, 1990; Blanchette *et al.*, 1990). Typically, lignin removal by preferential white rot fungi is more expansive, occurring over much greater distances across cell wall away from hyphae, usually in the cell lumen. However the nature of removal of both the secondary cell wall and the middle lamella (ML) around distal hyphal regions and where tunnels had coalesced was more consistent with simultaneous white rot i.e. uneven erosion of the tunnel wall with few cell wall residues in the gap between the hypha and the wall. Furthermore there was no

evidence of defibrillation caused by more expansive lignin removal from the ML as is typical for preferential white rot.

The ability to "peel away" wood layers in order to reveal underlying features was very useful for gaining a 3D perspective of decay micromorphology or for revealing partly obscured features, for example of a hypha penetrating a bore hole. Typically with conventional sectioning for LM, many attempts would be needed to encounter features within a thin section i.e. bisected close enough and at the correct angle to ensure good resolution. Whilst the glutaraldehyde-N-*Rh*-PE fluorescent staining technique provided superior contrast between tunnelling bacteria and the wood cell wall compared to aniline blue stain used for LM, the 3D capabilities of CLSM did not provide improved insight into the nature of tunnelling. The LM micrographs discussed in chapter 4 provided greater insight into the orientation and nature of penetration. For example, cross walls were clearly visible in LM micrograph which helped gain insight in previous positioning of the bacterium in distal tunnel regions. Furthermore, the depth of field of the LM optics used also enabled observation of the angle of tunnels relative to the cell wall layers. Also the pleomorphic nature of the bacteria was revealed in LM micrographs but not in CLSM micrographs. This may be due to the quality or freshness of the samples examined which is a reflection of the time taken to stain and embed sections for CLSM. Examination of fresh, non-embedded sections was not a viable option for CLSM because of the distortion introduced during sectioning. Also with LM many hundreds of sections can be examined in the time taken to prepare a much smaller number for CLSM and inevitably this enables images to be captured for wood at different stages of decay. Comparison of many images of different orientations from wood in various stages of decay improves objective analysis.

TEM is currently the most powerful tool for elucidation of the fine details of decay micromorphology (Blanchette et al., 1990; Zabel and Morrell, 1992; Eaton and Hale, 1993). However the LM images produced in chapter 4 provided information concerning the spatial relationship between the decay microorganism and the wood cell wall that cannot be obtained using TEM. For example, as discussed in chapter 4 the alignment of tunnelling bacteria with the cellulose microfibrils, typically those of

the S2 layer but also the S1 layer, is not apparent in high magnification TEM images. Tunnelling bacteria attack is typically described as random within the cell wall (Singh and Butcher, 1991). Since alignment was more common that non-alignment in the early stage of decay at all sites were tunnelling bacteria occurred, it seems unlikely that is was a feature peculiar to this study. The avoidance mechanism adopted by bacteria in regions of wood with a high tunnel density probably overrides alignment with microfibrils in later stages of decay and this has probably resulted in the significance of alignment being largely overlooked. Clearly alignment is considered a major diagnostic feature of soft rot caused by deuteromycetes and ascomycetes. This is significant for two reasons. Firstly, if as the literature suggests, alignment with cellulose microfibrils is an important indication of the nature of decay initiation in terms of fungal anatomy and decay biochemistry, then its significance for tunnelling bacteria should also be considered. Secondly, if alignment is a common feature amongst most decay microorganisms as is suggested by observations of chapter 4, then its significance as a diagnostic feature requires revision.

The tendency of hyphae associated with several types of decay, including soft rot and tunnelling type T4, to align with concentric wall layers has been reported by a relatively small number of authors (Nilsson and Daniel, 1983; Sulaiman and Murphy, 1995). Its common observation in this study was probably due to the high resolution over a broad depth of field achieved with the LM optics used in this study. Typically such concentric alignment would be confirmed by examination of transverse sections under TEM. However in this study features aligned with the curvature of the cell wall could be seen in relatively thick longitudinal sections. These observations illustrate the advantage of combining microscopic techniques during wood decay investigation. It also illustrates the ability of good LM optics to produce images with a reasonable 3D perspective, especially when coupled with the iterative approach facilitated by rapid multiple sectioning that is not possible with other microscopic techniques, including epifluorescent CLSM.

The potential of computer assisted manipulation coupled with continued improvements to imaging techniques was shown to be considerable. In the time available it was not possible to develop improved fluorescent techniques or make full

use of the capabilities of mathematical interpolation software used to construct 3D images. In theory once these two features of epifluorescent CLSM have been perfected and married together optimally it should be possible to produce near holographic images of decay features. For example, a complete network of tunnelling hyphae, or mucopolysacharide lined TB tunnels, could be shown within a predefined volume of wood cell wall. The hyphae or TB tunnels could be looked at from any angle, or any position within the network, and the surrounding layers of wood cell wall could be replaced digitally in stages. Similar procedures are used in medical science to examine the 3D perspectives of diseased vascular systems and surrounding tissues (Mills *et al.*, 1994). Perhaps future discussion of the details of new findings will be assisted by use of holographic projections introduced into the midst of those within an auditorium.

Section 7.4

Conclusion

In principle, CLSM has the same resolution as traditional light microscopy but CLSM can produce images of higher quality at greater magnification due to its blur-free imaging and strong laser illumination. However this advantage can only be fully exploited if fluorescent stains with appropriate wavelength band emission and appropriate microorganism specificity are used. Protracted staining procedures, the need to embed samples, and the time taken to manipulate images using computer assisted 3D modeling software makes the CLSM-epifluorescent technique time consuming and unsuitable for examination of large numbers of samples. The LM optics used in chapter 4 provided insight into the 3D nature of decay micromorphology for large numbers of samples and this capability may have been under exploited to date. The future potential of computer assisted image manipulation coupled with continued improvements to imaging techniques is likely to provide significant new insight into decay micromorphology, offering exciting opportunities for future research.

SECTION 7: Appendix

Section 7.5.1 - CLSM Micrographs of white and brown rot

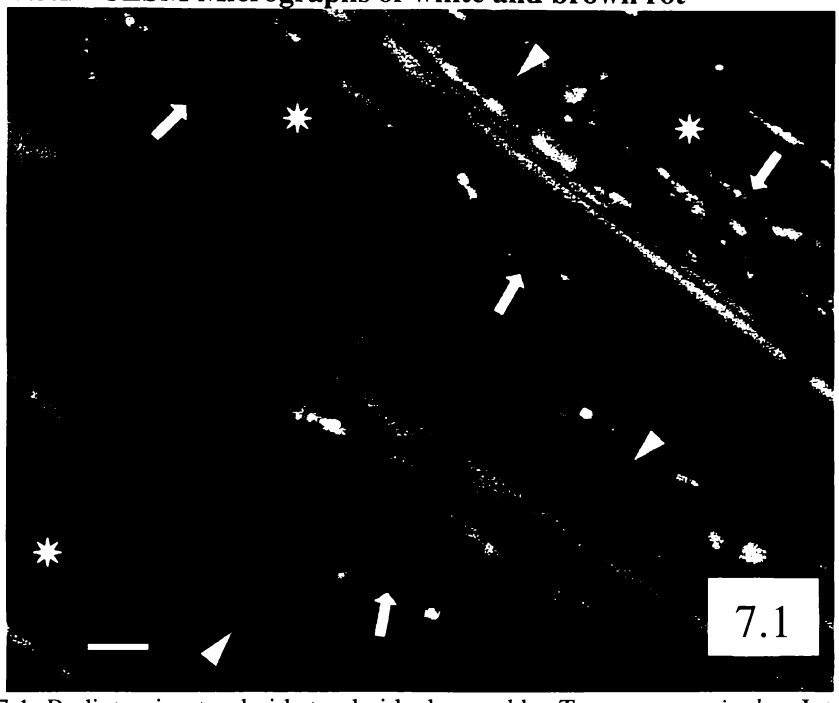


Figure 7.1: Radiata pine tracheids tracheids decayed by *Trametes versicolor*. Intensity of fungal fluorescence caused by glutaraldehyde fixation (arrows) is similar to the autofluorescence of wood cell walls (arrowheads). Asterisks = wood cell lumen. Bar = 10µm.

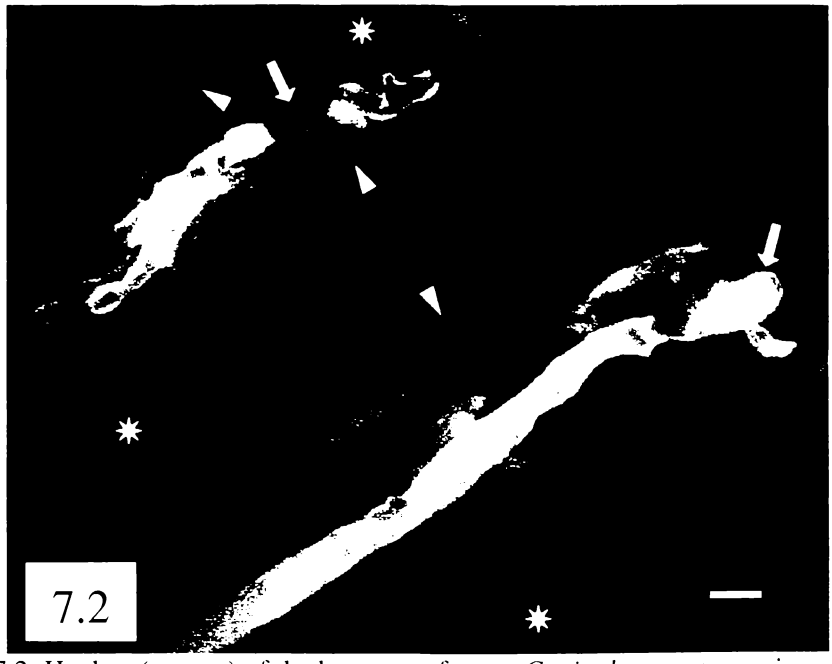


Figure 7.2: Hyphae (arrows) of the brown rot fungus *Coniophora puteana*, in a wood section treated with glutaraldehyde and WGA. Arrowheads = wood cell walls; asterisks = wood cell lumen. Bar = 10µm.

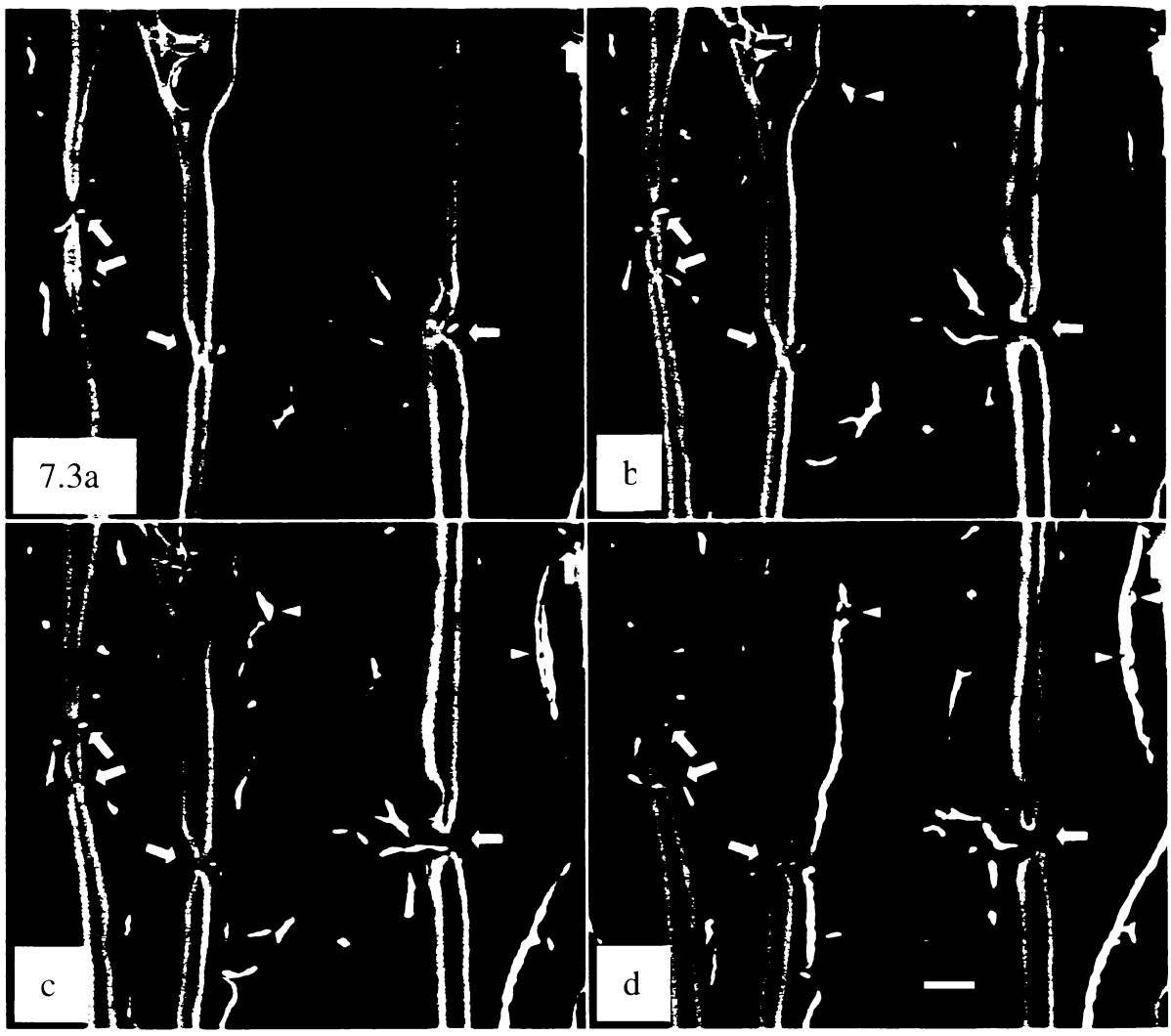


Figure 7.3a-d: Wood attacked by a white rot fungus, *Trametes versicolor*, treated with glutaraldehyde and WGA and embedded in Spurr's resin. Four images (a – d), 1.25 µm apart through the depth of a 5 µm section. Arrows = fungal hyphae penetrating wood cell walls. Note the erosion of wood cell walls around the hyphae. Arrowheads = clamp connections.

Section 7.5.2
CLSM micrographs of tunneling type 1 (T1)

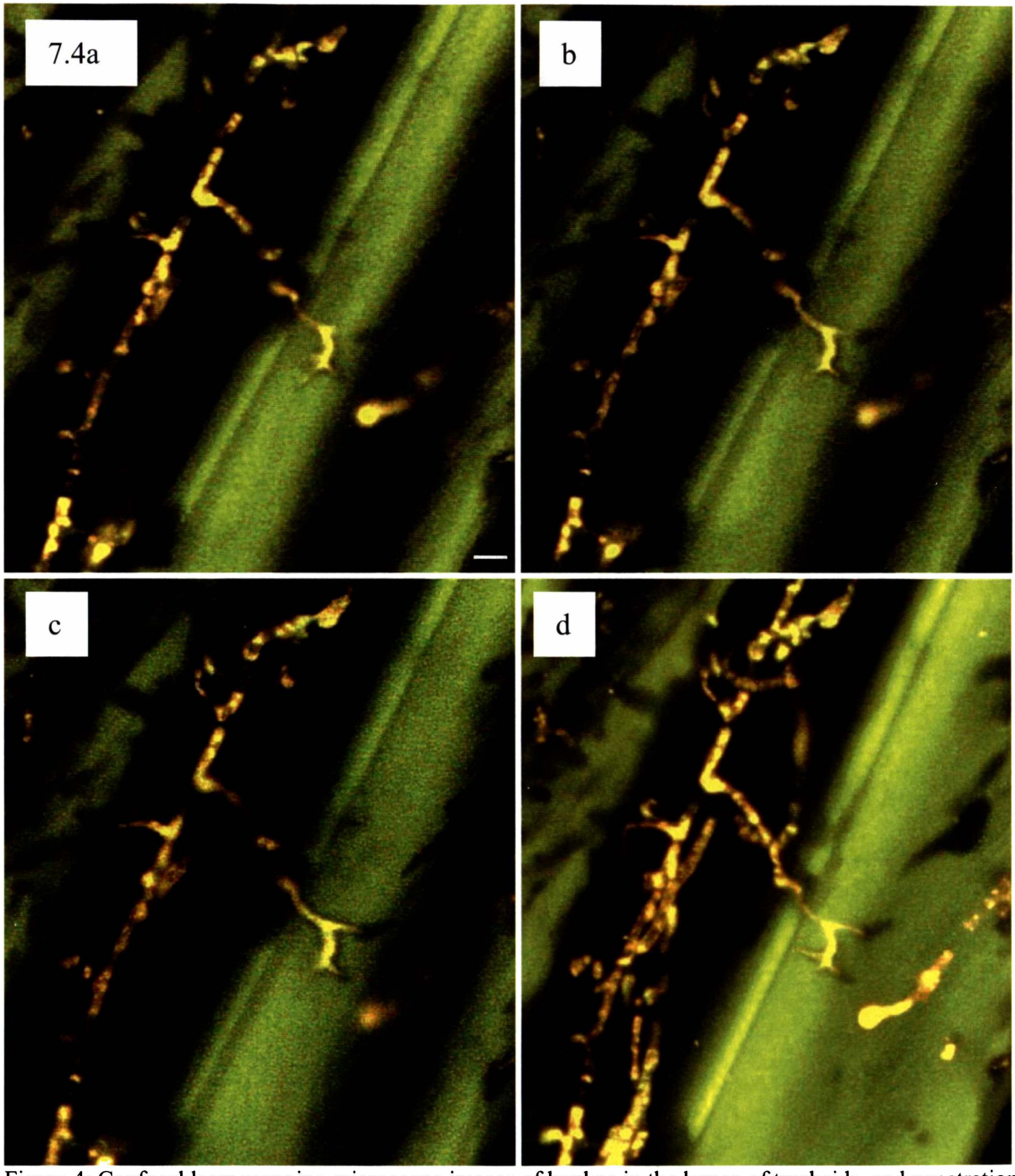


Figure 4: Confocal laser scanning microscope images of hyphae in the lumen of tracheids, and penetration of tracheid walls by hyphal branches (arrows). a - c are images of individual sequential optical sections showing hyphal penetration into the tracheid wall. d is a composite projection of a – c. Bar = 5 μm.



Figure 7.5: Transverse section through axial tracheids showing the presence of tunnels (T) in the secondary walls as well as middle lamellae. The points of fungal entry into tracheid walls are indicated by arrowheads. The asterisks show the location of hyphae in the lumen. Bar = 5 μm.

Section 7.5.3
TEM and SEM micrographs of T1

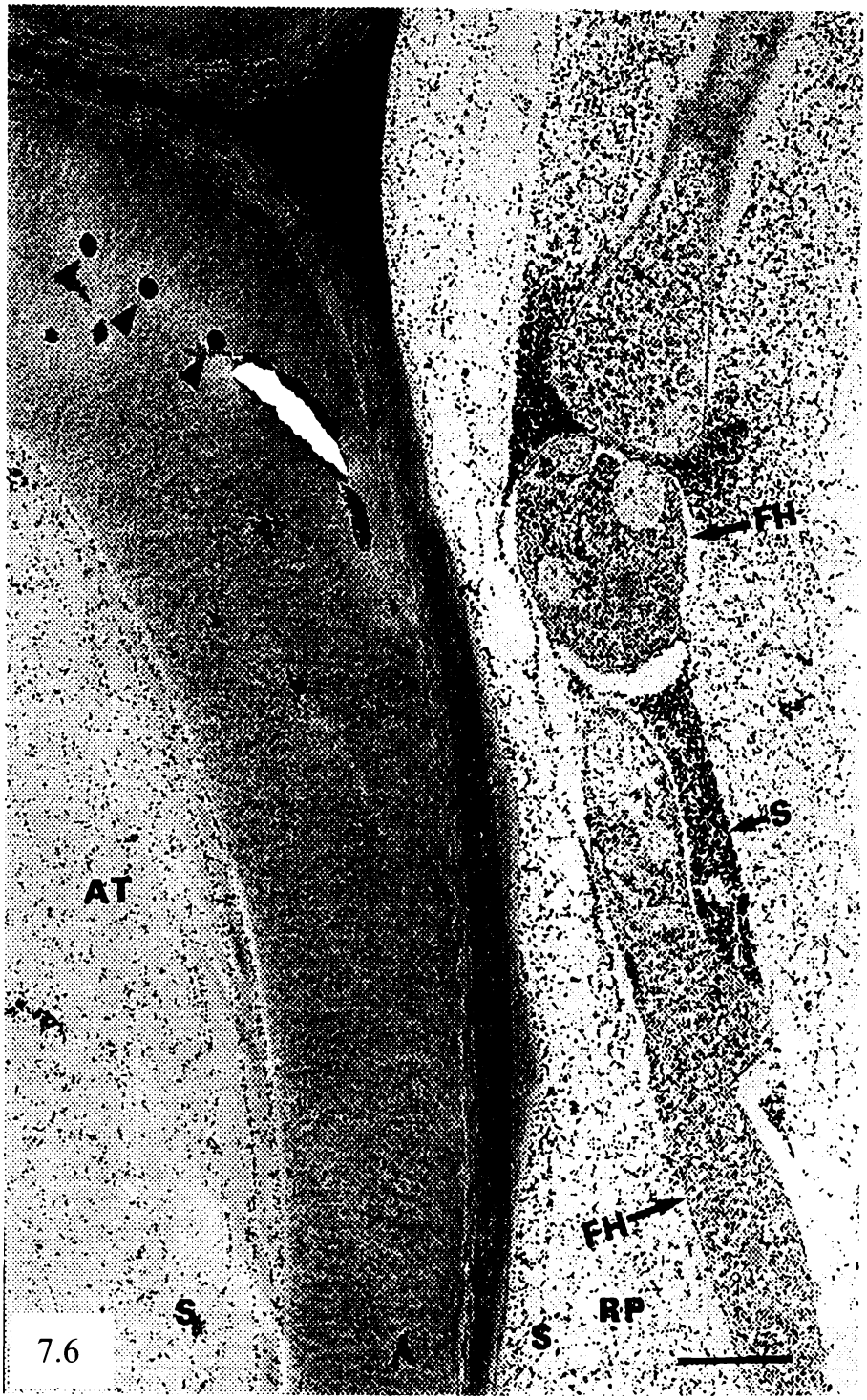


Figure 7.6: Transverse section through an axial tracheid (AT) and a ray parenchyma cell (RP). Large fungal hyphae (FH) are present in the ray parenchyma cell and small hyphae which are cut transversely, are present in the S2 layer of the tracheid (arrowheads). S = slime. Transmission electron micrograph (TEM). Bar = 2μm.

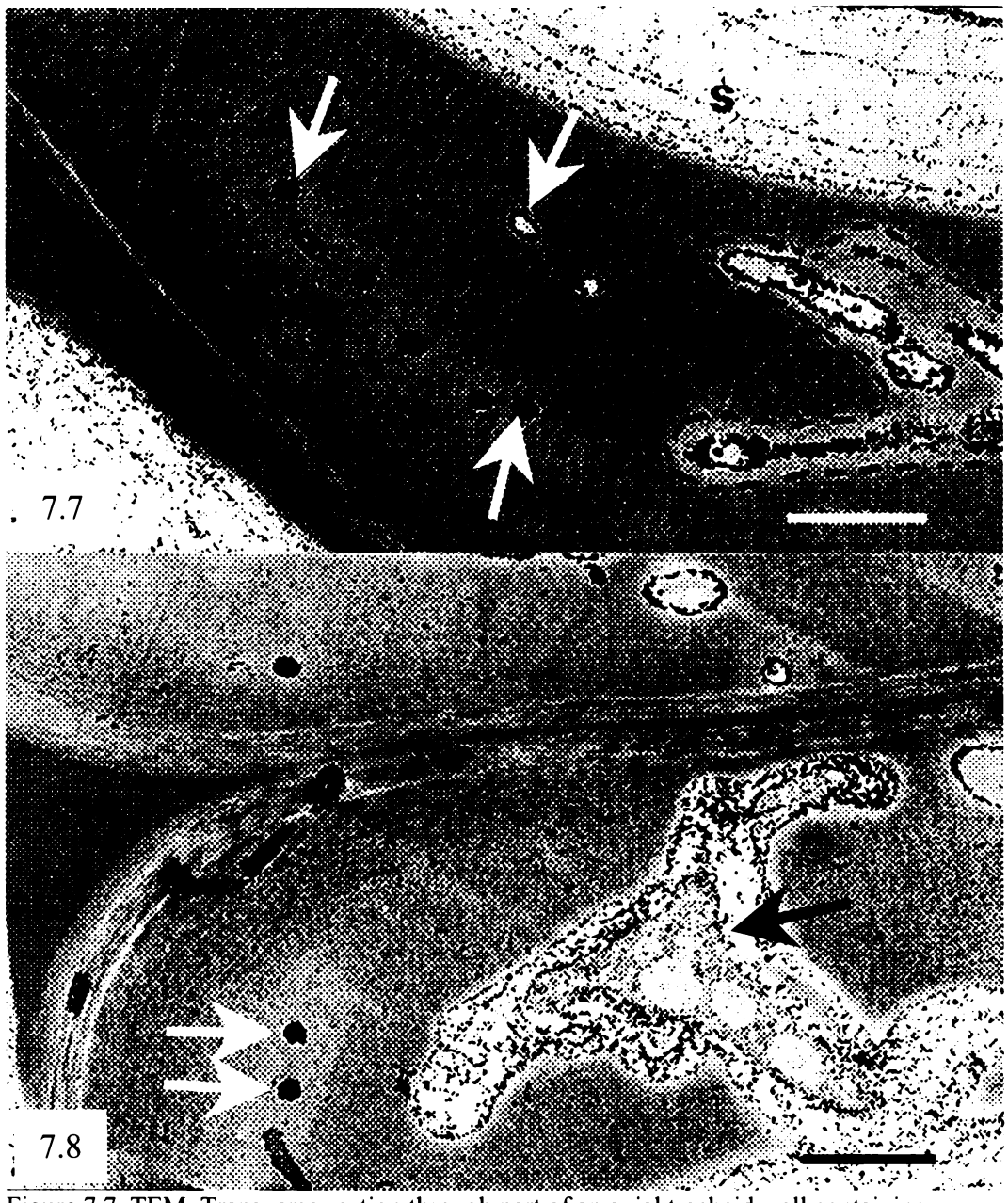


Figure 7.7. TEM. Transverse section through part of an axial tracheid wall containing fungal hyphae in the S2 layer, which vary in their size and orientation. A translucent zone present in the S2 wall around hyphae is marked by broken lines. S = slime. Bar = 2 μm.

Figure 7.8. TEM. Transverse section through part of an axial tracheid. White arrows = longitudinal sections of hyphae in tunnels. Black arrows = transverse sections through hyphae in tunnels. Bar = 2 μm.



Figure 7.9. SEM. Hyphae (arrowheads) and tunnels (arrows) in wood cell walls. Bar = 20 μm.

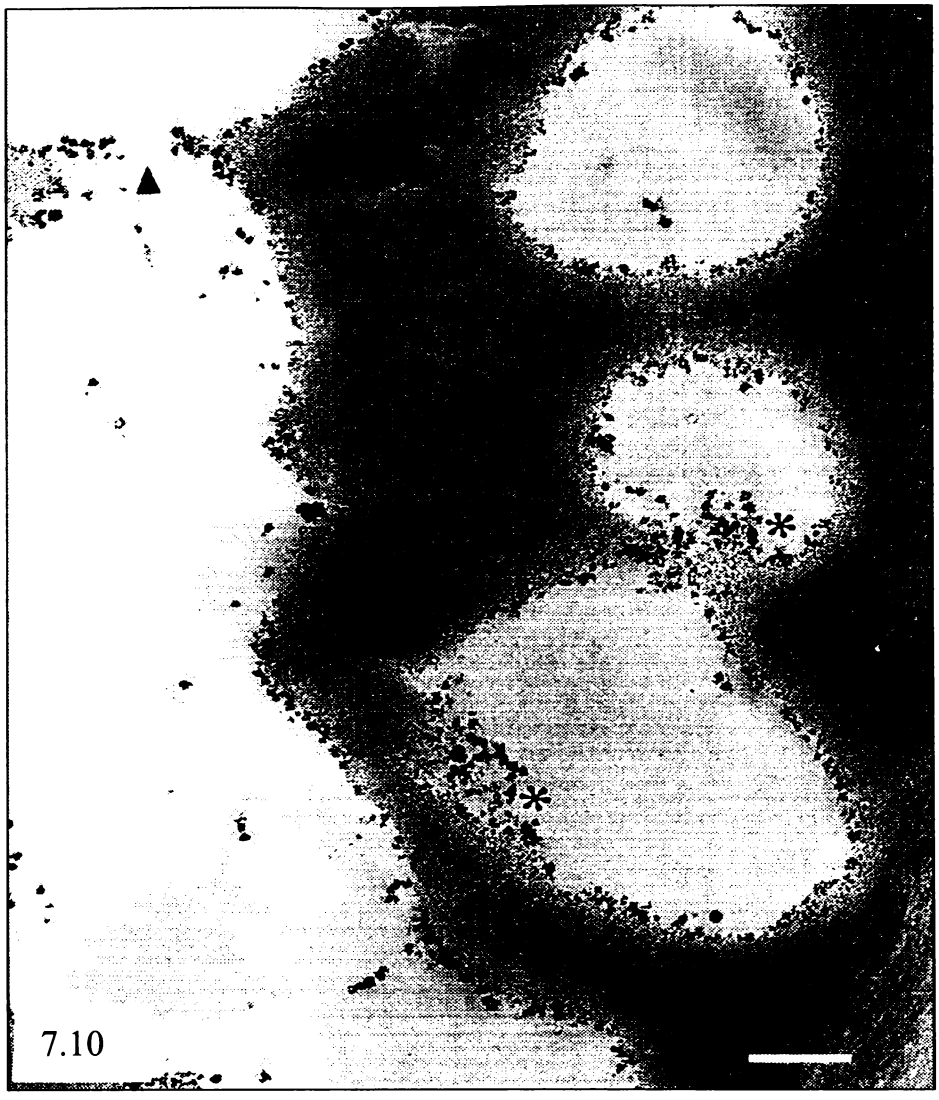


Figure 7.10. TEM. Transverse section through part of an axial tracheid wall containing tunnels. The arrowhead indicates the region of coalescence between adjacent tunnels. Dense, granular material present in the tunnels is indicated by asterisks. Bar = 0.25μm.

Section 7.5.4
CLSM micrographs of tunneling bacteria

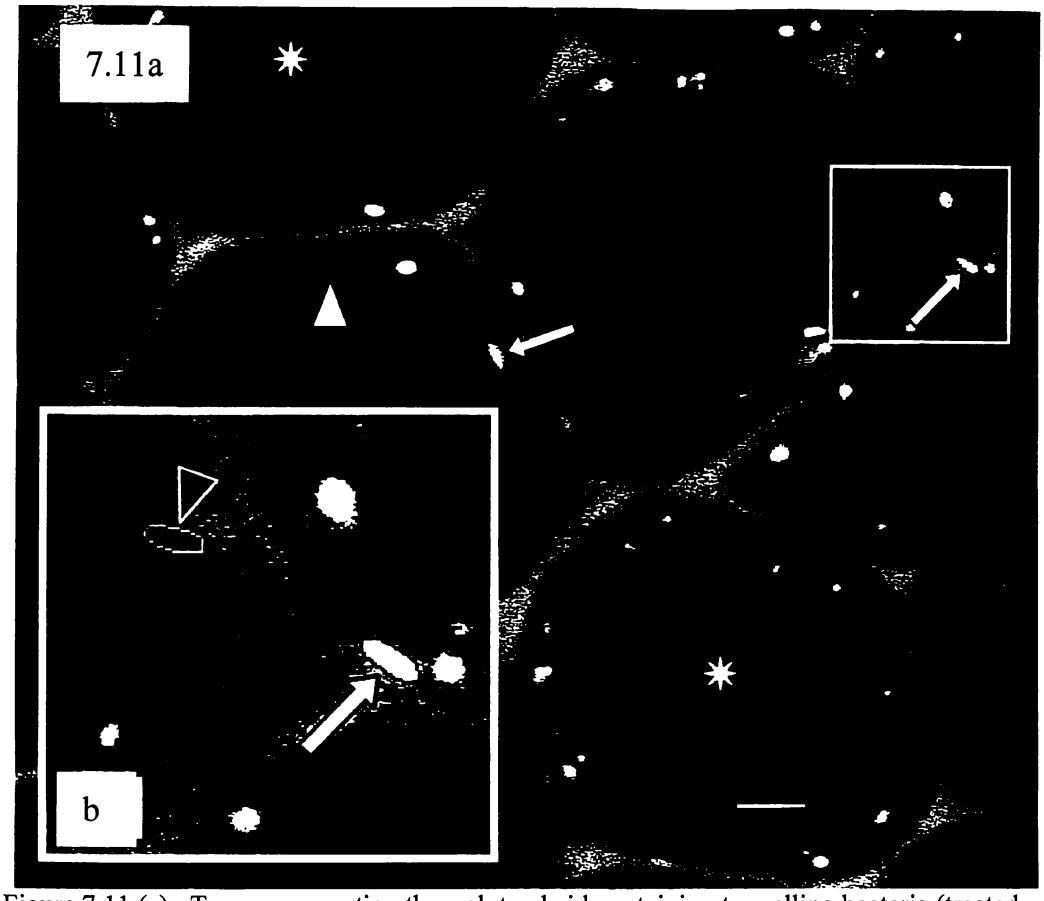


Figure 7.11 (a): Transverse section through tracheid containing tunnelling bacteria (treated with glutaraldehyde and N-Rh-PE and embedded in Spurrs'. Bacteria (arrows) are differentiated from the wood cell wall (arrowhead). All the punctate fluorescent areas in wood cell walls are bacteria. Asterisks = wood lumen. (b) Detail from (a). Arrowhead = tunnel in wood cell wall (outlined with a white line) with very similar shape and size to a bacterium (outlined in black line, arrow). Bar = 5 μm.

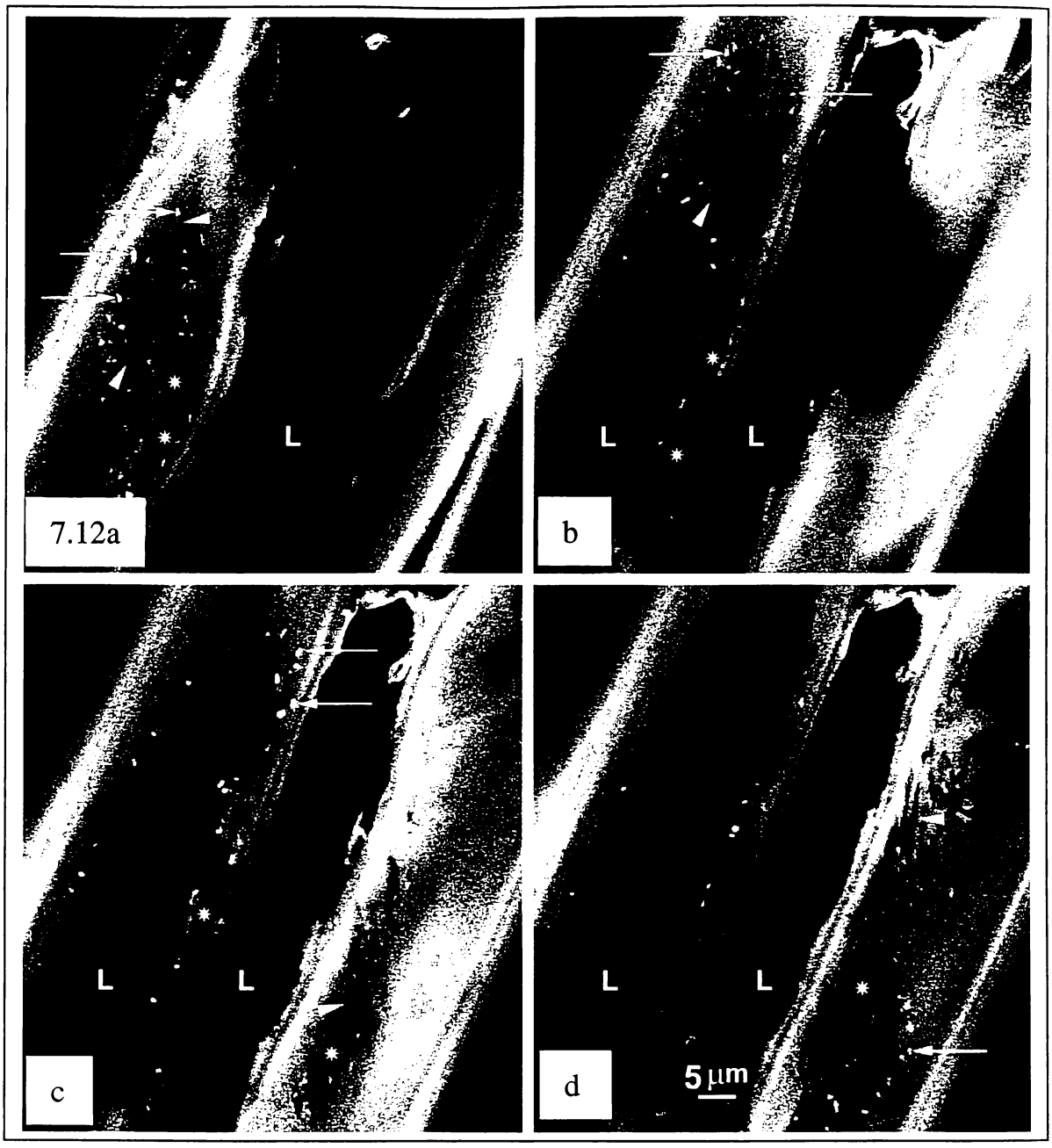


Figure 7.12 a-d: Four confocal images 2.15 µm apart (out of 16 image slices) across a 8.5 µm thick longitudinal wood section of a tracheid containing tunneling bacteria. Arrows = bacteria in tunnels; arrowheads = tunnels; asterisks = collapsed wood cell wall areas; L = wood lumen. Bar = 5μm.

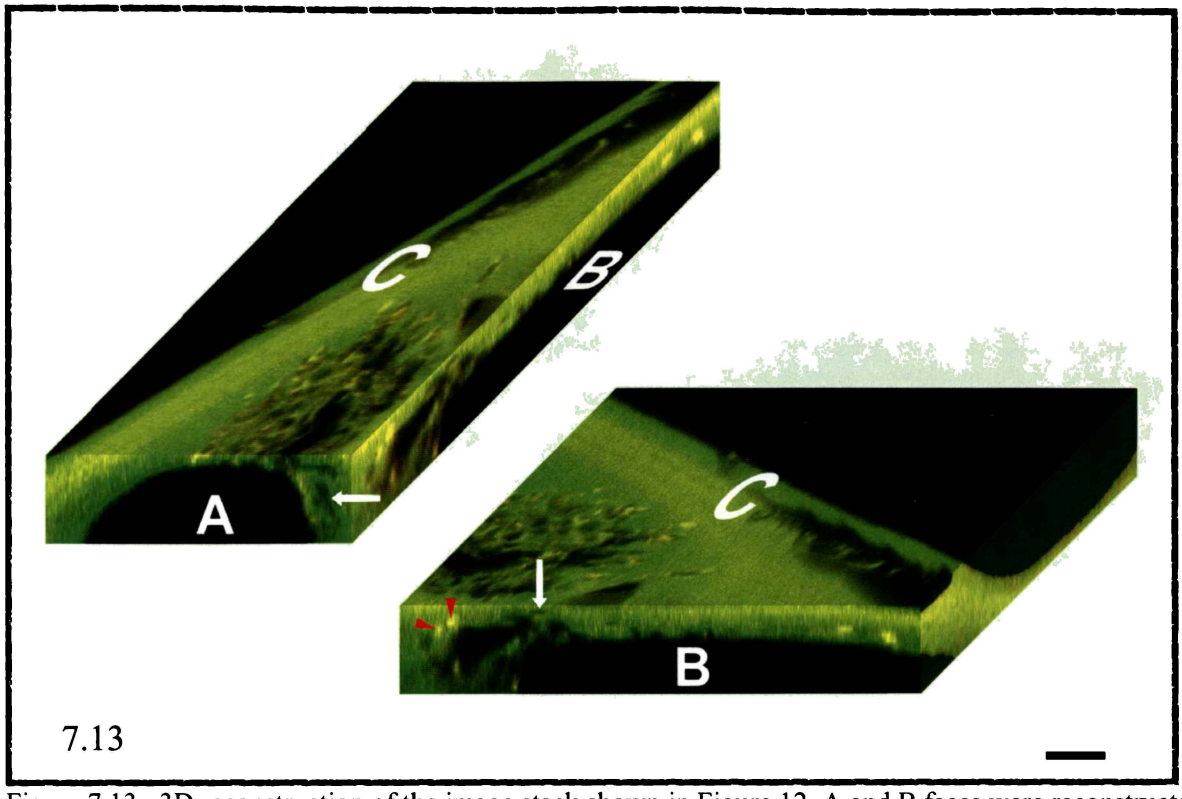


Figure 7.13: 3D reconstruction of the image stack shown in Figure 12. A and B faces were reconstructed by mathematical interpolation computer software. C face was the original imaging face. The two pieces of tracheid are identical but one is rotated at 90° to show the B face more clearly. Arrows = tunnels produced by bacteria; arrowheads = bacteria at the end of tunnels. Bar = 5μm.

Section 7.5.5.
TEM micrographs of tunnelling bacteria



Figure 7.14: Tunnelling bacterium (white arrow) shortly after penetration though the lumen wall into the S2 layer of a tracheid. Bands of mucopolysacharide (black arrow) remain attached to the lumen wall giving insight into earlier bacterium position. Bar = 2μm.



Figure 7.15: Pleomorphic tunnelling bacterium (arrow) closely associated with tunnel wall in pine tracheids. Bar = 2μm.



Figure 7.16: Tunnel produced by tunnelling bacterium (arrow) penetrating the middle lamella (star). Bar = 2μm.



Figure 7.17: Tracheid wall containing many closely associated tunnels (arrows) produced by tunnelling bacteria. Bar = 2μm.

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