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VARIATIONS IN DECAY SUSCEPTIBILITY AND THEIR ASSOCIATION WITH WOOD PROPERTIES OF *PINUS RADIATA*

BY

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"Studies of the chemical and physical properties of wood in relation to its resistance to decay are necessary to understand the protective mechanisms involved in these naturally durable wood substances, and the reasons for their variation."

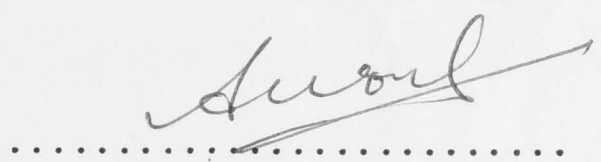
Robert A. Zabel

(Bulletin of the New York State College of Forestry at Syracuse University, December 1948)

Dedicated to my parents for their endless support...

DECLARATION OF ORIGINALITY

Unless otherwise acknowledged in the thesis, this research was conducted by the author without collaboration



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ABSTRACT

A series of experiments examined the possible causes of variations in the natural susceptibility of the 13-year-old juvenile sapwood stems of *Pinus radiata* to decay *in vitro* by a white-rot (*Fomes lividus*) and brown-rot (*Gloeophyllum trabeum*) organism. In addition to decay susceptibility, a number of wood structural properties (wood density, percentage late wood and tracheid length) and chemical properties (contents of lignin, available sugars, nitrogen, phosphorus, potassium, calcium, magnesium, sodium, zinc and extractives) were determined radially within stems from three provenances.

Within stems, decay susceptibility was significantly ($P < 0.05$) greater in the outer than inner sapwood. For one or both fungi, this variation was significantly and positively correlated with density and percentage late wood, and also with the contents of nitrogen, phosphorus and potassium. The lack of a causal influence of gross wood structure on the variations in decay susceptibility was therefore suggested, while the influence of the three elements (and possibly also, calcium and magnesium) was more likely stimulatory (a form of nutrient source). These hypotheses were in part supported by a study of the progressive changes in certain chemical properties of the wood (contents of lignin, nitrogen, phosphorus and potassium, and solubility properties) during the decay process for each fungus.

CHAPTER 1

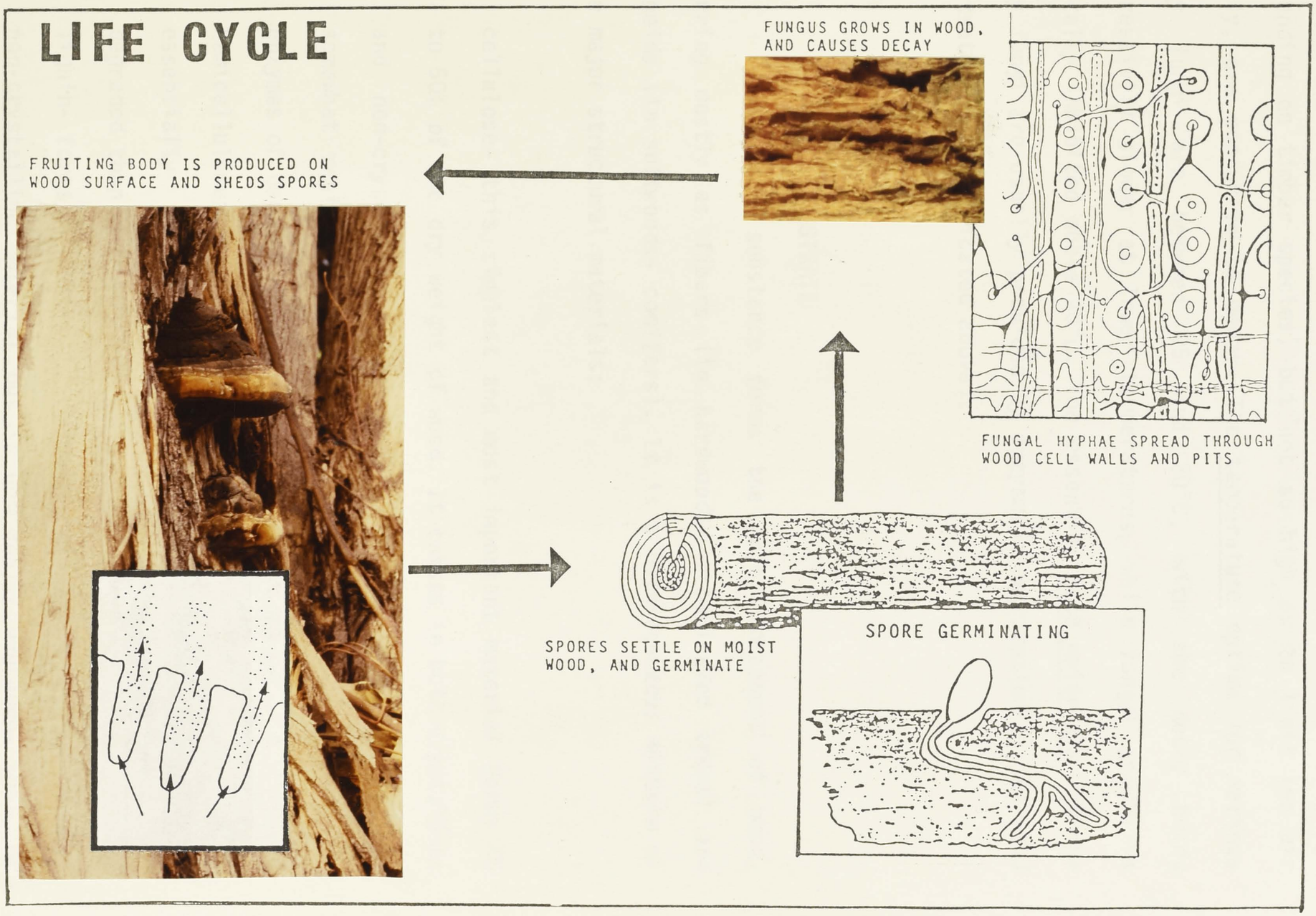
GENERAL INTRODUCTION

1.1 THE NATURE OF DECAY

All timbers, whether as living trees or as processed materials, are susceptible to decay by a wide variety of bacteria, fungi and insects. By far the most destructive of these organisms are the wood-destroying fungi (termed 'wood decay' fungi) which belong to the class *Basidiomycetes*. These fungi cause either a white- or brown-rot type of decay resulting in severe loss in both the weight and strength of the timber.

Fungi responsible for decay in timbers produce spores from fruiting bodies which form occasionally on the surface of the wood. Under favourable conditions (discussed below), spores germinate and the fungus forms a network of branching hypha which extends rapidly through the wood, passing from one cell to another by penetrating the pits or cell walls. By the action of enzymes secreted from the hyphae the wood material is degraded into simpler substances which provide readily available nutrients for fungi. As a result the weight of wood can decrease appreciably. A typical cycle of development of decay fungi on wood is shown in Figure 1-1.

FIGURE 1-1: Generalized life cycle of a wood-destroying fungus



Wood is rendered degradable under certain conditions which favour the growth of suitable decay fungi. An adequate supply of oxygen together with a wood moisture content of at least 30 to 40% (depending on timber species) but not so high as to limit the air supply, are conducive to decay. The temperature optima for various decay fungi are between 25 and 30°C, with the decay being progressively slower at lower temperatures, while fungal growth is typically inhibited above 40°C. The essential food materials for the decay fungi are usually provided by enzymatic degradation of the wood substrate itself (indicated above).

1.2 THE WOOD SUBSTRATE

Cell wall substance forms the major component of wood, occurring mostly as fibers (in hardwoods- broadleaved trees) and tracheids (in softwoods- conifers). It is a heterogeneous mixture of three major structural materials:

(a) cellulose- this simplest and most important material forms 40 to 50% of the dry weight of wood. It exists in both crystalline and non-crystalline form. The crystalline regions may be somewhat resistant to penetration by the large molecules of enzymes of decay fungi.

(b) hemicelluloses- form 20 to 30% of cell wall substance. Being essentially non-crystalline, is material is more readily degraded than cellulose.

(c) lignin- forms 15 to 30% of cell wall substance. It is a complex non-crystalline material but is not easily degraded. Thus, by encrusting the cellulosic and hemicellulosic components, lignin

offers some protection against the degradative fungal enzymes.

The minor components of the wood substrate, often comprising from 1 to 4% per cent of dry weight, are the cytoplasmic and reserve food materials (i.e. starch, simple sugars, lipids, proteins, peptides, amino-acids, nucleic acids, vitamins, etc.), and other extraneous substances (i.e. waxes, fats, fatty acids, organic acids, alkaloids, oils, resinous and phenolic compounds, etc., extractable with neutral organic solvents or water) which are formed and/or stored in parenchyma cells. The cytoplasmic and reserved food materials occur in greatest amounts in the living parenchyma cells of the sapwood. Near the sapwood-heartwood boundary in living trees, amounts of these materials gradually decrease and extraneous compounds are formed in parenchyma cells and deposited in the cell walls and cell lumina. Such substances account for the typically darker colour of the heartwood.

The materials in parenchyma of sapwood may act as a source of readily available foods for fungi, and such tissues are potentially susceptible to decay. In the heartwood, certain types of extractives, particularly some phenolic substances which are strongly anti-microbial, provide the major source of resistance of the heartwood to decay (Section 1.4).

1.3 ASSESSMENT OF NATURAL DURABILITY (NATURAL DECAY RESISTANCE)

Timbers vary enormously in the natural resistance of their heartwood to degradation by wood-destroying fungi. Such variations are ascribed to differences in the structure and chemical composition between timbers (see Section 1.4). In particular, it is the

variations in the quantity and quality of heartwood extractives which are largely responsible for the observed differences in durability. For practical purposes many of these timbers have been officially assigned durability ratings based on in-ground assessments of the service life (in years) of untreated heartwood (e.g. SAA[1], 1980). Timbers are thus graded into one of the general resistance classes, as exemplified by the SAA (1980):- Class 1 (very durable): at least 24 years service; Class 2 (durable): 15 to 24 years; Class 3 (moderately durable): usually less than 15 years; and Class 4 (non-durable): a few years or less. Clearly, in this type of testing, timbers are often decayed by a variety of microorganisms occurring under natural conditions.

In the laboratory the assessment of natural durability involves the exposure of small sterilized wood samples to decay by specific test fungi growing in pure culture under controlled conditions, and over a period of one to four months. Here, loss in weight of samples is used to classify timbers into their resistance classes [e.g. ASTM[2] D 2017 (ASTM, 1984)):- Class 1 timbers: decayed up to 10% weight loss; Class 2: 11 to 24% ; Class 3: 25 to 44%; Class 4: 45% or more.

It is not known whether the laboratory testing can provide a reliable estimate of the durability of wood under natural conditions, although such testing has been used quite successfully to rank timbers for natural decay resistance (e.g. Clark, 1969; Highley and Scheffer, 1970; Da Costa, 1979), and to estimate the influence of

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1. SAA: Standards Association of Australia
2. ASTM: American Standards for Testing and Materials

selected treatment variables (e.g. the content of preservatives) on the resistance of wood to decay.

1.4 THE CAUSES OF VARIATIONS IN NATURAL DECAY RESISTANCE AND SUSCEPTIBILITY OF WOOD

Fungitoxic or fungistatic extractives deposited during the formation of heartwood are the principle cause of decay resistance in wood. For example, the relatively high durability of the heartwood of certain *Eucalyptus* timbers, e.g. *Eucalyptus microcorys* F. Muell. (Da Costa and Rudman, 1958) and *E. wandoo* Blakely (Rudman, 1965), is due to the amounts and toxicity of phenolic extractives (Da Costa and Rudman, 1958; Rudman, 1965). The most outstanding variation found in a standing bole is an increase in decay resistance from inner to outer heartwood, paralleling variations in the quantity and/or quality of extractives *in situ* (Rudman, 1964; Scheffer and Cowling, 1966; Rao, 1982).

The dominant influence of extractives in these more resistant timbers largely obscures any secondary influences of other factors. By studying timbers of low decay resistance (i.e. sapwood and non-durable heartwoods), the contributing influence of these factors can be elucidated. Such factors, largely ignored in the durable species, may explain much of the variation in natural decay resistance in non-durable woods. Although the variations may be characteristically small in absolute terms, they are attracting

increasing attention as efforts are made to better understand the bio-deterioration processes in wood and to develop methods for preventing wood decay.

Factors, other than extractives, likely to account for variation in decay resistance in non-durable timbers are both anatomical/physical and chemical in nature.

1.4.1 Wood Structure

The influence of wood structure on decay has received little attention. If, however, it is assumed that fungi grow most rapidly through the void spaces in wood, i.e. cell lumina and pit apertures/chambers, then, the expansion of hyphae may be delayed when wood substance has to be traversed. Owing to the orientation of most of the lumina in the axial direction, the speed of invasion of tissues could be enhanced in this direction. Here, the number of end walls which must be penetrated per unit distance would be important. Further, the diameter of cell lumina is probably important in determining the surface area of cell wall substances exposed to the action of decaying fungal enzymes (Southam and Ehrlich, 1943; Toole, 1972). Deposited in vessel lumina in the heartwood of some hardwoodss are membrane-like structures (tyloses) which could present an additional barrier to the vertical extension of hyphae (Hosli and Osusky, 1978). Apart from direct penetration of cell walls by forming bore holes (Proctor, 1941), fungi also depend on the pittings of cell walls to colonize neighbouring cells (Greaves and Levy, 1965). Thus if these pits are aspirated or do not contain membrane openings wide enough for hyphae to pass through, some barrier/resistance to the

spread of hyphae from one cell to another might be expected.

The moisture content and air supply in wood which are so vital for decay (discussed), are related to wood structure. In light woods the wide lumina may permit both the rapid uptake of water and the movement of air within, providing conditions favourable for decay; in relatively impermeable (usually dense) woods, the movement of air and/or water may limit decay (Southam and Ehrlich, 1943).

1.4.2 Chemical Components

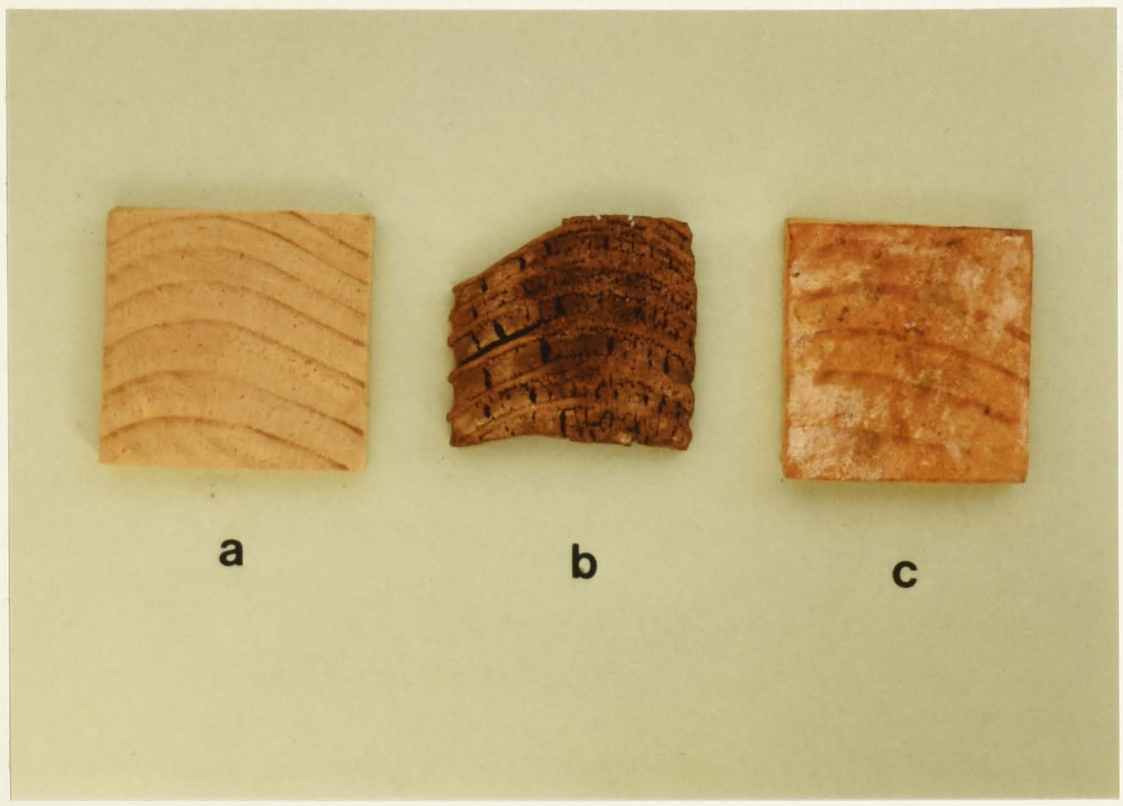
1.4.2.1 Structural Materials

Since cellulose, the hemicelluloses and lignin differ in their susceptibility to microbial degradation, variation in the proportions of these components within and between trees could be expected to contribute to variations in natural decay resistance in non-durable woods. Such variation in decay resistance or susceptibility of cell wall substance may be related to preferential degradation of these components by various decay organisms. Indeed, it has long been shown that an important difference between white-rot and brown-rot type of fungi lies in the relative ability of the two groups to utilize lignin. Thus white-rot species metabolize both the structural carbohydrates and lignin of wood (Savard and Andre, 1956; Cowling, 1961; Kirk and Highley, 1973), generally leaving the wood a light colour (Plate 1-1), while with the latter fungi, the lignin is left largely undegraded (Bray and Andrews, 1924; Cowling, 1961; Seifert, 1968; Kirk and Highley, 1973). The decayed wood in this case is usually a darker brown colour (Plate 1-1). Perhaps, with the

brown-rot fungi, the relative proportions of lignin which obstructs the access of fungal enzymes to cellulose in wood, is critical (Wilcox, 1965).

It is possible (Pew and Wyna, 1962; Wilcox, 1965) that the bonding arrangements between lignin and cellulose could have a greater influence on fungal degradation than the absolute amounts of the various materials. The degree of crystallinity of cellulose, which varies considerably between and within trees [e.g. the increase in crystallinity with distance from the pith (Wellwood *et al*, 1974)], could also be important since the crystalline regions are more resistant to fungal hydrolysis than are their amorphous counterparts (Scheffer and Cowling, 1966). Variations in components of lignin (Highley, 1982) and the hemicelluloses (Highley, 1977) also deserve consideration as possible causes of variations in natural decay resistance.

Plate 1-1: Appearance of *Pinus radiata* sapwood blocks decayed in the laboratory by brown- ('b': *ca.* 60% weight loss) and white-rot ('c': *ca.* 36% weight loss) decay fungi (*cf.* 'a': sound wood)



1.4.2.2 Non-structural Materials

The food materials such as starch and simple sugars which occur in the parenchymal tissues of sapwood, constitute a more readily available source of carbon nutrition for invading decay fungi than do the major cell wall substances (Hulme and Shields, 1970). Indeed, an abundance of these materials may encourage rapid establishment of decay fungi on wood even before any appreciable degradation of the wood occurs. Variations in the distribution of minute amounts of nitrogen and various inorganic elements could also govern rates of decay in non-durable woods. Nitrogen, which is essential to all decay fungi, has been shown to have a direct influence on decay susceptibility (Merrill and Cowling, 1965). The various macro- (e.g. potassium, calcium and magnesium) and micro- (e.g. manganese, zinc and iron) nutrients, may be similarly important.

1.5 AIMS OF THE PRESENT INVESTIGATION

The present investigation was initiated to further elucidate the roles of several structural and non-structural factors as determinants of variations in the relative susceptibility to decay of non-durable woods. Juvenile stems (aged 13 years), of *Pinus radiata* D.Don are used for this purpose. Samples are taken from various positions in a number of provenances in an attempt to incorporate a wide variation in wood properties of the species. A secondary aim is to determine the amount of variation in selected wood properties (e.g. density and tracheid length) with distance from

the pith, and between provenences, as this area of work has attracted considerable interest in recent years (e.g. Matziris, 1979; Nicholls and Eldridge, 1980; Benyon, 1984). The present study provided an opportunity to augment current knowledge in this area.

CHAPTER 2

MATERIALS AND METHODS

2.1 WOOD MATERIAL

2.1.1 Field

2.1.1.1 The Site- Boboyan Provenance Trial

Juvenile wood material of *Pinus radiata* were obtained from the CSIRO[1] Provenance Trial established in 1969 at Boboyan Plains in the Gudgenby Nature Reserve near Canberra, ACT (Figure 2-1).

The seeds for the trial were collected during the mid-1960's from the five recognized natural occurrences of the species (Libby *et al*, 1968). These provenances are located on the mainland (Ano Nuevo, Monterey and Cambria) and insular (Cedros and Guadelupe) regions of California (USA) and Baja California (Mexico),

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1. CSIRO: Commonwealth Scientific and Industrial Research Organization, Australia

respectively (Figure 2-2). Each provenance was divided into a number of stands based on topography, altitude, edaphic factors and distance from the coast. The trial was planted with seeds from five stands per provenance. Each stand was represented by two families (and each family by two trees). The trial, replicated twice, thus consisted of 20 trees per provenance. A random block design using single tree plots, was adopted.

The trial was established with two main objectives:

(i) to refine the taxonomy of the species complex, especially in relation to the two-needled island forms, and

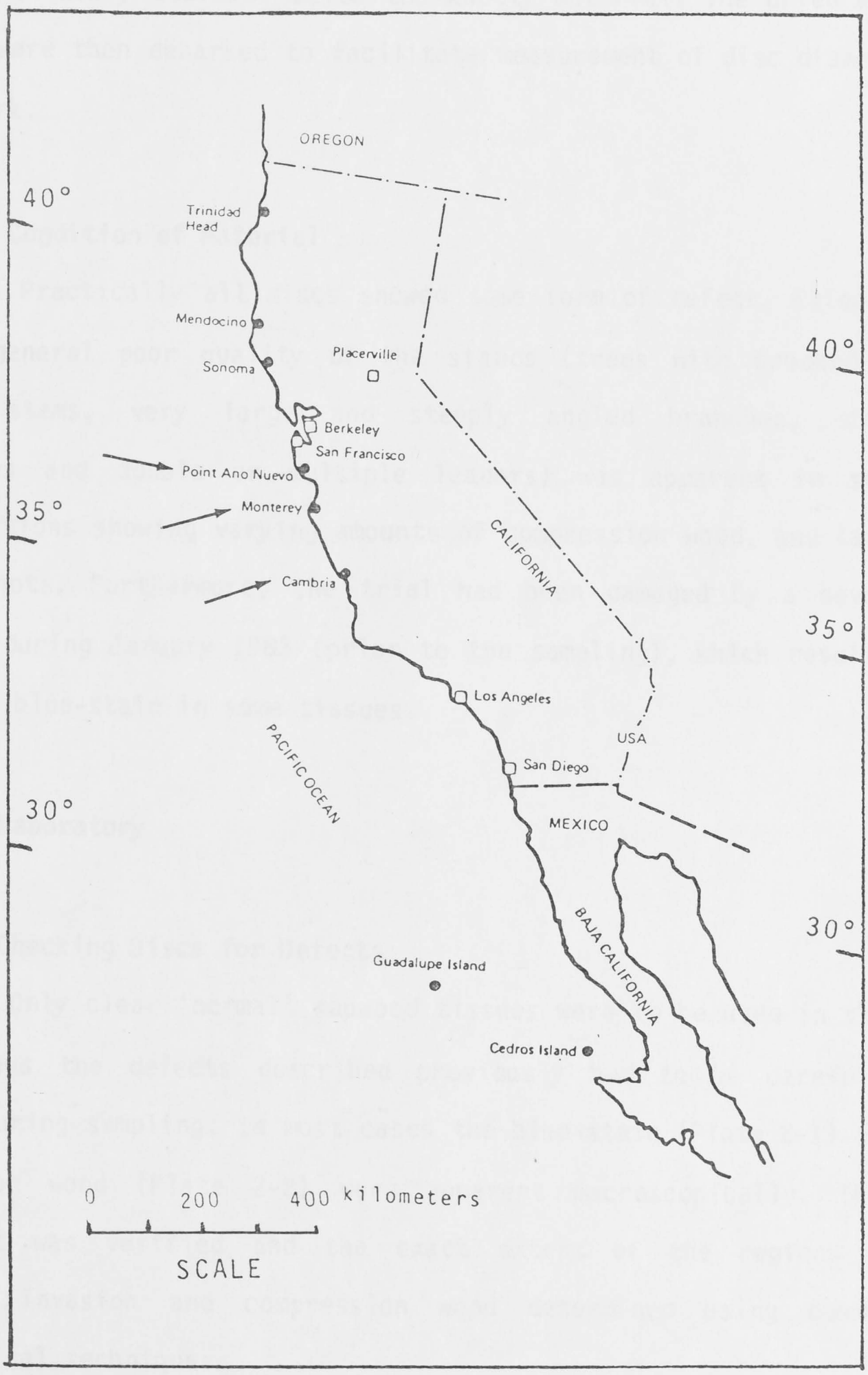
(ii) to establish a broad genetic base of the species for the purpose of gene conservation (CSIRO, Unpublished).

2.1.1.2 Sampling of Material

From the trial 24 stems were selected for assessment. At the time of sampling the trees were 13 years old and thus contained only sapwood (*P. radiata* does not normally form heartwood before 15 years of age). The wood specimens were selected only from the mainland provenances (Ano Nuevo, Monterey and Cambria), since many of the trees representing the insular provenances were missing in the trial. Eight stems were selected from each of the three provenances so as to incorporate an appreciable range of tree heights (6.0 to 13.2 m) and diameters (diameter breast height under bark: 14.0 to 36.0 cm). A greater replication was not possible because of the limited time available for this independent study.

Figure 2-1 Location of Boboyan Provenance Trial in the Gudgenby Nature Reserve, near Canberra (ACT) where samples of stemwood of juvenile *Pinus radiata* were obtained

Figure 2-2: Natural occurence of *Pinus radiata* in California (USA) and Baja California (Mexico) (origin of wood material is shown by arrows)



The trees selected were butt-pruned to a height of 1.6 m, felled, and their height recorded to the nearest decimetre. A disc 15 cm thick was cut at breast height (1.3 m above ground level) from each tree, and immediately transported to the laboratory and air dried. Where a branch whorl was present at breast height the disc was taken immediately above or below the knotty material. The dried wood samples were then debarked to facilitate measurement of disc diameter under bark.

2.1.1.3 Condition of Material

Practically all discs showed some form of defect. Evidence of the general poor quality of the stands (trees with crooked or twisted stems, very large and steeply angled branches, short internodes and double or multiple leaders) was apparent in stem cross-sections showing varying amounts of compression wood, and large branch knots. Furthermore, the trial had been damaged by a severe bushfire during January 1983 (prior to the sampling), which resulted in fungal blue-stain in some tissues.

2.1.2 Laboratory

2.1.2.1 Checking Discs for Defects

Only clear 'normal' sapwood tissues were to be used in this study. Thus the defects described previously had to be carefully avoided during sampling. In most cases the blue-stain (Plate 2-1) and compression wood (Plate 2-2) were apparent macroscopically. This assessment was verified and the exact extent of the regions of microbial invasion and compression wood determined using common histochemical techniques.

Plate 2-1: Examples of transverse sections ('a', 'b' and 'c') of *Pinus radiata* with visible fungal blue-stains (sections were wetted to enhance the visualization of the stain)

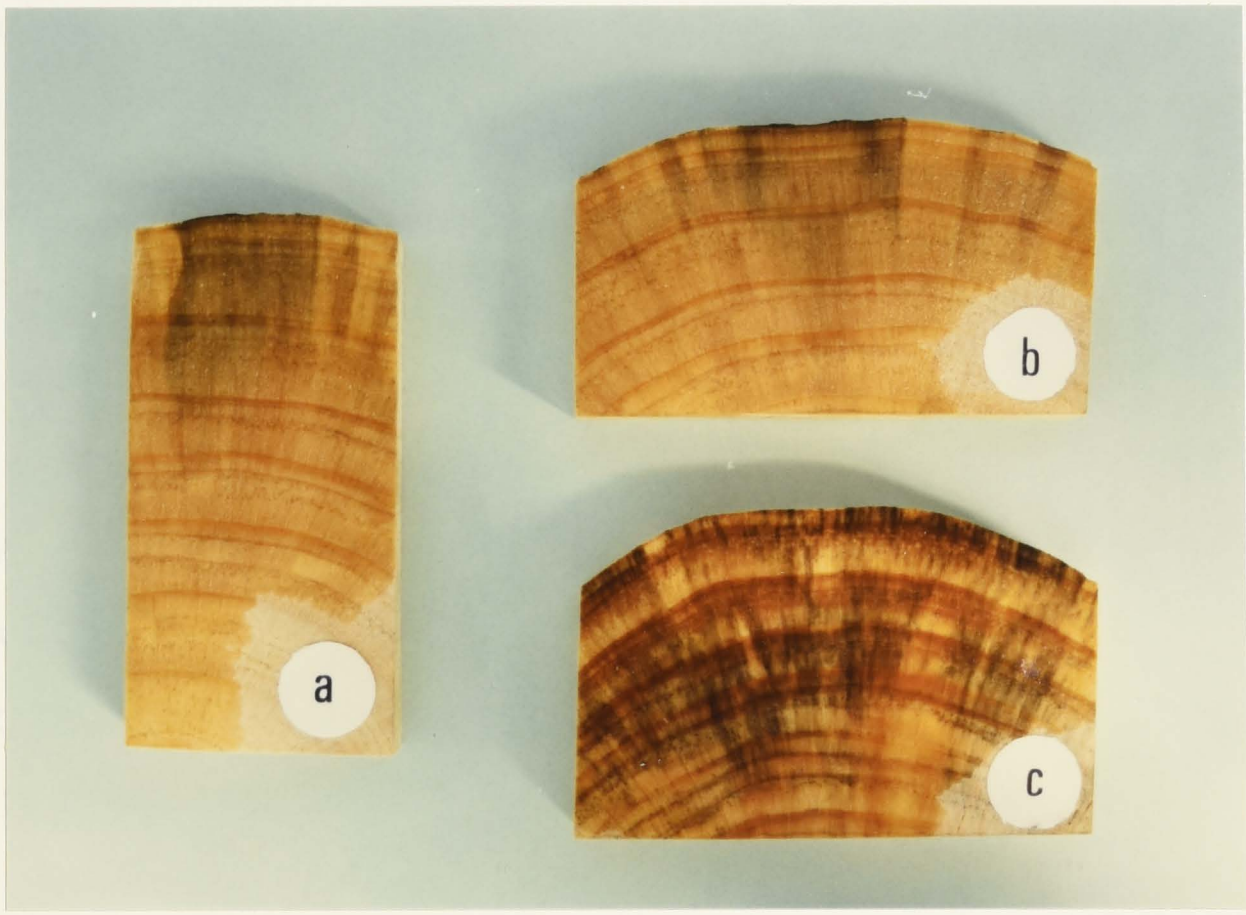
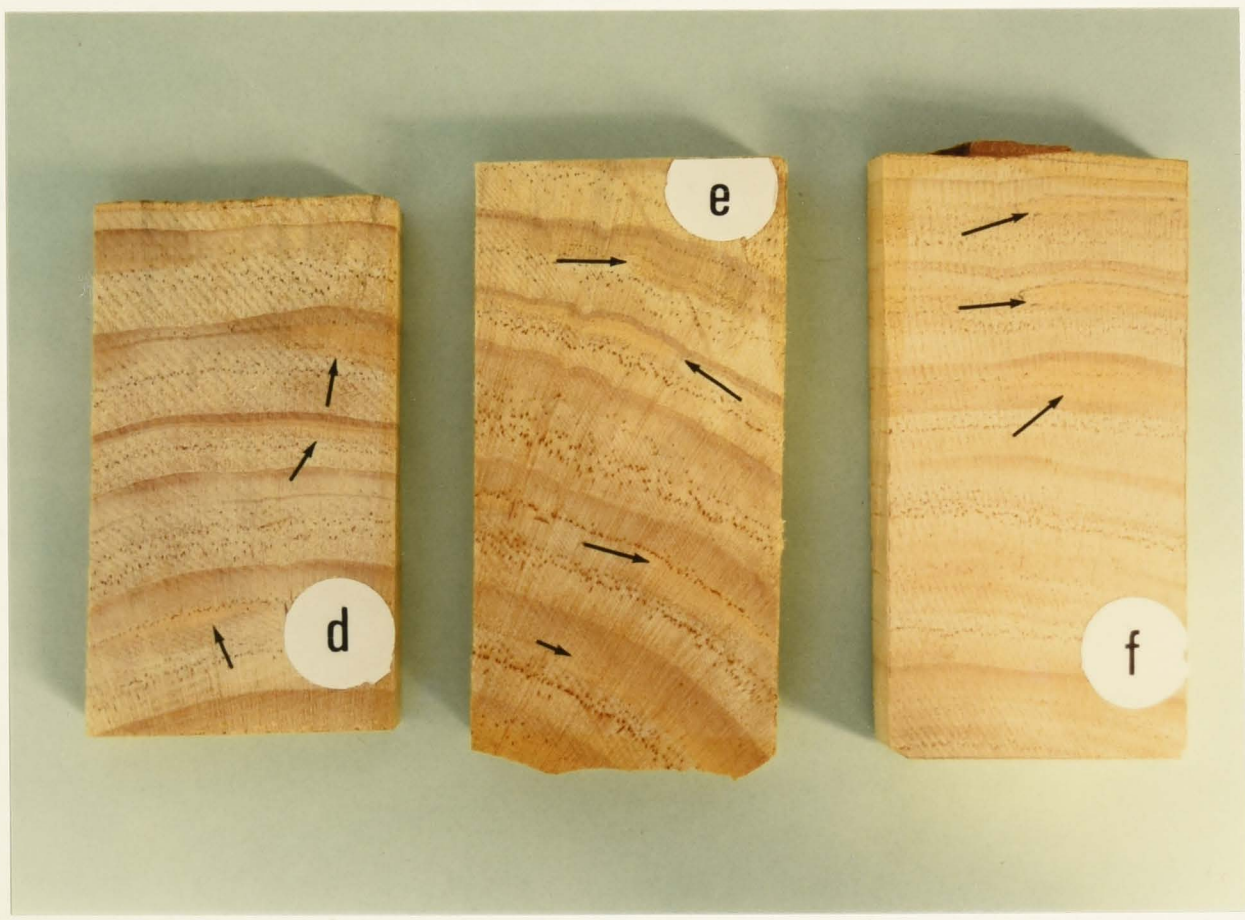


Plate 2-2: Examples of transverse sections ('d', 'e' and 'f') of *Pinus radiata* showing varying grades of compression wood (shown by arrows) usually forming a narrow strip adjoining the late wood bands, and may even be largely indistinguishable from the late wood bands



2.1.2.1.1 Detection of Compression Wood (Phloroglucinol-HCl Test)

Features which commonly distinguish the most obvious form of compression wood from normal tissue include: a relatively dark red-brown colour; a lack of contrast in colour between early and late wood; an unusually high lignin content, and tracheids which, in cross-section, are not close-fitting and angular but rather rounded and with intercellular spaces. However, these features alone may not be enough to distinguish compression wood. Thus a phloroglucinol-hydrochloric acid test (Purvis *et al*, 1966, p. 184) which stains preferentially for lignin was employed frequently. Microtomed preparations of transverse sections were stained on a slide in a 1% aqueous solution of phloroglucinol for one to two minutes. The superfluous stain was drained away and a few drops of concentrated hydrochloric acid added. Within a few minutes lignified tissue stained red. The acid was rinsed off immediately the red-violet colouration appeared. Using this test, many regions of compression wood of intermediate form were detected, e.g. where tracheid lumina were unusually rounded, but where intercellular spaces were absent.

2.1.2.1.2 Detection of Blue-Stain (Safranin-Picro Aniline Blue Test)

Blue discolourations (known as 'sapstains') are most commonly caused by fungi which develop coloured hyphae in wood, stimulate the formation of coloured deposits in the ray cells, and/or secrete soluble pigments which stain the cell wall (Bolland, 1983). The stains are often most obvious in green material. Fungal hyphae were detected by the double staining technique of Cartwright (1929).

Microtomed radial longitudinal sections were stained in 1% aqueous safranin, and washed with water, leaving the tissues slightly overstained. These sections were then covered in picro aniline blue (formulated by adding 100 ml saturated aqueous picric acid to 25 ml saturated aqueous aniline blue), and warmed over a flame until the solution began simmering. Finally, the sections were washed in water, in a few changes of methylated spirit, and ethanol (in that order). Lignified cell wall stained red, while fungal mycelium if present, appeared blue.

2.1.2.2 Sampling Within Discs

As required, sections 1.4 cm thick were cross-cut from the original discs (collected in the field). On a radius selected randomly on sound tissues, two radial positions designated outer and inner sapwood respectively were delineated (Plate 2-3). Each position was 1.5 cm wide radially, the outer boundary of the outer sapwood area being the last formed tissues of ring 13 (based on number of rings from the pith), and the inner boundary of the inner-sapwood area being the first formed early wood of rings 5, 6, or 7, depending on tree diameter. The outer and inner positions covered *ca.* three and two rings respectively (Table 2-1). Tissues were carefully dissected from the delineated radial positions and used as wood blocks (for assessment of decay resistance and wood density) or ground to woodmeal (for chemical analyses).

Plate 2-3: Circumferential sampling at two radial sapwood positions for wood blocks (and woodmeal)

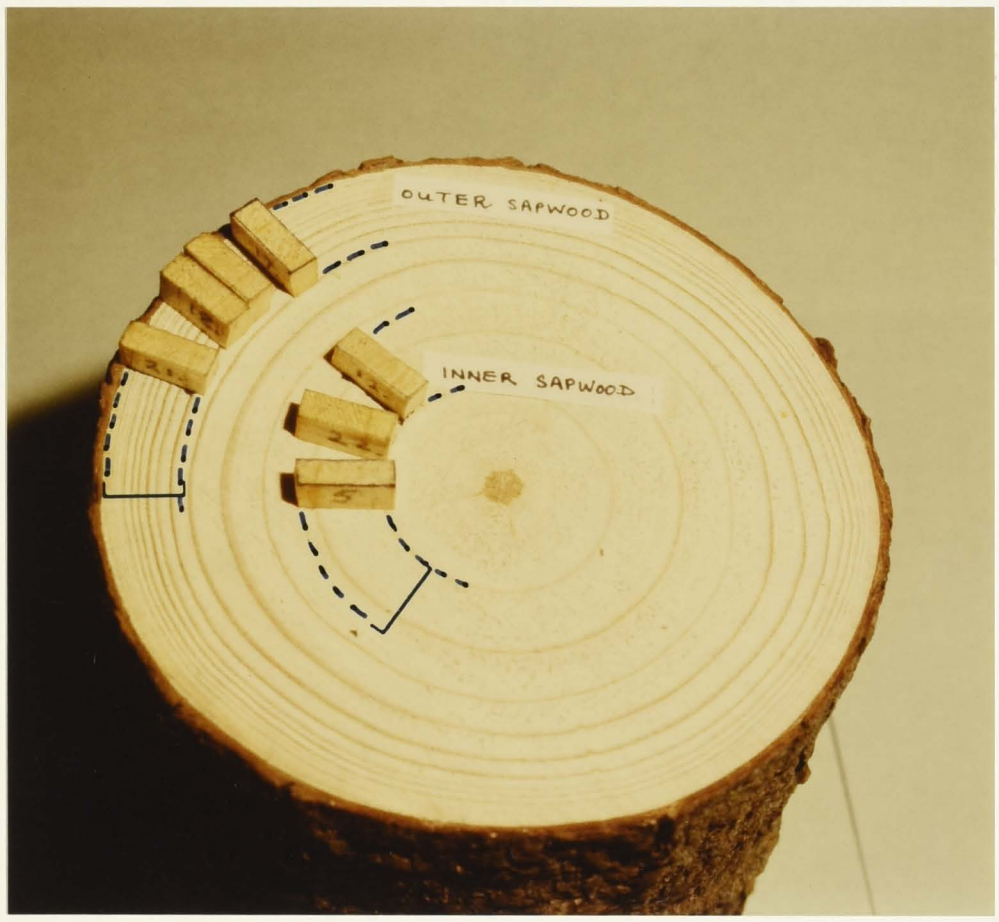


Table 2-1: Identification of tree samples, growth and radial sampling positions at breast height in juvenile sapwood (13 years old) of 24 progenies from the 3 natural occurrences[1] of *Pinus radiata* in California, USA

Provenance	Tree Identification[2]			Growth Measurements		Radial Sampling Positions[4] (included ring positions from pith)	
	Tree No.	Libby	CSIRO	Height (m)	Diameter[3] (cm)	Inner sapwood	Outer sapwood
Ano Nuevo	1	015001	815	5.0	15.7	4 - 6	10 - 13
	2	011001	703	6.6	17.4	5 - 6	10 - 13
	3	014002	795	9.1	20.7	5 - 6	10 - 13
	4	016002	743	10.5	20.2	7 - 8	11 - 13
	5	011002	653	10.9	22.1	6 - 7	11 - 13
	6	018002	615	11.3	21.4	4 - 5	11 - 13
	7	016002	706	11.4	28.8	5	11 - 13
	8	011002	617	12.4	27.1	7	12 - 13
Monterey	9	021001	746	6.9	17.4	4 - 5	11 - 13
	10	029001	719	8.0	22.1	4 - 5	12 - 13
	11	029002	647	9.5	23.3	5 - 6	11 - 13
	12	025001	607	11.4	27.8	6	12 - 13
	13	027001	672	11.8	35.8	7	12 - 13
	14	025002	720	13.2	30.2	6 - 7	12 - 13
	15	027001	601	6.2	14.8	6 - 8	11 - 13
	16	025002	732	12.4	33.2	6	12 - 13
Cambria	17	037002	744	6.0	20.5	5	11 - 13
	18	030001	678	6.7	22.1	5	11 - 13
	19	036001	667	6.9	17.2	5	11 - 13
	20	030002	766	7.9	20.1	7	11 - 13
	21	034001	634	8.5	23.1	7 - 8	12 - 13
	22	036002	734	9.4	14.0	5 - 8	11 - 13
	23	032002	621	10.0	32.7	6	12 - 13
	24	034001	747	11.8	30.8	6 - 7	12 - 13

1) Origin of the 3 provennces are indicated in Figure 2-2

2) Libby- a 6-digit code number introduced by Professor W.J. Libby (University of California, Berkeley) of which the first two (01-16) identify the provenance, the next one (0-9) the stand within the provenance, and the last three (1-149) designate the individual mother trees
CSIRO- a 3-digit number provided by CSIRO Division of Forest Research for the Boboyan Provenance Trial

3) Diameter measurements recorded at breast height under bark

4) Each of the two radial positions was sampled circumferentially on a transverse section and confined to a 1.5 cm radial distance

2.2 EXPERIMENTAL METHODS

2.2.1 Methods of Laboratory Assessment of Natural Decay Susceptibility

2.2.1.1 Preparation of Wood Blocks

Test blocks of fixed dimensions, 15 mm (rad.) x 5 mm (tang.) x 14 mm (long.) were cut from the selected radial positions and cleaned of loose fragments with a scalpel. The blocks were subsequently oven dried (85°C) to constant weight for 48 hours and the weights recorded to the nearest milligram. Fumigation with propylene oxide gas (Hansen and Snyder, 1947) was used to sterilize the dry blocks, 4 ml per litre air space, in sealed containers. In this case, the containers were stored for three days in a sterile environment to ensure complete evaporation of the gas. The total numbers of test blocks prepared in this way is calculated from the following general design:-

numbers of trees	a
numbers of radial positions	b
numbers of test fungi	c
replications	d
Total numbers of test blocks	a.b.c.d

Additional blocks were similarly prepared to serve as 'control', and 'sacrificial' (inspection) blocks during the assessment of decay susceptibility (explained in Section 2.2.1.2.2).

2.2.1.2 Wood-Block/Soil-Jar Method (Chapters 3 and 4)

2.2.1.2.1 Preparation of Soil-Jar Decay Chambers

Preparation of jars and subsequent facets of the test adhered to the general method of ASTM D 2017 (ASTM, 1984). Approximately 100 ml (*ca.* 54 g air dry) of a sandy loam top soil from Uriarra Forest (ACT) was added to the test bottles (cylindrical, 375 ml screw-top glass jars). Enough deionized water (*ca.* 12 ml) was then added to the soil in each jar, to raise the moisture content to field capacity. A nutrient-enriched 5.5 cm Whatman No.1 filter paper 'feeder' was carefully laid over the soil surface. The nutrient solution, in which the feeders had been soaked, had the following composition: asparagine, 2 g; potassium dihydrogen orthophosphate (KH_2PO_4), 1 g; magnesium sulphate ($MgSO_4.7H_2O$), 0.5 g; cation iron (Fe^{3+}), 0.2 mg; cation zinc (Zn^{2+}), 0.2 mg; cation manganese (Mn^{2+}) 0.1 mg; biotin, 5 ug; thiamine, 100 µg; distilled water, 1000 ml (Lilly and Barnett, 1951).

The test bottles, with screw-caps untightened, were steam sterilized in an autoclave at 121°C (steam pressure: 103 kPa) for 30 minutes. When cool, the feeders were inoculated centrally with malt-yeast agar (MYA) [composition: Difco-Bacto malt extract, 10 g; Difco-Bacto yeast extract, 2 g; Difco-Bacto agar, 20 g; distilled water, 1000 ml (Shigo, 1976)] (approximately 5 mm diameter, 2 mm thick) containing actively growing mycelium of the fungus under test. The inoculated bottles, with caps now closed tightly, were incubated in the dark at 25°C until the feeders were covered with a mat of active mycelium. Extra soil jars were prepared so that at this stage those found to be contaminated could be replaced.

2.2.1.2.2 Decay Procedure

The sterilized blocks were aseptically planted on the feeder strips (usually two blocks per jar), and the assemblies, with caps closed loosely to facilitate aeration, were incubated in the dark at 25°C and 95 to 99% relative humidity for a suitable period of time. The incubation period was usually determined after assessment of the weight losses sustained by 'sacrificial' blocks. These blocks were periodically retrieved from incubation and weight losses measured, so giving an indication of the progress of the decay with time. The test was terminated when the loss in weight of the 'sacrificial' blocks had reached a value considered satisfactory, usually greater than 20%, 'Control' blocks (i.e. blocks not exposed to fungal deterioration), were also included in the decay tests.

Blocks retrieved at the end of the incubation period, were carefully brushed free of surface mycelium, oven dried (85°C) and weighed. The percentage weight losses of the test blocks (based on initial equilibrim dry weights) after correction using the 'control' values, provided a measure of the natural decay susceptibility of tissue. A typical soil-jar decay assembly is shown in Plate 2-4.

Plate 2-4: Wood-block/soil-jar decay assembly containing moist sandy-loam top soil (nutrient-rich base) covered with a filter paper feeder strip which is overgrown by vigorous mycelial mat of the test fungus. The wood blocks are decayed by white-rot organisms

Plate 2-5: Wood-block/foam-jar decay assembly with polyurethane foam (nutrient-depleted base) soaked in sterile deionized water. The wood blocks are decayed by a white-rot (A) and a brown-rot (B) organism

2.2.1.3 Wood-Block/Foam-Jar Method (Chapters 5 and 6)

2.2.1.3.1 Preparation of Foam-Jar Decay Chambers

Polyurethane foam sheets (2.5 cm thick) were cut to squares, 5 cm x 5 cm, and soaked in repeated changes of hot water for five days to ensure the material was free of fungal nutrients and antimicrobial compounds. Each foam piece was placed in a 375 ml glass jar to which 15 ml of deionized water was added. The units, sterilized as described in Section 2.2.1.1, were then ready to receive inoculated wood blocks. A foam-jar decay assembly is shown in Plate 2-5.

2.2.1.3.2 Inoculation of Wood Blocks

The pure cultures used to inoculate wood blocks were prepared by sub-culturing the test fungi three times, on a low nutrient malt extract agar (MEA) medium (Nobles, 1965) but with the content of malt extract lowered [composition: Difco-Bacto malt extract, 1 g; Difco-Bacto agar, 10 g; distilled water, 1000 ml] to help deplete the mycelium of stored nutrients. The final subculture was made on to a 12 cm Whatman No.1 filter paper overlaying the low nutrient MEA in a 14 cm Petri dish.

Sterilized wood blocks were placed transverse face downwards, on the feeders once these had been covered with mycelium. The units, sealed in polythene bags, were then incubated at 25°C until the fungus grew over the blocks. The blocks were then ready to be placed in foam jars.

2.2.1.3.3 Decay Procedure

Inoculated blocks were aseptically transferred to the foam jars (two or more blocks per jar). The assembly was incubated in the dark at 25°C and 95 to 99% relative humidity until satisfactory block weight losses were obtained. Loss in weight was again the measure of decay susceptibility.

2.2.1.4 Wood-Block/Petri Dish Method (Chapter 3)

2.2.1.4.1 Preparation of Petri Dish Chambers

Malt yeast agar (MYA) was dispensed into 9 cm disposable Petri dishes, 20 ml per dish. A filter paper (5.5 cm Whatman No.1), was placed over the agar and inoculated with the fungus under test. Fungal mycelium covered the surface of the paper within two weeks of the units being placed in a sealed polythene bag and incubated in the dark at 25°C. The dishes were aerated every week by lifting the lids, for a period of 10 seconds, under aseptic conditions. This procedure was assumed to provide an adequate supply of oxygen for the decay of the blocks. Decay susceptibility was assessed, as described previously (Section 2.2.1.1.3), at the end of the incubation period.

2.2.2 Determination of Physical Characteristics (Chapter 4)

2.2.2.1 Wood Density

A water displacement method (Desch ,1977, p. 152) was used to determine 'unextracted' basic density of test blocks, *ca.* 5 mm (tang.) x 15 mm (rad.) x 14 mm (long.). The blocks were weighed oven

dry (85°C) to the nearest milligram before boiling in water for five days to ensure swelling to maximum gross volume. Paper towelling was used to absorb adhering water droplets from each sample which was subsequently lowered (attached to a long needle) into a tared beaker of water resting on a top loading balance. The recorded weight (grams) of water displaced when the block was just submerged was regarded as its volume (cm^3); thus 'unextracted' basic density [grams (oven dry) cm^{-3} (swollen)] was calculated.

Duplicate samples of ground woodmeal which had been passed through a 425 um (40 mesh) or 250-um (60 mesh) sieve per radial position were extracted with alcohol-benzene mixture following the procedure of ASTM D 1107 or APPITA[1] P7m-65 (see Section 2.2.4.3), and the content of extractives used to calculate 'extracted' basic density of the above wood blocks.

2.2.2.2 Tracheid Length

2.2.2.2.1 Sampling of Tissue

A sliver of wood (0.5 to 1.0 mm thick tangentially, 14 mm axially) was split from a 1.5 cm radial surface, and used for the determination of average tracheid length for that radial position.

2.2.2.2.2 Maceration of Slivers

To effect cell separation, the sliver was boiled in a mixture of glacial acetic acid and hydrogen peroxide (volume ratio

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1. APPITA: Standards issued by the Technical Association of the Australian and New Zealand Pulp and Paper Industry.

1:1) in a labelled test tube. The resulting bleached material was shaken vigorously. When cool, the supernatant was drained off, and the macerated pulp remaining in the tube was carefully rinsed, two or three times with distilled water. The pulp was held overnight in a 0.01% safranin solution to stain the tracheids.

Some of the pulp was transferred to a microscope slide using a pipette and any clumps of cells were carefully teased apart to ensure that a large proportion of the individual tracheids were distinguishable. When dry, the preparation was covered with D.P.X. neutral mounting medium (Koch-Light Laboratories Ltd.) and a cover slip affixed.

2.2.2.2.3 Assessment of Cell Length

A pilot count was made on a slide representing the inner sapwood of one tree. This was to determine the approximate number of cells which had to be measured to provide an estimate of the average tracheid length at each radial position with an error not exceeding 5% of the mean. Measurement was undertaken with the aid of a projection microscope (final magnification 50X). Lengths of randomly selected whole tracheids were determined using a map measuring wheel calibrated to read in millimetres. A formula (Freese, 1962) was used to calculate the required sample size (see Appendix 2-1). It was thus decided to measure 200 tracheids were measured for each sample. Two slides used were for this purpose (i.e. 100 tracheids measured per slide). Average tracheid length (mm) for the radial position was thus obtained.

2.2.2.3 Percentage Late Wood

The percentage late wood at each position was assessed, by expressing the sum total of the radial widths of late wood bands as a proportion of the total radial distance radially. Measurements to the nearest 0.1 mm were made using vernier calipers and a dissecting microscope. The transverse surface of the wood was wetted and cleaned with a scalpel to assist identification and measurement of the late wood bands. Where there was any doubt as to the distinction between early and late wood within a ring, freehand transverse thin sections were viewed at 40 or 100X magnification. Tissues in which the majority of tracheids had a Runkel ratio (2 x cell wall thickness / lumen diameter) > 0.5 were regarded as late wood (Bamber and Burley, 1983).

2.2.3 Determination of Chemical Characteristics (Chapters 5 and 6)

2.2.3.1 Lignin Content

The general method of ASTM D 1106 was adopted. Wood was ground in a Wiley mill fitted with a 1 mm sieve. The resulting woodmeal was subsequently ground to pass through a 250 µm (60 mesh) sieve. This woodmeal was placed in a thimble and extracted with an ethanol-benzene solution (volume ratio 1:2) in a Soxhlet for 24 hours based on the general procedure of ASTM D 1107 or APPITA Standard P7m-65 (see Section 2.2.4.3). A further extraction involved holding the sample in boiling water for 48 hours.

Each of two replications of 'extracted' woodmeal (both 0.5 g oven dried at 85°C) per position was left to stand in 15 ml of 72%

sulphuric acid for two hours at 18 to 20°C, with frequent stirring. The wood/acid mixture was washed into a one litre beaker and diluted to a 3% concentration of sulphuric acid by adding 560 ml of distilled water. This mixture was boiled for four hours, with the occasional addition of hot water to maintain the original volume, and then filtered through a pre-weighed sintered glass filtering crucible (porosity no.2). The brown residue was washed repeatedly with hot distilled water and then oven dried (85°C). The residue was regarded as lignin, i.e. the loss in weight of the extracted sample was assumed to reflect the removal of the holocelluloses (polysaccharides). The lignin content of the samples was expressed as a percentage of the 'unextracted' moisture-free weight.

2.2.3.2 Total Available Sugars

The method for determining total available sugars was that of Lambert (1978) and Yemm and Willis (1954). Duplicate woodmeal samples [each 2 g oven dry (85°C)], which had been ground to pass a 250 μm (60 mesh) sieve, were weighed into a thimble and extracted for four hours with 100 ml of 80% ethanol[1], in a Soxhlet. The alcohol/water extract was then evaporated slowly on a water bath to about 5 ml, care being taken not to reduce extract to dryness. This solution was quantitatively transferred to a 100 ml volumetric flask with distilled water, then 5 ml of 0.25N barium hydroxide[2]

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1. 80% ethanol: prepared by diluting 860 ml redistilled absolute alcohol to one litre with distilled water.
2. 0.25N $BaOH_2.8H_2O$: 39.4 g of the reagent-grade alkali was dissolved in one litre distilled water.

($BaOH_2.8H_2O$) added and the resultant solution thoroughly shaken. After 5 ml of 0.25N cadmium sulphate[1] ($CdSO_4.8H_2O$) had been added, the solution was again shaken vigorously. The flask was then filled to volume with distilled water, the mixture centrifuged, and the extract removed from the white precipitate.

Ten millilitres 0.1% anthrone reagent[2] was pipetted into a McCartney bottle and chilled in ice water. A 2 ml aliquot of the extract was carefully layered onto the anthrone reagent and the unit was again held in ice water for a further five minutes. The solution was thoroughly mixed (by swirling), with the lower portion of the bottle still immersed in ice water. The bottles, loosely capped, were then held in a hot water bath (*ca.* 80°C) for exactly five minutes. After 30 minutes cooling at room temperature, the absorbance at 625mu was read in a spectrophotometer (Bausch and Lomb, Spectronic 20), against a blank prepared with distilled water. Analytical grade D-glucose and D-fructose were used to prepare the standard curve. The concentration of sugars in the extract (ppm) was converted to percentage (w/w) of total (available) sugars in unextracted wood weight.

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1. 0.25N $CdSO_4.8H_2O$: 32.1 g of the reagent-grade salt was dissolved in one litre distilled water.
2. 0.1% anthrone reagent: prepared by dissolving 0.2 g of anthrone in 100 ml of H_2SO_4, made by adding 500 ml of conc. acid to 200ml of water. The reagent was allowed to stand for 30-40 min. with occasional shaking until perfectly clear. It was freshly prepared each day and used within 12 hours.

2.2.3.3 Nitrogen, Phosphorus and Mineral Elements

Tissues were chipped and ground to pass through a 2 mm screen. This woodmeal was thoroughly homogenized and duplicate samples of it, oven dried at 85°C, were used in the following determinations of nitrogen, phosphorus and mineral elements:-

2.2.3.3.1 Nitrogen and Phosphorus

About 0.5 g of woodmeal was wet digested in 5 ml of a digestion mixture [composition: sulphuric acid-phosphoric acid mixture (volume ratio 20:1), 10 ml; potassium sulphate, 5 g; selenium, 5 mg; 1000 ml water, 1000 ml (Technicon Industrial Systems, 1977)]. Digestion was effected in two stages: 150°C for 1½ hours, then 370°C for a further 1½ hours. Digestion was satisfactory if the solution turned clear yellow. The two stages were repeated if this final colour was not obtained. After cooling, 50 ml distilled water was added to the yellow solution while agitating it vigorously. When cool again, the volume of the solution was made up to 75 ml with distilled water and the container inverted repeatedly to ensure complete mixing. A 'TECHNICON' Autoanalyser II (Technicon Industrial Systems, 1977) was used to determine nitrogen and phosphorus concentrations in 1 ml aliquots. The reference standards for total phosphorus and total nitrogen were solutions of potassium dihydrogen orthophosphate (KH_2PO_4) and ammonium sulphate [$(NH_4)_2SO_4$] respectively. The values obtained were expressed in ppm.

2.2.3.3.2 Mineral Elements

Duplicates of about 0.25 g of the woodmeal were digested by boiling in 5 ml 3N hydrochloric acid and 15 ml 7N nitric acid combined. After digestion the mixture was evaporated to a final volume of 1 ml. The extract was transferred quantitatively with distilled water through a Whatman No.42 (ashless) filter paper into a 50 ml volumetric flask. A 'VARIAN TECHTRON' Atomic Absorption method (Amos *et al*, 1975) was used in the determination of levels of sodium, potassium, calcium, manganese, magnesium, zinc and iron. The values were expressed in ppm.

2.2.4 Solubility of Wood (Chapter 6)

2.2.4.1 Water Solubility

Determination of the water solubility of wood followed closely the methods described in ASTM D 1110. Cold water solubility provides a measure of the wood sugars, free mineral matter, free acids, small quantities of gums and/or polyphenolic materials, and other cytoplasmic contents of parenchyma. Hot water extracts wood starch, in addition to the above materials.

2.2.4.1.1 Cold Water Solubility

About 1 g oven dried (85°C) woodmeal [particle size: < 250 µm (60 mesh) sieve] was placed in 300 ml distilled water in a 400 ml beaker or glass jar. The mixture, at room temperature (25°C), was stirred frequently over a period of 48 hours and then filtered through a pre-weighed sintered glass filtering crucible (porosity

no.2) under suction. The residue was dried to constant weight at 85°C. The difference between the pre- and post-extraction weights of the sample (i.e. the weight of cold water soluble matter) was expressed as a percentage of the pre-extraction weight.

2.2.4.1.2 Hot Water Solubility

In the extraction phase of this procedure the material was placed in a 250 ml conical flask covered with aluminium foil, and heated on a hot plate for four hours with frequent stirring. Retrieval of woodmeal and subsequent determination of hot water soluble content was as described above.

2.2.4.2 Dilute Alkali Solubilty

The test method of ASTM D 1109 covers the determination of the solubility of wood in a hot dilute alkali solution [1% sodium hydroxide[1] (NaOH)]. Hot dilute alkali removes low molecular-weight carbohydrates (predominantly hemicellulose and degraded cellulose) and lignin degradation products, as well as the non-structural materials.

About 0.6 g of oven dried (85°C) woodmeal [particle size: < 250 μm (60 mesh) sieve] was placed in a 250 ml conical flask and 100 ml of 1% sodium hydroxide solution added. After stirring well, the flasks were covered with foil and placed in a boiling water bath for exactly one hour, the contents being stirred by swirling after 10, 15

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1. 1% sodium hydroxide: prepared by dissolving 10 gram of reagent-grade NaOH in 1 litre of boiled (i.e. free of carbon dioxide) distilled water.

and 25 minutes of heating. The mixture was subsequently filtered through a pre-weighed sintered glass filtering crucible (porosity no.2), and the residue was washed first with 100 ml distilled water, then with 50 ml of 10% acetic acid[1]. The woodmeal was finally thoroughly washed with hot water until, as indicated by a litmus test of the last drop or two of the filtrate from the crucible, the sample was acid-free. The contents were dried in the crucible to constant weight at 85°C. The loss in weight, as a percentage of the unextracted oven dry wood weight, was taken as dilute alkali solubility.

2.2.4.3 Ethanol-Benzene Solubility (also Chapter 5)

Substances soluble in ethanol-benzene are those not generally considered part of the wood substance. These include waxes, fats, resins, some gums, and water soluble materials. The determination of this solubility follows the general method of ASTM D 1107 or APPITA P7m-65.

About 1 g of oven dry (85°C) woodmeal [particle size: < 250 µm (60 mesh) sieve] was weighed in a thimble and extracted with a mixture (volume ratio 1:2) of 95% ethanol[2] and chemically pure benzene for 24 hours, keeping the liquid boiling briskly (to ensure about five to six siphonings per hour in a Soxhlet). The difference in weight of the extracted woodmeal provided the amount of ethanol-

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1. 10% acetic acid: prepared by diluting 10 ml glacial acetic acid (AR) with distilled water to 100ml.
2. 95% ethanol: prepared by mixing 19 volumes of redistilled absolute ethanol and one volume of distilled water.

benzene soluble matter expressed as a percentage of the unextracted oven dry weight.

2.2.5 Hydrogen-ion Concentration (Chapter 6)

All pH determinations of aqueous extracts from wood were made on a 'Townson and Mercer (Model PT75) pH meter, with readings taken to within 0.01 of a pH unit. Extracts which contained insoluble materials had to be filtered beforehand, and the determinations were made within 48 hours of the time of extraction.

CHAPTER 3

SELECTION OF PROCEDURES FOR ASSESSING NATURAL DECAY SUSCEPTIBILITY

3.1 INTRODUCTION AND LITERATURE REVIEW

Before a major programme involving the assessment of resistance or susceptibility to decay under laboratory conditions can be initiated several decisions must be made regarding laboratory procedures, since there is as yet no universally acceptable decay system. Among the important considerations in designing a decay test are the choice of test fungi, decay chamber and assembly, and method of sterilization of wood blocks prior to decay testing.

3.1.1 Test Fungi

Only a small proportion of the wood-destroying fungi perform satisfactorily (percentage weight loss > 10% at 6 - 10 weeks decay) under laboratory conditions. Additionally, some fungi which perform adequately under one set of conditions may fail in other situations. For instance, in decay tests using Petri dishes and small wood blocks, Bravery *et al* (1982) found variation in the decay

capacity of one strain of *Coniophora puteana* (Schum. ex Fr.) Karst., which proved to be more vigorous if blocks were planted directly on the mycelium, than if they were placed on sterile polyethylene mesh lying over the mycelium. Again, using *Trametes lilacino-gilva* (Berk.) Lloyd, there was much greater decay if the wood blocks were placed in glass jar decay chambers filled with soil as medium, than if they were in Petri dishes with agar medium. Decay fungi also show marked host species preferences as exemplified by the obvious tendency for brown-rot species to attack softwoods and white-rot species to attack hardwoods. Even within the softwoods or hardwoods decay organisms frequently show striking preferences for different wood species. Thus, the heartwood of *Thuja plicata* D.Don which is highly resistant to degradation by most *Basidiomycetes* is decayed relatively rapidly by *Poria weirii* (Murr.) Sacc. and Trott. which apparently has an unusual tolerance to the polyphenol thujaplicin present in large quantities in the timber (Wagener and Davidson, 1954).

Perhaps the most widely used test organisms are *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. syn *Lenzites trabea* Pers. ex Fr., *Poria placenta* (Fr.) Cke. syn *Poria monticola* Murr., and *Coriolus versicolor* L. ex Fr. syn *Polyporus versicolor* L. ex Fr.. *G. trabeum* and *P. placenta* are recommended for the testing of softwoods and all three organisms for the testing of hardwoods (ASTM D 2017). However these three organisms have not been found entirely satisfactory for the assessment of natural decay resistance of many Australian-grown timbers, and other species, e.g. *Coniophora olivacea* (Fr.) Karst., *Trametes lilacino-gilva* (Berk.) Lloyd. and *Fomes lividus* (Kalch.) Sacc., have gained wider acceptance or may even be

preferred (Da Costa, 1979; Thornton, 1979; Keirle, 1985). An added complication in the selection of test fungi lies in the fact that different strains or isolates of a single species (Bravery *et al*, 1982; Amburgey, 1967) or even different subcultures of the same strain (Wazny and Greaves, 1984) can differ markedly in their capacity to attack a given wood.

3.1.2 Decay Assembly

A large assortment of chambers in which wood blocks are exposed to conditions favouring degradation by pure cultures of test fungi have been tested (e.g. Humphrey, 1916; Scheffer, 1935; Leutritz, 1946; Duncan, 1958; Behr, 1973). Most commonly used is the wood-block/soil-jar technique (Leutritz, 1946), a form of which is described in detail in Chapter 2 (Section 2.2.1.2). The following aspects require particular attention in selecting a suitable form of decay assembly.

3.1.2.1 The Medium for Fungal Growth

This is usually soil or agar, overlaid with a nutrient feeder strip of a decay susceptible timber. Soil is often preferred to agar because of its ability to regulate moisture uptake by test organisms (Leutritz, 1946). A soil moisture content approximating field capacity usually results in a block moisture content between fibre saturation point (28 to 35%) and saturation (often 100 to 300%), thus allowing active mycelial growth and decay throughout the sample. The very high moisture content frequently attained by blocks placed directly on agar may restrict aeration within the samples, and

thus limit decay (Bravery *et al*, 1982). Where soil is selected it must have a high water-holding capacity if the incubation period and evaporation of moisture from the chambers are appreciable.

The nutrient status of the medium also requires consideration. While the medium should be capable of supporting luxuriant growth of the test fungus, an oversupply of readily assimilable carbohydrates (simple sugars) appears to inhibit attack on test samples (Hulmes and Shields, 1970). Highley (1973) has suggested that this effect reflects diversion or repression of enzymatic hydrolysis of wood cellulose in the presence of readily available nutrients. In this respect, the choice of nutrient feeders is important (Highley and Scheffer, 1970; Highley, 1978), particularly if they are to be further artificially enriched with nutrients.

3.1.2.2 Nature of Decay Chamber

The decay chamber is usually a glass jar or Petri dish. The larger the chamber the greater the number of blocks which can be incubated under the single set of environmental conditions within a jar, but the greater the loss of useful data should the blocks become contaminated, and have to be discarded. As a precaution against such losses, containers accommodating two to four blocks are often used. Petri dishes have several advantages which facilitate large replications. They can be prepared relatively quickly, they need little space for storage during incubation, and the blocks are easily placed in them and retrieved. However, because of the relatively small air space within a Petri dish gradual depletion of the oxygen

supply occurs. Additionally, the increased moisture content of blocks in contact with agar, may largely account for the relatively low block weight losses (< 10%) usually recorded when wood is decayed over an extended period in Petri dishes (e.g. Wong, 1983; Bravery *et al*, 1982). Artificial aeration (e.g. periodic ventilation by lifting covers of dish) may be required in such situations.

3.1.3 Method of Sterilization

The results of decay tests are regarded as meaningful only if the sterilization kills all microorganisms in wood, without altering the inherent characteristics of the wood in the process. Sterilization procedures for laboratory decay tests commonly involve fumigation of the wood blocks with volatile toxic chemicals or application of heat. There is also increasing interest in the use of gamma radiation (e.g. Smith and Sharman, 1971; Hansen, 1972).

3.1.3.1 Fumigation

The most widely used fumigant is propylene oxide (1,2-epoxypropane). This chemical is commonly thought to have little effect on the natural decay resistance of wood (Hansen and Snyder, 1947; Klarman and Craig, 1960). However Smith (1965) found that prior fumigation of blocks of *Pinus sylvestris* L. and *Fagus sylvatica* L. severely retarded the activity of *Lentinus lepideus* Fr., despite the complete removal of the gas (by ventilation) prior to inoculation. Further, propylene oxide, when used to sterilize wood blocks impregnated with creosote, increased the fungitoxicity of the creosote (Da Costa and Osborne, 1969). The chemical nature of these

effects has yet to be determined.

Other fumigants have been tested but have not gained wide acceptance. Acetic acid fumes (Fritz, 1930) are effective only as a surface sterilant of wood blocks. Ethylene oxide gas, though more highly active and more readily dissipated from wood than propylene oxide (Hansen and Snyder, 1947), is not favoured because it has a marked ability to attack certain components of the wood, such as vitamins and some amino-acids, which are important in microbial degradation of tissues (Wallhauser, 1967; cited Hansen, 1972).

3.1.3.2 Heating

Both dry and wet (steam) heat are known to alter the chemical composition of the wood substrate. Scheffer and Eslyn (1961) found that heating apparently decreases the resistance of wood cellulose to decay. Steaming and autoclaving (100 or 118°C) have been shown to reduce the decay resistance of sapwood and/or heartwood of many species (Chapman, 1933; Scheffer and Eslyn, 1961), although this effect was not detected by Boyce (1950) working with certain northern hemisphere softwoods. The presence of water appears to be critical, since, dry heat is less deleterious to decay resistance than is wet heat. Regardless, where lowering of the natural decay resistance does occur, it can usually be attributed to an increase in the assimilability of cell wall substances by the fungal hyphae, rather than to a denaturing of toxic extractives (Glassare, 1970).

3.1.3.3 Gamma Irradiation

This method would appear to be highly effective, and to

have little effect on the natural decay resistance of samples. Cellulose polymers are apparently highly resistant to the radiations in part because of protection by lignin (Hansen, 1972). However, radiation facilities are not widely available at present.

3.2 AIM OF THE STUDY

The present experiment was designed to allow selection of test organisms, decay assemblies and a method of sterilization suitable for the study reported in Chapter 4. Time did not permit testing of other factors of some importance in the decay procedure, e.g. the most appropriate temperature for incubation of each test fungus, and the most suitable dimensions of the test blocks. In this respect, standard decay testing methods (see ASTM D 2017) and/or those previously used successfully in this laboratory (Chapter 3 of Wong, 1983) were followed.

3.3 MATERIALS AND METHODS

3.3.1 Sampling Wood Blocks

Discs were removed at breast height from a single 13-year-old *Pinus radiata* stem, air dried, then dissected to provide wood blocks- each 5 mm (tangential) x 15 mm (radial) x 10 mm (axial). The last formed late wood of the growth ring adjacent to the cambial region (13th ring) marked the outer tangential boundary of the blocks. Altogether 336 test blocks, 10 control blocks, and 64

'sacrificial' (inspection) blocks were prepared. The blocks were labelled, dried to constant weight at 85°C and weighed before the application of various treatments (see below). The three treatment variables (i.e. test organisms, decay chambers, and method of sterilization) selected for the design of the decay test are described as follows:-

3.3.2 Organisms

Seven strains/species of wood-destroying fungi, three causing brown-rot and four causing white-rot were used;

Brown-rot Fungi

- *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. syn. *Lenzites trabea* Pers. ex Fr. [strain DFP[1] 7520; Madison[2] 617]
- *Poria placenta* (Fr.) Cke. syn. *Poria monticola* Murr. [DFP 7522; Madison 698]
- *Trametes lilacino-gilva* (Berk.) Lloyd [DFP 1109]

White-rot Fungi

- *Coriolus versicolor* (L. ex Fr.) Quel. syn. *Trametes versicolor* (L. ex Fr.) Lloyd [DFP 2666]
- *Coriolus versicolor* L. ex Fr. syn *Polyporus versicolor* L. ex Fr. [DFP 7521; Madison 697]
- *Fomes lividus* (Kalch.) Sacc. [DFP 7904]
- *Phellinus gilvus* (Schw.) Pat [DFP 2442]

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1. DFP: CSIRO Division of Forest Products (Australia) culture accession number.
2. Madison: USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin (USA) culture accession number.

These strains/species were selected on the basis of their wide use elsewhere and/or other studies in this laboratory (Wilkes and Wong, Unpublished).

3.3.3 Decay Assemblies

- Soil-jar (see Section 2.2.1.2), with two blocks per jar (Total: 168 blocks).
- Agar-Petri dish (see Section 2.2.1.3), with two blocks per dish (Total: 168 blocks).

3.3.4 Methods of Sterilization

- Propylene oxide gas (Sections 2.2.1.1) (112 blocks treated).
- Wet Heat: Blocks were steam sterilized in an autoclave in sealed metal containers at 121°C (steam pressure: 103 kPa) for 30 minutes, and then cooled (112 blocks treated).
- Dry Heat: Blocks were placed in sealed metal containers heated in an oven, at 105°C for 48 hours, and then cooled (112 blocks treated).

The general design of the decay test with replications per variable combination (fungus/chamber/sterilization) was therefore:-

number of test fungi	7
number of decay assemblies	2
number of sterilization methods	3
number of replications	8
TOTAL	336 wood blocks

Decay susceptibility (absolute and percentage weight loss) of the blocks was assessed after these had been inoculated with the test fungi and incubated for six weeks. The number of the retrieved wood blocks with contamination visually apparent (e.g. as coloured mold) was also recorded.

3.4 STATISTICAL ANALYSIS

For statistical purposes (Cunningham, pers. comm.), it was desirable to separate data for the two fungal groups (white- and brown-rot species) and also for the two types of decay chamber. The four resulting data sets were subjected to a two factor analysis of variance (ANOVA), applying the following relationship:-

$$E(Y_{ijk}) = u. + S_i + F_j + S_iF_j + e_{ijk}$$

where

$E(Y_{ijk})$- expected value of decay susceptibility

$u.$- overall mean (constant term)

S_i- effect of method of sterilization

F_j- effect of test organism

S_iF_j- interaction term (fungus x sterilization)

e_{ijk}- random error (replications) independent and normally distributed

Assumptions regarding homogeneity of variances and normality (Neter and Wasserman, 1974) were satisfied, usually by the transformation of data. Multiple comparisons were made using least significant difference values. All tests were run at the two-tailed 0.05 level of significance.

3.5 RESULTS

None of the 112 blocks fumigated with propylene oxide were subsequently found to be contaminated (Table 3-1). This contrasts with the heat treatments, where the proportion of blocks contaminated was *ca.* 2.7% for the autoclaved blocks, and 12.5% for the dry heated samples. However from comparisons between the replicate samples, it was apparent that contamination had no appreciable effect on weight losses due to decay. Thus, in view of the obvious advantages in having a balanced set of data for statistical analysis, the results from the contaminated samples were not excluded in the case of this preliminary experiment.

In some cases it was necessary to transform the values for percentage weight loss to achieve homoscadesticity and normality of the variance of the data. For this purpose, modifications of the basic logarithmic transformations (see Table 3-2) were found suitable. For the purposes of the discussion following the term 'decay susceptibility' is used, regardless of the transformation applied.

The ANOVA results suggest that both differences in the method of sterilization and in the test organism were both significant ($P < 0.05$) determinants of decay susceptibility in three of the four fungal group/decay assembly combinations (Table 3-2). However the significant two-way interactions between method of sterilization and test organism (Table 3-2) indicate that any such effects are not entirely consistent. Thus the results need to be interpreted with considerable caution, and comparisons of individual cell means (per treatment combination) using least significant difference (LSD) values are particularly appropriate (Table 3-3).

Table 3-1: Number and pecentage of sapwood blocks of *Pinus radiata* initially 'sterilized' by either of the 3 methods, but found contaminated during the laboratory testing of decay susceptibility

Method of Sterilization	Visual Apparent Contamination	
	Number of Blocks Affected	Percentage of Blocks Affected
Fumigation with propylene oxide[1]	0	0
Steaming[2]	3	2.7
Dry heat[3]	14	12.5

1) 4 ml per litre of air space for 72 hours

2) 121°C, 103 kPa for 30 minutes

3) 105°C for 48 hours

Each method of sterilization used data from 112 replications of wood blocks from the 7 test organisms and 2 decay assemblies

Table 3-2: Variance ratios obtained from analysis of variance of weight loss[1] occurring in sapwood blocks of *Pinus radiata* decayed for 6 weeks under laboratory conditions

Organism	Assembly	Required Transformation[4]	Source of Variation		
			Method of Sterilization	Test Organism	Two-way Interaction[5]
Brown-rot fungi[2]	Agar-Petri dish	log[(WL + k)/IW]	3.7*	1.1	3.7*
	Soil-jar	% WL	21.0*	755.4*	35.6*
White-rot fungi[3]	Agar-Petri dish	log[(WL + k)/IW]	9.7*	35.8*	6.1*
	Soil-jar	log(WL + k)	1.2	70.4*	0.8

1) The analysis was performed on the basis of 8 test blocks for each treatment (fungal species-decay chamber-method of sterilizaton) combination

2) Brown-rot organisms- *Trametes lilacino-gilva, Gloeophyllum trabeum* and *Poria placenta*

3) White-rot organisms- *Coriolus versicolor* strain DFP 2666, *C. versiclor* strain DFP 7521, *Fomes lividus* and *Phellinus gilvus*

4) log- logarithm (base 10); WL- weight loss; IW- initial dry weight; k- constant;

5) 'Test organism' x 'method of sterilization' interaction

* Variance ratios significant at $P<0.05$

Table 3-3: Mean weight losses[1] in sterilized sapwood blocks of *Pinus radiata* decayed by 7 test organisms in 2 types of decay assemblies

Assembly	Organism	Method of Sterilization: Fumigation with propylene oxide	Steaming (121°C)	Dry heat (105°C)	LSD[4] ($P<0.05$)
Agar-Petri dish	Brown-rot fungi				
	Gloeophyllum trabeum (DFP 7520)	0.8[2](-1.38)[3]	3.6(-1.33)	0.6(-1.44)	
(A)	*Poria placenta* (DFP 7522)	-0.1(-1.48)	0.3(-1.43)	0.5(-1.37)	0.11
	Trametes lilacino-gilva (DFP 1109)	-1.3(-1.49)	0.1(-1.42)	4.0(-1.30)	
Agar-Petri dish	White-rot fungi				
	Coriolus versicolor (DFP 2666)	1.1(-1.38)	14.4(-0.96)	13.9(-0.99)	
	Coriolus versicolor (DFP 7521)	-0.5(-1.49)	-1.3(-1.48)	1.4(-1.41)	
(B)	*Fomes lividus* (DFP 7904)	2.7(-1.31)	1.2(-1.40)	2.4(-1.33)	0.14
	Phellinus gilvus (DFP 2442)	-0.7(-1.51)	-0.7(-1.46)	0.6(-1.40)	
Soil-jar	Brown-rot fungi				
	Gloeophyllum trabeum (DFP 7520)	68.6(-1.23)	68.4(-1.20)	63.0(-1.31)	
(C)	*Poria placenta* (DFP 7522)	12.7(-2.87)	3.0(-4.27)	3.4(-4.17)	0.24
	Trametes lilacino-gilva (DFP 1109)	32.1(-2.03)	39.1(-1.76)	38.0(-1.84)	
Soil-jar	White-rot fungi				
	Coriolus versicolor (DFP 2666)	19.7(19.7)	21.7(21.7)	16.0(16.0)	
	Coriolus versicolor (DFP 7521)	1.0 (1.0)	1.1 (1.1)	1.3 (1.3)	
(D)	*Fomes lividus* (DFP 7904)	11.5(11.5)	14.9(14.9)	12.6(12.6)	5.0
	Phellinus gilvus (DFP 2442)	3.4 (3.4)	3.3 (3.3)	3.2 (3.2)	

1) Each mean value was based on 8 test blocks, i.e. including contaminated blocks
2) Values unbracketed are mean percentage weight losses. A negative value indicates a weight gain
3) Values in brackets are those on which statistical comparisons can be made, these being the transformed values for A, B and C. No transformation of the data was required in the case of D
4) LSD- least significant difference value for making multiple comparisons of differences between mean values unbracketed within a data set (A,B,C or D) at $P<0.05$

When these are made, the inconsistency of the method of sterilization effect (where the latter exists) is immediately apparent. Thus, for example, where the soil-jar technique was used, susceptibility to decay by *Tramates lilacino-gilva* was significantly greater in blocks autoclaved, than in those fumigated. The reverse applies for *Poria placenta*.

Considerable variation occurred between test organisms in their ability to degrade the blocks under the conditions applied (Tables 3-2 and 3-3). This was most obvious where the soil-jar technique was used, a situation in which *Gloeophyllum trabeum* was strikingly active (mean weight loss > 60%), but where certain other organisms (*Coriolus versicolor* [DFP 7521] and *Phellinus gilvus*) induced only relatively small weight losses (usually < 5%, Table 3-3). The two strains of *C. versicolor* differed markedly in their ability to attack the samples in the soil-jar chambers, strain DFP 2666 being very much more active than DFP 7521. In the case of the agar-Petri dish technique, weight losses were typically small (< 5%) and differences between the decay organisms were thus minimal. *C. versicolor* was, however, reasonably active on blocks which had been heat sterilized.

3.6 DISCUSSION

Marked variations in the ability of test fungi to decay wood samples are apparent in Table 3-3, and are recorded widely elsewhere (Eslyn and Highley, 1976; Da Costa, 1979). The activation of fungal enzyme systems on various components of the wood substrate

is receiving increasing attention (Cowling, 1961; Koenigs, 1972; Highley, 1973). At present inter- and intra-specific effects of the type recorded here cannot be explained in detail.

Because of limitations in equipment and time, only two decay fungi (i.e. a brown-rot and white-rot species) could be used in the experimentation described in the following chapter.

The outstanding activity of *G. trabeum* in the soil-jar assemblies makes this species an obvious choice. For the white-rot organisms, the choice is between *C. versicolor* [DFP 2666] and *Fomes lividus* (Table 3-3D). Although *C. versicolor* [DFP 2666] usually produced greater weight losses in soil-jars, and is possibly the most widely used of the white-rot fungi, *F. lividus* was selected, since there are indications (Thornton, 1979; Keirle, 1985) that this organism could be receiving more attention in future.

The generally greater decay of wood blocks in the soil-jars than in the agar-Petri dishes suggested selection of the former technique despite the greater practical difficulties associated with it (e.g. the more time-consuming preparation of the soil medium). The lack of appreciable decay of blocks in the agar-Petri dishes probably reflects excessive uptake of water (Bravery *et al*, 1982). Certainly the samples retrieved from the agar-Petri dishes appeared much wetter than did those removed from the soil-jars.

The method of sterilization had no consistent effect on the subsequent decay of wood samples (Table 3-3). Further any effects which did emerge were dependent on the test organism used for decay. Clearly this area warrants attention, and detailed discussion of the topic must await further studies. The important finding here is that,

on the basis of the weight loss data, no one method of sterilization was consistently superior or inferior to the other two. Even though effects of contamination on the activity of the decay organisms were not appreciable in this study, it is still desirable they be minimized. On this basis, propylene oxide sterilization would be selected.

3.7 CONCLUSIONS

For the major decay test reported in Chapter 4, the following conditions are appropriate:

1. sterilization of wood blocks by fumigation with propylene oxide,
2. the use of *Gloeophyllum trabeum* and Fomes lividus as test organisms, and
3. the use of the soil-jar decay assembly.

CHAPTER 4

VARIATIONS IN PHYSICAL CHARACTERISTICS OF SAPWOOD OF *PINUS RADIATA* AND THEIR ASSOCIATION WITH DECAY SUSCEPTIBILITY

4.1 INTRODUCTION

While the quantity and quality of fungitoxic extractives are recognized factors in the natural durability of many timbers (Scheffer and Cowling, 1966), the influence of other wood properties on decay susceptibility has not been resolved. Limited evidence from certain non-durable timbers suggests some effect of wood density (Garren, 1939; Wong *et al*, 1983). Other physical factors of possible importance include cell length and type, and the proportions of various cell types (Chapter 1).

In the present study juvenile sapwood of *Pinus radiata* from three Californian mainland provenances (see Chapter 2) was selected in order to elucidate possible relationships between certain physical properties and decay susceptibility of wood. Sapwood of *P. radiata* was considered suitable for the study in that, as with sapwood of most timbers, it is non-durable because of a lack of inhibitory materials. Additionally, information obtained on the fundamental

physical properties of *P. radiata* is valuable in augmenting existing knowledge (Bamber and Burley, 1983) in this area. The selected properties were wood density, tracheid length, and percentage late wood; it was considered that if decay susceptibility of this wood species was influenced appreciably by gross structure, good correlations would be obtained between one, or more, of these factors and decay *in vitro*.

This chapter reports investigations on the following aspects of the quality of sapwood of *P. radiata*:

(a) within stem, between tree and between provenance variation in certain physical wood properties and decay susceptibility, and

(b) the possible role of the physical properties as determinants of decay susceptibility.

4.2 LITERATURE REVIEW

4.2.1 Variations in Decay Resistance and Susceptibility of Non-durable Woods

4.2.1.1 Non-resistant Heartwood

An increase in natural decay resistance from the inner to outer heartwood has been found in many moderately durable or non-durable species [e.g. *Eucalyptus citriodora* Hook. (Reis, 1973); *E. grandis* Hill ex. Maid. (Nelson and Heather, 1972); *E. delegatensis* (Wong *et al*, 1983)], although for *Pinus sylvestris* L., no major variation in this direction was found by Schulz (1957). It is thought

that such radial gradients in the heartwood are largely attributable to variation in the toxicity and/or amounts of extractives (Rudman, 1963; Wong *et al*, 1983), which might overshadow the possible influences of other factors in relation to decay resistance.

4.2.1.2 Sapwood

No pronounced radial variation in the decay susceptibility of sapwood of *Pinus sylvestris* (Schulz, 1957), and *P. radiata* (Arentz, 1970) was detected. However sapwood of *P. radiata* which was kept 'fresh' to induce the production of inhibitory materials before testing, subsequently showed greater decay susceptibility in the inner than the outer region (Arentz, 1970). Greater decay susceptibility in the inner sapwood was also recorded in juvenile *P. elliottii* var *elliottii* Engelm. (Schmidtling and Amburgey, 1977), *P. taeda* L. (Schmidtling and Amburgey, 1982) and 44-year-old *P. radiata* (Neville, 1969).

4.2.1.3 Inter-provenance Variation

While decay resistance varies appreciably among trees of many species (Scheffer and Hopp, 1949; Zabel, 1956; Rudman *et al*, 1967), the extent of variation between provenances appears to have received little attention. Findlay (1940) compared the natural decay resistance of the heartwood and sapwood of *P. sylvestris* from a number of different localities, and found Russian specimens to be slightly more durable than their more southerly counterparts. However, resistance in both heartwood and sapwood of this species did not vary among the Scandinavian and German sources (Schulz, 1957).

It is generally believed that variations of the type detected by Findlay are, at least in part, attributable to genetic as well as environmental variation (Zabel, 1956; Clark and Scheffer, 1983).

4.2.2 Variation in Physical Characteristics

4.2.2.1 General

Tree age when wood is being formed, i.e. number of rings from the pith, exerts a major influence on many physical properties of both softwoods and hardwoods. A considerable proportion of the studies of wood quality in plantation-grown *P. radiata* therefore involve assessment of radial variation in wood properties such as densitometric characteristics and tracheid dimensions (Bamber and Burley, 1983). The present study of the progeny from three mainland (Californian) provenances includes assessment of variations in wood density, tracheid length and percentage late wood. This section thus collates information on these three physical properties of wood.

4.2.2.2 Wood density

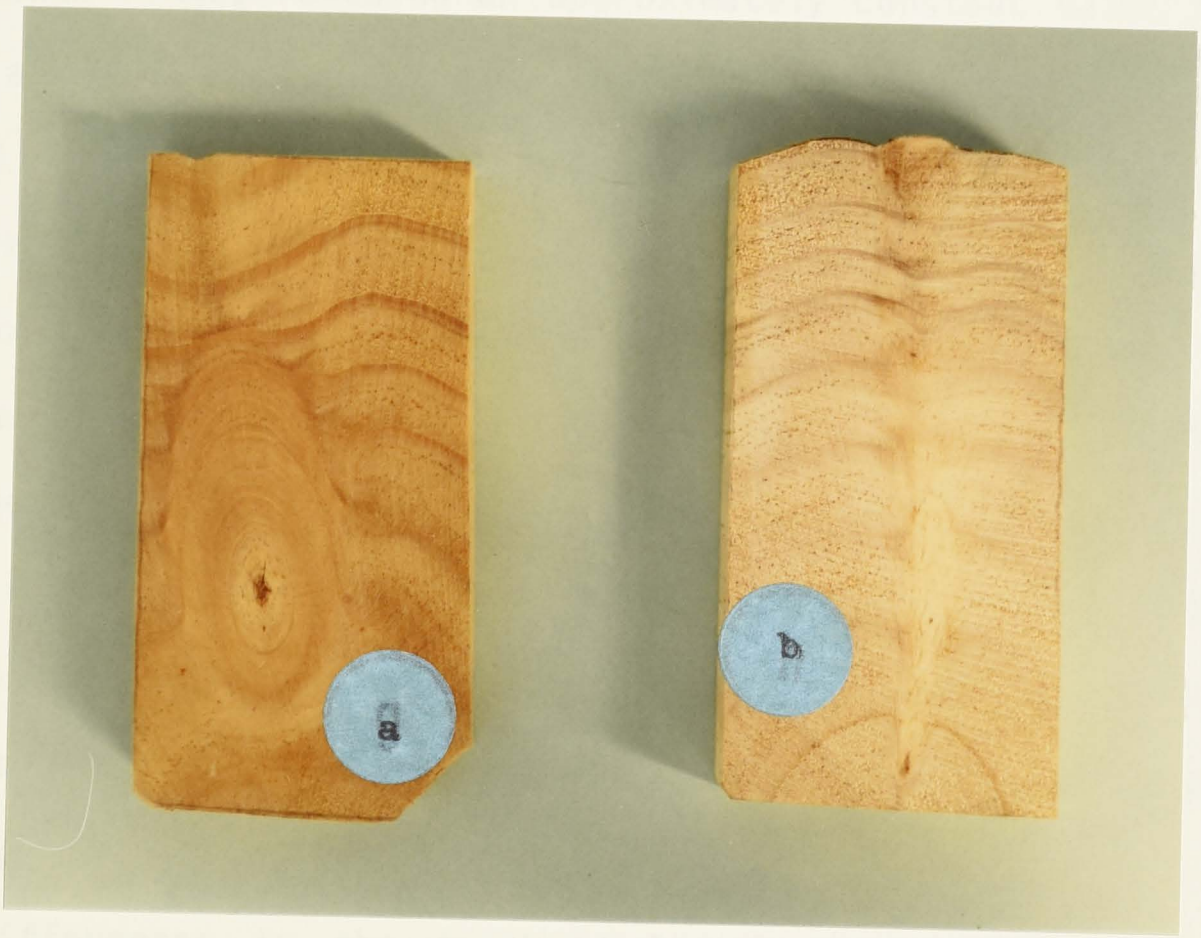
This property is a complex reflecting "...the effects of several growth and physiological variables compounded into one fairly easily measured wood characteristic" (Elliott, 1970). Density, which is a measure of the amount of wood substance per unit gross volume, is affected by cell dimensions and the amount of extractives in wood. In mature stems of many woods, wood density increases outwards from the pith within the juvenile zone (covering five to fifteen rings)

and plateaus thereafter in mature wood. The increase wthin the juvenile zone of *P. radiata* is considerable, changes from 0.36 to 0.45 g cm^{-3} being common (Bamber and Burley, 1983). Considering only the first five rings from the pith, Burdon and Bannister (1973) found average basic density for both Monterey and Ano Nuevo provenances to be quite similar (0.334 and 0.329 g cm^{-3}, respectively), while in Cambria, it was lower (0.320 g cm^{-3}). A similar ranking was obtained by Nicholls and Eldridge (1980). In the mature wood of stems growing in field trials near Canberra, Ano Nuevo material was least dense (0.436 g cm^{-3}), while the densest samples were obtained from Monterey trees (0.503 g cm^{-3}) (Nicholls and Eldridge, 1980).

4.2.2.3 Percentage Late Wood

The proportion of late wood, as visible on the transverse face, could be expected to show some correlation with density in that the late wood tracheids are thicker walled than their early wood neighbours. Such a relationship has been observed in species showing a clear distinction between early and late wood zones (Elliott, 1970), e.g. Douglas fir (*Pseudotsuga menziesii* Mirb.) and the Southern pines (*Pinus elliottii*, *P. taeda*, etc.). Where the distinction is less obvious as in *P. radiata*, *Picea sitchensis* (Bong) Carr., the correlation between whole-ring density and percentage late wood is often poor (Elliott, 1970), although the association was close in the *Pinus radiata* examined by Nicholls and Dadswell (1965). The relative difference in the growth rings between *Pseudotsuga menziesii* and *Pinus radiata* is shown in Plate 4-1.

Plate 4-1: Distinction between growth rings of *Pseudotsuga menziesii* ('a': distinct-ringed wood) and *Pinus radiata* ('b': diffuse-ringed wood)



The amount of late wood in *P. radiata* increases strongly from about 5% of the ring near the pith to about 30% *ca.* 20 rings from the pith. Thereafter, the proportion of late wood is reasonably constant (Bamber and Burley, 1983).

In *P. radiata* the denser insular provenances (Nicholls and Eldridge, 1980; Benyon, 1984) tend to have a higher late wood ratio (analogous expression of percentage late wood), than do the mainland populations. Of the three mainland populations, the highest late wood ratio probably occurs in Monterey (Nicholls and Eldridge, 1980).

4.2.2.4 Tracheid Length

As in the case of density, cambial age strongly influences cell length in many sftwoods and certain hardwoods (Dinwoodie, 1961). In *P. radiata*, tracheid length increases markedly outwards through successive growth rings, reaching an approximately constant value in mature wood about 15 rings from the pith (Nicholls and Dadswell, 1965). Marked differences in the period of juvenile wood formation and in the cell lengths attained at maturity occurred between four Australian-grown populations (Nicholls and Dadswell, 1965).

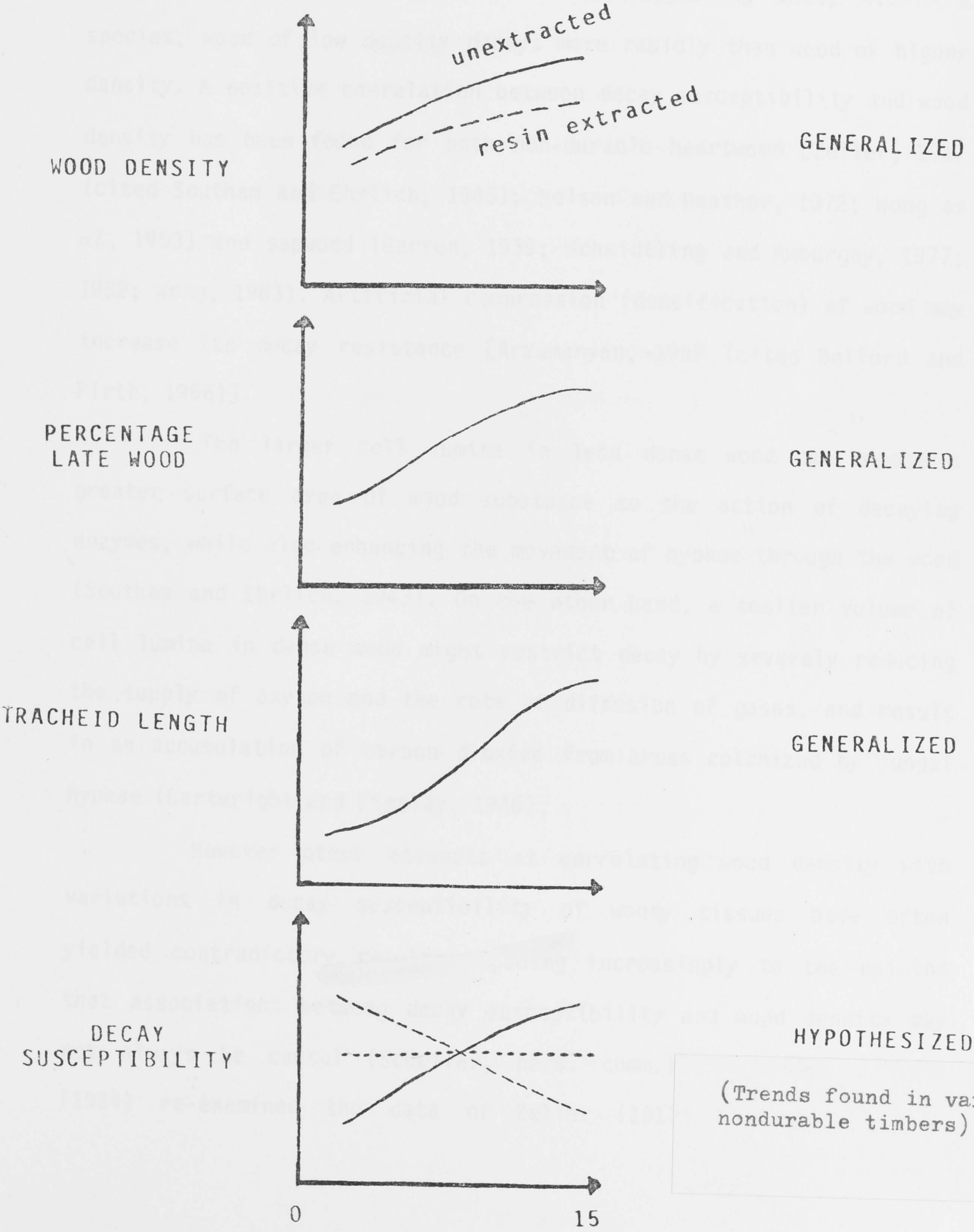
A number of studies have indicated variation in tracheid length with geographic location of a species (Bissett *et al*, 1951). For example, it was found (Zobel *et al*, 1960) that in natural stands of *Pinus taeda* growing in the south-eastern United States, tracheid length increased from south to north. Nevertheless, when progenies of *P. radiata* provenances were grown under uniform environmental conditions, differences in the tracheid length between the three mainland, and also between insular provenances, were not significant (Nicholls and Eldridge, 1980).

The softwood tracheid wall is a composite structure consisting of an array of predominantly cellulosic microfibrils, enveloped by the polyoses and bonded into a coherent mass by lignin (Page, 1976). Tracheid length has been positively correlated with cellulose content in *P. radiata* (Nicholls and Dadswell, 1965; Uprichard, 1971).

4.2.2.5 Summary of Radial Variations

To summarise the review presented in Sections 4.2.1 and 4.2.2, radial variation in wood density, tracheid length, percentage late wood and decay susceptibility in juvenile wood of P. radiata are shown graphically in Figure 4-1.

Figure 4-1: Generalized and hypothesized radial variations in decay susceptibility and some physical properties of juvenile *Pinus radiata* sapwood



4.2.3 Influence of Physical Characteristics of Wood on Variations in Decay Susceptibility

4.2.3.1 Wood Density and Percentage Late Wood

There is a body of evidence suggesting that, within a species, wood of low density decays more rapidly than wood of higher density. A positive correlation between decay susceptibility and wood density has been found for both non-durable heartwood [Zeller, 1917 (cited Southam and Ehrlich, 1943); Nelson and Heather, 1972; Wong *et al*, 1983] and sapwood (Garren, 1939; Schmidtling and Amburgey, 1977; 1982; Wong, 1983). Artificial compression (densification) of wood may increase its decay resistance [Arzumanyan, 1959 (cited Belford and Firth, 1966)].

The larger cell lumina in less dense wood can expose a greater surface area of wood substance to the action of decaying enzymes, while also enhancing the movement of hyphae through the wood (Southam and Ehrlich, 1943). On the other hand, a smaller volume of cell lumina in dense wood might restrict decay by severely reducing the supply of oxygen and the rate of diffusion of gases, and result in an accumulation of carbon dioxide from areas colonized by fungal hyphae (Cartwright and Findlay, 1946).

However other attempts at correlating wood density with variations in decay susceptibility of woody tissues have often yielded contradictory results, leading increasingly to the opinion that associations between decay susceptibility and wood density may not always be causal (Scheffer, pers. comm.). Thus when Buckman (1934) re-examined the data of Zeller (1917) statistically, no

correlation between the two factors was apparent; in fact some tendency towards a negative association was detected. The decay susceptibility of leached heartwood and sapwood blocks of *Thuja plicata* D.Don showed no correlation with density (Southam and Ehrlich, 1943). Studies of *Picea abies* L. Karst. and *Pseudotsuga menziesii* (Courtois, 1970) and of *Pinus sylvestris* (Rydell, 1982) have led to a similar conclusion.

Intra-incremental studies have not resolved the issue. Decay resistance was greater in late wood than early wood blocks of *E. delegatensis* (Wong *et al*, 1984) and *P. radiata* (Wong, Unpublished). Microscopic observation under polarized light (Meier, 1955) revealed the late wood of spruce (*Picea excelsa* L.) to be more resistant to brown-rot [*Merulius domesticus* Falck Syn. *Merulius lacrymans* (Wulf) Fr., and *Polyporus betulinus* (Bull.) Fr.] attack than the early wood. However, when Schulze and Theden (1938) subjected both *Pinus* and *Picea* species to decay by brown-rot organisms [*M. domesticus*, *Coniophora puteana* (Fr.) Karst. Syn. *Coniophora cerebella* Pers., and *Poria vaporaria* (Fr.) Cooke.], the reverse trend was found. Similarly, early wood of *Picea excelsa* was more resistant to white-rot than late wood (Meier, 1955). Thus it appears that intra-incremental variation in decay susceptibility may depend substantially on both the fungal species and tree species used.

Percentage late wood, a factor commonly associated with density, is often not correlated with decay resistance (Baxter, 1935; Garren, 1939; Southam and Ehrlich, 1943), although in one case (Zeller, 1917), a positive association was detected. It has been

suggested that where wood density and decay resistance are found to be positively related, any influence of density would not be direct, but would reflect the association between density and the ratios of water and/or air to cell wall substance in wood. Within critical moisture limits (e.g. 25 - 300% moisture content, depending on wood density and block dimensions), the rate at which moisture is adsorbed into wood influences the percentage weight losses (Courtois, 1968; Wong, Unpublished). Determination of the exact relationship between moisture content, water potential, and wood decay under laboratory conditions is made difficult by the fact that metabolic production of water by wood decay fungi (Peterson and Cowling, 1973) can alter significantly the water content of wood (Griffin, 1977). In reviewing the subject Southam and Ehrlich (1943) concluded that any tendency towards greater initial decay resistance in wood of high density is nullified, and may even be reversed as decay progresses, and so is of little practical value.

4.2.3.2 Cell Length

The possible relationship between fibre length and decay susceptibility has apparently not been studied. However it might be postulated that with shorter fibres or tracheids, fungal invasion of wood could be slower than where the elements are longer, in that a greater number of cell walls must be traversed per unit distance of invasion in the longitudinal direction. The end walls of cells could hinder fungal development, particularly where pits are scarce and/or impermeable (e.g. if aspirated). Any such effect would be transitory, but perhaps evident with most decay fungi (Wilcox, 1970) which rarely

penetrate cell walls directly (by forming bore holes) in the early stages of decay. The rate of invasion in the transverse direction, e.g. along rays, would presumably be independent of cell length.

4.2.3.3 Other Physical Factors

The relative resistance to fungal degradation of the various wood elements and the different cell wall layers is not uniform (Meier, 1955; Cowling, 1961; Wilcox, 1968). However, these important anatomical considerations fall outside the scope of this study.

4.3 MATERIALS AND METHODS

4.3.1 Wood Specimens

The method of sampling for wood blocks (and woodmeal) from the inner (rings 4 - 7) and outer (rings 10 - 13) sapwood has been described in Chapter 2 (Section 2.1.2.2). Only the three Californian mainland provenances were sampled, eight trees per provenance.

4.3.2 Decay Susceptibility Test

The ASTM soil-block decay procedure was adopted (Section 2.2.1.2). Based on preliminary assessment (Chapter 3), sapwood blocks were sterilized with propylene oxide (1,2-epoxypropane), and decayed by *G. trabeum* (a representative of the brown-rot fungi) and *F. lividus* (white-rot). The number of sapwood blocks required for the testing of decay susceptibility was defined as follows:

24 trees x 2 radial positions x 6 replications x 2 decay fungi = 576 blocks

Each jar was planted with two blocks, one from each radial position of each tree. The design also incorporated 64 'sacrificial' and 7 'control' blocks.

4.3.3 Determination of 'Unextracted' and 'Extracted' Wood Density

Water displacement (Section 2.2.2.1) was used to determine the 'unextracted' basic density of four blocks from each radial position of each tree. 'Extracted' basic density was computed by subtracting from the 'unextracted' density data, values of total extractives content [solvents: ethanol-benzene (Section 2.2.4.1) followed by hot water (Section 2.2.4.2)], determined with duplicate woodmeal samples (each *ca.* 1 g) from these positions.

4.3.4 Determination of Tracheid Length

The procedure for determination of average tracheid length per radial position (n = 200) of each tree, has been described (Section 2.2.2.3).

4.3.5 Determination of Percentage Late Wood

For each radial position, eight replicate measurements were used to determine the average percentage late wood (Section 2.2.2.3).

4.4 STATISTICAL ANALYSIS

Data were analysed statistically on the 'UNIVAC' 1100

System, using the computer package programs GENSTAT (Alvey *et al*, 1982) and GLIM (Baker and Nelder, 1978).

4.4.1 The Analysis of Variance Model

When applying analysis of variance (ANOVA), data were tested for homogeneity and normality of the residual variances (Neter and Wasserman, 1974). Where necessary, transformations were applied to satisfy these requirements. The ANOVA regression model for each wood property and decay susceptibility, incorporated two treatment effects, i.e. provenance and radial position, as expressed by the following relationship:

$$E(Y_{ij}) = \mu. + p_i + r_j + p_i r_j + e_{ij}$$

where $E(Y_{ij})$- expected value of wood property or decay susceptibility

$\mu.$- mean value

p_i- provenance effect

r_j- radial position effect

$p_i r_j$- interaction (provenance x radial position) term

e_{ij}- random error (number of trees and replications) are independent and normally distributed

Of main interest in this study was the variation between radial positions and between provenances. Thus differences among trees were included in the analysis as random variations (Cunningham, pers. comm.) However, for completeness, between-tree (within-provenance) differences were subsequently assessed using the *t*-test (see Appendix 4-1). The presence of interaction between provenance

and radial position does not preclude the discussion of the significance of the main effects in a fixed effect ANOVA model (Winer, pers. comm.).

4.4.2 Differences Between Means

Where either of the main effects (radial position, provenance) were significant ($P<0.05$) determinants of variation in data for decay susceptibility or the other wood properties, mean values were compared between radial positions and/or between provenances, by the two-tailed t-test at 0.05 level. Comparisons between radial positions were made using data from each provenance separately, and by pooling across all provenances. Similarly, provenance differences were determined for inner-sapwood, outer-sapwood, and for the two positions combined.

4.4.3 Simple Correlation Analysis

Correlation coefficients (r values) were computed for simple regressions between decay susceptibility and each physical property. Similar regressions examined the association between wood density (extracted or otherwise) and percentage late wood. The analysis assumes a normal bivariate distribution for the pair of factors correlated.

Correlations for single provenances used data from both radial positions, i.e. 16 paired observations. For general correlations (all provenances) three separate categories of data were considered: inner sapwood, outer sapwood, and the inner and outer positions combined. The significance of the r values was tested at the 0.05 level.

4.4.4 Additional Regression Analyses

Combining paired data from both radial positions and all provenances (n = 48), an extension of simple regression analysis (model 1; Figure 4-2) was developed, which determined:

(a) the contribution of any provenance effect to the relationship between decay susceptibility and a particular wood property (model 2), and

(b) the additional contribution of the provenance x wood property interaction effect to variation in decay suceptibility (apart from the effects of provenance and wood property (model 3).

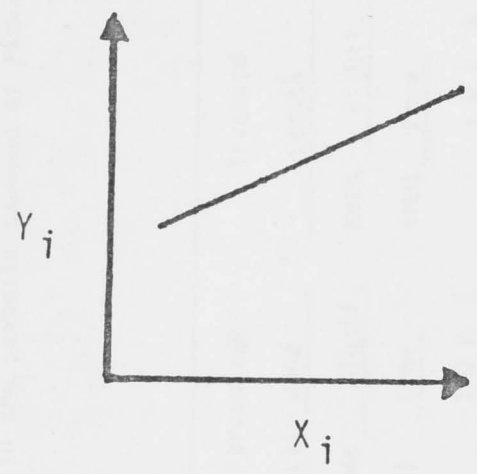
Higher order regression models were successively compared with lower order models using *F*-tests.

4.5 RESULTS

4.5.1 Within-tree Variation in Decay Susceptibility and Wood Properties

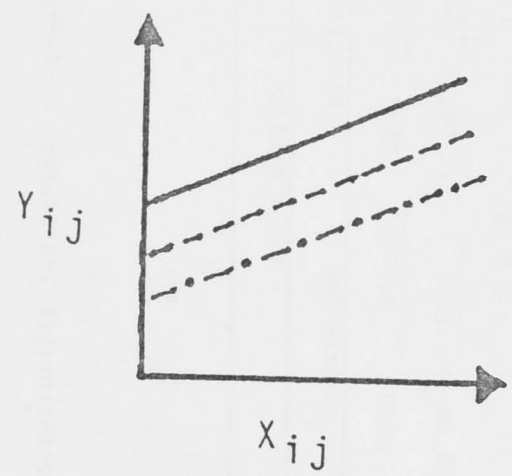
With one notable exception, differences in mean values, for each wood property including decay susceptibility, between inner and outer sapwood, were significant (Table 4-1) (Radial variation in susceptibility to decay of Cambria material by *F. lividus* was not significant).

Figure 4-2: Regression models of the effects of each wood property and individual provenances on variations in decay susceptibility



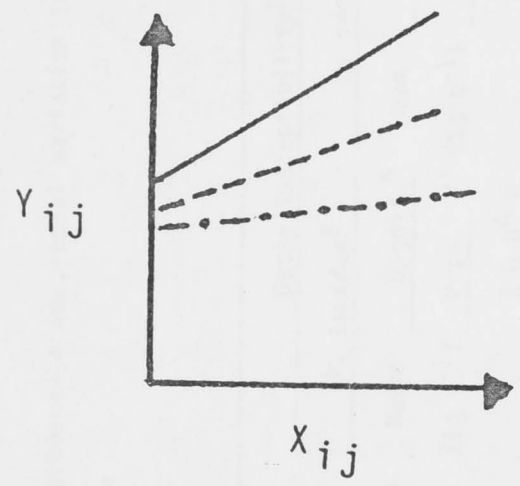
MODEL 1

$$Y_i = u. + X_i + e_i \qquad i=1,...48$$



MODEL 2

$$Y_{ij} = u. + X_i + P_j + e_{ij} \qquad i=1,...48 \quad j=1,2,3$$



MODEL 3

$$Y_{ij} = u. + X_i + P_j + X_iP_j + e_{ij} \qquad i=1,...48 \quad j=1,2,3$$

$E(Y_i)$ or $E(Y_{ij})$: expected value of decay susceptibility

$u.$: constant term

X_i : wood property effect

P_j : provenance (covariance) effect

X_iP_j : provenance x wood property (interaction) effect

e_{ij} : random error (independent and normal)

Table 4-1: Inter-provenance and radial variation in decay susceptibility and certain physical properties in the juvenile sapwood of *Pinus radiata*

Provenance (Prov)	Radial Position (R)	Decay Susceptibility[1] *F. lividus*[2] Mean (%g g^{-1})	Signif.[5] Prov I O B[4] R	Decay Susceptibility[1] *G. trabeum* Mean (%g g^{-1})	Signif. Prov I O B R	Basic Density Unextracted Mean (g cm^{-1})	Signif. Prov I O B R	Basic Density Extracted[3] Mean (g cm^{-1})	Signif. Prov I O B R	Percentage Late Wood Mean (%)	Signif. Prov I O B R	Tracheid Length Mean (mm)	Signif. Prov I O B R
Ano Nuevo	I[4]	2.6	a - - a	47.5	b - - a	0.339	a - - a	0.308	a - - a	4.7	a - - a	1.56	a - - a
	O[4]	3.7	- a - b	53.0	- a - b	0.392	- b - b	0.357	- b - b	20.4	- a - b	2.49	- a - b
Monterey	I	2.3	a - - a	37.4	a - - a	0.312	a - - a	0.312	a - - a	3.0	a - - a	1.56	a - - a
	O	3.2	- a - b	49.3	- a - b	0.370	- a - b	0.332	- a - b	17.1	- b - b	2.43	- a - a
Cambria	I	2.5	a - - a	46.7	b - - a	0.330	a - - a	0.298	a - - a	4.5	a - - a	1.45	a - - a
	O	3.0	- a - b	51.0	- a - b	0.364	- a - b	0.331	- a - b	15.2	- a - b	2.37	- a - b
Ano Nuevo	Both positions	3.1	- - a -	50.2	- - a -	0.365	- - a -	0.332	- - a -	12.5	- - b -	2.02	- - a -
Monterey		2.7	- - a -	43.3	- - a -	0.357	- - a -	0.322	- - a -	10.0	- - a -	1.99	- - a -
Cambria		2.7	- - a -	48.9	- - a -	0.347	- - a -	0.314	- - a -	8.8	- - a -	1.91	- - a -
All provenances	I	2.4	- - - a	43.8	- - - a	0.338	- - - a	0.306	- - - a	4.1	- - - a	1.52	- - - a
	O	3.3	- - - b	51.1	- - - b	0.375	- - - b	0.340	- - - b	16.9	- - - b	2.43	- - - b

1) Mean percentage weight loss after 6 weeks decay by the soil-jar method

2) Analysis based on square root transformed data

3) Analysis based on logarithm (base 10) transformed data

4) I- inner sapwood; O- outer sapwood; B- both radial positions

5) Mean values for a wood property were compared for differences between provenances in each of the 3 categories of data (I,O or B) and between 2 radial positions (R) at 0.05 level of significance. Within each of I,O or B column, provenances assigned the same letter do not differ significantly for that wood property, the same applying for the radial position (R) column

The susceptibility to decay by *G. trabeum*, was significantly greater in outer [mean weight loss: 49.3 - 53.0%] than inner [mean weight loss: 37.4 - 47.5%] sapwood. *Fomes lividus* also showed this trend, although weight losses were much lower: outer sapwood [mean weight loss: 3.0 - 3.7%]; inner sapwood [mean weight loss: 2.3 - 2.6%]. Mean values were also greater in outer than inner sapwood for basic density [mean unextracted: 0.364 - 0.392 g cm^{-3} *cf.* 0.330 - 0.339; extracted: 0.331 - 0.357 g cm^{-3} *cf.* 0.298 - 0.312], tracheid length [2.37 - 2.49 mm *cf.* 1.45 - 1.56], and percentage late wood [13.2 - 20.4% *cf.* 3.0 - 4.7].

4.5.2 Between-provenance and Between-tree Variations in Decay Susceptibility and Wood Properties

The differences between provenances, for mean values of each wood structural property and decay susceptibility, were not consistent and depended on the radial position sampled (Table 4-1), i.e. provenance x radial position interactions were present. In the outer sapwood, material from the Ano Nuevo provenance showed greater wood density, extracted (0.357 g cm^{-3}) or unextracted (0.392 g cm^{-3}) and greater percentage late wood (20.4%) compared with the other mainland material. These provenance differences were less marked when the data for both radial positions were combined, although the percentage late wood did remain significantly greater in the Ano Nuevo material. Mean values of each wood property for each tree are presented in Appendix 4-1. Differences between trees within a provenance were not usually significant for tracheid length and percentage late wood. Consistent, and significant, differences in

density were detected for the progeny from the Monterey and Cambria provenances although, in relative terms, the variation between means was often less pronounced than was the case for tracheid length and percentage late wood. Appreciable variation between trees in the susceptibility of sapwood to decay was detected, even where *F. lividus* was used as the test organism.

4.5.3 Correlation and Regression Analyses

Correlation coefficients for regressions between decay susceptibility and each of the other wood properties, and for regressions between wood density and percentage late wood, are presented in Tables 4-2 and 4-3, respectively.

While susceptibility to decay by *G. trabeum* was significantly and positively correlated with both tracheid length (Monterey, all provenances combined) and percentage late wood (all provenances combined), the contribution of each of these variables to the variation in decay susceptibility is apparently minor, i.e. tracheid length and percentage late wood each explained about 10% of the variation. Where *F. lividus* was the test organism, decay susceptibility was significantly correlated with wood density [r = 0.38 (extracted) - 0.42 (unextracted)], and percentage late wood (r = 0.33) when the data for all provenances was combined. Susceptibility to decay by *F. lividus* was also significantly correlated with density for the inner sapwood blocks (r = 0.44 - 0.46).

Table 4-2: Correlation coefficients (r values) for regressions between decay susceptibility and certain physical properties in the juvenile sapwood of *Pinus radiata*

Causal Organism	Wood Property	Both Radial Positions[1]				All Provenances[2]	
		Ano Nuevo	Monterey	Cambria	All	Inner Sapwood	Outer Sapwood
Gloeophyllum trabeum	Unextracted Basic Density	0.10	-0.07	0.15	0.05	-0.36	0.06
	Extracted Basic Density	0.10	-0.12	0.14	0.03	-0.36	0.00
	Percentage Late Wood	0.20	0.48	0.23	0.32*	0.03	0.07
	Tracheid Length	0.20	0.55*	0.18	0.31*	-0.13	0.04
Fomes lividus	Unextracted Basic Density	0.28	0.55*	0.32	0.42*	0.44*	0.12
	Extracted Basic Density	0.31	0.43	0.31	0.38*	0.46*	0.03
	Percentage Late Wood	0.40	0.12	0.47	0.33*	-0.19	0.30
	Tracheid Length	0.43	0.00	0.20	0.22	-0.24	-0.19

1) Correlations based on 16 paired observations for each provenance and 48 for 3 provenances (All), when data from both radial positions were combined

2) Correlations based on 24 paired observations for each radial position, when data from 3 provenances were combined

*) Correlations significant at 0.05 level

Table 4-3: Correlation coefficients (r values) for regressions between percentage late wood and wood density in the juvenile sapwood of *Pinus radiata*

Correlation between	Both Radial Positions[1]				All Provenances[2]	
Percentage Late Wood and...	Ano Nuevo	Monterey	Cambria	All	Inner Sapwood	Outer Sapwood
Unextracted Basic Density	0.93*	0.37	0.78*	0.69*	0.45*	0.47*
Extracted Basic Density	0.92*	0.28	0.77*	0.65*	0.42*	0.39

1) Correlations based on 16 paired obsrvations for each provenance and 48 for 3 provenances (All), when data from both radial positions were combined

2) Correlations based on 24 paired observations for each radial position, when data from 3 provenances were combined

*) Correlation coefficients significant at 0.05 level

It is evident from Table 4-3 that percentage late wood does not account significantly for variation in wood density in the Monterey material, (r^2 = 0.08 - 0.14, i.e. 8 - 14% of the variation in density is possibly attributable to variation in percentage late

wood). At the other extreme, strong correlations between density and percentage late wood were recorded for Ano Nuevo (r > 0.90) and Cambria (r > 0.75). Moderate correlations between the two factors were obtained when data for all provenances were combined for inner-sapwood (r = 0.42 - 0.45), outer sapwood (r = 0.39 - 0.47) and for both positions combined (r = 0.65 - 0.69). No consistent differences were observed in the correlations between percentage late wood and unextracted or extracted density (Table 4-3).

Table 4-4: Statistical modelling of the importance of a wood property (X_i) in explaining variation in decay susceptibility, with stepwise comparison for possible provenance effects (P_j) and provenance x wood property interaction effects (P_jX_i)

Causal Organism	Model[1]	Change in Variance Ratio due to the stepwise addition of 'P_j' and 'P_jX_i'			
		Unextracted Basic Density	Extracted Basic Density	Tracheid Length	Percentage Late Wood
Gloeophyllum trabeum	u. + bX_i	0.12	0.04	5.10*	5.50*
	u. + bX_i + P_j	2.03	2.03	2.36	2.01
	u. + bX_i + P_j + X_iP_j	0.19	0.29	1.07	0.54
Fomes lividus	u. + bX_i	9.68*	7.77*	2.23	5.42*
	u. + bX_i + P_j	0.33	0.31	0.67	0.38
	u. + bX_i + P_j + X_iP_j	0.51	0.08	0.86	0.50

1) Analysis performed on 48 paired observations (2 radial positions of all provenances)

*) Variance ratio significant at 0.05 level

The step-wise regression analysis (Table 4-4) showed that, in addition to the above findings for correlations between decay susceptibility and wood properties, neither the effects of provenances nor the interactions between provenance and wood property, provided a significant explanation for the variations in decay susceptibility.

4.6 DISCUSSION

4.6.1 Variation in Physical Wood Properties

4.6.1.1 Within-tree Variation

A consistent pattern of increasing average basic density, percentage late wood and tracheid length from the innermost to the outermost sapwood in juvenile *P. radiata* is consistent with other observations for many softwoods [tracheid length (Dinwoodie, 1961), wood density and percentage late wood (Elliott, 1970)]. Radial gradients in these wood properties will predictably level off at about 15 years in the case of *P. radiata* (Dadswell, 1958). Thereafter values are often approximately constant in mature wood. The gradient in the juvenile wood studied was steepest for percentage late wood (312% increase from inner to outer sapwood, meaned across provenances), followed by tracheid length (60%) and basic density (11% unextracted; 11% extracted). This result affirms other findings in *P. radiata* (Bamber and Burley, 1983; Nicholls and Eldridge, 1980) showing a minimal radial variation in basic density as compared to

percentage late wood, with variation in tracheid length being intermediate.

In this work, the low average tracheid length of the outer sapwood position (< 2.5 mm, Table 4-1), suggests that juvenile wood formation, at least regarding this feature, may have continued well beyond 15 years in the Boboyan Provenance Trial. Harris (1965) has suggested that owing to the small radial changes in density no significant genetic improvements can be expected by selecting for seed sources with higher density juvenile wood. Nevertheless, most tree-breeding programs which include wood quality selection criteria aim to increase the uniformity of the resource. Clearly this aim is justified if timber is to be converted and utilized most efficiently.

4.6.1.2 Between-provenance and Between-tree Variations

The variation in wood properties between progenies originating from the three geographical localities in California depends on the position sampled (radial position x provenance interaction). For example, wood density for Ano Nuevo provenance was significantly greater than for either Monterey or Cambria only for the outer sapwood (Table 4-1). With the two radial positions combined, the average percentage late wood was significantly greater for Ano Nuevo than for the other mainland provenances but it is likely that this is primarily a reflection of the between-provenance variation at the outer sapwood position. Since only outermost sapwood showed some provenance variation in wood properties it seems that the expression of genetic control of variation in these properties between-trees is influenced by the maturity of the cambium as wood is formed.

The present study of biodeterioration in timber was not designed to assess, in detail, the between-provenance differences in the wood properties. The replication of eight trees per provenance, which was chosen as a compromise between what was desirable and what was practicable, approximates that used by other workers (Fielding and Brown, 1960; Nicholls and Eldridge, 1980). However, one recent study on *P. radiata* found such a sample size (6 - 12 trees) to be insufficient to provide conclusive evidence of differences (or otherwise) in density parameters between provenances (Benyon, 1984). Statistical analysis of the present results has suggested the need for assessment of at least 16 trees per provenance (Cunningham, pers. comm.). Thus, detailed conclusions regarding genetic variations cannot be drawn from the results obtained. Nevertheless, it is noteworthy that the data are broadly consistent with those obtained by other workers who examined the three mainland provenances growing in the Boboyan Provenance Trial (Benyon, 1984), and in a different location (Nicholls and Eldridge, 1980). For example, between-provenance variation in both density and tracheid length is consistently minimal. In agreement with the results of this study (Appendix 4-1), it is found that significant variations in wood density (Matziris, 1979; Nicholls and Eldridge, 1980) and tracheid length (Zobel, 1961; Nicholls and Eldridge, 1980) occur between trees within the provenances both for *P. radiata*, and for other tree species. These variations are thought to be great enough to largely negate any benefit in selecting between provenances for the two wood properties (Zobel, 1961). Whether the between-tree differences reflect environment or genetic differences is contentious.

4.6.2 Relationship Between Density and Percentage Late Wood

Of the various component cells in *P. radiata* (mainly axial tracheids, ray tracheids and ray parenchyma), it is the axial tracheids which contribute most of the tissue volume (*ca.* 90% of volume, Bamber and Burley, 1983). It could be expected then that the relative proportions of early wood and late wood tracheids would have a major influence on density. Generally, in softwoods of "normal" ring frequency, the tendency is for high wood density to coincide with high ring frequency (Panshin and de Zeeuw, 1980). The correlation between these two factors probably reflects an increasing proportion of dense late wood cells in the years of slower growth. However, where late wood is not clearly defined from early wood, as in *P. taeda*, a weakening of the correlation could be expected (Paul, 1939). *P. radiata* can also be placed in this category, and this may account partly for the moderate to low correlations between basic density and percentage late wood obtained in some cases, e.g. for the Monterey provenance (Table 4-3; Nicholls and Dadswell, 1965). In the Ano Nuevo material, where the late wood appeared to be clearly defined (relatively less diffused), the correlations were closer (Table 4-3).

In correlating density and percentage late wood no account is taken of the effect of radial gradients on the density of early wood and late wood. For example, Wong *et al.* (1983) found gross density to increase from inner to outer heartwood in 45-year-old stems of *Eucalyptus delegatensis*. This increase was subsequently related to an inter-incremental increase in the density of both early and late wood while only a small rise occurred in the percentage late

wood (Wong *et al*, 1984). Thus, correlations between basic density and percentage late wood in *E. delegatensis* could be weakened if more than one radial position were included in the analysis, and the same may apply in *P. radiata*.

4.6.3 Variation in Decay Susceptibility

4.6.3.1 Influence of Test Fungus

In common with the findings of the present study others (Zabel, 1948; Cowling, 1961; Scheffer, 1964; Eslyn and Highley, 1976) have found brown-rot fungi to cause very high weight losses in sapwood as compared to white-rot species. However it cannot be suggested that sapwoods of all wood species are generally more resistant to decay by white-rot fungi; a general survey (Peterson and Cowling, 1964; Scheffer, 1964) reveals an apparent preference of white-rot species for hardwoods, and brown-rot fungi for softwoods. The present results for sapwood of *P. radiata* augments this general finding.

4.6.3.2 Within-tree Variation in Decay Susceptibility

Zabel (1948) in his work on white oak (*Quercus alba* L.), showed the sapwood closest to the cambium to be more susceptibile to decay than that closest to the heartwood. This is probably the only direct evidence to support the similar pattern recorded in the present study (Table 4-1). The reverse pattern has been more widely detected (Neville, 1969; Schmidtling and Amburgey, 1977; 1982). Despite the greater decay caused by *G. trabeum*, *F. lividus* showed a

greater relative sensitivity to radial position- a 38% increase from inner to outer sapwood (data meaned across provenances) *cf.* 17% for *G. trabeum*. This is not surprising given that *F. lividus* has such a limited capacity to decay the wood; small variations (e.g. radially) in the wood property or properties most strongly controlling susceptibility to decay in general could profoundly affect the activity of the organism. Discussion of possible causes of variation in decay susceptibility in sapwood is reserved for Section 4.6.4.

4.6.3.3 Between-provenance and Between-tree Variations

Several studies which have considered variations between geographic locations in the decay resistance of heartwood (Scheffer and Duncan, 1947; Scheffer and Hopp, 1949; Scheffer *et al*, 1949, Clark and Scheffer, 1983) have ascribed apparent differences to genetic variation. It is thought that the large variations between localities in the decay resistance of the heartwood of redwood [*Sequoia sempervirens* (D.Don) Endl.], are due to the population in a given locality developing a common level of resistance by inter-breeding. Thus, individuals within a population tend to produce heartwoods more alike in decay resistance than trees from different localities (Clark and Scheffer, 1983). Whether such genetic differences apply to sapwood, is not known. However Scheffer (pers. comm.) has found that some correlation exists between the resistance of sapwood and that of the associated heartwood in a stem.

The susceptibility of inner sapwood of *P. radiata* to decay by *G. trabeum* (see Table 4-1) showed greatest resistance for the Monterey material. Provenance differences, with this fungus, were not

significant for outer sapwood. Perhaps, since the between-provenance variations in the physical characteristics investigated were minimal in inner sapwood, other factors of a chemical nature, e.g. the relative amounts of potential microbial nutrients, could account for the greater average resistance of the inner tissues of the eight Monterey trees studied. For example, Fossum *et al* (1972) found amounts of inorganic metals (calcium, magnesium and manganese) in inner sapwood of *Pinus* species to vary between geographical locations.

Since the decay resistance of untreated *P. radiata* heartwood and sapwood is generally low, this wood property is not used in grading the timber for various end uses. It would appear that the present results (Appendix 4-1) support this practice, at least for sapwood, in that, under appropriate conditions, (e.g. those of the soil-jar test, with *G. trabeum* as the test organism), decay of the blocks from all trees and all provenances was very rapid-consistently > 20% weight loss in six weeks. The gains obtainable by selecting between provenances and/or between trees under these conditions are not likely to be of major practical significance.

4.6.4 The Relationships Between Physical Characteristics and Variations in Decay Susceptibility

4.6.4.1 Association With Wood Density

The present results show decay resistance in juvenile sapwood of *P. radiata* to be sometimes inversely correlated with wood density when the test fungus is the white-rot species *F. lividus*.

Using both white-rot and brown-rot organisms, Zabel (1948) found a similar relationship in the heartwood of *Quercus alba*. This association, being the reverse of the correlation usually detected (Garren, 1939; Schmidtling and Amburgey, 1977; 1982), is not easily explained and is unlikely to be causal. Nelson and Heather (1972) have cautioned that where samples are taken on a radial course, a significant relationship between decay susceptibility and basic density could simply reflect the strong individual correlations of these factors with distance from the pith. For example, decay susceptibility can be closely linked to the content or toxicity of heartwood extractives, which often increases with distance from the pith in a similar fashion to basic density (Rudman, 1965; Wong *et al*, 1983). Thus it is notable that a significant positive relationship between density and weight losses induced by *F. lividus* was detected for inner sapwood tissues alone (Table 4-2), i.e. where only slight variations occurred between trees in the sampling position (see Table 2-1). Nevertheless, it cannot be ignored that no such significant relationship was obtained for the outer position using *F. lividus*, or for either position using *G. trabeum* (Table 4-2), and it must be assumed that density has a relatively minor influence on the decay susceptibility of the pine sapwood studied.

Even where significant relationships were obtained (using *F. lividus*; inner sapwood or both positions combined), variation in basic density accounted for less than 22% of the variation in the weight loss data. On limited experimental evidence (Southam and Ehrlich, 1943; Courtouis, 1968; 1970), it is widely regarded that even in woods where there is some apparent influence of wood density

on decay resistance (the effect of which is usually transitory-) the tendency for wood of high density to decay more slowly initially is nullified, and possibly even reversed as decay progresses (Southam and Ehrlich, 1943). The absence of significant correlations between resistance to decay by *G. trabeum* and wood density in the present study (Table 4-2) is probably not unexpected if indeed density does have some type of transitory influence, since the decay had advanced substantially (> 37% mean weight loss) when the blocks were retrieved.

4.6.4.2 Association With Percentage Late Wood (and Ring Frequency)

Both a lack of correlation (Baxter, 1935; Garren, 1939; Southam and Ehrlich, 1943), and inverse relationships (Zeller, 1917) between weight losses and ring frequency or percentage late wood have been recorded. In his investigation, Garren (1939) employed *Pinus taeda* which has poorly defined late wood bands (Paul, 1939). It is perhaps not surprising then, that he found no significant relationship between decay and the late wood ratio despite the existence of a positive correlation between wood density (associated with percentage late wood) and decay resistance. *P. radiata* also lacks distinct late wood, but here (Table 4-2), percentage late wood was often positively correlated to decay susceptibility (Table 4-2). Just as for wood density, such significant correlations could merely reflect close individual associations between these wood properties and another more important factor, such as wood chemistry.

4.6.4.3 Association With Tracheid Length

As postulated previously (Section 4.2.3.2), longer cells may facilitate more rapid invasion of timber in the axial direction in that fewer end walls need be traversed. This hypothesis is supported by the significant correlations between tracheid length and percentage weight loss attributable to *G. trabeum*, where the data for both positions is included in the analysis (Table 4-2). However, no significant correlations are apparent when the inner and outer positions are considered separately, or when *F. lividus* is the decay species. This suggests the lack of any major influence of tracheid length on decay.

4.7 CONCLUSIONS

In the juvenile sapwood of *P. radiata* studied, the decay susceptibility, wood density, tracheid length, and percentage late wood are greater in outer than inner sapwood. There is some evidence of variation in wood density, percentage late wood and decay susceptibility between the three provenances (Ano Nuevo, Monterey and Cambria). Correlations between wood density and percentage late wood are sometimes poor, perhaps because the late wood bands are not clearly defined in *P. radiata*. Although significant positive correlations between decay susceptibility and tracheid length, density, and percentage late wood can be found, such relationships are quite likely non-causal, e.g. there may be the parallel variations in a number of wood properties, including chemical factors not studied here, with distance from the pith. It is probable that

the true effects (if any) of late wood ratio and/or tracheid length on relative decay susceptibility in the sapwood are obscured by other important factors such as variations in cell wall chemistry and amounts of reserve food materials. The influence of these factors on the decay susceptibility of the sapwood requires closer investigation.

CHAPTER 5

VARIATIONS IN CHEMICAL CHARACTERISTICS OF SAPWOOD OF *PINUS RADIATA* AND THEIR ASSOCIATION WITH DECAY SUSCEPTIBILITY

5.1 INTRODUCTION

The investigations into the possible relationship between the physical characteristics of juvenile sapwood of *Pinus radiata* and decay susceptibility which was reported in Chapter 4 suggest that either the physical properties examined have no major influence on variations in decay susceptibility, or that their exact roles are overshadowed by other factors. In particular, variations in chemical components which are stimulatory or inhibitory to growth of fungi may be more important.

In this Chapter variations in decay susceptibility of the sapwood of *P. radiata* are considered in relation to the possible influences of certain chemical characteristics of wood. The selected characteristics include amounts of lignin, nitrogen, minerals, carbohydrates, and extractives. The radial variation in these chemical properties *per se* is emphasized here in the light of general

interest in these chemical components affecting wood quality in this tree species.

5.2 LITERATURE REVIEW

5.2.1 Survey of Chemical Components of Wood

When not otherwise indicated, the review in this section is taken from Fengel and Wegener (1983) and Kirk (1973a).

5.2.1.1 Structural Materials

The main structural components of wood are the polymeric substances: cellulose, the hemicelluloses and lignin. Generally, softwoods contain between 25 and 35% lignin and between 40 and 50% cellulose, with hemicelluloses comprising the remainder.

5.2.1.1.1 Holocellulose

This is the total carbohydrate fraction of wood, which is commonly isolated by extracting a sample of woodmeal to remove extractives and then applying chemical treatments (e.g. chlorination) to remove lignin. The isolated substance constitutes 60 to 80% of the dry weight of wood. Holocellulose is mainly characterized as two major fractions: α-cellulose and the hemicelluloses.

α-cellulose is a linear high molecular-weight polymer of exclusively D-glucose units linked by 1,4-β-glucosidic bonds, with a degree of polymerization (DP) ranging from about 200 to 3000 or more. Chains of these units are aggregated laterally by hydrogen bonds into

a linear, micellar structure (microfibril). The strong aggregation and nearly perfect alignment of the chains gives rise to *ca.* 70% crystallinity of wood cell wall cellulose, with regions of the more randomly oriented non-crystalline (amorphous or para-crystalline) material interspersed in microfibrils. Filling the spaces within and between the microfibrils are the hemicelluloses and the lignin.

The hemicelluloses of wood occur as relatively short, and usually branched, chains of sugar units. The polymers are of low DP (*ca.* 15 to 200), and are essentially non-crystalline. The major hemicelluloses of wood are polymers of five monosaccharides: the hexoses glucose, mannose and galactose, and the pentoses xylose and arabinose. As well as the linear polymers of each single monosaccharide unit (termed glucans, mannans, galactans, xylans, and arabans), two or more of these sugar units may be included in a given hemicellulose molecule, e.g. gluco-mannans, arabo-galactans, and arabo-xylans. Uronic acids are additionally present. More hemicelluloses are found in hardwoods (20 - 30% w/w) than softwoods (15 - 20% w/w) and the sugar composition varies between the two types of woods (Hamilton and Thompson, 1959). The main constituent of hardwood hemicellulose is xylose, whereas in softwoods mannose predominates.

It is most likely that the hemicelluloses, together with lignin, surround the cellulose fibrils as a matrix, forming an interpenetrating complex, bonded covalently.

5.2.1.1.2 Lignin

This is an encrusting material of wood cell walls. Although

relatively poorly defined structurally, lignin is known to be an amorphous, complex, highly branched, three-dimensional aromatic polymer of phenylpropane (e.g. guaiacyl, syringyl and p-hydroxyphenyl) units. Hardwood lignin contains both guaiacyl and syringyl residues, while in softwoods, the lignin is essentially a guaiacyl type. About 70% of the lignin in wood cells is located in the secondary wall layers, while most of the remainder is located in the middle lamella regions where it is the main constituent.

5.2.1.2 Non-structural Materials

These components usually are not very important quantitatively. They include the water-soluble hemicelluloses (Timell, 1967), nitrogenous materials, pectins, starch, low molecular-weight sugars, minerals, and a variety of other extractives, e.g. lignans, terpenes and phenols.

5.2.1.2.1 Starch and Pectins

Starch is the main reserve polysaccharide of living trees, functioning as storage compounds for glucose when needed by the tree. This polysaccharide consists of two components, amylose and amylopectin, both of very high molecular weight. Starch granules are found in phloem and parenchyma and, in hardwoods, the vessel tissues as well. Most softwoods have little starch but instead have fatty-acid and fatty-acid esters, which are functionally similar to starch in hardwoods. Starch levels in tree species fluctuate seasonally, reflecting variation in both vegetative growth and other physiological activity throughout the year.

5.2.1.2.2 Nitrogen

In stemwood, nitrogen usually constitutes only 0.03 to 0.10% by weight (contrasting with 1 - 5% in herbaceous tissues). It occurs as protein in cell walls and cytoplasm and is located particularly in ray tissues (Merrill and Cowling, 1966). The number of living parenchymal cells in wood is apparently related to nitrogen content, and thus a far greater amount of nitrogen occurs in sapwood than heartwood. This difference suggests an internal recycling mechanism through which trees conserve their meagre amounts of this element (Wardell and Hart, 1973). However some of the proteins within the cell walls would be resistant to this recycling as sapwood matures (Cowling and Merrill, 1966). It appears that there are also seasonal variations in levels of this element (Levi and Cowling, 1968).

5.2.1.2.3 Minerals

These are the inorganic components of the wood and are summarily contained in ash. The content of minerals in stemwood is low, most elements showing a 10 to 100-fold increase in herbaceous tissues. The timber of temperate zone species usually contains 0.1 to 1.0% ash. Many of the minerals are essential for the biochemical activities of the developing cambial cells. The main mineral elements of wood ash is calcium, which comprises 50% or more of the total ash of many woods. The next most common mineral elements, in order of decreasing content, are potassium, magnesium, manganese, sodium and phosphorus. These are termed macro-elements, since they normally occur in wood in quantities of more than 50 ppm (dry wood weight

basis) Among the trace elements, aluminium, zinc and iron are predominant. Estimation of trace elements is relatively difficult, although more than 50 have been detected in *Picea* species (Young and Guinn, 1966).

The minerals, together with the hemicelluloses, lignin and wood extractives occupy the amorphous regions of cellulose microfibrils as well as the compound middle lamella and the tertiary (S3) wall layer (Cowling, 1961). At least calcium, magnesium and manganese were found in all types of cells in sapwood of *Pinus sylvestris*, the amounts per unit weight being greater in the late than early wood (Fossum *et al*, 1972). It is believed that recycling mechanisms (as postulated for nitrogen) maintaining higher concentrations of nitrogen in sapwood than heartwood, also apply to certain minerals (Wardell and Hart, 1973; Bamber, 1976).

5.2.2 Radial Variations in Chemical Characteristics of Wood

5.2.2.1 Structural Carbohydrates and Lignin

The cellulose content increases from the pith to bark although the pattern of this increase may vary appreciably. In mature trees the increase in actual percentage values approximates 3% in *Pinus contorta* Dougl. and *P. taeda* L, 6 to 10% in *Pseudotsuga menziesii* (Mirb.) Franco, and as much as 8 to 20% in *Pinus radiata*. Most of this increase lies in the first 12 to 20 years of growth (i.e. in juvenile wood). In hardwoods the radial trends in cellulose content are more variable (Panshin and de Zeeuw, 1980), although in some cases, they are similar to those in softwoods, e.g. in *Populus x*

euramericana (Dode) Guinier (Ferrari, 1966).

The amount of hemicellulosic components in wood is commonly determined by analysis of simple sugars produced from hydrolysis of the polysaccharide fraction of the wood. In *P. radiata*, the amount, based on simple sugars expressed as pentosans (Harwood, 1971; Uprichard, 1971), decreases about 3% from pith to bark. Further, in *Pinus resinosa* Ait. (Larson, 1966) and *P. radiata* (Harwood and Uprichard, 1969; Harwood, 1971), glucose and mannose contents increase with distance from the pith in a flat curve, while for xylose and galactose the reverse trend is true. A general reduction in the hemicelluloses from pith to bark was also described in the *Populus x euramericana* (Ferrari, 1966; 1967).

The lignin content in softwoods shows a general decrease of 1.5 to 3% from the pith to bark. In hardwoods such variations have received little attention, but have been shown to differ from those of softwoods, e.g. *P. x euramericana* (Ferrari, 1966).

5.2.2.2 Available Carbohydrates

In general, for the genus *Eucalyptus*, the content of starch varies from 1.4 to 2.7% (w/w) in the phloem to nil at the sapwood-heartwood boundary, with mid-sapwood peaks of 5 to 7% (Doimo, 1984). Approaching sapwood-heartwood boundary starch in both the rays and axial parenchyma is progressively lost. However, levels in sapwood of living trees decrease generally from the cambium to the sapwood-heartwood boundary (Doimo, 1984). Little is known about variation in amounts of other non-structural carbohydrates in sapwood.

5.2.2.3 Nitrogen and Minerals

The nutrient composition of wood varies considerably within a tree, with clear though inconsistent radial patterns being recorded by many workers. Previous work on *P. radiata* (Orman and Will, 1960) showed that highest concentrations of nitrogen, phosphorus and potassium occurred in the outer growth ring of the sapwood. A very sharp decrease in the content of these nutrients then occurred towards the heartwood. By contrast, the level of calcium was low in outer sapwood, rising to a maximum in the heartwood. Similar variations have been found in Scots (*P. sylvestris* L.) and Corsican (*P. nigra* var *poiretiana*) pines (Wright and Will, 1958). Decreasing radial gradients of calcium, sodium, magnesium and potassium from the pith in *P. taeda* have also been reported (McMillin, 1970). In *Quercus alba* L., amounts of chlorine, sulphur, calcium and manganese were similar across the sapwood (Wardell and Hart, 1973). The content of phosphorus decreased, and that of potassium increased, significantly from the outer to inner sapwood position. It has been suggested that where an increase in calcium occurs inwards from the bark it is associated with this element forming a necessary part of the enzyme system for translocation of carbohydrates, and is directly concerned with starch-sugar changes. Calcium is unlike other elements in that once it is deposited it appears to remain immobile (Orman and Will). Apparently phosphorus and potassium are normally re-locatable and usually accumulate in regions of greatest metabolic activity, i.e. in outer sapwood *cf.* heartwood.

Gradients in nitrogen content have been investigated for stems of both hardwoods and softwoods [e.g. *Populus grandidentata*

Michx., *Fraxinus americana* L., *Quercus alba* L., *Pinus strobus* L. and *Picea abies* (L.) Karst. (Merrill and Cowling, 1966a); *P. radiata* (Orman and Will, 1960); *Tsuga canadensis* L. (Grozdits and Ifju, 1973)]. In the stems containing heartwood, the levels of nitrogen consistently appear highest in the outer sapwood, remain relatively stable (at lower values) from inner sapwood to heartwood (Figure 5-1). nitrogen content decreases progressively from the cambium to the pith in species without heartwood (Merrill and Cowling, 1966b).

The change in nitrogen content radially within sapwood probably results from:

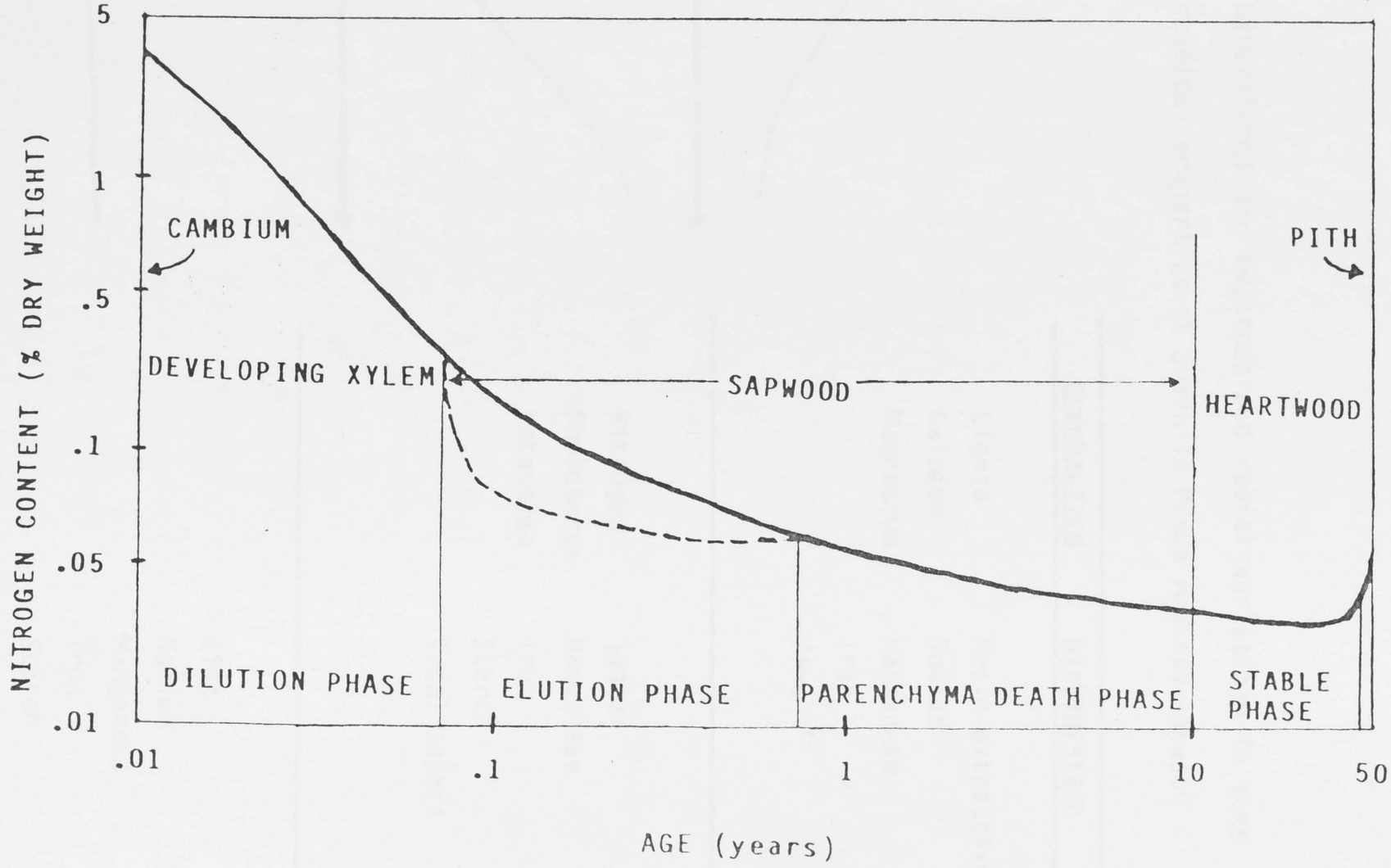
(a) apposition of cellulose and lignin in cell walls (dilution phase),

(b) elution of the elements from the vascular cells in the transpiration stream (elution phase), and/or

(c) retrieval of nitrogen from dying parenchymal cells progressively from the first (outer) annual increment across the sapwood (parenchyma death phase) (Cowling and Merrill, 1966; Grozdits and Ifju, 1973).

Radial variation in various proteinaceous nitrogenous substances was assessed in *P. sylvestris* (Laidlow and Smith, 1965). The amino-acid composition of the proteins did not appear to vary significantly across the sapwood.

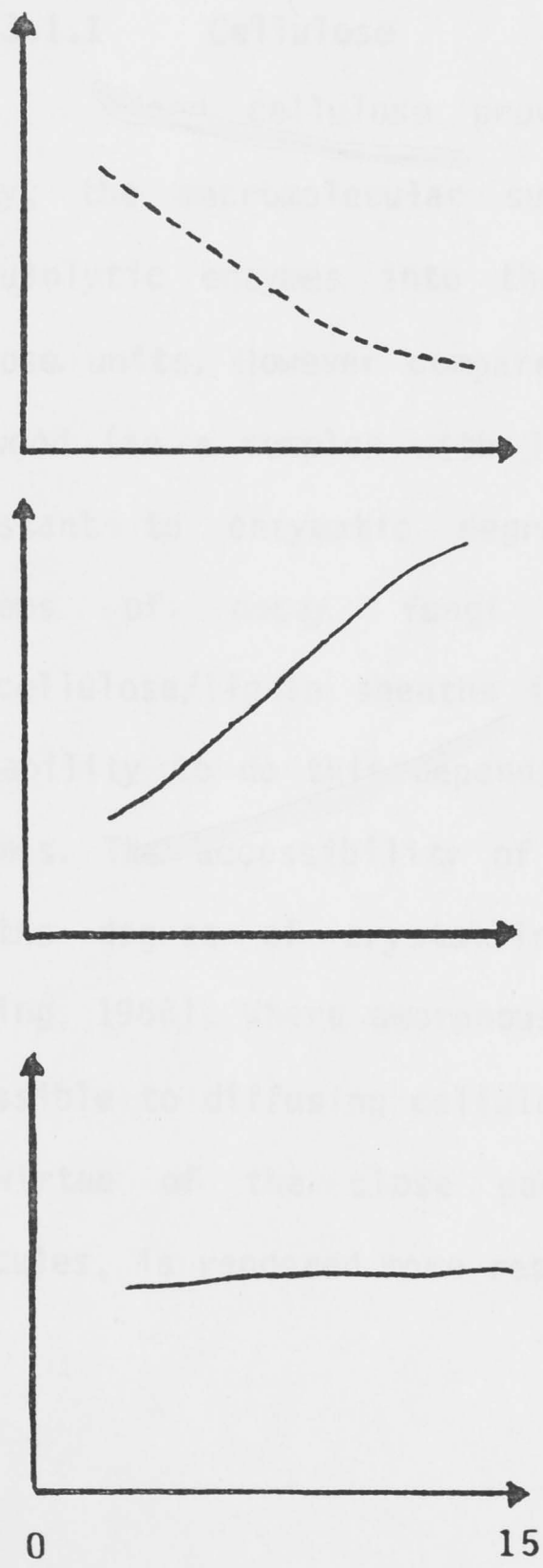
Figure 5-1: Hypothetical graph of radial variation in nitrogen content of a wood species and its association with four phases of ageing of the cambial and xylary cells



5.2.2.4 Summary of Radial Variations

By way of orientation and as a summary for Section 5.2.2, generalized and hypothesized gradients of properties selected for the present study are represented graphically in Figure 5-2.

Figure 5-2: Generalized and hypothesized radial variations in some chemical properties of juvenile *Pinus radiata* sapwood



GENERALIZED	HYPOTHESIZED
Lignin	Resin extractives
Calcium	Sodium
Magnesium	Manganese
	Iron
	Zinc
Nitrogen	Sodium
Phosphorus	Manganese
Potassium	Iron
	Starch
	Total sugars
	Zinc
	Sodium
	Manganese
	Iron
	Starch
	Total sugars

5.2.3 Nutritional Requirements of Fungi

The optimal food base varies among wood-decay fungi, but the wood substrate alone is usually adequate. The nutrients are derived from the polysaccharide fractions (holocellulose, starch and sugars), and in the case of some fungi, particularly the white-rot types, lignin. Important also for fungal growth is the supply of nitrogen and various macro-elements, trace elements and vitamins.

5.2.3.1 Structural Carbohydrate-Lignin Complex

5.2.3.1.1 Cellulose

Wood cellulose provides the main source of carbon for decay, the macromolecular substrate being eventually degraded by cellulolytic enzymes into the readily absorbed, smaller monomeric glucose units. However compared with that in a pure form, cellulose in wood (in a complex with lignin and the hemicelluloses) is more resistant to enzymatic degradation (Cowling, 1958). The enzyme systems of decay fungi are required to penetrate the hemicellulose/lignin sheaths in order to utilize the cellulose, and the ability to do this depends on the size and diffusibility of the enzymes. The accessibility of wood cellulose is further complicated by the degree of crystallinity of the cellulose (Scheffer and Cowling, 1966). Where amorphous arrangements occur the microfibril is accessible to diffusing cellulolytic enzymes; the crystalline region, by virtue of the close packing and bonding of the cellulose molecules, is rendered more resistant to enzymatic attack.

5.2.3.1.2 The Hemicelluloses

These components of wood are readily degraded into monomeric sugar units. It is likely that prior removal of certain hemicellulose components enhances cellulose degradation by promoting a cellulolytic enzyme system [and possibly also a non-enzymatic system (Koenigs, 1972)]. This process is important in the brown-rot type of decay (Kirk and Highley, 1973). In white-rot decay the hemicelluloses are apparently degraded at approximately the same rates as cellulose (Highley and Kirk, 1979).

5.2.3.1.3 Lignin

Lignin degradation by many fungal species, particularly those belonging to the white-rot type, yields carbon dioxide, but it is doubtful if any useful energy or assimilable lignin products are obtained. However these particular fungi carry out this process as a necessary step to enhance the utilization of the cellulose and other polysaccharides of wood. The specific degradative enzymes involved in lignin metabolism are poorly understood although apparently, the polymer is first depolymerized by oxygenases yielding low molecular-weight products which are absorbed by the hyphae and further metabolized. By contrast, brown-rot fungi leave the lignin essentially undigested, although they do modify the polymer (Kirk, 1975). Lignin was unexpectedly shown in a recent study to be degraded to carbon dioxide to an appreciable extent by brown-rot fungi [Haider and Trojanowski, 1979 (cited Highley and Kirk, 1979)].

5.2.3.2 Non-structural Materials

5.2.3.2.1 Available Carbohydrates

Starch and low molecular-weight carbohydrates (sugars) are important in the decay of wood, since these substances are readily available to fungi, and thus constitute the initial simple carbon source for microorganisms invading tissues (Hulme and Shields, 1970). In the presence of these nutrients fungal hyphae spread rapidly through the host substrate prior to any decomposition of wood structure (Wilcox, 1968).

5.2.3.2.2 Minerals

The wood-decay fungi require the same nutrient elements as the tree for their biochemical activities (Kirk, 1973a). At least a third, and perhaps half, of the elements found on the tree are also found in fungus cells (Lilly, 1965), and some have been demonstrated *in vitro* to be essential for growth and enzymatic activity of fungi.

The requirement for minerals has been studied in some detail (see Jennison *et al*, 1955; Cochrane, 1958; Lilly, 1965). The main macro-nutrients required at concentrations of 10^{-4} to 10^{-3} M, are potassium, phosphorus, magnesium, nitrogen, sulphur and calcium. Other elements, e.g. iron, zinc and manganese are needed at much lower levels ($< 10^{-6}$ M). With the possible exception of calcium, the macro-nutrients are generally accepted as being required for the growth of all fungi. Although some fungi cannot be shown to require calcium (Steinberg, 1948), a reduction in growth in the absence of this added element (Steinberg, 1948), or the improvement in growth

when it is added to certain liquid media (Shigo, 1970) has nevertheless been demonstrated.

The iron (or other transition metal) present in wood is probably required by decay fungi as a "precursor" to make the wood accessible to cellulolytic enzymes (Koenigs, 1974a; b). The metallic element is possibly associated with the development of a non-enzymatic system involving hydrogen peroxide, which serves to increase the permeability of wood to the decaying enzymes.

5.2.3.2.3 Nitrogen

Like all other living organisms, wood decay fungi require nitrogen for their growth and metabolism since it is a constituent of extra-cellular and intra-cellular enzymes, nucleic acids, lipoprotein membranes, chitin, and other constituents of fungus cells (Cowling and Merrill, 1966). Studies *in vitro* of 42 wood decay *Basidiomycetes* revealed that none require nitrogen in organic form, although growth is usually greater with organic nitrogen than with ammoniun salts (Jennison *et al*, 1955). Thus, among the three utilizable forms of nitrogen (i.e ammonia, nitrate, and amino forms) growth is most rapid with amino nitrogen. In wood, decay fungi presumably satisfy their nitrogen requirements at least partially by hydrolysis of the proteins, since the proteins with their preformed peptides, may support greater growth than their constituent amino-acids (Cowling and Merrill, 1966). No information is available to suggest that nitrogen in wood in the form of nucleic acids, imides, indoles, alkaloids, and other non-amino nitrogen compounds can be utilized as sources of nitrogen by wood-decay fungi (Cowling and Merrill, 1966).

5.2.3.2.4 Vitamins/Growth Factors

Available nitrogen cannot generally be utilized in the absence of vitamin B_1, thiamine. Hence this vitamin is required by most or all fungi, and in only a few instances can biotin (vitamin H, co-enzyme R) be successfully substituted for thiamine (Jennison *et al*, 1955). Most of the known vitamins function as catalysts or co-enzymes. By definition such organic molecules are required in small amounts and are not normally used as sources of energy or for structural framework of protoplasm (Cochrane, 1958). Other vitamins are apparently required by fewer fungi, if any.

5.2.4 The Role of Chemical Characteristics of Wood in Variations in Decay Susceptibility

5.2.4.1 Influence of Structural Materials

5.2.4.1.1 Cellulose

In general the relatively high crystallinity of cellulose in cell walls of wood contributes to the resistance of timber to biodeterioration (Scheffer and Cowling, 1966; Section 5.2.3.1.1). Thus the decay resistance of wood could be expected to increase from early wood to late wood, and from pith to bark, in parallel with increases in the crystallinity of cellulose (Wellwood *et al*, 1974). However this topic has apparently received little attention.

There is however, some evidence that cellulose crystallinity has a greater influence on resistance to brown-rot than to white-rot deterioration (Cowling, 1961). With the brown-rot

fungus, *Poria placenta* (Fr.) Cke., the amorphous part of the cellulose was partly depolymerized before the crystalline cellulose was affected substantially. By contrast, the white-rot fungus, *Polyporus versicolor* L. ex Fr., degraded both the amorphous and crystalline regions simultaneously.

5.2.4.1.2 The Hemicelluloses

The relationship between the decay resistance of wood and the quantity and/or quality of the hemicelluloses present is largely unknown. It is nevertheless recognized that the hemicelluloses may act as 'inducers' of cellulolytic activity, especially in brown-rot fungi (King, 1968; Highley, 1976; 1977; Section 5.2.3.1.2). The mannans, in particular, appear to promote such an effect, and this may account for the generally greater susceptibility of softwoods to decay by brown-rot than white-rot fungi in nature (Scheffer, 1964).

5.2.4.1.3 Lignin

Cell-wall lignification apparently accounts for a significant proportion of the natural decay resistance of wood (Scheffer and Cowling, 1966). Lignin encrusts the refractory polysaccharides in wood, thus acting physically and/or chemically as a barrier to the enzymatic degradation of the polysaccharides (Pew and Wyna, 1962). Thus Wilcox (1965) made the following microscopical observations of brown-rotted sapwood in sweetgum (*Liquidambar styraciflua* L.) and a southern pine (*Pinus* species):

(a) the order of attack of cell wall layers of sweetgum fibres was S2, S1 and S3, which corresponds with their increasing lignification,

(b) in comparison with sweetgum wood, a more uniform resistance to decay was exhibited across secondary wall layers of *Pinus* tracheids, apparently reflecting the lignin distribution, and

(c) the high lignin content both in the walls of ray parenchyma cells and vessels and in the compound middle lamellae of all cells, was associated with greater decay resistance of these areas to brown-rot fungi (see also Toole, 1972).

There is also reason to speculate a lignin-correlated resistance to decay by white-rot fungi, even though these organisms are capable of utilizing lignin. The ray cell walls in both the sweetgum and pine and the vessel walls in sweetgum, were highly resistant to degradation by *P. versicolor* (Wilcox, 1965).

It is probable that differences in the type and quantity of lignin between softwoods (guaiacyl lignin) and hardwoods (guaiacyl-syringyl lignin) contribute to a generally greater resistance of softwoods to white-rot attack (Peterson and Cowling, 1964; Highley, 1982).

However, the lack of any relationship between lignin content and decay resistance has also been reported [Buckley, 1931 (cited Cartwright and Findlay, 1946)]. Others (e.g. Kirk, 1973b) have refuted the common assumption that lignin 'protects' the polysaccharide in wood from decay, when instead, the cellulose is removed by certain white-rot fungi without a proportional removal of lignin (Kirk, 1973b). In the case of brown-rot decay, degradation of polysaccharides may occur by non-enzymatic means (Koenigs, 1974a; b), whereby substances much smaller than decaying enzymes are able to

penetrate the lignin and degrade polysaccharides. It is even postulated that in some cases the presence of lignin in the complex wood substrate might induce the synthesis of cellulases by brown-rot fungi (Highley, 1975; Nilsson, 1974).

5.2.4.2 Influence of Non-structural Materials

5.2.4.2.1 Starch and Available Carbohydrates

It has long been held that the presence of a readily utilizable carbon source in the parenchyma cells of wood increases its liability to decay (Wilson, 1935; Hulmes and Shields, 1970). These materials are thought to promote rapid hyphal growth before any significant loss in weight of wood has occurred. Rapid utilization of starch during decay of sweetgum sapwood by *Poria placenta* was observed (Wilcox, 1968). However, no such result was obtained with *Polyporus versicolor* and further study is required in this area.

5.2.4.2.2 Nitrogen and Minerals

The role of nitrogen in wood decay has been extensively investigated. Initially it was found that the addition of a suitable nitrogen source to wood increased its susceptibility to decay (Findlay, 1934; Schmitz and Kaufert, 1936). Further studies showed a strong positive correlation between decay susceptibility and natural variation in nitrogen content in *Populus grandidentata* (Merrill and Cowling, 1966a), in which a three-fold difference in nitrogen content among the annual increments was associated with a two-fold difference in block weight losses in a laboratory decay test. The correlations

(*r* values) were 0.95 (for decay by *Gloeophyllum trabeum*) and 0.98 - 1.00 (for *Polyporus versicolor*). Broadly similar results were established using sapwood of *Quercus falcata* Michx. (Levi and Cowling, 1968), Norway Spruce (*Picea* species) and *Pinus sylvestris* (Cowling *et al*, 1969). Reis (1972; 1973) reported that sapwood decay susceptibility within Brazilian species was directly related to their nitrogen content, but only when these species were considered separately.

A wide variety of wood decay fungi are able to adapt the nitrogen content of their mycelium to the low levels of nitrogen in wood. The fungi conserve the meagre supply of nitrogen in wood by the following means:

(a) preferential allocation of available nitrogen to metabolic substances and pathways that are highly efficient in the utilization of wood constituents (Levi and Cowling, 1969),

(b) re-utilization of available nitrogen by autolysis of less active fungus cells, and re-use of nitrogen constituents by more active mycelia (Cowling and Merrill, 1965), and

(c) a possible utilization of nitrogen from sources externally, e.g. nitrogen-fixation from the atmosphere as observed in some wood-inhabiting microorganisms (Seidler *et al*, 1972; Sharp and Millbank, 1973).

Surprisingly little has been reported on the influence of various minerals on decay susceptibility, considering the importance of some elements in fungal nutrition (Section 5.2.3.2.2).

5.2.4.2.3 Vitamins

Thiamine appears to be the main vitamin essential for fungal growth (Section 5.2.3.2.4). Apparently the removal of this factor (de-thiaminization), effectively improves decay resistance in wood (Gjovik and Baechler, 1968; Highley, 1970).

5.3 MATERIALS AND METHODS

5.3.1 Wood Specimens

Only the eight trees of the Monterey provenance (Table 2-1) were used in this study since provenance variation in susceptibility to decay was previously found to be minimal (Chapter 4). The inner and outer sapwood positions were sampled, six blocks being removed form each position in each tree for each fungal treatment. Sample blocks were prepared in the manner previously described (Section 2.1.2.2).

5.3.2 Decay Susceptibility Test

The main modification of the decay procedure was the substitution of polyurethane foam cubes (2.5 cm thick) for soil (Section 2.2.1.1). The foam was considered to be a more standard base material, which would provide only small quantities of nutrients to the decay organisms. Sterilized wood blocks were subjected to attack by *Gloeophyllum trabeum* and *Fomes lividus*, with six blocks (replications) allocated randomly to each treatment combination (radial position x tree x fungal species). The test was supplemented

with 64 'sacrificial' blocks and 7 'control' samples. Blocks were inoculated as detailed in Section 2.2.1.3.2, by placement in Petri dishes bearing the culture growing on low nutrient MEA. The control blocks were planted with culture-free MEA.

5.3.3 Determination of Lignin Content

Two replications (each 0.5 g) of woodmeal [particle size: < 250 μm (60-mesh)] per radial position per tree were assessed for lignin content as described in ASTM D 1106 (Section 2.2.3.1).

5.3.4 Determination of Minerals

Woodmeal was analysed for the minerals sodium, potassium, calcium, magnesium, manganese, iron and zinc by the 'VARIAN TECHTRON' atomic absorption method (Amos *et al*, 1975) (Section 2.2.3.3.2). Facilities were not available for the analysis of sulphur. For each radial position per tree, two replications (each 0.25 g) of woodmeal were used.

5.3.5 Determination of Nitrogen and Phosphorus

For each tree and radial position, duplicate 0.5 gram woodmeal samples were assessed for nitrogen and phosphorus (in ppm) by the 'TECHNICON' Autoanalyser II procedure (Technicon Industrial Systems, 1977) (Section 2.2.3.3.1).

5.3.6 Determination of Available Carbohydrates

A colorimetric anthrone method for assessing total sugars (Yemm and Willis, 1954; Lambert, 1978; Section 2.2.3.2) was used with

duplicate 2.0 gram samples [particle size: < 250 μm (60-mesh)] per radial position per tree. The staining of radial longitudinal sections with iodine/potassium iodide revealed the presence of only negligible amounts of starch. Thus no attempt was made to quantify this factor using the colorimetric iodine procedure (Humphreys and Kelly, 1961; Lambert, 1978).

5.3.7 Determination of Alcohol-benzene Solubility

As described in Section 2.2.4.3, duplicate 0.5 gram woodmeal samples [particle size: < 250 μm (60-mesh)] per radial position per tree were extracted in a soxhlet using an ethanol-benzene mixture and the average percentage weight loss on extraction (unextracted dry weight basis) was determined.

5.4 STATISTICAL ANALYSIS

Variability in percentage weight loss data in blocks decayed by *F. lividus* and *G. trabeum*, was assessed by descriptive statistics (standard error, minimum and maximum values and mean value) computed with subprogram CONDESCRIPTIVE of SPSS (Nie *et al*, 1975).

For the purposes of comparing radial positions, data representing all eight trees were pooled as random observations in analyses of variance (ANOVA). Data for phosphorus, potassium, calcium, magnesium and sodium were transformed (square root or logarithmically) to meet the assumptions of ANOVA (Neter and Wasserman, 1974).

Correlations (r value) between mean values of decay susceptibility and the chemical properties were computed using least squares linear regression on the GENSTAT Statistical Package (Alvey *et al*, 1982). Data for the correlations was organized into three categories: inner sapwood (eight paired observations), outer sapwood (eight paired observations), and two positions combined (sixteen paired observations). In all tests, a two-tailed 0.05 was accepted as the level of significance.

5.5 RESULTS

5.5.1 Variation in Decay Susceptibility and Chemical Characteristics

After an incubation period of 10 weeks the white-rot fungus, *F. lividus*, had generally induced higher weight losses in the outer than inner sapwood samples (Table 5-1). The difference in percentage weight losses (outer wood (OSW): 12.5%; inner wood (ISW): 7.5%) was significant. No consistent radial variation in resistance to decay by *G. trabeum* was detected (Table 5-2). Decay by this organism was erratic, as suggested by the large standard error values. Hence further statistical analysis of data was not attempted.

Among the chemical wood properties listed in Table 5-3, significant increases from the inner to outer sapwood were detected for phosphorus (OSW: 157 ppm; ISW: 64 ppm), potassium (OSW: 1490 ppm; ISW: 488 ppm) and nitrogen (OSW: 1484 ppm; ISW: 897 ppm). The reverse trend was obtained for calcium (OSW: 1967 ppm; ISW: 2982 ppm),

magnesium (OSW: 954 ppm; ISW: 1626 ppm), lignin content (OSW: 22.1%; ISW: 24.0%), and ethanol-benzene solubility (OSW: 0.60%; ISW: 0.86%).

The remaining properties studied did not vary significantly in the radial direction [zinc (OSW: 2.8 ppm; ISW: 3.3 ppm), sodium (OSW: 33 ppm; ISW: 23 ppm), available sugars (OSW: 0.17%; ISW: 0.16%)]. Iron and manganese were not present in detectable quantities.

5.5.2 Correlations between Decay Susceptibility and Chemical Characteristics

In view of the erratic nature of decay by *G. trabeum* (Table 5-2), correlations involving weight losses caused by this species were considered impossible to interpret reliably; thus the regression coefficients are not presented. The overall correlations, i.e. with radial positions combined, for decay with *F. lividus* are provided in Table 5-4. Here nitrogen accounted for a greater proportion (up to 72%) of variation in percentage weight losses (r = 0.85; sig. $P < 0.05$) than did potassium (61%; r = 0.78; sig.) or phosphorus (56%; r = 0.75; sig.). Other factors were not associated significantly with decay by *F. lividus*. In the inner sapwood, significant corelations were obtained only for calcium (88%; r = 0.94), nitrogen (58%; r = 0.76) and magnesium (62%; r = 0.79), while significant correlations were absent in the data for outer sapwood. It is noteworthy however, that for the individual positions, at least 'moderate' correlations ($r > 0.35$) were always obtained for nitrogen, phosphorus, potassium, calcium and magnesium. Neither lignin nor ethanol-benzene solubility was shown to be significantly correlated with decay susceptibility.

Table 5-1: Variation in susceptibility of sapwood blocks to decay by *Fomes lividus* after incubation for 10 weeks in foam-jar decay chamber

	Inner Sapwood			Outer Sapwood		
Tree No.[2]	MWL[1] (s.e.)	Minimum WL	Maximun WL	MWL (s.e.)	Minimum WL	Maximum WL
9	7.0[3] (0.2)	6.2	7.4	11.5 (0.8)	9.7	13.7
10	6.5 (0.9)	4.8	11.1	11.0 (0.9)	7.7	13.4
11	4.9 (1.0)	1.7	8.4	15.1 (1.2)	10.8	19.1
12	10.6 (1.5)	6.7	17.4	12.0 (2.3)	6.3	21.3
13	7.6 (1.2)	2.9	11.4	13.5 (1.0)	10.2	17.1
14	7.5 (0.6)	5.4	9.3	12.5 (2.2)	7.2	22.4
15	13.6 (3.4)	5.0	29.4	13.8 (0.8)	12.4	16.9
16	6.6 (1.4)	1.4	11.4	14.3 (1.1)	11.5	18.1

1) MWL- mean percentage weight loss; s.e.- standard error of the mean; WL- percentage weight loss

2) Numbers correspond to the tree samples described in Table 2-1

3) Values mean of 6 wood blocks for each radial position

Table 5-2: Variation in susceptibility of sapwood blocks to decay by *Gloeophyllum trabeum* after incubation for 10 weeks in foam-jar decay chamber

	Inner Sapwood			Outer Sapwood		
Tree No.[2]	MWL[1] (s.e.)	Minimum WL	Maximum WL	MWL (s.e)	Minimum WL	Maximum WL
9	4.9[3] (3.5)	1.1	22.2	10.1 (6.1)	0.2	36.6
10	35.4 (6.8)	8.9	49.3	26.0 (7.7)	1.0	48.0
11	11.6 (10.1)	1.2	61.9	2.3 (0.3)	0.9	3.0
12	2.1 (0.5)	1.0	4.4	1.5 (0.2)	0.9	2.3
13	30.1 (11.0)	1.9	62.6	41.5 (8.8)	-0.6	57.6
14	17.4 (7.8)	0.2	42.6	39.3 (9.1)	0.5	60.6
15	52.2 (5.2)	36.0	68.7	39.9 (8.4)	0.7	56.9
16	3.0 (1.3)	0.8	9.6	21.4 (9.2)	1.3	47.3

1) MWL- mean percentage weight loss; s.e.- standard error of the mean; WL- percentage weight loss

2) Numbers correspond to the tree samples described in Table 2-1

3) Values mean of 6 wood blocks for each radial position

Table 5-3: Radial variation in decay susceptibility and certain chemical properties in the juvenile sapwood of *Pinus radiata*

Wood Property	Mean Values		Variance
	Inner Sapwood	Outer Sapwood	Ratio
Decay Susceptibility (%g g^{-1})[1]			
Fomes lividus	7.5	12.5	24.1*
Gloeophyllum trabeum	19.6	22.8	n
Ethanol-benzene Solubility (%g g^{-1})[2]	0.86	0.60	7.7*
Available Sugars (%g g^{-1})[3]	0.16	0.17	0.31
Lignin (%g g^{-1})[3]	24.0	22.1	10.5*
Calcium (ppm)[3,4]	2982	1967	25.6*
Magnesium (ppm)[3,4]	1626	954	24.3*
Nitrogen (ppm)[3]	897	1484	48.7*
Phosphorus (ppm)[3,5]	64	157	166.3*
Potassium (ppm)[3,4]	488	1490	99.4*
Sodium (ppm)[3,5]	23	33	0.9
Zinc (ppm)[3]	3.3	2.8	0.7

1) Mean percentage weight loss for each fungus after incubation for 10 weeks in a foam-jar decay test. Each value the mean of 48 observations
2) Mean of 32 observations
3) Mean of 16 observations
4) Analysis performed on square root transformed data
5) Analysis performed on logarithm (base 10) transformed data
*) Variance ratio significant at 0.05 level
n) Assumptions of Analysis of Variance not satisfied

Table 5-4: Correlation coefficients (*r* values) for regressions between susceptibility to decay by *Fomes lividus* and certain chemical characteristics of juvenile sapwood of *Pinus radiata*

Wood Property	Inner[1] Sapwood	Outer[1] Sapwood	All[2] Positions
Ethanol-benzene Solubility	0.13	-0.17	-0.43
Available Sugars	0.18	0.06	0.19
Lignin	0.11	0.12	-0.33
Calcium	0.94*	0.36	-0.17
Magnesium	0.79*	0.35	-0.27
Nitrogen	0.76*	0.69	0.85*
Phosphorus	0.71	0.55	0.75*
Potassium	0.40	0.36	0.78*
Sodium	-0.23	-0.05	0.02
Zinc	0.46	-0.15	0.02

1) All intra-positional correlations based on 8 paired observations
2) Inter-positional correlations based on 16 paired observations
*) Correlation coefficients significant at 0.05 level

5.6 DISCUSSION

5.6.1 Variation in Chemical Characteristics

5.6.1.1 Lignin

The decrease in lignin content from pith to bark, which characterises many softwoods (Panshin and de Zeeuw, 1980), is shown here for *Pinus radiata*. The decrease is similar in magnitude to that recorded in other studies on this species (Harwood, 1971; Uprichard, 1971), it contrasts with the 1.5 to 3% change generally recorded in other softwoods (Section 5.2.2.1). Differences in sample positions, both radially and axially, could partially account for differences in findings (Harwood, 1971; Uprichard, 1971).

5.6.1.2 Minerals and Nitrogen

The literature (Section 5.2.2.3) available on radial gradients in the elements studied here, both for *Pinus* species (Wright and Will, 1958; Orman and Will, 1960; McMillin, 1970; Fossum *et al*, 1972) and other woods (Merrill and Cowling, 1966b; Grozdits and Ifju, 1973; Basham and Cowling, 1976) agrees broadly with the present results. A few exceptions are apparent however, e.g. levels of calcium and magnesium are reportedly similar across the sapwood of *Quercus alba* (Wardell and Hart, 1973), contrasting with the decrease from inner to outer sapwood of *P. radiata* (Table 5-6).

Radial gradients for the elements magnesium, calcium and zinc are generally different from those for nitrogen, phosphorus and potassium. The latter three elements are known to be loosely bound to

plant cell wall material and are highly mobile in phloem (Epstein, 1972) and xylem. Indeed, rapid radial transport of potassium from the xylem to phloem in tree stems has been shown (Stout and Hoagland, 1939). Mobility of these elements is associated with their increased concentration in regions of greatest metabolic activity (Orman and Will, 1960), e.g. in outer sapwood relative to inner sapwood, where parenchyma cells are beginning to lose vitality (Frey-Wyssling and Bosshard, 1959; Nair and Chavan, 1983). If, as suggested by Wardell and Hart (1973), these elements are concentrated in the ray cells, the opportunity for radial translocation would be improved. Mobility of these elements is critical in their recycling and retrieval during heartwood formation, thus providing trees with a survival and competitive advantage (Wardell and Hart, 1973). Sodium is also highly mobile, but its recycling appears to be of lesser importance.

The relative immobility of the bivalent metals is evidenced by the fact that the internal recycling (operating for nitrogen, phosphorus and potassium) did not appear to include these metallic elements. It has been suggested that interactions between cations and cell wall material, and the lack of source-sink pathways through the phloem, could restrict recycling of these elements (Basham and Cowling, 1976). However, increasing concentrations of these elements with tissue age (distance from the cambium) is not easily explained. Possibly it reflects a continuing centripetal diffusion of cations from recently formed xylem elements to the sink provided by anionic sites in cell wall material (Basham and Cowling, 1976). Alternatively, it may be that no re-location of these minerals occurs but that, as the tree ages, lower concentrations of the elements are present in the differentiating xylem.

5.6.1.3 Solubility Characteristics

Estimates of ethanol-benzene solubility have been recorded for a number of woods (Scheffer, 1936; Cowling,1961), but specific chemicals were not characterized. It is known, however, that the non-structural components rendered soluble include waxes, resins, fats and some water-soluble matter (ASTM D 1107). Quite conceivably in pines, oleoresins form the bulk of extractives removed with this and other solvents (Lloyd, 1978; Uprichard and Lloyd, 1980). The study wood material contained numerous vertical resin canals particularly towards the last-formed early wood zone of each annual ring. If the radial gradient in ethanol-benzene solubility in *P. radiata* found in this study does reflect the amount of oleoresins, it conforms with the general decrease in oleoresins (Lloyd, 1978; Uprichard and Lloyd, 1980) and in the numbers of vertical resin canals [Oros, 1971 (cited Bamber and Burley, 1983)] from pith to bark for this species.

In mature stems of *P. radiata* increased resin content of the heartwood is probably due to an enrichment process whereby resins flow inward through the transverse resin canals long after the original heartwood has been formed (Harris, 1965). Whether substantial centripetal movement of resins occurs across normal sapwood is unknown, but the present results (i.e, increased solubility in inner sapwood) offer circumstantial evidence for this.

The absence of any pronounced radial variation in the content of available carbohydrates, from 80% ethanol extracts, is not surprising in view of the low absolute levels of these materials present (< 0.2%, Table 5-3). *P. radiata* has apparently not been studied in this respect, although several sugars have been

identified/isolated in sapwood from *Pinus* species (Brasch and Wise, 1956; Smith and Zavarin, 1960). It is possible that the levels of sugar in *P. radiata* would have been different if sampling had occurred at a different time of the year.

5.6.1.4 Practical Implications

The studies on variations in the chemical composition of *P.radiata* are particularly relevant in the chemical pulping of wood. Various chemical properties of the raw material substantially affect pulping processes and paper-making, in addition to the more obvious physical properties of wood. The lignin (and holocellulose) content of pine wood will influence the rate of delignification and pulp yield. The extractives content is crucial since the acidic or phenolic fractions consume alkali (and/or other chemicals) needed for the delignification process, while the quantity and nature of wood extractives will be reflected in the yields of by-products such as turpentine, tall oil and ethanol. The mineral content of wood, affects such processes as pulp-bleaching (McMillin, 1970).

The present results thus supplement the pool of existing knowledge used to develop and/or refine aspects of pulping and paper-making processes using *P. radiata*, and to select raw material suited to various paper uses. Detailed discussion of the findings in this respect, and in relation to other wood properties, e.g. resistance to weathering, paintability and capacity for preservative treatment, are beyond the scope of this study.

5.6.2 Variation in Decay Susceptibility

The finding in this chapter that susceptibility to decay by *F. lividus* increases from the inner to outer sapwood conforms with the results of Chapter 4. Possible reasons for this trend are considered in the following section. Absolute values of decay are somewhat greater in this work [average weight losses usually > 6% (Table 5-1) *cf.* < 4% in the previous Chapter (Table 4-1)], perhaps reflecting the longer period of decay (10 *cf.* 6 weeks) and/or the differing conditions of incubation (foam-jar *cf.* soil-jar). It is not known why *G. trabeum* behaved so erratically here, but some evidence was obtained to suggest that in the foam-jar test many of the blocks became too wet for effective decay by *G. trabeum* (moisture contents > 300%). White-rot organisms have a greater capacity to cope with this situation in that they are able to regulate (to some extent) moisture levels in their wood substrate (Shields, 1967; Peterson and Cowling, 1973).

5.6.3 The Role of Chemical Characteristics in Decay Susceptibility

5.6.3.1 The Non-significant Correlations

It has long been suggested that lignification enhances the natural decay resistance of wood by providing a barrier to enzymatic degradation of polysaccharides (Scheffer and Cowling, 1966; Section 5.2.4.1.3). However, the main correlation between decay susceptibility and lignin content (this study), though negative, was not significant (r = -0.33; not sig.). The weakness of this correlation may, in part, be attributed to one or both of the

following possibilities:

(a) an insufficient sample size (paired observations), and/or

(b) an inability of the lignification process to confer resistance to decay by certain white-rot fungi.

Although *F. lividus* is capable of attacking the lignin-polysaccharide complex in the wood, it remains uncertain whether lignin is removed preferentially, simultaneously, or later in the decay of wood substrate. Perhaps *F. lividus* removes the components simultaneously whereas another white-rot species, *Polyporus versicolor* (Cowling, 1961), removes the polysaccharides preferentially and this residual lignin reduces the rate of deterioration. In any case, better correlations could be expected with brown-rot organisms.

It was postulated (Section 5.6.1.3), that resinous compounds, extractable in selected organic solvents, comprise the bulk of extractives in *P. radiata*. These compounds, when formed as a reaction to wounding and fungal invasion, can inhibit the development of fungal growth (Schuck, 1982; Shrimpton and Whitney, 1979). In the study material ethanol-benzene solubility was found to be negatively correlated with decay susceptibility; however, the association is not significant (r = -0.43; not sig.), perhaps due in part to the small sample sizes.

Levels of each of sodium, zinc and available sugars were relatively constant radially, and therefore it was to be expected that these factors would correlate poorly with decay susceptibility (Table 5-4).

5.6.3.2 The Significant Correlations

This study is apparently the first report of correlations between decay susceptibility and the macro-nutrients other than nitrogen (Table 5-4). The various positive correlations support the view (e.g. Cochrane, 1958) that these elements are indeed essential to fungal growth and metabolism. Many of the moderate correlations which are not significant (particularly those for nitrogen, phosphorus and potassium) might be improved if replication was increased. The significant associations between decay by *F. lividus* and levels of calcium and magnesium for the inner sapwood position must, however, be questioned as possibly being non-causal, since, when data for the two positions is combined, no such significant correlations are evident (Table 5-4).

5.6.3.3 Potential Role of Other Chemical Properties

Other wood characteristics not examined in this study (e.g. variations in properties of wood cellulose such as crystallinity, the hemicellulosic components and vitamins/growth factors) could govern appreciably variations in decay susceptibility in the non-durable wood. Some literature concerning the influence of these factors was reviewed earlier (Section 5.2.4).

5.7 CONCLUSIONS

Using eight juvenile trees of the Monterey provenance of *P. radiata*, determinations were made of gross radial variations in various chemical characteristics and decay susceptibility of sapwood.

The following conclusions are drawn:

1. the foam-jar decay test successfully demonstrated a distinct radial variation (a centripetal decrease) in susceptibility to decay by *F. lividus* (white-rot) but was not suitable for assessment of decay susceptibility in the case of *G. trabeum* (brown-rot),
2. contents of nitrogen, phosphorus and potassium were greater ($P < 0.05$) in outer than inner sapwood. The trends for the contents of calcium, magnesium, ethanol-benzene solubility and lignin were the reverse. Levels of sodium, zinc and available sugars varied little radially. Manganese and iron were not present in the sapwood in detectable quantities,
3. correlations between decay susceptibility and each of nitrogen, phosphorus and potassium (and perhaps also calcium and magnesium) suggest a stimulatory influence of these elements on the decay susceptibility, and
4. there is still considerable need for further research on the relative contributions of these properties and others (e.g. the quality of the cellulosic and hemicellulosic components) to the natural decay resistance or susceptibility of sapwood of *P. radiata*.

The findings, taken in conjunction with the results in Chapter 4, suggest that the supply of fungal nutrients (other than carbon) is probably a critical factor determining variations in the decay susceptibility of the sapwood of *P. radiata*.

CHAPTER 6

QUANTITATIVE CHANGES IN CHEMICAL CHARACTERISTICS OF THE SAPWOOD OF *PINUS RADIATA* DURING DECAY BY WHITE-ROT AND BROWN-ROT FUNGI

6.1 INTRODUCTION

Few investigations have been made of the relative rates of change in the chemical properties of wood during decay (Kirk, 1973b). This is true for both softwood and hardwood species. Those studies which have been made have generally concentrated on the structural components (cellulose, hemicelluloses and lignin) [e.g. *Betula verucosa* Ehrh., *Pinus sylvestris* L. and *Picea abies* (L.) Karst. (Ander and Eriksson, 1975); *Liquidambar styraciflua* L. (Scheffer, 1936; Cowling, 1961); *Picea glauca* var. *albertiana* (S. Brown) Sarg., *Picea sitchensis* (Bong.) Carr., *Pinus monticola* Dougl., *Pinus taeda* L. and *Tsuga heterophylla* (Raf.) Sarg. (Kirk and Highley, 1973); and *Betula alleghaniensis* Britton (Kirk, 1973b).

Surprisingly the rates of depletion of nitrogen and various minerals in decaying wood have received little attention despite the importance of these factors in growth of fungi (Sections 5.2.3.2.2; 5.2.3.2.3) and their likely role in the decay of wood (Sections

5.2.4.2.2 and 5.6.3.2)

In Chapter 5, a study of the influence of selected chemical characteristics (lignin, nitrogen, minerals, sugars and total extractives) on decay susceptibility was reported. the present study examines the progressive effects of a white- and a brown-rot organism on such chemical constituents of the sapwood of *Pinus radiata* in the hope of achieving the following objectives:

(a) to further investigate the effects of lignin, nitrogen, phosphorus and potassium on the susceptibility of the timber to decay, and

(b) to evaluate solubilization of wood during decay in order to better understand the influence of the cell wall polysaccharides on the decay process.

Achievement of these goals would help determine the causes of variations in decay susceptibility of *Pinus radiata*.

6.2 LITERATURE REVIEW

6.2.1 Changes in Solubility Characteristics in Wood During Decay

6.2.1.1 Determinations of Solubility of Decayed Wood

Solubility in wood is a measure of the amount of material which is soluble in a particular solvent. Solubility of decayed wood can thus provide an index of the extent of degradation in wood. This is particularly true for solubility in dilute alkali (1% sodium

hydroxide) which increases substantially in brown-rotted wood, whereas that in white-rotted timber is only slightly greater than in sound wood (Campbell, 1952; Cowling, 1961).

The extracting solvents commomly employed in wood analyses are partially selective for particular degradation products. The common solvents and the general components of wood which are removed are as follows:

(a) **dilute alkali** - carbohydrate (ASTM D 1109) and to a lesser extent, lignin degradation products (Cowling, 1961) as well as the materials removed in b, c and d,

(b) **cold water** - soluble sugars, free minerals, free acids, certain phenolic materials and cytoplasmic contents (ASTM D 1110) and some lignin degration products (Cowling, 1961),

(c) **hot water** - materials solubles in cold water, wood starch (ASTM D 1110) and lignin degradation products (Cowling, 1961), and

(d) **Ethanol-benzene** - resins and other extractives such as phenols, waxes, fats, etc. (ASTM D 1107).

Additionally, methyl cellosolve (Cowling, 1960) and dioxane-water mixtures (Kirk, 1975) are solvents selective for lignin degradation products.

6.2.1.2 Interpreting Trends in Solubility

Changes in solubility of wood during decay are best visualized by graphical representation of the data. The time-course in solubility can then be described in one of three ways (Cowling, 1961):

(a) an ascending line would indicate a more rapid extra-cellular depolymerization of the insoluble material than metabolism of these products by intra-cellular respiration by the decay fungi,

(b) a descending line indicates the reverse of the two processes in (a), and

(c) a horizontal line depicts a stage of equilibrium being maintained between the two processes, i.e. both the depolymerization and the respiratory reactions occur at similar rates.

6.2.1.3 A Case Review- Sapwood of *Liquidambar styraciflua* Decayed by White-Rot and Brown-Rot Fungi

6.2.1.3.1 Preliminary Considerations

Probably the only intensive study of changes in solubility characteristics in decayed wood relevant to this study, is that on sapwood of the hardwood *Liquidambar styraciflua* by Cowling (1961). A selective review of Cowling's results (Figure 6-1) will therefore provide a basis for comparison with the present study.

6.2.1.3.2 Progressive Effects of *Polyporus versicolor* (White-Rot Organism) on Solubility

Solubility in dilute alkali declined gradually as decay progressed, i.e. the rate of intra-cellular respiration of the materials soluble in dilute alkali exceeded the rate of their production by depolymerization of the wood substance (Figure 6-1A). Solubility in the other solvents (hot and cold water, and ethanol-benzene) varied little as decay progressed.

6.2.1.3.3 Progressive Effects of *Poria placenta (= Poria monticola)* (Brown-Rot Organism) on Solubility

In this case, the alkali solubility increased rapidly during the deterioration process up to *ca.* 20 - 25% weight loss, after which, a slight decrease occurred (Figure 6-1A). The quantity of materials soluble in hot and cold water, and ethanol-benzene increased gradually up to 32% weight loss.

6.2.1.3.4 Differences in Dissolving Capacity of Solvents

Dilute alkali had the greatest capacity to solubilize wood in all stages of either type of decay, while solubility in hot water was greater than in cold water. The amount of material dissolved in ethanol-benzene was generally equal to, or slightly greater than that removed by cold water.

6.2.1.3.5 Sugars in Water Extracts

The sugars were characterized as "potential" and "available" (apparent) reducing substances as determined, respectively, with or without treatment of hot water soluble extracts in 3% (w/v) sulphuric acid. As a broad generalization, the levels of potential and available reducing sugars decreased gradually during decay by the white-rot fungus up tp 35% weight loss (Figure 6-1B). In brown-rotted wood, amounts of potential reducing sugars increased rapidly with the decay (Figure 6-1B_1). The increase for available sugars was more gradual. In both types of decay, potential reducing substances constituted the bulk of the hot water extracts.

6.2.1.3.6 pH of Water Extracts

Progressive changes in pH of the water extracts as shown in Figure 6-1C (*Polyporus versicolor*) and Figure 6-1C_1 (*Poria placenta*), revealed higher values for cold than hot water extracts at all stages of decay. The drop in pH of the brown-rot extracts was substantial (> 1.5 units) and occured over the weight loss range of 0 - 20%. In contrast, the pH of extracts from the white-rotted wood showed an initial drop and a subsequent increase. Such a trend was probably due to a rapid extra-cellular production of relatively non-volatile organic acids that were subsequently metabolized (Cowling, 1961).

6.2.2 Changes in Chemical Composition of Wood During Decay

6.2.2.1 Lignin

The work of Cowling (1961) (see Figure 6-2) and others (Seifert, 1968; Kirk and Highley, 1973) showed that lignin is either consumed in small amounts or remaining fairly constant during decay by brown-rot fungi. White-rot organisms utilize lignin at an essentially constant rate during decay.

The slight removal of lignin by brown-rot fungi [e.g *ca.* 2% of the lignin present in sound wood (Cowling, 1961)] has been ascribed to removal of methoxyl groups (Bray and Andrews, 1924; Campbell, 1952). White-rot fungi apparently are not only able to degrade the lignin in sound wood, but have the capacity to metabolize most of the lignin degradation products (Cowling, 1961).

FIGURE 6-1: Solubility properties of the sapwood of *Liquidambar styraciflua* in progressive stages of decay by a white-rot (*Polypurus versicolor*) and brown-rot (*Poria placenta*) fungus

LEGEND: SOLUBILITY

- ■ 1% SODIUM HYDROXIDE
- ◆ HOT WATER
- ◇ COLD WATER
- □ ETHANOL-BENZENE

LEGEND: REDUCING SUGARS

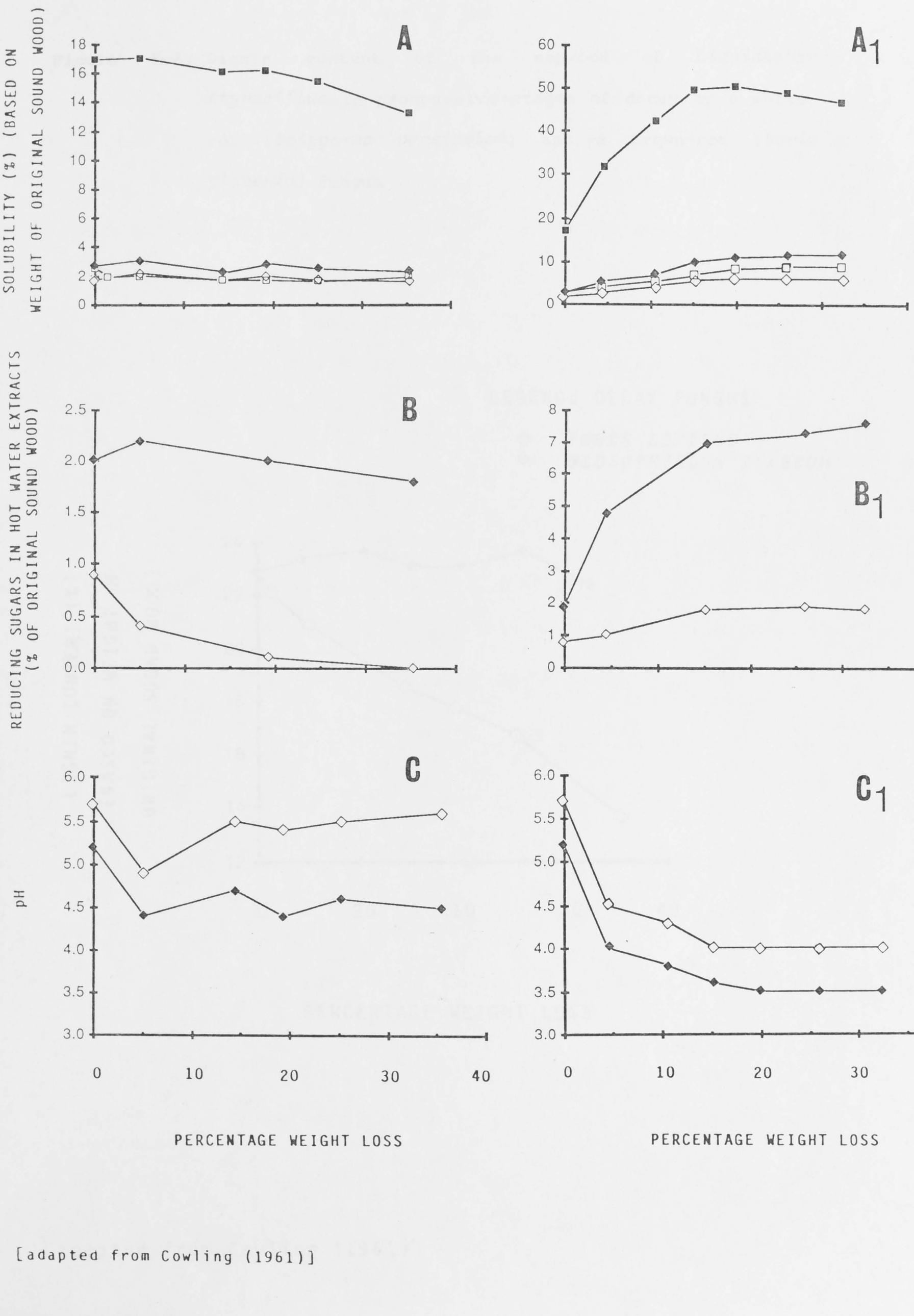
- ◆ POTENTIAL REDUCING SUGARS
- ◇ AVAILABLE REDUCING SUGARS

LEGEND: pH

- ◇ COLD WATER
- ◆ HOT WATER

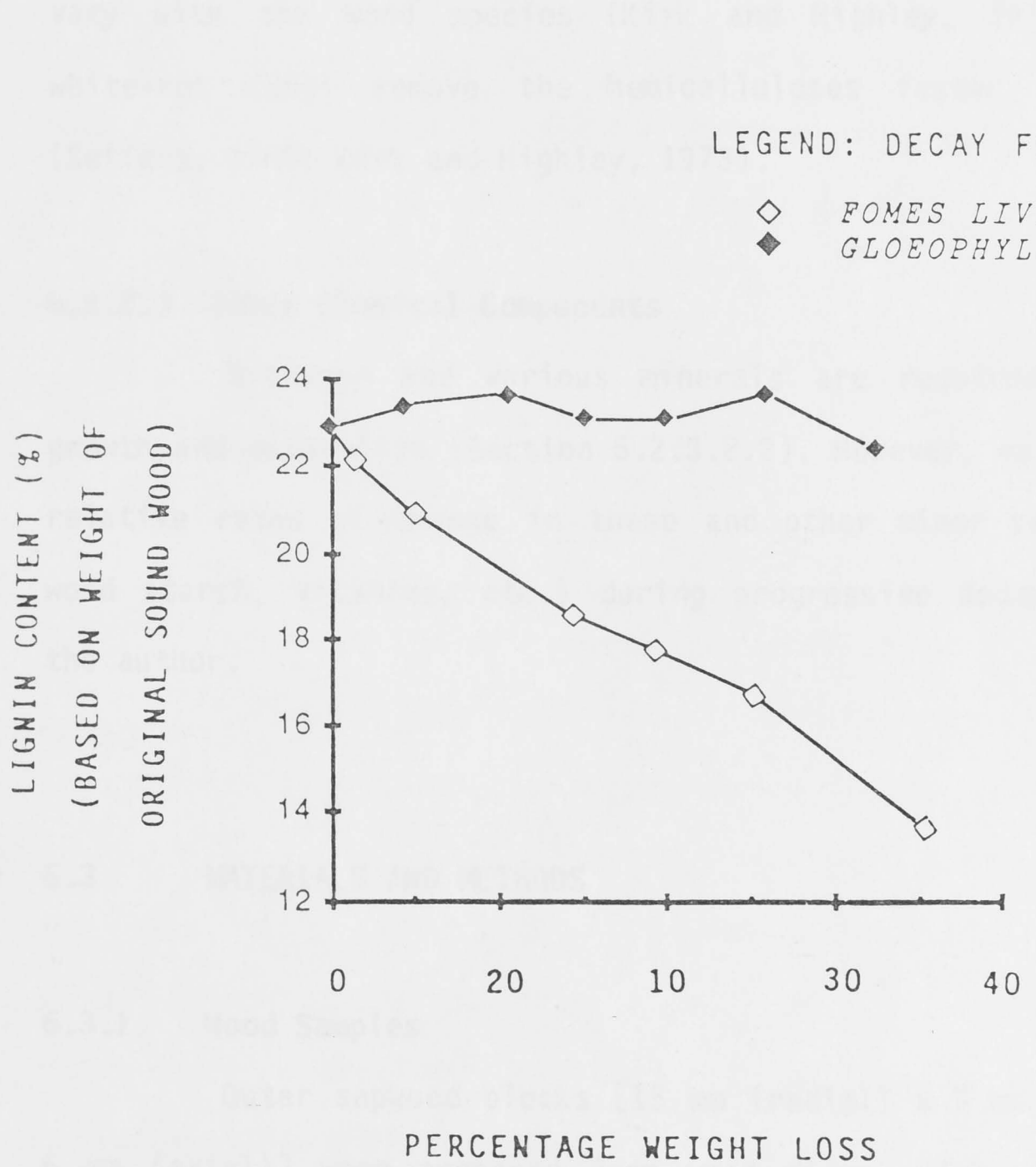
POLYPORUS VERSICOLOR

PORIA PLACENTA
(= PORIA MONTICOLA)



[adapted from Cowling (1961)]

Figure 6-2: Lignin content of the sapwood of *Liquidambar styraciflua* in progressive stages of decay by a white-rot (*Polyporus versicolor*) and a brown-rot (*Poria placenta*) fungus



[adapted from Cowling (1961)]

6.2.2.2 Polysaccharides

Loss in weight during decay by brown-rot fungi reflects, almost entirely, the loss of polysaccharides (Cowling, 1961; Kirk and Highley, 1973). It appears that the various components of the polysaccharides are not necessarily removed at the same rate by the brown-rot organisms (Kirk and Highley, 1973). For example, in certain softwoods decayed by *Poria placenta*, mannan is removed more quickly than cellulose, while the extent of removal of xylan would seem to vary with the wood species (Kirk and Highley, 1973). Generally, white-rot fungi remove the hemicelluloses faster than cellulose (Seifert, 1968; Kirk and Highley, 1973).

6.2.2.3 Other Chemical Components

Nitrogen and various minerals are required by fungi for growth and metabolism (Section 5.2.3.2.2). However, no studies of the relative rates of change in these and other minor components (e.g. wood starch, vitamins, etc.) during progressive decay are known to the author.

6.3 MATERIALS AND METHODS

6.3.1 Wood Samples

Outer sapwood blocks [15 mm (radial) x 5 mm (tangential) x 6 mm (axial)] were prepared from wood discs which were removed at breast height from the two largest diameter trees made available for the study [Tree 13, dbhub 35.8 cm; Tree 16, 33.2 cm (Table 2-1)].

6.3.2 Progressive Decay

Blocks were selectively decayed to progressively increasing weight losses using the foam-jar decay assembly (Sections 2.2.1.3; 5.3.2). One white-rot (*Fomes lividus*) and one brown-rot (*Gloeophyllum trabeum*) organism were used to inoculate wood blocks from Tree 13 and Tree 16 respectively. For each test organism, six blocks were randomly allocated to each of 140 jars (840 test blocks altogether). In addition, 100 'sacrificial' and 7 'control' samples were subjected to deterioration. Blocks were selectively retrieved at various periods of the decay between 2 and 24 weeks. Blocks were subsequently ranked in order of increasing percentage weight losses irrespective of the time taken to achieve the required weight losses. They were then grouped by arbitrary partitioning, such as to provide at least 15 grams of material in each group (weight loss class) for chemical analyses. Seven groups were obtained for *G. trabeum* and eight for *F. lividus*.

6.3.3 Determination of Chemical Characteristics

For each category of decay, the following properties were determined:- lignin content; solubilities in hot water, cold water, ethanol-benzene and 1% sodium hydroxide; total sugars in water extracts; pH of water extracts; and concentrations of nitrogen, phosphorus and potassium. Other minerals were not assessed in view of their poor correlation with decay obtained in Chapter 5. Duplicate measurements were always obtained. All methods have been described in detail in Chapter 2.

The analysis of sugars was performed on hot and cold water

extract solutions rather than on 80% ethanol extracts as used in the general procedure of Lambert (1978) (Section 2.2.3.2). It was noticed during the determination of water solubility that with increasing extents of decay above 32% weight loss (particularly in brown-rotted samples), that a greater proportion of woodmeal particles tended to float on the surface of the water instead of sinking in the solution. This problem was not apparent with the less decayed samples. Therefore to prevent this problem arising in the present study, a 0.5% (w/v) solution of a wetting agent as suggested in a previous study (Cowling, 1960), polyoxyethylene sorbitan monolaurate (Tween 20) (Sigma Chemical Co.) in this study, was used to determine solubility at all stages of decay.

6.4 RESULTS

6.4.1 Variation in Progressive Decay

The average percentage weight losses, which defined each increasing stage of decay by *Fomes lividus* and *Gloeophyllum trabeum*, and corresponding to the variabilities in weight losses (i.e. standard error and standard deviation of the average value, and range) are presented in Table 6-1. The arbitrary grouping of test blocks to provide at least 15 grams of material in each weight loss class for chemical analyses (Section 6.3.2) was a necessary compromise with the need, also, to ensure that blocks providing approximately comparable weight losses were grouped into each weight loss class. Therefore, inconsistencies in variations between each

weight loss class (Table 6-1) were unavoidable. The average weight losses for *F. lividus* were 0, 2.7, 6.5, 10.6, 14.2, 18.4, 23.0 and 30.5%, and for *G. trabeum* 0, 2.0, 2.9, 4.8, 8.9, 16.2 and 38.2%. As could be expected, variability is greatest for the upper weight loss classes.

6.4.2 Changes in Solubility

To permit a more ready visualization of the changes in solubility, the results are represented on the basis of the oven dry weight of the original sound wood (Figure 6-3A, A_1).

Clearly, dilute alkali removed the greatest amount of wood materials in all stages of both types of decay [solubility range: 10 - 15% (white-rot, Tree 13); 6 - 26% (brown-rot, Tree 16)]. This was followed by hot water [range: 4 - 10% (white-rot); 3 - 9% (brown-rot)] while the cold water [range: 1.9 - 4.4% (white-rot); 1.6 - 4.6% (brown-rot)] and ethanol-benzene [range: 1.6 - 5.0% (white-rot); 1.9 - 9.0% (brown-rot)] differed little.

Solubility of white-rotted wood in both dilute alkali and hot water peaked weakly at 18% weight loss (Figure 6-3A). This was in marked contrast to the brown-rotted material, in which solubility in dilute alkali increased rapidly in the initial stage of deterioration and then more gradually later in the decay process (Figure 6-3A_1). Hot water solubility of brown-rotted material peaked at 9 - 16% weight loss.

For both types of deterioration, the amount of material extractable in cold water and ethanol-benzene varied relatively little as weight losses increased (Figure 6-3A, A_1).

Table 6-1: Variability in average percentage weight losses for the sapwood samples of *Pinus radiata* in the various weight loss classes

Type of Decay and *Causal* Organism	Variability in Percentage Weight Loss			
	Percent	Standard error	Standard deviation	Range
White rot caused by	0.0	-	-	-
Fomes lividus	2.7	0.130	0.946	3.40
	6.5	0.147	1.157	3.90
	10.6	0.127	1.109	3.90
	14.2	0.179	1.540	4.50
	18.4	0.145	1.164	3.90
	23.0	0.183	1.476	4.80
	30.5	0.540	4.552	5.70
Brown rot caused by	0.0	-	-	-
Gloeophyllum trabeum	2.0	0.037	0.321	1.04
	2.9	0.039	0.293	0.98
	4.8	0.112	0.933	2.90
	8.9	0.184	1.422	4.87
	16.2	0.340	2.888	5.20
	38.2	0.905	11.558	4.90

Figure 6-3: Solubility properties of the sapwood of *Pinus radiata* in progressive stages of decay by a white-rot (*Fomes lividus*) and brown-rot (*Gloeophyllum trabeum*) fungus

LEGEND: SOLUBILITY

- ■ 1% SODIUM HYDROXIDE
- ◆ HOT WATER
- ◇ COLD WATER
- □ ETHANOL-BENZENE

LEGEND: TOTAL SUGARS IN WATER EXTRACTS

- ◆ SUGARS SOLUBLE IN HOT WATER
- ◇ SUGARS SOLUBLE IN COLD WATER

LEGEND: pH

- ◇ COLD WATER
- ◆ HOT WATER

POMES LIVIDUS

GLOEOPHYLLUM TRABEUM

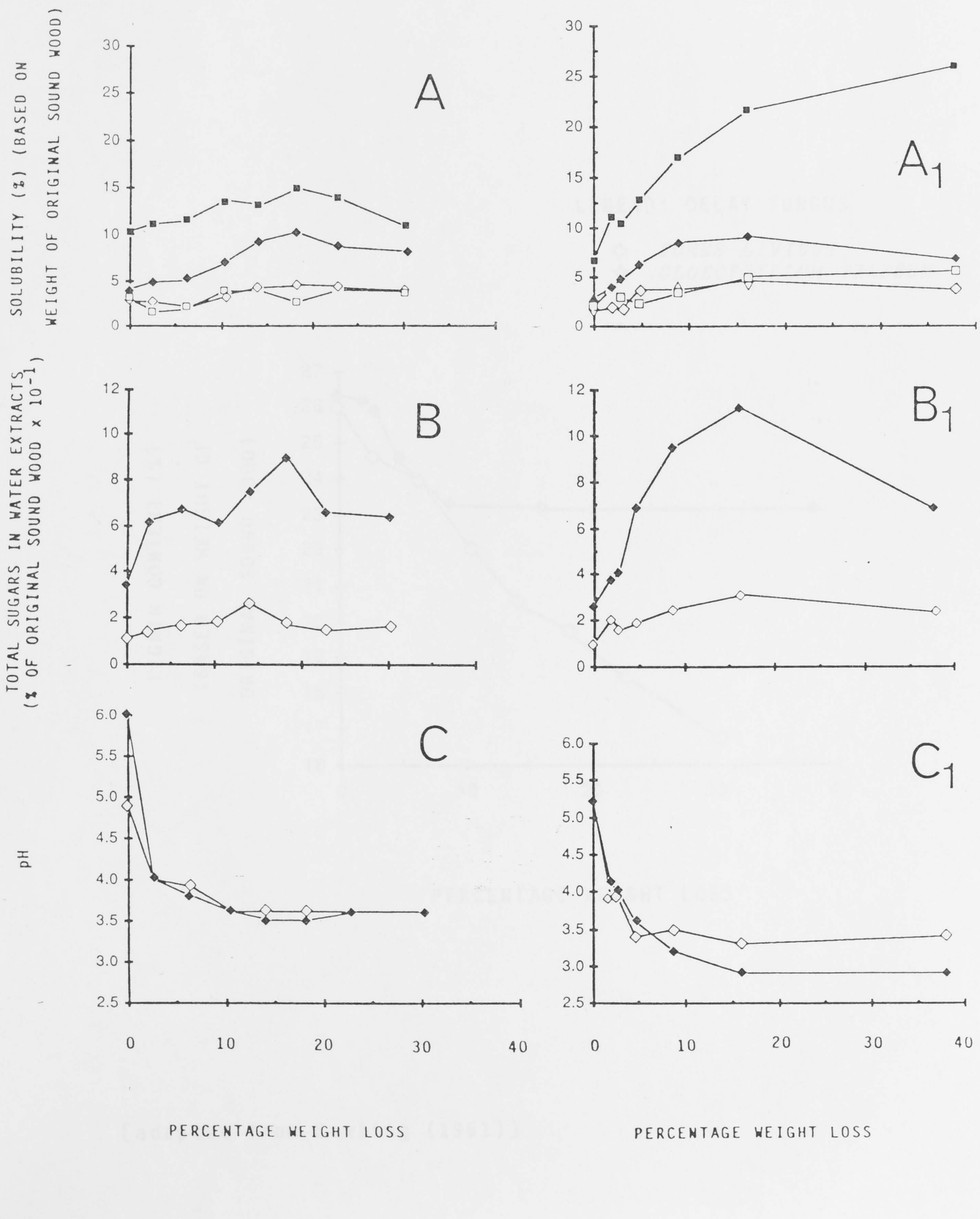
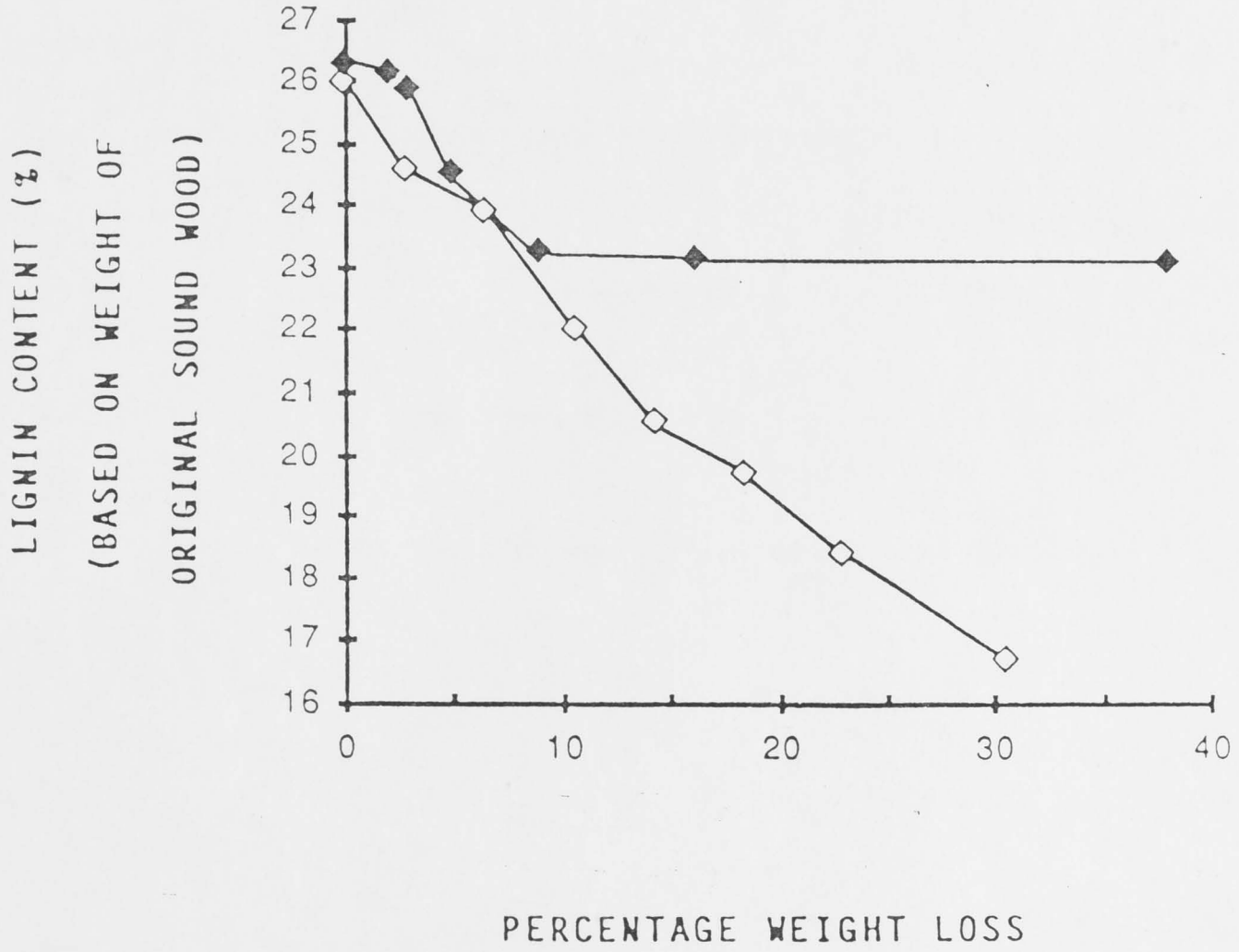
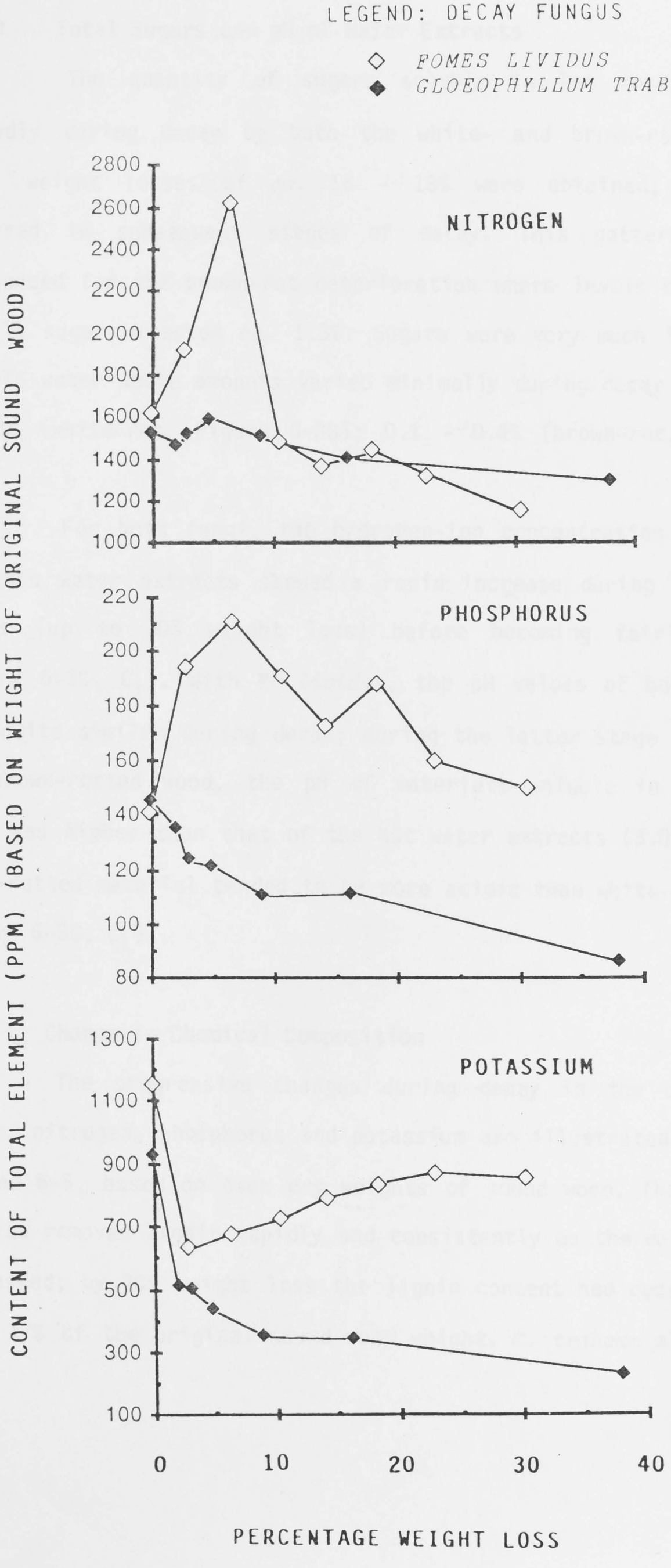


Figure 6-4: Lignin content of the sapwood of *Pinus radiata* in progressive stages of decay by a white-rot (*Fomes lividus*) and brown-rot (*Gloeophyllum trabeum*) fungus



[adapted from Cowling (1961)]

Figure 6-5: Contents of nitrogen, phosphorus and potassium of the sapwood of *Pinus radiata* in progressive stages of decay by a white-rot (*Fomes lividus*) and brown-rot (*Gloeophyllum trabeum*) fungus


LEGEND: DECAY FUNGUS
◇ FOMES LIVIDUS
◆ GLOEOPHYLLUM TRABEUM
NITROGEN
PHOSPHORUS
POTASSIUM
CONTENT OF TOTAL ELEMENT (PPM) (BASED ON WEIGHT OF ORIGINAL SOUND WOOD)
PERCENTAGE WEIGHT LOSS
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
220
200
180
160
140
120
100
80
1300
1100
900
700
500
300
100
0
10
20
30
40

6.4.3 Total Sugars and pH of Water Extracts

The quantity of sugars soluble in hot water increased markedly during decay by both the white- and brown-rot organisms until weight losses of *ca.* 16 - 18% were obtained; a decrease occurred in subsequent stages of decay. This pattern was most pronounced for the brown-rot deterioration where levels of hot water soluble sugars reached *ca.* 1.3%. Sugars were very much less soluble in cold water where amounts varied minimally during decay [range: 0.1 - 0.3% (white-rot, Figure 6-3B); 0.1 - 0.4% (brown-rot, Figure 6-$3B_1$)].

For both fungi, the hydrogen-ion concentration in the hot and cold water extracts showed a rapid increase during the initial stages (up to 10% weight loss) before becoming fairly constant (Figure 6-3C, C_1). With *F. lividus*, the pH values of both extracts were quite similar during decay; during the latter stage of decay in the brown-rotted wood, the pH of materials soluble in cold water (3.5) was higher than that of the hot water extracts (3.0). Overall, brown-rotted material tended to be more acidic than white-rotted wood (Figure 6-3C, C_1).

6.4.4 Change in Chemical Composition

The progressive changes during decay in the contents of lignin, nitrogen, phosphorus and potassium are illustrated in Figures 6-4 and 6-5, based on oven dry weights of sound wood. The white-rot organism removed lignin rapidly and consistently as the deterioration progressed; by 30% weight loss the lignin content had decreased from 26 to 17% of the original sound wood weight. *G. trabeum* also removed

lignin early in the decay process; the lignin content decreased by *ca.* 3% at 10% weight loss. However, further losses in lignin were minimal.

For the white-rotted wood, the content of nitrogen increased sharply from *ca.* 1600 to *ca.* 2800 ppm at 7% weight loss, before declining to values less than 1800 ppm in the later stages of decay (Figure 6-5A). In contrast, decay by *G. trabeum* had little effect on nitrogen levels. Phosphorus was similarly dependent on the test fungus; thus, during decay by *F. lividus*, levels of phosphorus increased rapidly before gradually declining while *G. trabeum* induced only a lowering in amounts of phosphorus. Early in the decay process, both fungi lowered the potassium content of the samples. In the case of *G. trabeum*, the decline continued, while a weak reversal in the trend was evident for *F. lividus*.

6.5 DISCUSSION

6.5.1 Change in Solubility Characteristics

Comparisons between the classical study by Cowling (1961) (Figure 6-1) and the present results (Figure 6-3) on the progressive changes in solubility during decay reveal some general similarities. Overall, the observed trends might well be, in part, specific for a particular association between a host wood and decay fungus. Nevertheless, broad distinctions can be made between the white- and brown-rot types of action on the wood substrate. A well known example is the greater solubility of brown- than white-rotted wood in dilute

alkali (Campbell, 1952; Cowling, 1961; Figure 6-3A, A_1).

In studying deterioration of *Liquidambar styraciflua*, Cowling (1961) suggested that some aspect of depolymerization reactions of *Polyporus versicolor* could be rate-limiting, wheareas it was the respiration process which determined weight losses in the case of *Poria placenta*. The present results (Figure 6-3A, A_1) generally support these conclusions. Thus with *G. trabeum* (brown-rot), the polysaccharides are apparently depolymerized faster than the products, including simple sugars, can be utilized. It is possible, that the essentially constant solubility of both white- and brown-rotted sapwood in ethanol-benzene and cold water (Figure 6-1A, A_1; 6-3A, A_1) indicates that the original materials in sound wood (e.g. resins and certain low molecular-weight sugars) remain unchanged during decay. However Cowling (1961) has suggested that it is more likely that the two relevant processes (depolymerization and metabolism) were in equilibrium during decay.

6.5.2 Change in Sugars

It is evident that sugars comprised only a small portion of materials extractable in both hot [amount present in extract: 8 - 11% (white-rot); 9 - 15% (brown-rot)] and cold [4 - 7% (white-rot); 6 - 8% (brown-rot)] water, although relatively greater amounts were present in hot water. It thus appears that the bulk of the soluble materials in water extracts of both types of rotted wood are the partially degraded polysaccharides, and some degraded lignin products may also have been present, particularly in hot water extracts of brown-rotted samples (Cowling, 1961).

Although methods of sugar analysis differed between this study and that of Cowling (1961), it is apparent (Figures 6-1 and 6-3) that the production of sugars varies appreciably for the two decay systems (*Liquidambar styraciflua* decayed by *Polyporus versicolor* and *Poria placenta*; *Pinus radiata* decayed by *Fomes lividus* and *Gloeophyllum trabeum*). Such variability is to be expected. It is noteworthy that the general difference between the white- and brown-rot effects is consistent.

6.5.3 Change in pH

The sharp increase in acidity of pine sapwood early in the process of decay by both decay organisms (Figure 6-3C, C_1) was noted previously only in brown-rotted sapwood (Figure 6-1C_1; Cowling, 1961). In the case of the decay by *P. placenta* (and perhaps also *G. trabeum*), lignin is modified causing a rapid release of certain acidic lignin derivatives. Perhaps *F. lividus* has a similar effect on pine sapwood, i.e. some lag exists in the utilization of acidic lignin breakdown products. Alternatively, the drop in pH in both the white- and brown-rotted pine sapwood (Figure 6-3C) may be explained by the release of acidic (probably acetyl) fractions bonded to the cellulose (Reese, 1956). The fact that both the hot and cold water extracts showed this same dip in pH indicates that some, if not all, of the soluble acids produced were relatively non-volatile.

6.5.4 Change in Chemical Composition

6.5.4.1 Lignin (and Structural Carbohydrates)

The progressive depletion of lignin by white-rot fungi may

be nearly linear (Figures 6-2 and 6-4) or vary with the extent of decay (Kirk and Highley, 1973). The latter was observed when the sapwood of *Pinus monticola* was decayed by *Polyporus versicolor* (Kirk and Highley, 1973).

The patterns of removal of lignin and structural polysaccharides from a given wood is apparently quite similar for various white-rot fungi, whereas variable effects are observed when the same fungus is tested on different woods (Kirk and Highley, 1973). Such variability is particularly evident between hardwoods and softwoods (Richards, 1962; Kirk and Moore, 1972). With brown-rot fungi, the lignin of hardwoods is probably more susceptible to "decay" than is that of softwoods (Richards, 1962; Highley and Murmanis, 1985), while the patterns of removal of cellulose and certain hemicelluloses are apparently similar irrespective of the woods and fungi involved (Kirk and Highley, 1973). The greater removal of lignin from *P. radiata* by *G. trabeum* than from *L. styraciflua* by *P. placenta* fails to support this generalization, and is in fact, more in agreement with the commonly observed tendency for brown-rot fungi to attack softwood in preference to hardwood timbers (Peterson and Cowling, 1964; Scheffer, 1964).

By contrast to the rapid early decline in the lignin content of *P. radiata* subjected to decay by *G. trabeum* (Figure 6-4), Kirk and Highley (1973) observed a much more gradual decrease in lignin in another softwood-brown rot combination (*Picea sitchensis-Poria placenta*). Differences of this type are probably attributable to varying rates of removal of the methoxyl group of lignin (Bray and Andrews, 1924; Campbell, 1952).

6.5.4.2 Chemical Elements

This component of the study was included in an attempt to learn more of the importance of nitrogen, phosphorus and potassium as sources of fungal nutrients during decay of sapwood of *Pinus radiata*, i.e. it was hoped that the rates of depletion of these elements during decay would give some indication of their relevance in the deterioration process. Such an aim entails the following three assumptions:

(a) the absence of appreciable contamination of the blocks with nitrogen, phosphorus or potassium from external sources. In this regard foam was used in place of soil in the decay chambers, and all glassware was washed exceptionally thoroughly,

(b) the absence of extensive leaching of the elements from the blocks, and

(c) the translocation of a large proportion of these elements utilized by microorganisms to mycelium external to the wood block, i.e. to the extensive mycelial mat covering the block and penetrating the foam base. The methods used here do not differentiate between the elements in the wood which have not been utilized, and those within mycelium in the wood.

Against this background, the patterns of change in levels of nitrogen, phosphorus and potassium (Figure 6-5) can be assessed. It is apparent that concentrations of all three nutrients declined during decay by *G. trabeum*, i.e. nitrogen, phosphorus and potassium are apparently utilized during decay by this fungus. Levels of

potassium decreased markedly before weight losses reached 5%, perhaps reflecting the tendency for this element to occur in wood as free, highly mobile ions (Tattar *et al*, 1972; Shortle, 1982), allowing rapid leaching and/or aquisition by the fungus. *G. trabeum* could possibly accentuate any leaching effect by promoting ionization of the potassium (Shortle, 1982). Quite different trends are apparent for the white-rot organism *F. lividus* (Figure 6-5), although the distinct early increase in levels of nitrogen and phosphorus in the initial stages of decay suggest that contamination, perhaps from the foam base, was a problem in this case. Nitrogen fixation is known to occur in some wood inhabiting microorganisms (Seidler *et al*,1972; Sharp and Millbank, 1973), but it would be expected that if *F. lividus* was capable of obtaining nitrogen in this way, levels of the element would remain substantially elevated throughout decay *cf.* the decline evident in Figure 6-5. The differing patterns in levels of these elements between the white-rot and brown-rot organisms (Figure 6-5) may relate in some way to the differing types of growth of the two fungi over the wood blocks. *F. lividus* tends to form a much thicker mycelial mat extending deep into the foam base.

6.5.5 Natural Decay Susceptibility of Sapwood of *Pinus radiata*

A major aim of this study was to gain further insights into the causes of variations in the natural decay susceptibility of pine sapwood. For both *F. lividus* and *G. trabeum*, weight loss is apparently determined largely by the rate of metabolism of sugars and other breakdown products of the polysaccharides, i.e. the low molecular-weight carbohydrates are produced (enzymatically) faster

than they are utilized by the fungi (Figure 6-3). Certainly, possible problems in the accessibility of the breakdown products to fungal hypha (especially for the brown-rot fungus) cannot be ignored, but it appears unlikely that the rate of decay in the sapwood of *P. radiata* is determined by the chemistry of the polysaccharide component of the cell wall.

The possible importance of lignin and the elements as determinants of the rate of decay can probably be best judged when the percentage change in each is correlated with the percentage change in the weight of the test blocks (Appendix 6-1). Here, it is apparent that the relatively refractory lignin is no impediment to the decay by *F. lividus* and if anything, the removal of lignin occurs at a faster rate than does that of the polysaccharides. The extent to which decay by *G. trabeum* is limited by the presence of lignin is unknown. However, any such effect is probably minimal since the rate of loss in weight caused by this organism is at a maximum late in the decay process when the proportion of lignin in the substrate is greatest (Wong, Unpublished).

It has been suggested (Cowling and Merrill, 1966) that the meager content of nitrogen may limit decay in some timbers. However, during decay by *G. trabeum*, the nitrogen content of the pine sapwood varied relatively little (Appendix 6-1), and even for *F. lividus*, the percentage loss in nitrogen failed to exceed the percentage loss in weight of the decayed samples. This situation possibly reflects efficient re-use of nitrogen by the fungi as decay progresses (Cowling and Merrill, 1965). Nevertheless, the results cannot be taken as evidence of the absence of an effect of nitrogen levels on

the decay process. Similarly, most (in the case of *G. trabeum*) or all (*F. lividus*) of the phosphorus remained in the wood blocks at the end of the decay period (Appendix 6-1), but it is not known whether the levels (*ca.* 100 - 200 ppm) were sufficiently low to adversely affect deterioration. Perhaps it is the content of potassium which is most likely to become rate limiting- more than 40% of potassium in the sapwood was lost before weight losses reached 3%, and over the full course of decay by *G. trabeum*, *ca.* 75% of this chemical component was eventually removed. Potassium is required in relatively large quantities (at least 10^{-3} M in nutrient solution) for satisfactory growth of many fungi (Cochrane, 1958; Lilly, 1965).

6.6 CONCLUSIONS

The changes in the solubility properties and lignin/polysaccharide content of *Pinus radiata* subjected to decay by *Fomes lividus* (white-rot) and *Gloeophyllum trabeum* (brown-rot) were found to agree reasonably well with the trends obtained using other timber-fungus combinations. Most importantly, *G. trabeum* tended to depolymerize the polysaccharides at a greater rate than they could be utilized, resulting in a substantial rise in the solubility of brown-rotted samples in hot water and dilute alkali. Levels of nitrogen, phosphorus and potassium in the wood generally declined during the brown-rot decay process, but showed variable trends in the samples subjected to attack by *F. lividus*.

These results suggest that variations in the natural decay susceptibility of pine sapwood are not determined to any major extent

by variations in the nature and/or amounts of cell wall structural components. The extent in which nitrogen, phosphorus and potassium are rate limiting was not ascertained, but the depletion in levels of potassium during decay, especially by *G. trabeum*, could be of importance.

CHAPTER 7

THE CAUSES OF VARIATIONS IN DECAY SUSCEPTIBILITY- SUMMARY AND GENERAL CONCLUSIONS

7.1 ACHIEVEMENTS IN RELATION TO OBJECTIVES

Based on limited evidence (e.g. Garren, 1939; Schmidtling and Amburgey, 1982; Wong *et al*, 1983), it was considered likely that variations in the natural decay resistance and susceptibility of relatively non-durable woods may be best explained by the influence of physical characteristics of the wood and/or chemical factors other than the quantity and/or quality of extractives. Certainly in sapwood, which generally lacks fungitoxic compounds, it is likely that variations in decay susceptibility (Zabel, 1948; Schmidtling and Amburgey, 1982; results of this study) may be associated with other wood properties. This possibility was examined here in a series of experiments which investigated several possible causes of variations in the decay susceptibility in juvenile sapwood of 13-year-old *Pinus radiata* attacked by a white-rot (*Fomes lividus*) and a brown-rot (*Gloeophyllum trabeum*) organisms *in vitro*.

The study (Chapter 4) of the role of certain attributes of

gross wood structure (wood density, percentage late wood and tracheid length) on the variations in susceptibility to decay (soil-jar decay test) suggested that the tendency towards greater decay in outer than inner sapwood was probably not related causally to corresponding variation in the physical factors.

The possible influence of a number of chemical characteristics (contents of lignin, available carbohydrates, nitrogen, various mineral elements and resinous extractives) on the radial variation in decay susceptibility was therefore examined (Chapter 5). Of these factors, the contents of nitrogen, phosphorus and potassium were often significantly, and positively, correlated (r = 0.7 - 0.9) with decay, indicating a likely stimulatory effect of these chemical elements on the rate and amount of decay.

In an attempt to consolidate the findings in Chapter 5, the progressive changes in the contents of lignin, nitrogen, phosphorus and potassium as well as the relative rates of production and utilization of available carbohydrates (originating from cell wall polysaccharides) were charted during the course of decay by both fungi (Chapter 6). Overall, the changes in solubility characteristics (indicative primarily of carbohydrates) and the lignin content during decay agreed reasonably well with the findings from previous studies (Cowling, 1961; Kirk and Highley, 1973) using other wood-fungus combinations. Of particular significance, was the rapid depolymerization of the structural carbohydrates by the brown-rot fungus *G. trabeum*, being far greater than the utilization of the resultant degradation products. This suggests that weight losses are limited at the level of fungal metabolism, and not by the ability of

the organism to degrade cell wall substance. In this case, structural factors may be relatively unimportant as determinants of decay susceptibility. The contents of nitrogen, phosphorus and potassium in the sapwood generally decreased during decay by *G. trabeum*, but showed variable trends in white-rotted samples (Chapter 6).

The overall findings of these studies have suggested that variations in the natural decay susceptibility in the juvenile pine sapwood are not likely to be substantially influenced by gross physical characteristics nor the amounts and/or nature of cell wall structural materials. Of greater importance may be the amounts of fungal nutrients such as nitrogen, phosphorus and potassium.

7.2 OPPORTUNITIES FOR FUTURE RESEARCH

An exhaustive examination of all aspects of the variations in the natural decay susceptibility of woods containing little or no fungitoxic extractives was certainly not possible within the time-frame for this study. However, observations from this and other related studies do suggest avenues for further investigations. Perhaps, most importantly would be the study of:

- the comparative influences of the soluble and insoluble fractions of nitrogen and minerals (particularly phosphorus and potassium), rather than the total amounts of these elements, on the variations in decay susceptibility
- the probable inhibitory role of resinous extractives in decay susceptibility. Recent evidence (Shrimpton and Whitney, 1979; Schuck, 1982) suggests this may be marked in woods containing abundant resinous substances (e.g. *Pinus radiata*)

- Differences in the decay susceptibility between early wood and late wood tissues. Such differences could possibly be related to variations in the chemical or physical characteristics between the closely neighbouring intra-incremental positions.

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Appendix 2-1: Computational formula for calculating required sample size (number of measurements of tracheids) to restrict the error to within 5% of the mean tracheid length

The computational formula taken from Freese (1962) considers that for a number of measurements on one smple having values x_1, x_2, ..., x_a, the sample mean value ($\bar{x}$) is denoted by,

$$\bar{x} = \frac{1}{a} \sum_{i=1}^{n} x_i$$

with standard deviation,

$$\sigma = \frac{1}{\sqrt{a-1}} \sum_{i=1}^{n} (x_i - \bar{x})^2$$

Estimate of the coefficient of variation (c) is given by,

$$c = \frac{\sigma \times 100}{\bar{x}}$$

For a required error ($e\%$) of the mean, the student's t distribution is then applied to calculate the required sample size (n) as follows:-

$$n = \left(\frac{ct}{e}\right)^2$$

where,

n = number of individual measurements required,

c = coeficcient of variation,

e = percentage error of the mean,

t = student's t value for small sample sizes at a selected $e\%$ level.

Appendix 4-1: Between-tree variation in decay susceptibility and physical properties within each provenance of *Pinus radiata*

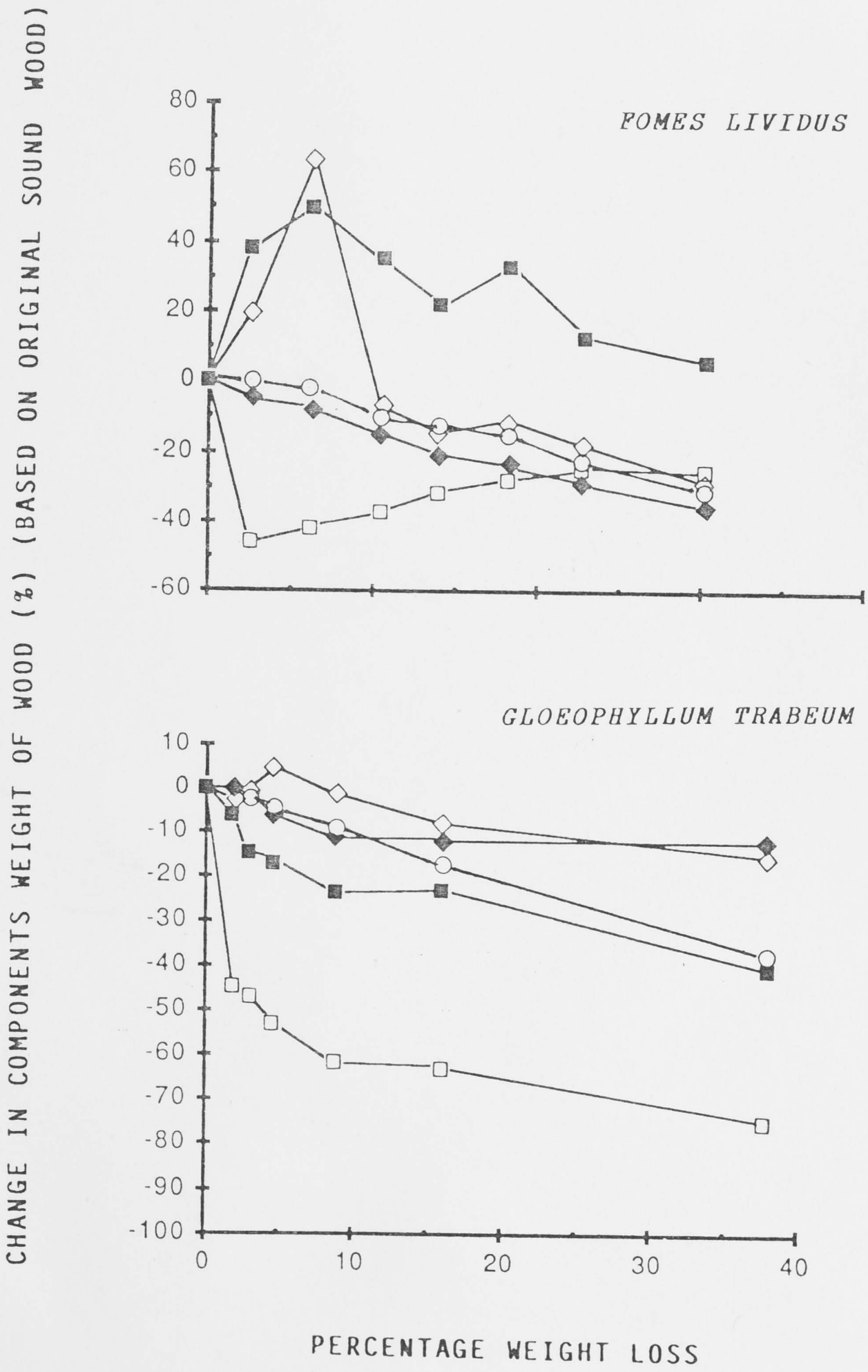
Wood Property	Provenance	Variance Ratio (Between-tree)		Between-tree Significance ($P < 0.05$) of Mean Values							
Decay susceptibility	Ano Nuevo	4.5*	Tree:[2]	3	8	6	2	5	7	1	4
			Mean (%):	1.2	2.5	3.2	3.2	3.3	3.6	3.9	4.7
Fomes lividus	Monterey	1.0n	Tree:	16	15	13	12	11	9	14	10
			Mean (%):	2.1	2.4	2.4	2.6	2.6	2.8	3.0	4.0
(x[1] = 12)	Cambria	1.6n	Tree:	24	18	20	17	21	19	23	22
			Mean (%):	1.9	2.2	2.3	2.7	3.0	3.1	3.4	3.5
Decay susceptibility	Ano Nuevo	14.2*	Tree:	1	8	3	4	2	6	5	7
			Mean (%):	28.9	49.4	49.5	50.2	50.3	55.2	58.7	59.4
Gloeophyllum trabeum	Monterey	5.7*	Tree:	10	16	9	15	12	11	14	13
			Mean (%):	34.1	35.0	36.0	39.2	45.4	47.4	53.4	57.4
(x = 12)	Cambria	5.0*	Tree:	20	23	22	24	21	17	18	19
			Mean (%):	39.6	43.6	45.9	46.8	47.2	48.4	59.2	60.5
'Unextracted' Wood Density	Ano Nuevo	1.0n	Tree:	6	4	7	3	8	1	5	2
			Mean: ($g\ cm^{-3}$)	.347	.348	.356	.370	.374	.374	.376	.377
(x = 8)	Monterey	11.7*	Tree:	13	15	12	16	9	11	14	10
			Mean: ($g\ cm^{-3}$)	.303	.322	.348	.350	.367	.378	.385	.394
	Cambria	4.5*	Tree:	17	24	19	18	23	21	22	20
			Mean: ($g\ cm^{-3}$)	.318	.326	.342	.351	.358	.358	.360	.364
'Extracted' Wood Density	Ano Nuevo	1.0n	Tree:	6	4	7	3	8	1	5	2
			Mean: ($g\ cm^{-3}$)	.317	.318	.325	.333	.337	.341	.342	.344
(x = 8)	Monterey	23.3*	Tree:	13	15	12	16	9	11	14	10
			Mean: ($g\ cm^{-3}$)	.259	.292	.316	.318	.336	.345	.355	.358
	Cambria	4.4*	Tree:	17	24	19	18	23	21	22	20
			Mean: ($g\ cm^{-3}$)	.286	.297	.311	.315	.319	.327	.330	.332
Percentage Late Wood	Ano Nuevo	0.6n	Tree:	6	7	4	8	2	3	1	5
			Mean (%):	9.2	10.1	11.2	13.2	13.6	13.9	14.0	15.2
(x = 16)	Monterey	1.0n	Tree:	12	9	10	13	15	11	16	14
			Mean (%):	7.6	8.4	8.6	9.1	9.4	10.8	12.7	13.3
	Cambria	3.6*	Tree:	17	21	20	19	18	24	23	22
			Mean (%):	6.2	6.9	7.6	8.2	8.2	8.6	10.1	14.9
Tracheid Length	Ano Nuevo	0.2n	Tree:	5	1	2	6	7	3	8	4
			Mean (mm):	1.83	1.95	1.96	1.99	2.03	2.06	2.09	2.27
(x = 4)	Monterey	0.6n	Tree:	12	10	9	13	15	11	14	16
			Mean (mm):	1.74	1.75	1.86	1.92	2.08	2.10	2.23	2.26
	Cambria	0.4n	Tree:	23	20	19	18	22	21	17	24
			Mean (mm):	1.75	1.78	1.81	1.82	1.85	1.93	2.07	2.23

1. Number of replications (readings) combining both radial positions for each tree
2. Trees number 1, ..., 24 correspond with those in Table 2-1

*. Variance ratio significant at 0.05 level; n- not significant

Trees joined by the same line do not differ significantly at 0.05 level

APPENDIX 6-1: Percentage changes in weight of wood, the contents of lignin, nitrogen, phosphorus and potassium in progressive stages of decay of the sapwood of *Pinus radiata* by a white-rot (*Fomes lividus*) and brown-rot (*Gloeophyllum trabeum*) fungus


LEGEND: COMPONENTS
WEIGHT OF WOOD
LIGNIN
NITROGEN
PHOSPHORUS
POTASSIUM
FOMES LIVIDUS
GLOEOPHYLLUM TRABEUM
CHANGE IN COMPONENTS WEIGHT OF WOOD (%) (BASED ON ORIGINAL SOUND WOOD)
PERCENTAGE WEIGHT LOSS
80
60
40
20
0
-20
-40
-60
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40

CORRIGENDA

Throughout - 'early wood' and 'late wood' should preferably read 'earlywood' and 'latewood'

- 'weight' should preferably read 'mass'

P xxi, para 2, line 7
- after 'suggested' insert '(other workers have usually found negative relationships)'

P 3, para 1, line 6
- '25' should read '20'

P 3, para 1, line 8
- after '40°C.' insert 'The upper limit may, however, be as low as 27°C'

P 3, para 2, line 2
- 'as fibres' should read 'in fibres'

P 3, para 2, line 3
- 'is a heterogeneous mixture of' should read 'has'

P 3, para 4, line 2
- 'essentially' should read 'largely'

P 5, para 1, line 8
- '24 years service' should read '25 years service'

P 8, para 2, line 2
- 'light' should read 'low density'

P 9, caption to Plate 1.1, line 3
- after 'decay fungi' insert '(*Gloeophyllum trabeum*, *Fomes lividus*)'

P 10, para 1, line 2
- 'parenchymal' should read 'parenchymatous'

P 10, para 2, line 5
- 'are taken' should read 'were taken'

P 10, para 2, line 8
- 'is' should read 'was'

P 18, para 1, line 5
- after 'not' insert 'always'

P 18, para 1, line 9
- after 'frequently' insert 'to help locate the less obvious areas of reaction wood'

P 25, caption to Plate 2.4, line 5
- 'white rot organisms' should read 'the white rot organism *Fomes lividus*'

P 25, caption to Plate 2.5, line 4
- after '(A)' and '(B)' insert '(*Fomes lividus*)' and '(*Gloeophyllum trabeum*)' respectively

P 30, para 1, line 3
- delete 'radially'

P 35, para 2, line 3
- 'heated' should read 'the water boiled'

P 42, para 2, line 6
- delete 'interest in the'

P 45, under '*White-rot fungi*'
- after '*Fomes lividus* (Kalch.) Sacc. (DFP 7904)' insert 'syn. *Perenniporia tephropora* (Mont.) Ry.'

P 91, para 2, line 3
- 'essentially' should read 'largely'

P 106, para 2, line 1
- 'speculate' should read 'suggest'