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Heartwood can have properties that are distinct from sapwood, including resistance to insect and microbial attack. Despite the practical importance of heartwood formation in trees, a review of the literature revealed that little was known about the effects of environmental factors on heartwood quality or how variations in heartwood properties may influence natural durability. Growth rate variations in six Douglas-fir trees coincided with variations in the extractive concentration in annual rings estimated to have become heartwood at the same time. An experiment to examine the mechanisms for this phenomenon involving thinning or pruning treatments with 30 young Douglas-fir trees produced no consistent relationships between environmental changes, growth rate and heartwood extractive content. An examination of the stable carbon isotope patterns in the acetone/water-soluble wood extractives and the cellulose of Douglas-fir wood indicated that some of

the sapwood extractives within an annual ring were formed at the time the ring was formed. Extractives and cellulose $\delta^{13}C$ values within annual rings were correlated ($R^2 \approx 0.50$) across the radius of six trees, which suggested that sapwood extractives were relatively immobile over time and made an important contribution to the heartwood extractives that were subsequently deposited in the same rings. This suggests that influences on a wood ring at the time of formation will affect heartwood properties many years later when the ring becomes heartwood. An analysis of 24 young western redcedar trees from a silvicultural trial that involving thinning and fertilization treatments found no consistent effect of increasing growth rate on heartwood quality, as represented by various measures of extractive content. Tests of the natural durability of western redcedar and Alaska cedar heartwood against termites and fungi revealed that methanol-soluble extractives were crucial for resistance; however, correlations among extractive components were weak and extractive concentrations explained relatively little of the variation in natural durability. These studies suggest a potential for influencing heartwood quality through silviculture, but considerable research will be needed to understand the relationships between environmental factors and heartwood formation, and between heartwood variability and natural durability.

Environmental Effects on Heartwood Extractive Content and Their Consequences for Natural Durability in Douglas-fir and Western Redcedar

by
Adam M. Taylor

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I understand that my dissertation will become part of the permanent collection of the Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Adam M. Taylor, Author

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CONTRIBUTION OF AUTHORS

Barbara L. Gartner and Jeffrey J. Morrell assisted with the writing of each manuscript. J. Renée Brooks assisted with the writing of Chapter 5. Kunio Tsunoda assisted with the writing of Chapter 7.

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DEDICATION

The work is dedicated to my grandfather, Rogers McVaugh.

ENVIRONMENTAL EFFECTS ON HEARTWOOD EXTRACTIVE CONTENT AND THEIR CONSEQUENCES FOR NATURAL DURABILITY IN DOUGLAS-FIR AND WESTERN REDCEDAR

CHAPTER 1: INTRODUCTION

Heartwood formation is a normal part of tree development and heartwood can have many distinct properties, including natural resistance to attack by insects and mircroorganisms. The natural durability of heartwood is mostly a result of the presence of special "extractive" chemicals that are formed when old sapwood becomes heartwood. Different tree species produce heartwood with different extractive types and concentrations, and thus different species have differing levels of natural durability. In addition to species differences, there is variation in the heartwood extractive profiles and natural durability within and among trees of the same species. A better understanding of the causes and implications of this intra-species variability is needed to help forest managers grow trees that will meet society's needs for high-quality wood products.

This objective of this thesis was to review and expand our knowldege of the patterns and causes of heartwood extractive variation within and between trees, and the implications of this variation for natural durability. Douglas-fir (*Pseudotsuga menziesii)* western redcedar (*Thuja plicata)* were the focus of much of the research, but the aim was to identify and develop general trends.

Douglas-fir is a widely planted species in the Pacific Northwest whose heartwood may be more difficult to pulp than the sapwood. Western redcedar heartwood has high natural durability.

The results of this study are presented here in a series of six manuscripts. The first of these, Chapter 2, is a comprehensive review of the literature relating to the heartwood formation process and natural durability. This review revealed that, whereas genetic and ontogenetic influences on heartwood formation were known, it had not yet been determined whether environmental factors played a role. The work described in Chapter 3 demonstrates that environmental influences on the living tree can influence the heartwood extractive content of Douglas-fir. Chapter 4 describes an investigation of the mechanism of this phenomenon.

Previous experience and research conducted as part of this thesis have shown that heartwood formation is a complex process that requires novel analytical techniques. Chapter 5 investigates an application of stable isotope analysis to determine the dynamics of carbon sources that contribute to the formation of heartwood extractives in Douglas-fir.

The durable heartwood of western redcedar is used for high-value wood products, thus an understanding of heartwood variation in this species is of particular interest to forest managers. Chapter 6 presents an evaluation the effects of silvicultural treatments on the heartwood extractives of young Western redcedar.

The influence of heartwood extractive variability on natural durability is the focus of Chapter 7. This study identifies the extractive fractions that are responsible for the high natural durability of Western redcedar and Alaska cedar *(Chamaecyparis nootkatensis)*, and goes on to evaluate the effect of naturally-occurring variations in these extractives on the resistance of the wood to termites and fungi.

All six of the research projects presented here share the goal of increasing our understanding of the how heartwood formation is affected by environmental changes, and how this can influence the natural durability of the wood.

CHAPTER 2: HEARTWOOD FORMATION AND NATURAL DURABILITY: A REVIEW

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2.1 ABSTRACT

This paper reviews recent literature on the formation of heartwood and on the components that affect natural durability. It includes discussion about the function of heartwood in living trees, factors influencing the natural durability of heartwood, the process of heartwood formation, and variations in heartwood quantity and quality. Heartwood formation is a regular occurrence in tree stems, and heartwood may have many different properties from sapwood, including natural decay resistance. A greater understanding of the heartwood formation process could allow control of heartwood production. Recent research involving enzymatic analyses have provided valuable insight into the biochemical processes involved in heartwood formation. Further study of the role natural durability plays in living trees would help to bring together many of the disparate strands of research relating to heartwood.

2.2 KEYWORDS

Decay resistance, extractives, heartwood, natural durability, sapwood.

2.3 INTRODUCTION

Heartwood is a normally occurring part of the xylem in trees. Heartwood has properties that can significantly influence its usefulness to the end user of wood products; notable among these is natural resistance to deterioration by insects, marine borers, and microorganisms. Understanding the formation of heartwood in trees may ultimately allow foresters to influence the heartwood formation process by using various silvicultural practices. It may also enable forest geneticists to enhance durability through selection and/or molecular biological techniques.

This review builds upon the reviews of Scheffer and Cowling (1966), Bamber and Fukazawa (1985), and Hillis (1987). In this review, we emphasize recent literature relating to the function of heartwood in the tree, the process of heartwood formation, and the factors that influence heartwood formation, while building upon previous reviews.

2.4 THE FUNCTION OF HEARTWOOD AND SAPWOOD IN TREES

It is important to consider wood quality in terms of wood as part of a living tree (Larson 1962). Thus, in order to understand heartwood formation and how it can be modified, it is first important to comprehend the role of heartwood and sapwood in the tree.

The International Association of Wood Anatomists [IAWA] defines heartwood as "the inner layers of the wood, which, in the growing tree, have ceased to contain living cells, and in which the reserve materials (e.g., starch)

have been removed or converted into heartwood substance" (IAWA 1964, p. 32). In some species, heartwood may be distinguished from sapwood by a darker color, lower permeability, and increased decay resistance. Heartwood often has different moisture content than sapwood; in conifers heartwood is usually drier than sapwood, but the relative moisture contents of the two regions in hardwoods are species specific (see Table 3.3 in USDA-FS Forest Products Laboratory, 1999).

Heartwood is formed in gymnosperm and angiosperm tree species, although in some cases it may be difficult to detect (eg. *Abies* spp.) or may only develop in very old sapwood (eg. *Alnus* spp.) (Panshin and de Zeeuw 1980) The presence of heartwood may optimize sapwood volumes, conserve resources, and provide structural support.

Sapwood is defined by the IAWA (1964) as "the portion of the wood that in the living tree contains living cells and reserve materials" (IAWA 1964, p. 43). It contains the wood that is part of the transpiration stream of the tree, and it generally has high moisture content. Sapwood also contains energy reserve materials such as starch. Its permeability is facilitated by the presence of un-aspirated, unencrusted pits. Sapwood contains few toxic extractives and is generally susceptible to decay.

The primary role of sapwood in a tree is to conduct water from the roots to the crown (Gartner 1995). According to the pipe model (Shinozaki 1964), sufficient sapwood is required to supply the foliage with water, and the amount

of foliage on a tree often is strongly correlated to the amount of sapwood (Berthier et al. 2001; Ryan 1989; Dean and Long 1986; Whitehead et al. 1984).

Sapwood also serves as a storage site for water and for energy reserve materials such as starch (Hillis 1987; Ryan 1989), and as a site for living cells that can respond to injury through production of more tissue or defensive compounds (Boddy 1992). However, there are also costs associated with maintaining sapwood in a tree. Living parenchyma cells respire and consume considerable amounts of "maintenance" energy [an estimated 5%–13% of annual net photosynthate in some species (Ryan et al. 1995)]. In addition, energy stored in sapwood parenchyma may be utilized in response to injury (Shain 1995). Thus, it may be hypothesized that sapwood in excess of the amount required to satisfy foliar demands is converted to heartwood to decrease energy demands.

Sapwood permeability is also a factor in the balance between the amount of foliage and sapwood. The concept of a "homeostatic balance" between these parameters has led people to speculate that the modification of one variable (e.g., leaf area) might result in changes in sapwood area (Margolis et al. 1988; Whitehead et al. 1984).

The "transition zone" between heartwood and sapwood has been defined as

"A narrow, pale-colored zone surrounding some heartwoods and injured regions, often containing living cells, usually devoid of starch, often impermeable to liquids, with a moisture content lower than the sapwood and sometimes also than the heartwood" (Hillis 1987, p. 16). This zone is also known as the "white zone" and "dry zone" (Nobuchi and Harada 1983). This zone is not apparent in all species, nor is it always observed in those species in which it occurs (Hillis 1987). The IAWA (1964) defines "intermediate wood" as "The inner layers of the sapwood that are transitional between sapwood and heartwood in color and general character" (IAWA 1964, p. 46). The term, "intermediate wood" is often confused with, or used interchangeably with, the term "transition zone" (Hillis 1987).

"Extractives" is a term used to describe the non-structural compounds present in wood. The term originates from the fact that many of these substances can be removed with neutral, organic solvents or water (Sjostrom 1993). However, Gang et al. (1998) point out that "extractives" may be a misnomer, because, in some cases, phenolic compounds present in heartwood are not easily removed.

Structural support

Wood properties, including density, vary from pith to bark (Panshin and de Zeeuw 1980). Wood density in conifers generally increases with distance from the pith. Hardwoods are more variable; density increases with distance from the pith in some, and remains unchanged or decreases in others.

Strength is closely correlated with density; thus strength differences may exist between heartwood and sapwood. However, heartwood does not differ structurally from sapwood; any significant strength differences result from radial changes in wood density and cell wall ultrastructure, not from whether the sample is heartwood or sapwood, per se (Panshin and de Zeeuw, 1980).

If the structure of a tree is considered to be a cantilevered cylinder, then the highest tensile and compressive stresses will occur in the outer growth rings. Thus, heartwood is less important than the sapwood for structural support. Indeed, it is not unusual to observe hollow trees that have stood for many years. However, there are indications that heartwood is necessary for structural support. Long et al. (1981) found that sapwood cross-sectional area was more or less constant below the crown of *Pseudotsuga menziesii* trees. They concluded that this sapwood was insufficient for the mechanical support of older trees, and that the additional xylem contained in the heartwood provided the necessary compressive strength. Mattheck (1995) showed that hollow trees will only break when the outer shell of wood is less than one-third of the total radius. Heartwood may compose part of this critical wood shell in thinner sapwood species. Mencuccini et al. (1997) found that heartwood made little contribution to the stiffness of *Pinus sylvestris* stems. However, this tree species maintains relatively thick sapwood bands, which would minimize the mechanical role of heartwood. Heartwood would extend farther from the neutral axis in trees with thinner sapwood, and would be composed of more of

the stronger, mature wood, simply because of radial changes in wood properties.

Recycling nutrients

A variety of species recycle nutrients from newly forming heartwood back into sapwood, in a process likened to nutrient resorption from senescing leaves (Bamber and Fukazawa 1985). Recycled nutrients can represent a significant nutrient source; Attiwell (1980) calculated that heartwood conversion supplied 31% of the entire phosphorous demand of *Eucalyptus obliqua* trees.

Andrews et al. (1999) studied nutrient recycling in the sapwood and heartwood of *Chamaecyparis thyoides* and found that nutrient levels in sapwood of trees growing on sites with lower soil nutrient concentrations were maintained at levels comparable with more nutrient-rich sites. Heartwood was relatively low in these elements in trees on nutrient-poor sites. Thus, heartwood formation may allow trees to concentrate nutrients in sapwood.

Repository for toxic substances

Stewart (1966) suggested that heartwood forms in response to a build-up of toxic by-products of metabolism. Death of the parenchyma cells in the heartwood could result from the build up of these toxins. For example, cambial cells are negatively affected by trace amounts of tannins (a common heartwood extractive) (Jacquiot 1947). Indeed, Carrodus (1971) measured

high levels of carbon dioxide (a waste product of respiration) in xylem, indicating that a build-up of respiratory wastes occurs in this zone.

However, in-situ formation of new chemical compounds at the sapwood/heartwood boundary, observations of the patterns of heartwood development, and the low toxicity of some heartwoods suggest that heartwood does not function primarily for waste storage (Bamber and Fukazawa 1985).

Factors influencing the natural durability of heartwood

"Natural" durability is commonly measured by exposing wood to decay fungi under accelerated conditions (e.g., ASTM 1993), although wood may also be tested for resistance to termites, beetles, and marine borers. The natural durability of sapwood is generally low, but the heartwood of some species can be very resistant to biodeterioration. There are also variations in natural durability between and within the heartwoods of individual trees, as will be discussed later.

Living sapwood has active and passive defense mechanisms. Active defenses are those that are induced by an attack or wound, while passive defenses are produced prior to infection. The CODIT concept has been proposed to describe the patterns of active and passive defenses in trees (Shigo 1984). When heartwood forms, death of the parenchyma eliminates the active defenses (Shain 1995), leaving only passive mechanisms of resistance to pathogens.

Extractives

Toxic extractive compounds in heartwood are recognized to be the most important factor in determining the natural durability of wood (Bamber and Fukazawa 1985; Hillis 1987; Scheffer and Cowling 1966). Durable wood from which extractives are removed becomes susceptible to decay (Scheffer and Cowling 1966; Smith et al. 1989). Similarly, adding heartwood extractives to normally decay-prone wood can render it decay-resistant (Kamden 1994; Onuorah, 2001; Smith et al. 1989). However, extractive concentrations in heartwood do not necessarily correspond to natural durability, as measured by standard methods (Kumar 1971; Hillis 1987). In some cases, decay resistance is poorly correlated with variations in the heartwood compounds that are largely responsible for preventing decay (DeBell et al. 1999; Hillis 1987). In other cases, extractives exhibit activities that are specific to the organisms that normally attack the living tree (Etheridge 1962; Ohsawa et al. 1992; Wilcox 1969). As well, some durable heartwoods may contain multiple low-toxicity extractive compounds that interact synergistically (Schultz et al. 1995; Shultz and Nicholas 2002). Finally, some species (e.g., *Larix* spp.) produce large amounts of extractive material that apparently provide little or no protection to the wood (Srinivasan et al. 1999).

Whereas total extractive content in a species is correlated with decay resistance, the micro-distribution of extractives also influences performance. Extractives that are impregnated into the cell wall are more effective in

deterring microorganisms than are those in the cell lumen (Hillis 1987; Kleist and Schmitt 1999).

The structure, toxicity, and specificity of various heartwood substances have been extensively studied (e.g., Anderson et al. 1962, 1963; Fitzgerald and Line 1990; Freydl 1963; Hart 1981; Hillis 1972, 1987; Kumar 1971; Ohsawa et al. 1992; Rao 1982; Rowe 1979; Rudman 1963; Scalabert 1991; Scheffer and Cowling 1966; Schultz et al. 1990, 1995). The micro-distribution of extractives has received less attention, largely because of the difficulty of accurately assessing extractive content in situ.

Reduced attractiveness to decay organisms

Heartwood may be less attractive than sapwood for some pathogens simply because it lacks the requisite nutrients, or because the nutrients it contains may be less accessible (Scheffer and Cowling 1966). For example, starch is required for the successful reproduction of *Lyctus* beetles in wood (Parkin 1938; Humphreys and Humphreys 1966; Wilson 1933). These insects rarely attack heartwood, which is free of starch. Sapstain and mold fungi feed on free sugars and starch in the sapwood, and their penetration into heartwood may be limited, in part, by the absence of readily assimilable carbohydrates (Findlay 1959). Wilson (1933) observed that sapwood with less starch suffered less discoloration. Similarly, Taylor and Cooper (2002) found that sapstain and mold fungi did not grow as well on *Pinus resinosa* sapwood with reduced starch content as on sapwood with normal amounts of starch.

Mechanical barriers

It also is logical to suppose that mechanical barriers to attack may prevent or slow wood decay in some species by physically blocking the penetration of insects or fungal hyphae. For example, aspirated and encrusted pits are also a feature of heartwood. Although wood-destroying fungi are capable of moving through pit membranes, aspirated pits reduce moisture movement, slowing wetting and presumably creating conditions less conducive to decay.

Verrall (1938) concluded that the resin exuded in response to wounding of *Pinus resinosa* reduced the risk of decay by its water repellency, rather than by inherent toxicity. DeVries and Kuyper (1990) suggest that decay resistance in *Taxus* spp. was determined more by physical characteristics (longitudinal permeability) than by its chemistry. Extractives clearly make important contributions to the natural durability of wood; however, the relative importance of extractives (like gums) as mechanical barriers vs. in their other roles (relating to toxicity, wettability, etc.) is poorly understood.

Tyloses are another factor that may impede wood decay. They are a regular feature of heartwood vessels in many hardwood species, but they also occur in the sapwood of these species and in some conifers (Gerry 1914). Gerry (1914) observed a correlation between the regular occurrence of tyloses and high decay resistance in hardwoods of the United States, although there were exceptions to this trend. Bell (1980) suggested that tyloses function as

part of the defensive strategy of trees by blocking the movement of pathogens along vessels and by allowing toxic extractives to accumulate without being diluted by the transpiration stream.

2.5 THE PROCESS OF HEARTWOOD FORMATION

The events associated with heartwood formation

Although heartwood transformation is not completely understood, many of the component processes have been studied. A number of changes may occur as sapwood becomes heartwood. Some of these events are clearly evident in the resulting heartwood (e.g., parenchyma death and extractive formation), while others are more ephemeral (e.g., changes in enzyme activity).

Death of parenchyma. — Complete parenchyma cell death, by definition, marks the transformation of sapwood into heartwood. Changes in parenchyma viability have been recorded by measuring respiration or by observing cytological changes. Shain and Mackay (1973) stated that most evidence suggests that parenchyma activity gradually declines with increasing distance from the cambium. However, their work demonstrated a spike of metabolism by parenchyma (by measuring oxygen consumption) in the transition zone during the dormant season. Shigo and Hillis (1973) concluded that there was a spike of metabolism in the transition zone related to the various processes involved with the transformation to heartwood. Nobuchi et al. (1979) described three patterns of parenchyma decline in sapwood of

various species. In Type I, all parenchyma survive from the cambium to the heartwood/sapwood boundary. In Type II, there is a gradual decline in parenchyma, beginning in the middle sapwood. In Type III, parenchyma cell death begins in the outer sapwood, and continues to the heartwood boundary; there is no sharp boundary at the heartwood/sapwood transition. Fukazawa et al. (1980) also defined three patterns of parenchyma "maturation," but further subdivided the groups by the season during which the changes took place.

Pruyn et al. (In press) observed greater CO_2 evolution in outer sapwood than in inner sapwood, and higher sapwood parenchyma respiration at the base and crown of the tree than at 1 m above the ground. They hypothesized that respiration was related to proximity to carbohydrate sources.

Changes in the nuclei of sapwood parenchyma have been related to heartwood formation in a number of species including *Cryptomeria japonica*, *Melia azedarach*, *Pinus sylvestris*, *Larix decidua*, *Pseudotsuga menziesii* and *Robinia pseudoacacia* (Hillis 1987). Changes in the shape of nuclei of the parenchyma before they disappear have been used to develop indices to describe the extent of parenchyma decline. These indices include the nuclear slenderness ratio (Frey-Wyssling and Bosshard 1959), the nuclear irregularity index (Yang 1992), and the nuclear elongation index (Yang et al. 1994).

Gas accumulation. -- Carrodus (1971) applied carbon dioxide to *Acacia mearnsii* sapwood and observed that heartwood-type extractive compounds were formed. High carbon dioxide levels and low oxygen levels have been

observed in heartwood, suggesting that heartwood extractive formation is related to the high level of carbon dioxide (a by-product of metabolism) within the trunk. However, Hillis (1987) reported that carbon dioxide inhibited polyphenol synthesis in various species. Shain and Hillis (1973) suggested that ethylene gas, and not carbon dioxide, was responsible for initiating the formation of heartwood extractives.

Desiccation. — Heartwood is often significantly drier than sapwood in conifers; in angiosperms, no consistent pattern exists, although there are often moisture content differences between the two tissues (Bamber and Fukazawa 1985; Hillis 1987). The transition zone may have lower moisture content than either heartwood or sapwood (Nobuchi and Harada 1983) and the transition in moisture content between these different tissues can be abrupt (reviewed in Hillis 1987). Often, desiccation of sapwood is associated with the formation of heartwood extractive compounds in sapwood (Jorgensen 1962; Jorgensen and Balsillie 1969; Shain and Hillis 1973; Torelli 1984).

The lower moisture contents of heartwood are not low enough to prevent fungal decay. Fungal activity is limited only when *free* water is completely absent from wood (moisture content < 30%). Heartwood moisture content is typically above this level (USDA-FS Forest Products Laboratory 1999), suggesting that moisture contents change when heartwood forms, but not enough to prevent fungal attack.

Lack of water transport. — The transport of water from the roots to the crown is one function of stems. All of this transport in living trees occurs in the sapwood. However, not all parts of the sapwood are equally important in conduction, nor is conduction the only function. Heartwood transports no water. Embolism of the water-conducting conduits and closure of the pits connecting cells are both associated with lack of conduction in wood.

Embolism. — Water movement through sapwood is generally explained by the "cohesion-tension" theory: A continuous string of water is pulled up the tree by evaporation at the leaves. Because of the tensile stress in the water column, the water in the cell lumens in the sapwood is under considerable tension. The entry of a gas bubble into any lumen—an "embolism"—will break the string and the conduction of water along that pathway will cease (Zimmermann 1983). Embolisms reduce the number of functioning sapwood cells producing more negative water potentials that further increase the risk of embolism.

Embolisms and their removal are believed to be continuous processes in sapwood maintenance. It has been proposed that the living cells of the sapwood are responsible for embolism reversal (Zwieniecki and Holbrook 1998; Wilson and Gartner, 2002), but there is also some evidence against this explanation (Borghetti et al. 1991).

Sperry et al. (1991) observed degraded pit membranes (allowing embolism) in a *Populus* species and concluded that their breakdown was the

cause of heartwood formation. However, similar processes have not been observed in other species. In fact, pit connections between cells often become tightly sealed during heartwood formation, greatly reducing wood permeability.

Shigo and Hillis (1973) suggested that changes in moisture content lead to the production of ethylene, which in turn stimulates heartwood formation (see below). However, Taylor and Cooper (2002) induced dramatic moisture content changes in the sapwood of *Pinus resinosa* through girdling, and heartwood production was not observed.

Pit closure. — Bordered pit pairs are specialized connections between adjacent xylem cells. The membrane separating the two cells contains a thickened torus in gymnosperms and some angiosperms (Carlquist 1988). "Aspiration" occurs when the torus moves to one side of the pit pair, blocking the connection and inhibiting fluid flow. Aspiration is believed to take place when free water is withdrawn from a lumen; the surface tension of the receding water layer draws the torus to the side (Panshin and de Zeeuw 1980). In the living tree, it is thought to occur when water tension becomes so high that the pit membrane can not exclude air bubbles. The bubble is sucked into a tracheid or vessel, the bubble expands to fill the tracheid or vessel, and that tracheid or vessel has less tension than the cells around it. The pit membranes are pushed away from the embolized cell because of the tension differences in the water column in an action that aspirates the pits.

Aspirated pits have been observed in a number of coniferous species (Fujii et al. 1997; Krahmer and Côté 1963) and in a few hardwoods (Dute and Rushing 1987, 1990). Harris (1954) observed bordered pits in *Pinus radiata* and found that the percentage of aspirated pits gradually increased from the cambium inward, reaching about 50% at the sapwood/transition zone boundary. At that point, the percentage of aspirated pits increased abruptly to about 90%. Fujii et al. (1997) found that the percentage of aspirated pits varied in the heartwood of samples of *Cryptomeria japonica* from different trees. They suggested that this variability was related to the relatively high moisture content of the heartwood in that species, although this correlation was not shown in their results.

Pits can be blocked by encrustation with extractives (Krahmer and Côté 1963; Panshin and de Zeeuw 1980) in the heartwood of some species in a process that can be independent of aspiration (Fujii et al. 1997). Encrustation occurs abruptly at the border between the sapwood and the transition zone (Yamamoto 1982).

Pit closure by aspiration and/or encrustation may reduce the ease of movement of decay organisms through the wood. Pits are the paths of least resistance to hyphal movement, and pit closure should make this movement more difficult in softwoods because the solid torus must be penetrated in aspirated pits, instead of the netlike margo (Boddy 1992).

Ethylene production. — Bamber (1976) suggested that the centripetal movement of a heartwood-inducing substance stimulated heartwood formation. Since then, numerous authors have investigated the possible roles of ethylene and various enzymes in heartwood formation. Ethylene is a phytohormone that can be produced by all plant tissues in small amounts (Hillis 1987) and is associated with such processes as fruit ripening, and flower and leaf senescence. Ethylene also has been linked to increases in enzyme activity and to the production of heartwood polyphenolic extractives (Hillis 1987). Greater ethylene production related to heartwood formation (at the heartwood/sapwood boundary or in the transition zone) has been observed in the dormant season in a number of species (Hillis 1987), but the relationship, if any, between ethylene production and the quantity or quality of extractives is unknown.

Enzyme activity. — Enzymes that have been associated with heartwood formation include malic and glucose-6-phosphate dehydrogenases (Shain and Hillis 1973); tyrosinase and peroxidase (Nelson 1977); acid phosphatase, adenosine triphosphatase, glucose-6-phosphatase, lipase, glucose-6-phosphate dehydrogenase, succinate dehydrogenase and peroxidase (Baqui and Shah 1985); phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) (Magel et al.1991); lipase and phosphclipases (Hillinger et al. 1996); sucrose synthase (SuSY) and neutral invertase (NI) (Hauch and Magel 1998); and 6-phosphogluconate dehydrogenase (Magel et

al. 2001). These various enzymes have been observed to be active in the vicinity of the transition zone or the sapwood/heartwood boundary, and have been implicated in the breakdown of the storage materials starch and fat and in the formation of heartwood-type extractives (e.g., Baqui and Shah 1985; Hillinger et al. 1996; Hauch and Magel 1998, Dehon et al. 2001).

Nair and Shah (1983) observed increased acid phosphatase, ATPase, and succinate dehydrogenase activities following the induction of heartwood by the application of the herbicide paraquat to *Azadirachta indica*. Succinate dehydrogenase and acid phosphatase were also observed during naturally occurring heartwood formation in the same species (Nair 1988).

Enzymes present in the sapwood have also been implicated in the non-microbial staining of sapwood after harvest. Forsyth and Amburgey (1992) noted that such staining often begins at the heartwood/sapwood boundary and progresses into the sapwood.

Depletion in storage compounds. — Heartwood is known to be low in energy reserve compounds such as sugars, starch, or lipids (Bamber and Fukazawa 1985; Hillis 1987). Gradual decreases in reserve materials in the sapwood from the cambium inwards are frequently observed (e.g., Saranpaa and Holl 1989; Magel et al. 1994, 1995). Nobuchi et al. (1987a) also observed gradual decreases in some species with wide sapwood, but found relatively constant levels throughout the sapwood of other species with narrow sapwood. Magel et al. (1994, 1995) observed gradual decreases in energy

compounds in the narrow sapwood zone of *Robinia pseudoacacia;* however, relatively high levels of these compounds were still at the heartwood boundary, although they were absent from the adjacent heartwood. Changes to—but not depletion of—lipid droplets in parenchyma cells have been observed in association with heartwood development in some species (Hillis 1987).

Removal or accumulation of elemental nutrients. — Trees may recycle nutrients from senescing sapwood back into living parts of the tree. Okada et al. (1993a, 1993b) identified three patterns of radial nutrient distribution: In Type 1, element concentrations increased outward from the pith across the heartwood/sapwood boundary. In Type 2, the pattern is reversed. In Type 3, there is a peak in element concentration at the heartwood sapwood boundary. Alkali metals and halogens typically follow Type 1 or 3 patterns, whereas alkaline earth metals follow a Type 2 pattern. Softwoods generally contain higher elemental concentrations in their heartwood, while the hardwoods studied contain higher concentrations in their sapwood.

Myre and Camire (1994) organized nutrient distribution in *Larix* species into three groups: "mobile" (P and K), "intermediately mobile" (Mg and Zn), and "immobile" (Ca and Mn). They observed that sapwood contained relatively high concentrations of mobile nutrients, while heartwood contained high concentrations of intermediately mobile and immobile nutrients. They

suggested that an increased number of exchange sites, changes in pH, and translocation of nutrients towards the pith may account for these differences.

Andrews and Siccama (1995) observed decreases in the concentrations of calcium and magnesium from the pith to the outer heartwood. They speculated that this pattern reflected changes in the availability of these nutrients in the soil over time. The significance of differences in elemental distribution between sapwood and heartwood is unclear. Substantial amounts of an element can make the wood harder and more resistant to insect or marine borer attack, and can alter the wood/moisture relationship. Generally, however, the differences noted in these studies were not of a magnitude that would produce these effects.

Formation of extractives. --Heartwood extractives are responsible for the distinctive color, odor, and luster of some heartwoods. Because color is evident to the naked eye, the presence of a color change in the xylem is often used as the indicator of heartwood formation, even though other changes associated with heartwood formation (e.g., parenchyma death) may not have occurred (Nobuchi et al. 1984).

Heartwood extractives form at the heartwood/sapwood boundary (or in the transition zone) using locally available compounds and materials translocated from the phloem and sapwood (Hillis 1987). The chemistry of extractive biosynthesis has been investigated in a variety of species (e.g., Burtin et al. 1998; Dellus et al. 1997; Magel et al. 1995). Magel and others

(reviewed in Magel 2000) have identified two types of heartwood formation, based on patterns in the production of phenolic extractives. In type 1, or *Robinia*-type, extractives accumulate in the transition zone. In type 2, or *Juglans*-type, the precursors to extractives gradually accumulate in the sapwood, and these precursors are transformed in the transition zone.

Streit and Fengel (1994) observed that extractives formed in the transition zone impregnated the cell walls, beginning in the middle lamella, and subsequently impregnated the secondary cell walls. They likened this process to lignification. Hergert (1977) coined the term "secondary lignification" for this phenomenon, and Jouin et al. (1988) reported more lignin in the heartwood than the sapwood of *Quercus*. However, Magel et al. (1995) and Gang et al. (1998) were careful to note that the formation of heartwood compounds is a different process than lignification, the process by which lignin is laid down in the formation of xylem cell walls. Magel (2000) refers to "secondary lignification" as "pseudo-lignification", and her analysis of this process has indicated that there are intimate chemical associations between heartwood extractives and the wood structural components.

It is clear that the pattern of extractive microdeposition plays a role in durability. For example, the impregnation of extractives from the heartwood of durable species into non-durable sapwood improved durability, but not to the extent of the original heartwood (Smith et al. 1989).

Formation of tyloses. — Tyloses are bubble-like projections from parenchyma into the lumens of adjacent vessels of some hardwoods, and occasionally in conifers (Gerry 1914), that are composed of material similar to the cell wall of adjacent cells (Chattaway 1949). Tyloses are believed to form as the result of enzymatic hydrolysis breaking down the pit membrane between a parenchyma cell and an adjacent vessel. This allows a portion of the parenchmya protoplast to extrude into the vessel lumen (Murmanis 1975). Tyloses inhibit fluid flow, which can limit moisture uptake and may physically impede the movement of pathogens through wood.

The relationships between heartwood formation processes

The temporal, spatial, and causal relationships between heartwood processes have received less attention than have the various processes themselves. Working with a number of species, Frey-Wyssling and Bosshard (1959) suggested that there is a gradual decrease in parenchyma activity in the sapwood going towards the heartwood. Aerobic respiration breaks down in the transition zone and anaerobic conditions develop. These conditions lead to the hydrolysis of starch, and once the starch is depleted, colored extractives are produced.

Fahn and Arnon (1962) studied the sapwood to heartwood transition in *Tamarix aphylla*. They noted that, when going from the cambium to the heartwood border in sapwood, starch grains disappeared first, followed by

inactivity of the parenchyma, and finally disintegration of the parenchyma nuclei.

Bamber (1976) suggested that parenchyma death was the result, not the cause of heartwood formation. This idea is supported by the observations of Nobuchi et al. (1984), who found that heartwood extractives were formed before parenchyma death in *Robinia pseudoacacia*.

When observing changes to parenchyma in *Pinus* species, Yamamoto (1982) determined that heartwood formation began with various changes to the parenchyma cells, followed by encrustation of bordered pits, and reductions in reserve materials and moisture content. Heartwood formation concluded with the degradation of the parenchyma nuclei.

Nobuchi, Harada, and their colleagues studied temporal and spatial relationships between some heartwood variables in their research. Moisture content decreases, and pits aspirate at the sapwood/transition zone boundary in *Cryptomeria japonica*. The parenchyma die in the transition zone, and extractives appear at the transition zone/heartwood boundary (Nobuchi and Harada 1983; Nobuchi et al. 1987b). Nobuchi et al. (1984) tracked temporal and spatial relationships between heartwood processes in *Robinia pseudoacacia* and found viable parenchyma inside the "heartwood" (defined as the colored region) for much of the summer and fall. Starch was depleted from sapwood adjacent to the heartwood, but only during the summer. A similar pattern was seen in the presence of large lipid droplets in the

parenchyma, and these observations imply that heartwood formation is gradual, not instantaneous.

The biochemistry of heartwood formation has been studied extensively in *Robinia pseudoacacia* by Magel and her coworkers, and their work has been summarized in Magel (2000). Magel (2000) describes heartwood formation as a programmed cell death that has similarities to the senescence of leaves.

The time of year of heartwood formation

The time of year that heartwood forms has been the subject of some debate. Difficulties in determining the time of heartwood formation include choosing which variable to measure, where to look for it, and how to sample the same individual repeatedly without affecting the measurement.

Much evidence suggests that heartwood forms in the dormant season. Shain and Hillis (1973) measured increased ethylene in the transition zone in the winter in *Pinus radiata*. Shain and Mackay (1973) found increased respiration and enzyme activity in the dormant season in the transition zone with the same species. Nelson (1977) studied ethylene concentration, enzyme activity, and nitrogen content in *Juglans nigra* and *Prunus serotina*, and concluded that heartwood formation occurs in the dormant season. Magel et al. (1991, 1995) and Hauch and Magel (1998) found that enzyme activity related to heartwood extractive synthesis in the transition zone was highest in the autumn in *Robinia pseudoacacia*.

Yamamoto (1982) studied the "maturation" processes in parenchyma in the inner sapwood and transition zone of *Pinus* species, concluding that "the season of heartwood formation is not the period when cambial growth is vigorous, but is the period when cambial activity declines" (Yamamoto 1982, p. 288). Similarly, Yang (1992) observed changes in ray parenchyma nuclei in the inner sapwood of *Pinus banksiana*, *Picea mariana*, and *Populus tremuloides* in the northern hemisphere. He found that the greatest change in these nuclei occurred in August, July-August, and August-October, respectively, and also concluded that heartwood formation was initiated at those times. However, Fukazawa et al. (1980) stated that, while some parenchyma necrosis took place in the dormant season, other parenchyma senescence patterns were independent of season.

Baqui and Shah (1985) did not observe seasonal patterns in heartwood-related enzyme activity in *Acacia auriculiformis*, and concluded that heartwood formation occurred throughout the year. Bergstrom et al. (1999) found no change in the concentration of a heartwood extractive at the heartwood/sapwood boundary in *Pinus sylvestris,* and thus concluded that there was no particular time of year for heartwood formation.

Most reports used color differences to mark the boundary between heartwood and sapwood. This demarcation is convenient, but it defines heartwood as the area where colored extractives are present, rather than the region of dead parenchyma. It is also a mistake to treat the colored zone as a

fixed target, since changes in the position of the colored boundary over time may better delineate "the season of heartwood development" than observations of activity in one fixed area of the xylem. Nobuchi et al. (1984) showed that different components of the heartwood formation process happen at different times, and in different locations in relation to the colored zone. The colored boundary of the heartwood in *Robinia pseudoacacia* (in the northern hemisphere) moves outward from July to September. Starch grains are depleted from, and lipid droplet patterns change in, the sapwood adjacent to the heartwood, just before the expansion of the colored zone. The parenchyma in the newly formed heartwood slowly die from September through the following spring. These studies indicate that heartwood formation is a stepwise process, occurring over much of the year, that ends with cell death.

2.6 VARIATIONS OF HEARTWOOD DISTRIBUTION AND QUALITY IN TREES

The amount of heartwood within and between trees and species has received considerable study. Patterns of variations in the quality—e.g., the chemical make up or natural durability—of heartwood are less well understood.

Macro distribution of heartwood

Heartwood volume. — Sapwood width, and consequently heartwood proportion, varies greatly between species. As extreme examples, *Catalpa*

speciosa has 1–2 annual rings of sapwood, whereas there are 80–100 sapwood rings in *Nyssa sylvatica* (Sargent 1926). Sapwood width also varies within species, due to genetic, environmental, and tree age differences.

Smith et al. (1966) observed decreasing sapwood thickness with height in *Pseudotsuga menziesii*. In contrast, sapwood widths have been observed to be constant from the base of trees to the crown (Brix and Mitchell 1983; Megraw 1986; Nobuchi et al. 1987b; Gominho and Pereira 2000), although the number of sapwood rings decreases with height. Thus the heartwood portion often tapers from the ground up (e.g., Yang et al. 1994); however, heartwood width in some *Pinus* species increases from the base to about 1–3 m, and then decreases to the top of the tree (Wilkes 1991; Stokes and Berthier 2001). Sapwood volume can have important consequences with regard to water transport and stem storage, but there appears to be no consistent heartwood/sapwood relationship among many species.

Extractives content. — The outer heartwood at the base of the tree in most species studied is the most decay-resistant heartwood (Anderson et al. 1963; Scheffer and Cowling 1966). This pattern has been associated with a decrease in extractive content towards the pith and up the tree (Hillis 1987).

There are exceptions to this trend, especially when individual components are considered. Hillis (1987) refers to work that found no trends in the radial distribution of extractives in the heartwood of *Pseudotsuga menziesii*, *Pinus ponderosa*, and *Pinus radiata*. Nobuchi et al. (1987b) found

that heartwood extractives were highest at the heartwood/transition zone boundary in boles of *Cryptopmeria japonica*. However, extractive concentrations in the crown area increased towards the pith. Chui and MacKinnon-Peters (1995) found relatively high concentrations of extractives in heartwood of young larch trees.

Morita et al. (1995) observed slightly increased concentrations of extractives in the upper portions of the heartwood of one *Cryptomeria japonica* tree. They also found that, although extractives concentrations generally decreased towards the pith, two of the component chemicals increased in concentration. Mosedale et al. (1996a) studied heartwood extractive patterns in *Quercus* species and observed that the concentration of total soluble ellagitannins showed a logarithmic decline from the heartwood boundary towards the pith, while individual ellagitannins varied in their response. Burtin et al. (1998) found that concentration of phenolic compounds in the wood of *Juglans* species peaked around the transition zone, whereas the remaining compounds had greater concentrations in the heartwood.

The pattern of lower extractives near the pith may reflect the degradation of extractives over time or an increase in extractive deposition with age. DeBell et al. (1999) found that lower extractive levels near the pith of *Thuja plicata* were associated with juvenile wood, and that aging of the wood seemed to have little effect Nault (1988) found similar patterns in the same species. Van der Kamp (1986) observed that staining fungi that occur naturally

in heartwood could reduce thujaplicin content in *Thuja plicata*, although this reduction was not associated with reduced decay resistance. Gartner et al. (1999) found no radial or horizontal patterns of decay resistance in young *Pseudotsuga menziesii* trees. However, the correlation between decay resistance and total heartwood extractive content can be poor, suggesting that more subtle differences in extractive composition or distribution may be important.

Micro distribution of heartwood extractives

Extractives are located mostly in the rays (Hillis 1972). Côté et al. (1966) observed that when arabinogalactan of *Larix occidentalis* was removed by extraction with water, the volume of the wood remained nearly constant, suggesting that almost all of the polysaccharide was located outside the cell wall (i.e., in the lumen). However, extractives can also form coatings on the cell wall and on the pits, and can penetrate the cell wall itself. In some cases, differences in heartwood dimensional stability have been attributed to the presence of cell wall extractives (Bosshard 1968; Hillis 1972).

Côté et al. (1966) found that earlywood in *Larix* contained more arabinogalactan than did latewood. They attributed this pattern to larger lumen size in the earlywood cells. Extractive levels peaked at the middle of the earlywood zone of *Pseudotsuga menziesii* and were minimal at the beginning and end of the annual ring (Squire et al. 1967). By contrast, Lloyd (1978) observed more extractives in the latewood portion of the annual ring of *Pinus*

radiata. This distribution was explained by Harris (1965) as a result of the exudation of resin from transverse resin canals after heartwood was formed, because latewood cells were less likely to aspirate when heartwood forms. Kuo and Arganbright (1980) reported that the proportion of heartwood extractives in the cell wall (as opposed to the lumen) increased in the inner portions of the heartwood of a *Sequoia sempervirens* tree and a *Libocedrus decurrens* tree. Pensar (1967) reported that the extractives in the earlywood of *Picea abies* contained a slightly higher proportion of extractives than the latewood. The resin acids and other terpenoids were concentrated in the resin canals of *Picea*, while fatty acids, glycerides, waxes, and sterols were located in the ray parenchyma cells (Kimland and Norin 1972). Although these reports imply differences in microdistribution of various extractives, there are few methods for delineating extractive distribution in situ. As a result, most relationships are inferred by extraction studies of whole wood sections.

2.7 CONTROL OF HEARTWOOD FORMATION AND DURABILITY

There has been relatively little work on factors controlling heartwood quantity and even less on heartwood quality (i.e., natural durability).

Sapwood area homeostasis

The concept of a dynamic relationship between sapwood area, sapwood permeability, and foliage area is not new (e.g., Gartner 1991; Margolis et al. 1988; Whitehead et al. 1984). Much of the research into this hypothesis has been directed toward estimating foliage biomass and modeling

stand and ecosystem dynamics (e.g., Margolis et al. 1995). If there is a homeostatic balance between sapwood area and leaf area, then it is a logical extension that the amount of sapwood (and thus the proportion of heartwood) can be influenced by the manipulation of the other variables.

Leaf-area-to-sapwood-area ratio (A_l:A_s) may vary with species (Grier and Waring 1974), soil water availability (White et al. 1998), average relative humidity (Mencuccini and Grace 1995), tree age (Dean and Long 1986), tree vigor (Sellin 1996), stand density (Shelburne et al. 1993), soil nutrient availability (Brix and Mitchell 1983), and height in the tree (White et al. 1998). The influence of each of these factors on heartwood formation will be addressed separately in the following discussion.

In most of the research into changes in A_l:A_s, it is not stated whether sapwood area changes when A_l:A_s ratios changes. Shelburne et al. (1993) suggested that leaf area changes are primarily responsible for the differences in A_l.As observed between stands of different basal areas. This assertion suggests that sapwood area (and hence heartwood area) is not easily modified by adjustments to the "homeostatic balance." Langstrom and Hellqvist (1991) observed reductions in the area of conducting sapwood in pruned young *Pinus sylvestris* trees. The reaction time was found to be quite slow; the homeostatic balance was not completed four years after the treatment. The authors were also careful to state that the sapwood that was no longer conducting was "immobilized sapwood" and not heartwood. It has not

yet been demonstrated clearly that heartwood volumes can be manipulated by alterations to the homeostatic balance.

Inter-specific variations in heartwood

Species differ widely in their relative amounts of heartwood, as well as in the quantity and composition of the extractives. The between-species differences in the durability of wood has long been of interest to users of wood products. The natural durability of wood of many species has been evaluated by a multitude of methods in many species (reviewed in Scheffer and Morrell 1998).

It is difficult to describe consistent trends in the natural durability of the wood of different species. Many tropical species, but not all, are durable with respect to biodeterioration, but there are also very durable species in temperate regions. It is reasonable to suppose that long-lived tree species would require durable heartwood that could last for many years. Indeed, many long-lived species have durable heartwood, but some do not (e.g., *Picea sitchensis*). These long-lived species with relatively low heartwood durability may adapt to longevity with highly active defense mechanisms in the sapwood parenchyma that limit wound invasion and thus exclude invaders from the decay-susceptible heartwood.

Factors influencing intra-specific variations in heartwood

Genetic control over heartwood formation. — Studies have been made of the genetic influence over both the quantity and quality of heartwood formed

in a number of different species. A review of the literature relating to genetic control of heartwood formation noted "reasonably strong genetic control" of heartwood area in mature trees of *Pinus* and some diffuse-porous species (Zobel and Jett 1995, p. 184). More recently, researchers have estimated the heritability of heartwood diameter of 25-year-old trees to be lower than that of 44-year-old *Pinus sylvestris* trees, with moderate values of 0.3 and 0.5, respectively (Fries and Ericsson 1998, Ericsson and Fries 1999). The authors cautioned that some work has overestimated the heritability of heartwood formation, but they suggested that breeding for heartwood was possible.

Fries (1999) found that variation in heartwood traits (number of annual rings and width) due to provenance was small compared with within-provenance variation in mature *Pinus sylvestris*. He suggested that family effects and stand characteristics had a greater influence on heartwood characteristics. Pâques (2001) measured high heritability levels for heartwood proportion in *Larix* sp. Woeste (2002) observed wide phenotypic differences in heartwood area in a progeny test of 35-year-old *Juglans nigra*. Much of the variation was attributable to differences in tree diameter, but statistically significant family effects for heartwood area were noted.

Overall, there appears to be significant genetic control of heartwood proportion within species, but environmental influences can be equally important.

There is often strong genetic control over the production of extractives in wood, particularly in older trees (Zobel and Jett 1995). It is unclear why this relationship becomes more evident in older trees. Rudman and DaCosta (1959) suggested that variations in the heartwood of *Tectona grandis* were more genetic than environmental, based on the observation that trees on the same site, with roughly the same growth rate, differed in decay resistance.

Rink (1987) found no genetic control over heartwood color in young *Juglans nigra* trees. Mosedale et al. (1996b) found that heartwood ellagitannin content in *Quercus* species was under strong genetic control, whereas heartwood color (the result of extractives) was under less genetic control.

The concentration of specific heartwood extractives was found to vary widely between individual trees and to be highly genetically correlated in *Pinus sylvestris* (Fries et al. 2000; Ericsson et al. 2001); however, these concentrations were not well correlated to the amount of heartwood.

Venalainen et al. (2001) studied the repeatability of heartwood characteristics in *Larix sibirica*, and observed moderate genetic influence over the decay resistance of heartwood, but less heritability of heartwood amounts. Harju et al. (2001) found low heritability of decay resistance to a brown rot fungus in *Pinus sylvestris*, and very wide phenotypic range in heartwood resistance. They concluded that genetic gains in breeding would only be possible in combination with the testing of environmental influences.

Genetic control of extractive production has considerable economic potential in some species. Squillace and Harrington (1968) reported a doubling of doubling of oleoresin yields as a result of selection and breeding in *Pinus* species. Franklin et al. (1970) predicted further genetic gains in extractive yield of about 12%. The possible effect of increased heartwood extractives on overall durability is difficult to predict, but one likely gain would be to create more uniform durability. In many cases, field tests of naturally durable species produce sporadic early failures, possibly as a result of heartwood samples with less extractives. Increased overall heartwood extractive content may help reduce the occurrence of these failures.

Tree age. -- Heartwood formation lags behind the growth of the pith and new sapwood layers by a time mostly governed by genetic differences between species (Hillis 1987). Once heartwood formation begins, heartwood is added on a more or less regular basis, gradually progressing out radially and up the tree (e.g , Hazenburg and Yang 1991a, 1991b).

Heartwood volumes are cumulative, whereas sapwood areas are not (rather, they are the sum of new sapwood from the new annual increments minus the loss to heartwood). Thus, the proportion of the bole that is heartwood increases with tree age (Sellin 1996). Various authors have developed regression equations relating age to the amount of heartwood or sapwood in different species (e.g., Hazenburg and Yang 1991a, 1991b; Yang and Hazenburg 1991a, 1991b; Sellin 1991, 1994, 1996).

Radial growth early in the life of the tree is a good predictor of heartwood diameter in some species (Climent et al. 1993; Hillis 1987; Wilkes 1991). Wilkes (1991) found that heartwood formation commenced earlier in the upper parts of *Pinus radiata* trees, even though the diameter of heartwood was less.

The amount of extractives in heartwood generally increases with distance from the pith. Thus, the age of the tree influences heartwood extractive content (Hillis 1987). Nault (1988) observed higher concentrations of extractives in the heartwood of older trees than in younger trees of *Thuja plicata*, and suggested that wood from young trees would be less resistant to decay. Krilov and Lasander (1989) found that wood of mature trees of *Eucalyptus* species had more gallic acid (a principle heartwood extractive) than did "regrowth" (the nature of the "regrowth" was not specified). In both these cases, however, the effect seems to be a result of the maturity (distance from the pith), rather than any consistent difference in the heartwood quality (see for example DeBell et al. 1999).

Gartner et al. (1999) studied young *Pseudotsuga menziesii* and found no radial or vertical variations in heartwood decay resistance. This result *may* indicate that the radial and vertical patterns in heartwood extractives present in older trees are not present in younger trees. However, given the sometimes-poor correlation between extractives content and decay resistance, this conclusion is not certain.

Tree vigor. — Tree vigor seems to have less predictable effects on heartwood volumes than do genetics or age. Hillis (1987) and Nair (1999) reviewed literature that demonstrated a positive relationship between early growth rate and heartwood proportion, as well as other studies showing that faster-grown trees produced less heartwood. This characterization can have important implications in aggressively managed plantations. Since Hillis' review, Wilkins (1991) and Gominho and Pereira (2000) have observed positive correlations between growth rate and heartwood volumes, but both studies were based on young trees.

Wilkes (1991) warned against misinterpreting those factors that stimulate growth of trees overall as influencing heartwood/sapwood proportions in *Pinus radiata*: "heartwood development is very much an age-related process, i.e., the heartwood boundary progresses outward at a more-or-less set fraction of an increment per annum, and where rings are wider more heartwood is produced" (Wilkes 1991, p. 89).

Kaufmann and Watkins (1990) studied old stands of *Pinus contorta* and found that the low-vigor trees had higher heartwood volumes than did the high-vigor trees. However, the age of the heartwood was similar in the high- and low-vigor trees. These observations indicate that the low-vigor trees in this study had grown more quickly in the past (overall volumes were similar), and the greater volumes produced in the past had since been transformed into heartwood. Sellin (1991; 1994) also found that suppressed *Picea* trees had a

greater percentage of heartwood than dominant trees had. Again, this pattern was due to the relatively narrow sapwood growth rings—the number of rings of sapwood was similar for the two groups (Sellin 1991). Yang and Hazenburg (1992) found that faster-growing *Picea* trees (as a result of wider spacing) in a 38-year-old plantation had more heartwood rings and area.

De Kort's (1993) study of relatively young (25–70 years old) *Pseudotsuga menziesii* gave contradictory results of the relative amount of heartwood in suppressed trees. In general, lower-vigor trees had higher heartwood volumes than did more vigorous trees. In non-vital trees (more than 60% needle loss) trees the number of sapwood rings was similar to that of the "vital" trees. However, trees with intermediate vigor ratings had higher heartwood proportions, even though the number of sapwood rings was *higher* than in the vital trees. Clearly, the relationship between tree vigor and heartwood formation merits further study.

Hillis et al. (1962) suggested that the amount of photosynthate available for translocation could influence the quantity of heartwood extractives produced. However, a poor understanding of allocation hierarchies in trees complicates the relationship between tree vigor and photosynthate supply: healthy trees might have more photosynthate than less vigorous trees, but they then may allocate more of that photosynthate into growth, reproduction, or other activities. Hillis's (1987) review cited examples of work that demonstrated decreased extractives in faster-grown trees of some species, as

well as other examples where no such relationship was observed. Wilkins and Stamp (1990) found that the outer heartwood of young, faster-grown (as a result of silvicultural treatments) *Eucalyptus grandis* trees was darker in color than that of more slowly grown trees. Magel (2000) has attributed annual differences in heartwood extractives in *Robinia pseudoacacia* to fluctuations in available sucrose caused by climate variations.

As foresters shift to more aggressive management of naturally durable species, understanding the potential effects of thinning, fertilization, and other stand manipulations will be essential for helping foresters make informed management decisions.

Wood structure differences. — Hillis (1987) reported on the lowered amounts of heartwood extractives in compression wood and tension wood than in normal wood, and suggested that this phenomenon may be due to reduced carbohydrate levels, increased lignin content (in compression wood), or changes in ethylene production. Blanchette et al. (1994) found that compression wood was more resistant to decay than was normal wood, but that tension wood was similar in decay resistance to normal wood.

Heartwood extractive synthesis occurs in the living cells in the sapwood (ray and axial parenchyma), thus there has been some speculation that the amount of parenchyma could influence the amount of extractives produced. Hillis et al. (1962) observed that there were fewer parenchyma and less heartwood extractives in the tension wood than in normal wood of *Angophora*

costata. Hemingway and Hillis (1970) also observed a positive correlation between parenchyma volume and heartwood extractives in *Pseudotsuga menziesii*. However, Nelson (1975) concluded that physiological conditions at the heartwood/sapwood boundary were more important than the amount of parenchyma in determining the amounts of extractives produced in two temperate hardwoods. More recently, Climent et al. (1998) found that *Pinus canariensis* trees with larger than predicted heartwood diameters had greater proportions of axial parenchyma.

Polge (1985) found less heartwood area in the tension wood regions of trees of a *Populus* species. Stokes and Berthier (2001) observed that the proportion of heartwood to sapwood stayed the same around the cross-section of *Pinus pinaster* trees containing compression wood, although more rings were incorporated into heartwood in the compression zone.

Site quality. — Site differences can influence the growth rate of trees and can be associated with differences in genetic stock. Thus, site considerations can easily be confounded with the tree vigor and genetic considerations described above.

Hillis (1987) refers to work that suggests that poor sites may delay the initiation age of heartwood formation or decrease heartwood proportion. A number of studies measured differences in the extractive contents of heartwood, but in those cases, the effect of site was not distinguished from genetic and other environmental factors. Harris (1954) found that adequate

water supply, and the absence of drying conditions favored heartwood development in *Pinus radiata*. Climent et al. (1993) determined that site characteristics (water balance and exposure to winds) related more strongly to sapwood width and diameter growth than to heartwood width in *Pinus canariensis*. Recently, however, Climent et al. (2002) concluded that climate is related to heartwood area, even after accounting for variations in early growth rate. Their models predict wider heartwood in drier climate sites versus wet, high altitude sites.

Guyette et al. (1992) observed that the mineral elements present in heartwood (and sapwood) were related to their abundance in the soil. It was even possible to observe changes in the environment in the past (e.g., the advent of lead smelting in the area) by observing changes in the elemental concentration between annual rings of *Juniperus virginiana*. As previously mentioned, soil nutrient availability influenced the degree of nutrient recycling from senescing sapwood back to the active sapwood in *Chamaecyparis thyoides* (Andrews et al. 1999). Pothier et al. (1989) found changes in sapwood conductance because of site differences, but observed that the number of rings of sapwood stayed the same regardless of site in *Pinus banksiana*. Phelps et al. (1983) studied influences on heartwood color in *Juglans nigra* and found that within-site differences were more important than between-site differences. Mosedale et al. (1996b) found that the extractive content in *Quercus* species varied little among ramets of the same clone

grown on two contrasting sites. Moraes et al (2002) have suggested that observed differences in resistance to termite attack among *Eucalyptus* sp. may be indirectly due to site differences, since trees growing on richer sites will be more vigorous and may produce more heartwood extractives. These studies indicate that site can influence wood mineral content and tree vigor, but suggest that other factors affect heartwood quality more directly.

Frost, Diseases, and Pollution. — Cold weather and extreme climate may induce irregular heartwood formation in some hardwood species (Krapiec 1999). Such heartwood zones are sometimes called frost heart or moon rings, and may or may not resemble true heartwood (Dujesiefken et al. 1984; Hillis 1987; Charrier et al. 1995).

Infestation of trees by the insect *Adelges piceae* has been shown to increase the area of heartwood in *Abies* species (Hillis 1987; Hollingsworth et al. 1991). Various pathogens that attack trees can induce a wound response in the sapwood, but, although wound tissue shares some characteristics with heartwood, this tissue is not true heartwood (Shigo and Hillis 1973), nor are the biosynthetic pathways the same (Magel 2000). Shigo (1984) also noted that wounding of a tree can cause localized delays in the development of heartwood. Rademacher et al. (1986) observed lower sapwood moisture content in *Picea abies* trees affected by pollution, and slight reductions in the sapwood proportion. However, Bauch (1990) reported that, while pollution may influence growth rates of trees, heartwood formation was not affected.

Overall, it appears that stress and traumatic events do not influence the normal heartwood formation processes. However, the noted localized disruptions to heartwood formation (associated with wounding or frost) may provide model systems for studying heartwood formation processes.

Stand age: Second growth versus old growth. — Old-growth trees may differ from second growth in a number of ways, including tree age, growth rate and genetic makeup. Moreover, the microenvironment experienced by the trees can differ greatly; old-growth trees more frequently live in environments with vertical patchiness of neighbors, and young-growth trees more frequently live in environments with horizontal patchiness. As discussed above, each of these factors can influence the amount and quality of heartwood. so it is difficult to generalize about heartwood quantity and quality as a function of stand age, per se.

Silvicultural treatments. — As with site differences, silvicultural treatments can influence vigor, and thus heartwood formation. Overall, treatment does not appear to induce dramatic changes in heartwood quality, although dramatic effects have been shown in sapwood area, and therefore in heartwood quantity (e.g., Gartner et al. 1999, Bergstom 2000).

Margolis et al. (1988) found that pruning reduced sapwood growth, but that severe pruning (0.2 live crown ratio compared with 0.8 for the controls) increased heartwood diameter. As noted previously, Langstrom and Hellqvist (1991) observed that pruning of young *Pinus sylvestris* decreased the relative

proportion of sapwood, but observed that the area identified as "heartwood" was more likely "immobilized sapwood" (Langstrom and Hellqvist 1991, p. 251). Morling and Valinger (1999) studied the effect of thinning and fertilization on *Pinus sylvestris*. They found that the treatments tended to increase tree and heartwood diameter, but that the number of heartwood growth rings was unchanged. Wilkins (1991) studied the effect of various treatments (ploughing, thinning, weeding, fertilization, and insecticide application) on a young *Eucalyptus grandis* plantation. The treatments increased the rate of growth overall and resulted in a higher proportion of heartwood. Yang and Hazenburg (1992) concluded that wider spacing in 38-year-old *Picea* sp. plantations resulted in more rings of heartwood and greater heartwood volumes. Bjorklund (1999) found that heartwood quantity in *Pinus sylvestris* was not well correlated with spacing, thinning, regeneration method, or site quality. Greater variations were found in heartwood quantity within, rather than between, sites. Thus, it was concluded that genetic manipulation would be more likely to alter heartwood proportions than would silviculture or site selection.

The application of the herbicide paraquat to standing trees, notably *Pinus* species, can induce "lightwood" (sapwood soaked with heartwood-like extractives, reviewed in Hillis 1987). Gref and Stahl (1994) found that mechanical wounding could also induce lightwood. Taylor and Cooper (2002) examined the ability of girdling to induce heartwood formation in live hardwood

and softwood trees. The results were inconclusive, although some heartwood-like changes were observed in the sapwood of girdled trees.

Although many aspects of heartwood formation and the natural durability of heartwood have been studied, the process of heartwood formation remains poorly understood. Attempts to improve heartwood durability will require a more thorough understanding of heartwood formation and the many factors by which it is influenced.

2.8 QUESTIONS

Heartwood is a significant portion of the xylem in mature individuals of many tree species. Heartwood tissues offer many characteristics that are different from sapwood, including lower moisture content, darker color and reduced permeability. Clearly, however, the process of heartwood formation remains poorly understood and requires further study in many commercially important, naturally durable species. Ultimately, the ability to manipulate the amount and quality of heartwood formed in trees would be of enormous practical value; however, many questions need to be resolved before control over heartwood formation is possible. Examples of such questions are as follows:

1. The relationships among the changes during heartwood formation. — Considerable research has been done on the various aspects of the heartwood formation process (i.e., enzyme activity, extractive formation,

parenchyma cell death). However, the sequence, and the temporal, spatial, and especially causal relationships between these factors must be addressed.

2. The importance of "natural durability" to the living tree. — All of the ratings of natural durability are based on tests of the wood once it is removed from the tree, and often under quite "unnatural" circumstances (i.e., gas, moisture compositions). This bias is a result of our desire to gauge how useful the wood will be in various applications, but does not necessarily reflect the tree's requirements for the wood. The often-poor correlation between extractives content and decay rating may reflect, in part, the manner in which the wood is tested. It would be instructive to consider heartwood durability in terms of the function of that durability in the living tree.

3. The costs and benefits of heartwood formation. — It has been proposed that trees require just enough sapwood to transport the water to the transpiring leaf surfaces and that there are metabolic costs of maintaining excess sapwood (Ryan et al. 1995). However, there is also evidence that these metabolic costs are relatively low in inner sapwood (Pruyn et al., 2002), while the costs of heartwood formation can be enormous (e.g., in species that accumulate large amounts of extractives in the heartwood). A cost/benefit analysis of heartwood formation could aid in understanding patterns of sapwood and heartwood in various species.

4. Triggers of heartwood formation. — Many possible initiators of heartwood development have been proposed in connection with the various

processes involved. Carbon dioxide accumulation (Carrodus 1971), ethylene production (Hillis 1987) and desiccation (Jorgensen and Balsillie 1969) have all been suggested as initiating the heartwood formation process; however, none of these satisfactorily explains the observed variety of heartwood formation patterns.

5. Ecological patterns of sapwood thickness and heartwood durability. — It is likely that there are suites of characteristics in heartwood and sapwood that have not yet been elucidated. For example, do diffuse-porous trees consistently have less sapwood than ring-porous ones, given that their hydraulic architecture is so different? Is there a relationship between storage levels in the sapwood and sapwood depth, or heartwood extractive content? Is the permeability of either bark or sapwood to air related to the amount of sapwood?

6. A more thorough understanding of how extractives interact to protect wood. — This knowledge might be used to foster development of trees that are more likely to produce extractives and could also aid in the development of more biorational wood preservatives.

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CHAPTER 3: CO-INCIDENT VARIATIONS IN GROWTH RATE AND HEARTWOOD EXTRACTIVE CONCENTRATION IN DOUGLAS-FIR

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3.1 ABSTRACT

Extractives can have a major impact on the properties of heartwood; however, our understanding of the process of heartwood formation and extractives production is limited and there are few data on how environment affects heartwood extractive content. This study assessed the relationship between growth ring width and extractive content of heartwood in Douglas-fir *(Pseudotsuga menziesii* [Mirbel] Franco) trees. The radial growth rates of the sampled trees were variable over their 53-61 years, in part because of recent stand thinning treatment. The year that each heartwood increment was formed was estimated by assuming that the trees maintained the same number of growth rings of sapwood in the past as it had at the time of sampling. Growth ring width increased after the recent thinning and there was an associated increase in the extractive content of the heartwood estimated to have been formed at the same time. In addition, there appeared to be a rough correlation between growth ring width and extractive content in the time before the thinning. These results suggest that silvicultural treatments that affect growth rate may affect wood durability in Douglas-fir.

3.2 KEYWORDS

Heartwood, growth, natural durability, Douglas-fir, extractives

3.3 INTRODUCTION

Wood extractive content varies from individual tree to individual tree, and probably varies among growth rings, but the relationship between growth and heartwood characteristics is poorly understood. Clearly there are genetic influences on extractive content (Hillis 1987); however, the effects of environmental factors on extractive formation during the conversion of sapwood to heartwood are unknown (Taylor et al. 2002).

Heartwood extractives can influence utilization both positively and negatively. For example, in Douglas-fir *(Pseudotsuga menziesii* [Mirbel] Franco), extractives (primarily dihydroquercetin) give the heartwood moderate durability (Scheffer and Morrell 1998), but can cause problems in the pulping process (Dellus et al. 1997).

Thinning (the selective removal of some individual trees) is a silvicultural practice that increases the resources (e.g. water, nutrients and sunlight) available to remaining trees in a stand. This increase in resources has commonly been observed to increase the growth rate of these remaining trees (Smith 1997). In principle, this increase in resources should increase a tree's net photosynthesis, creating the potential for production of not only more wood, but also additional carbon compounds that could contribute to heartwood extractive formation. The relationship, if any, between practices such as thinning and subsequent heartwood formation remains poorly

understood. The objective of this study was to assess the possible relationship between radial growth rate and heartwood extractive content in Douglas-fir.

3.4 METHODS

Douglas-fir trees were sampled from one site located in the Coast Range in western Oregon, USA (44°38'N, 123°12'W) designated as site class III (McArdle et al. 1961). The stand, consisting of even-aged trees about 60 years old (Table 3.1), was thinned in 1992 (about 12% reduction in basal area) and again in the fall of 2001. Following the thinning in 2001, six basal disks (30 mm thick) were removed from freshly cut stumps about 300 mm above the ground line. We selected this thinned stand to ensure that our samples had significant growth rate variations, not to assess the overall effect of thinning on heartwood extractive content; we will be investigating the effect of thinning on heartwood extractives in future work.

The disks were air-dried in the lab then sanded with a belt-sander. The width of each growth ring (nearest 0.001 mm) was measured along three radial transects from the pith to the cambium on each disk by viewing the sanded disks using a tree-ring measuring device. The three values for each growth ring were averaged.

Next, a radial strip 50 mm wide (tangential) by 30 mm thick (longitudinal) was cut from each disk using a band saw. The heartwood/sapwood boundary was located by staining with alizarin red

(Kutscha and Sachs 1962). The growth rings in the heartwood were then separated from one another using a chisel.

The heartwood annual ring samples were individually ground in a Wiley mill to pass a 20-mesh screen. Air-dried, weighed wood samples (about 1g) were wrapped in cellulose tissues (Kimwipes^{TM}) and the resulting "teabags" were fastened shut with a staple, and re-weighed. The teabags were extracted with 25ml per teabag of a 70:30 mixture of acetone and water (Dellus at al. 1997) for 72 hours on a rotary shaker table (100 rpm), with the solvent drained and replaced twice at 24-hour intervals. No wood flour was observed to leak out of the teabags. Comparison of ^{13}C NMR analysis spectra with that of Foo et al. (1992) indicated that the extractives removed were nearly pure dihydroquercetin (Foo et al. 1992), a biflavonoid that has been shown to be the principle heartwood extractive in Douglas-fir (Dellus et al. 1997). Our trials on wood-free controls indicated that the Kimwipes contained no measurable extractives.

After extraction, each bag was oven-dried (103°C) and re-weighed. Extractive content of the heartwood flour sample was calculated as the percent mass lost from the sample, with a correction for initial moisture content (as determined by oven-drying unextracted wood samples).

The year during which each growth ring in the heartwood region was converted from sapwood to heartwood was estimated by assuming that one

ring of heartwood was created per year; i.e. it was assumed that the trees had the same number of rings of sapwood in the past as it had at the time of harvest (Figure 3.1). The absence of heartwood extractive data prior to 1960 reflects the absence of heartwood in the stems of very young trees.

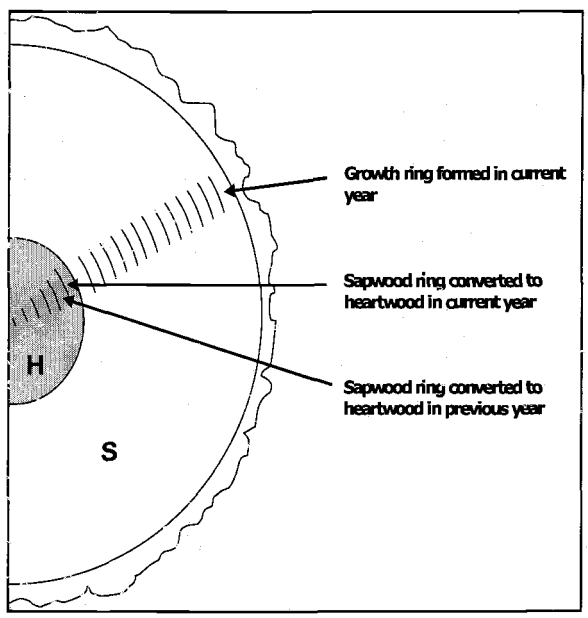

Figure 3.1. A simplified representation of the sampling method, showing how the year of heartwood conversion was estimated for growth rings along a radial transect.Not to scale. The inner, darkened region is heartwood (H) and the outer, lighter region is sapwood (S).

To isolate the general trends from background variability, five-year moving averages were calculated for both the growth ring width and extractive content.

3.5 RESULTS AND DISCUSSION

Although the history of the stand was not known, it appears that, in addition to the 1992 thinning, there were earlier disturbances that affected the radial growth rates of the trees (Figure 3.2) Both growth ring width and heartwood extractive content increased after the 1992 thinning in all six disks. Moreover, growth ring width and extractive content of the heartwood tended to rise and fall coincidentally (Figure 3.2), although this association was less pronounced in the older parts of the wood. We expect that our estimate of the year of heartwood formation was increasingly inaccurate when projected further into the past. All trends were most marked in the three largest trees (Trees A, B and C; Table 3.1).

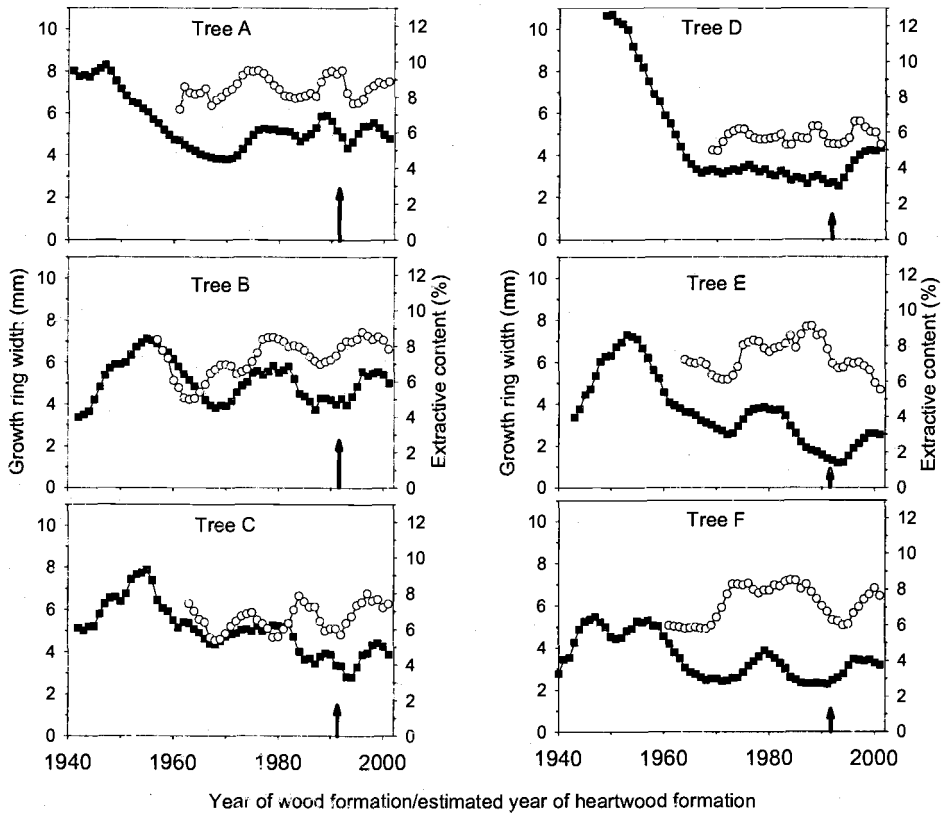

Figure 3.2. Annual growth ring width (-■-) and heartwood extractive content (-○-) vs. year for each of the six Douglas-fir wood samples.All data points are five-year moving averages. The arrows indicate the year of the thinning.

Table 3.1. Size and cambial age of the disks (300 mm above ground).

Tree label	Age (years)	Average radius (mm)
Tree A	61	334
Tree B	60	306
Tree C	60	303
Tree D	53	244
Tree E	59	218
Tree F	62	219

Accurate and precise estimates of the time of heartwood formation would improve our ability to assess the correlation between growth and extractive content. There were undoubtedly errors associated with our assumption that trees in the past had the same number of sapwood rings as in the present, and that one ring was converted from sapwood to heartwood annually; however, we are not aware of a more accurate method for estimating heartwood age in trees. Smith et al. (1966) developed a predictive equation for Douglas-fir sapwood thickness based on tree diameter, which could be used to estimate heartwood growth over time. Their equation predicted heartwood expansion of about one growth ring per year for our trees, but the precision of this estimate was low: ± 20mm, which corresponds to ± 2 to 10 annual rings.

The apparent positive correlation between radial growth rate and heartwood extractive content suggests that when a tree has more resources, it allocates more of the products of photosynthesis to heartwood extractive production. It is also possible that the same amount of photosynthate is being dedicated to extractive production, but that the volume of heartwood produced is decreased. More work is ongoing to assess the consequences of environmental disturbance and silvicultural practices on heartwood properties.

3.6 CONCLUSIONS

Annual variations in a tree's environment appear to influence its heartwood extractive content. These data suggest that there is a positive association between vigor, as expressed by annual growth ring width, and heartwood extractive content in Douglas-fir. This relationship could have important implications for manipulation of heartwood quality and for understanding the dynamics of tree decomposition in ecosystems.

3.7 ACKNOWLEDGEMENTS

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CHAPTER 4. EFFECTS OF GROWTH RATE ON EXTRACTIVE CONCENTRATION IN DOUGLAS-FIR HEARTWOOD

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4.1 ABSTRACT

Environmental changes around living trees can affect the heartwood formation process but the physiological basis of this relationship remains poorly understood. In an effort to understand the linkages between tree physiology and heartwood extractive formation in Douglas-fir, 30 young trees were subjected to one of three treatments: removal of competing vegetation (i.e. thinning), severe pruning or no treatment (control). Removal of the competing vegetation increased growth in the year following treatment, whereas the severe pruning treatment reduced tree growth relative to the controls. Heartwood, sapwood and phloem extractive content did not differ significantly among the treatment groups, nor were heartwood, sapwood or phloem extractive content correlated among the trees. These data suggest that heartwood formation is a complex process that involves a balance of many physiological processes.

4.2 KEYWORDS

Sapwood, phloem, defense, *Pseudotsuga menziesii*, thinning, pruning

4.3 INTRODUCTION

Heartwood is a normally occurring part of tree stems that can often be distinguished by its distinct color or odor. The special characteristics of heartwood are due, in large part, to the presence of non-structural, "extractive" chemicals formed when the oldest portions of the sapwood are converted to heartwood. Because extractives in heartwood can greatly affect the properties of the wood for processing into wood products, there is interest in understanding the nature of environmental and genetic controls over heartwood formation.

Previously we observed that radial growth fluctuations due to thinning in Douglas-fir (*Pseudotsuga menziesii*) trees were associated with differences in the extractive content of heartwood estimated to have been formed at the same time (Taylor et al. 2003). The following paper describes an experiment that sought to explain the physiological basis for this relationship between environmental change and heartwood extractives.

Bryant et al. (1983) proposed a link between environmental conditions and the deposition of carbon-based storage and defensive compounds in the living tissues of small boreal plants. According to their "carbon-nutrient balance hypothesis," carbon reserves either will be accumulated or depleted depending on the balance of nutrient absorption and carbon fixation (photosynthesis). Excess reserve compounds are then converted into carbon-

based defensive compounds that make the leaves, buds and twigs less palatable to the herbivores. Previous studies suggest that this carbon-nutrient dynamic may extend to trees and can be affected by silviculture, especially in cases where the "balance" is altered through fertilization (Sundberg et al. 1993, Von Fircks and Sennerby-Forsse 1998, Viiri et al. 2001, Cheng and Fuchigami 2002, Li et al. 2002).

Because sapwood generally contains free sugar, starch and lipids, whereas heartwood does not, it is generally assumed that these reserve materials in the sapwood are consumed in the heartwood formation process (Hillis 1987). Douglas-fir sapwood also contains phenolic glycosides and other phenolic compounds that are absent from the heartwood and that are believed to be precursors of the heartwood extractives (Dellus et al. 1997). Heartwood extractives also are formed from carbon-based compounds imported from the inner bark (living phloem) in *Eucalyptus sieberi* (Hillis and Hasegawa 1963). Thus it is plausible that the levels of carbon-based reserves in the inner bark and sapwood could influence directly the resulting concentrations of carbon-based extractives in the heartwood.

We hypothesized that tree vigor influences heartwood extractives formation because growth rate affects the levels of extractives in the living parts of the tree stem, and these sapwood and phloem extractives are the raw materials used to produce heartwood extractives. Based on this hypothesis,

we predicted that increasing tree growth by removing competing vegetation would increase sapwood and phloem extractives, which will in turn increase heartwood extractives concentration. Conversely, we predicted that reducing growth by severe pruning would decrease the subsequent production of sapwood, phloem and heartwood extractives.

4.4 MATERIALS AND METHODS

Douglas-fir trees were sampled from one site located in the Coast Range in western Oregon, USA (latitude 44°38'N, longitude 123°12'W, 75m elevation) designated as site class III (McArdle et al. 1961). Ten randomly-selected trees were subjected to each of the treatment regimes. The sample trees were as phenotypically similar as possible before the treatments (Table 4.1). For the "thinning" treatment, all trees and shrubs within 5m of the target tree were cut at ground level and left on the site. For the pruning treatment, all branches were removed from the tree to a height of 8.5m (or not more than an estimated 2/3 of the live crown). Control trees were not treated. All treatments were conducted in December of 2002.

Table 4.1. Age, height, height of live crown, and pruning height of treated Douglas-fir trees measured after felling in 2003 [average (standard deviation)]. N=10 for each treatment.

Treatment	Age (years at 1.3m)	Height (m)	Live crown (m above ground)	Pruned height (m above ground)
Thinned	17(2)	11(2)	2(1)	
Pruned	16(2)	13(2)	3(1)	8(1)
Control	16(1)	14(2)	4(2)	

Two increment cores (5mm diameter) extending well into the heartwood were taken from each tree at 1.3m from the ground. The first core was taken in March, 2003 to serve as a baseline ("2002 growth") before the growing season started. The second increment core was taken in October (the end of the growing season, "2003 growth"), at a 90° angle to the first core. For each increment core sample, additional samples of bark and phloem only were taken with the increment corer from directly above and below the increment core hole to provide sufficient material for the subsequent analyses. All samples were dried to constant weight in an oven at 50°C.

In December 2003, the trees were felled, and a wood disk containing the holes left by the increment corer was removed from the tree. After felling, the height of each tree was measured. The disks were air-dried in the lab and then sanded with a belt-sander. The width of each growth ring (nearest 0.001 mm) from the pith to the cambium was measured along four radial transects on each disk using a tree-ring measuring device consisting of a dissecting microscope mounted over a moving stage connected to a linear variable differential transformer (LVDT) displacement transducer (Acu-Rite Incorporated, Jamestown NY). The four values for each growth ring were averaged.

The heartwood/sapwood boundary on the increment cores and on the wood disk was located by staining with alizarin red (Kutscha and Sachs 1962).

The outermost heartwood ring contained in each increment core was separated at the annual ring boundaries from the rest of the core using a chisel. The outermost sapwood ring(s) (1-3 rings were required to provide enough material for the subsequent analysis) was cut off each increment core. The phloem samples were separated from the outer bark and combined to form the bulk phloem sample for each of the two sampling dates.

The heartwood, sapwood and phloem samples were ground to powder in a ball-type tissue pulverizer mill (Garcia Manufacturing, Visalia, CA) for 2 minutes. The powdered samples (~0.1g) were weighed and enclosed in heat-sealable polyester filter bags (mesh size 25 microns, ANKOM Technology, Macedon, NY), dried at 50°C for 12 hours and reweighed. The bags were then extracted according to the ASTM Standard Method for the Preparation of Extractive-free Wood D1105-84 (ASTM 1996), which involves successive extraction steps with toluene/ethanol (2:1), 95% ethanol, and hot water. The sample bags were then oven-dried at 103°C for 12 hours and reweighed. Total extractive content for each sample was determined as the mass lost from the oven-dried material. The precision of the method was +/-1.6%, measured as the average standard deviation of repeated measurements.

In all analyses, values for before and after the treatment for each parameter were compared for each tree by comparing samples from the two increment cores collected from each tree (i.e. before and after the 2003

growing season). Regression models were used to test for linear relationships among the variables measured (S-PLUS 6.1 for Windows. Lucent Technologies, Inc. Murray Hill, NJ).

4.5 RESULTS AND DISCUSSION

Removal of competing vegetation ("thinning") resulted in increased diameter growth following treatment, while severe pruning treatment reduced individual tree growth relative to the control (Figure 4.1).

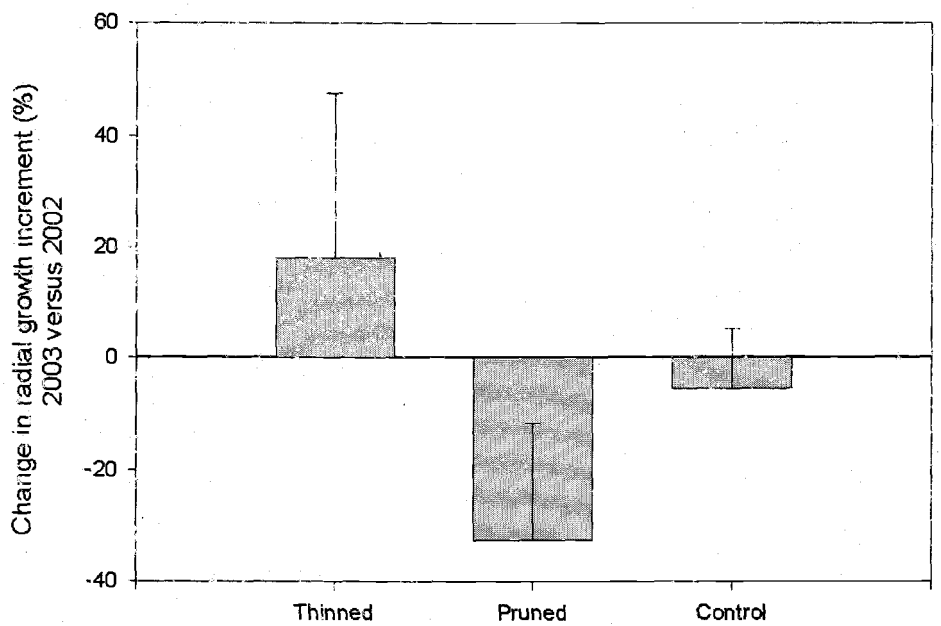

Figure 4.1. Average relative growth rate in 2003 versus 2002 at breast height (1.3m) for the trees in each treatment group. Error bars are one standard deviation.

No differences in heartwood, sapwood or phloem extractive content were found between treated and control trees (Table 4.2), nor were growth

rate and extractive content of the various stem tissues correlated for the individual trees. No significant relationships were found among the extractive content measurements (for linear regression coefficients, all p-values were>0.05).

Table 4.2. Extractive content values for stem tissues in the year after treatment, and change (%) in extractive content from previous year (2003 v. 2002) [average (standard deviation)]. N=10 for each treatment.

Treatment	Extractive content (%)					
	Heartwood		Sapwood		Phloem	
	2003	Change	2003	Change	2003	Change
Thinned	14(5)	5(54)	12(1)	-5(17)	89(23)	6(15)
Pruned	13(2)	0(20)	14(2)	17(22)	85(22)	9(31)
Control	14(3)	-8(26)	14(3)	18(34)	94(18)	7(20)

The results from this experiment differed from a previous study that showed a relationship between growth rate variations and heartwood extractive content in Douglas-fir (Taylor et al. 2003) as well as observations in western redcedar (*Thuja plicata*) that demonstrated a correlation between sapwood extractive levels and extractive concentrations in the adjacent heartwood (Taylor et al. submitted-a). The trees in the current study may not have had sufficient time to respond to the treatments, although this seems unlikely given the rapid response in terms of radial growth. The samples size may have been too small or the precision of the analytical method may have been too low to account for natural variability; however, the average values did not show any of the predicted trends (Table 4.2). It may also be that the young

trees (~16 years) used in this experiment behaved differently in terms of resource allocation and extractives accumulation than the older trees (~60 years) used the previous study with Douglas-fir (Taylor et al. 2003).

These data suggest that resource allocation following manipulation of Douglas-fir merits further study to better understand how these changes may ultimately affect wood quality. For example, a recent study involving stable isotopes analysis suggests that some extractives in an individual sapwood ring are formed when the wood ring forms, and remain *in situ* for many years before contributing to the heartwood formation process (Taylor et al. submitted-b). Thus, there may be a delay between changes in sapwood extractive content and effects on heartwood extractive formation.

4.6 CONCLUSION

Treatments that affect radial tree growth did not produce significant changes in the phloem, sapwood or heartwood extractive levels of young Douglas-fir trees. These data, combined with other recent studies, suggest that the link between tree physiology and heartwood extractive formation is complex

4.7 ACKNOWLEDGEMENTS

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CHAPTER 5. RADIAL PATTERNS OF CARBON ISOTOPES IN THE XYLEM EXTRACTIVES AND CELLULOSE OF DOUGLAS-FIR.

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5.1 ABSTRACT

Heartwood extractives are believed to be formed from a combination of extractives present in the adjacent sapwood and those imported from the phloem. Stable isotope analysis ($\delta^{13}C$) was used to assess the dynamics of sapwood extractives, and to estimate the relative importance of adjacent sapwood extractives and imported photosynthate in the formation of heartwood extractives. The cellulose and extractives from the outer 39 annual rings of six Douglas-fir (*Pseudotsuga menziesii)* trees were isolated and analyzed for their $\delta^{13}C$ composition. Although the extractives and the cellulose showed different absolute $\delta^{13}C$ values due to their different chemical compositions, the patterns of change over time (as represented by the annual rings) were similar in many cases. The carbon isotope ratios of extractives in an annual ring were correlated with the cellulose isotope ratio in the same ring (R^2=0.56 in sapwood; R^2=0.49 in heartwood). The relative stability of extractive carbon in an individual ring suggests that changes in growth conditions may have a limited effect on overall carbon storage in wood. These data suggest that some sapwood extractives are formed when the wood ring forms and remain *in situ* until they are converted to heartwood extractives many years later. Sapwood extractives appear to be important sources of materials for the biosynthesis of heartwood extractives in Douglas-fir.

5.2 KEYWORDS

Heartwood, sapwood, *Pseudotsuga menziesii,* $\delta^{13}C$, stable isotopes

5.3 INTRODUCTION

Xylem cell walls are composed of cellulose, hemicelluloses and lignin, but xylem also contains extractives: substances that are soluble in organic solvents or water (Sjostrom 1993). Sapwood extractives include materials such as sugar, starch and lipids, which are assumed to serve as energy reserves. Starch and sugar are absent from the heartwood and the extractives there are compounds such as phenols and terpenes, which are thought to act as part of a passive defensive system (Hillis 1987). Douglas-fir (*Pseudotsuga menziesii)* sapwood extractives also include phenolic glycosides and other low molecular weight phenolic compounds that are thought to be precursors for the heartwood extractives (Dellus et al. 1997).

Heartwood results from the senescence of old sapwood tissue, beginning in the oldest part of a tree stem, branch or root (i.e. surrounding the pith) It is believed that a new increment of heartwood is formed each year (e.g. Magel 2000, Nobuchi et al. 1984) but this pattern may vary from species to species. Heartwood extractives are formed at the heartwood/sapwood boundary, at least partly from carbon-based compounds imported from the inner bark (living phloem) (Hillis and Hasegawa 1963). Because sapwood extractives are different from heartwood extractives, it is assumed that extractives in senescing sapwood also contribute to the heartwood extractive formation process (Hillis 1987). The relative importance of these two carbon

sources (in the adjacent sapwood versus translocated from the phloem or other areas) for heartwood extractive formation is not known (Taylor et al. 2002).

Identifying and quantifying the carbon sources that contribute to heartwood extractive formation have not been undertaken because the biochemistry of the processes is uncertain (Magel 2000) and the location of heartwood within the living tree makes direct observation difficult. However, stable isotopes analysis should be of use. The relative natural abundance of the stable isotopes of carbon ($^{13}C/^{12}C$) in cellulose has been shown to vary among the annual rings of wood in trees in response to environmental changes (see review by McCarroll and Loader, 2004). Variation in ^{13}C abundance in cellulose reflects differences in the ^{13}C content of the atmosphere surrounding a leaf and differences in the conditions within the leaf during photosynthesis. Conditions within the leaf are a function of photosynthetic and transpiration rates, which can be influenced by many factors including water availability, irradiation, tree age and the position of the leaf in the crown (Barbour et al. 2002, McDowell et al. 2002, Brooks et al. 1998, Livingston and Spittlehouse 1996, Leavitt and Long 1986, Francey and Farquhar, 1982). Thus the $^{13}C/^{12}C$ balance in photosynthate changes over time within trees, and these changes are recorded in the increments of cellulose that are synthesized over time from that photosynthate.

Just as annual variations in photosynthetic conditions are reflected in the carbon isotope composition of the cellulose, there will be variations in the carbon stable isotope ratios of all wood components because all are ultimately derived from photosynthate. One would not necessarily expect the extractives and the cellulose made in the same year to have the same $\delta^{13}C$ signature, due to differences in ^{13}C discrimination along the pathways of their biosynthesis. However, one would expect that the carbon isotope values of these substances would be correlated over time because the differences in discrimination along the various biosynthetic routes should be consistent from year to year. Thus, stable isotopes analysis may be a useful tool for studying annual processes other than cellulose formation (such as extractive formation) in trees.

This study sought to apply stable isotopes analysis to the question of which carbon-based materials are used to manufacture heartwood extractives: Is it "new" carbon that is formed in the same year as the heartwood is formed, "old" carbon that has been stored in the annual growth ring that is converting to heartwood, or a mixture of these and/or other sources? We predicted 1) that the $\delta^{13}C$ of extractives and cellulose in Douglas-fir xylem would show radial variations, and 2) that the pattern of radial variation of $\delta^{13}C$ of extractives relative to the radial pattern of $\delta^{13}C$ of cellulose would provide insight into the process of heartwood extractive formation.

5.4 MATERIALS AND METHODS

Douglas-fir trees were sampled from one site located in the Coast Range in western Oregon, USA (latitude 44°38'N, longitude 123°12'W, 75m elevation) designated as site class III (McArdle et al. 1961). The stand, consisting of even-aged trees about 60 years old (Table 5.1), was thinned in 1992 (about 12% reduction in basal area) and again in the fall of 2001. Following the thinning in 2001, six basal disks (3cm thick) were removed from freshly cut stumps about 30cm above the ground line.

Table 5.1. Size and cambial age of the disks (300 mm above ground).

Tree label	Disk age (years)	Sapwood rings (no.)	Average radius (mm)	Sapwood extractives (% of dry wood)	Heartwood extractives (% of dry wood)	Sapwood to heartwood extractives ratio
A	61	20	334	6.0	15.2	0.40
B	60	12	306	7.4	14.6	0.51
C	60	19	303	6.9	13.7	0.51
D	53	19	244	6.1	13.7	0.44
E	59	18	218	5.8	12.6	0.46
F	62	16	219	5.7	11.7	0.49

The disks were air-dried in the lab and then sanded with a belt-sander. The width of each annual ring (nearest 0.001 mm) from the pith to the cambium was measured along three radial transects from the pith to the cambium on each disk using a tree-ring measuring device consisting of a dissecting microscope mounted over a moving stage connected to a linear variable differential transformer (LVDT) displacement transducer (Acu-Rite Incorporated, Jamestown NY). The three values for each annual ring were

averaged, and these values were summed to provide an average radius for the whole disk.

Next, a radial strip 50 mm wide (tangential) by 30 mm thick (longitudinal) was cut from each disk using a band saw. The heartwood/sapwood boundary was located by staining with alizarin red (Kutscha and Sachs 1962). Working from the cambium inward, groups of three consecutive annual rings were separated from the strip using a chisel. Thirteen sets of rings (comprising 39 annual rings in total) were taken from each disk.

The three-year ring samples were reduced to powder by grinding them in a ball-type tissue pulverizer mill (Kleco 4100, Garcia Manufacturing, Visalia, CA) for 2 minutes. Samples of the wood powder (0.15-0.20g) were weighed and enclosed in heat-sealable polyester filter bags (mesh size 25 microns, ANKOM Technology, Macedon, NY). The bags were extracted with 25ml per bag of a 70:30 mixture of acetone and water (Dellus et al. 1997) for 72 hours on a rotary shaker table (100 rpm). The extract solution was condensed in a centrifuge evaporator to remove the acetone. The aqueous extract solution was frozen and dried under vacuum. The resulting dried extract was weighed into tin cups for isotope analysis.

After extraction, cellulose was isolated from the wood powder samples using the method described by Leavitt and Danzer (1993), which involves

delignifying extractive-free wood powder using sodium chlorite. Any extractives that may have remained in the wood following the acetone/water extraction were removed from the wood by successive extraction steps with toluene/ethanol (2:1), 95% ethanol, and hot water. The samples were delignified in a 70°C sodium chlorite solution acidified with acetic acid to pH ~4.0. Fresh sodium chlorite and acetic acid were added over time to maintain pH~4.0 and a bright yellow solution color. Delignification was continued until the pH of the solution was stable (about 4 days). The bags were rinsed with distilled water and dried. The cellulose residue in the bags was weighed into tin cups for isotope analysis.

Extractive and cellulose samples were analyzed for $\delta^{13}C$ on an isotope ratio mass spectrometer (Delta Plus, Finnigan, Bremen Germany) interfaced with an elemental analyzer (ESC 4010, Costech, Valencia, CA) located at the Integrated Stable Isotope Research Facility at the Western Ecology Division of the US Environmental Protection Agency, Corvallis, Oregon. All $\delta^{13}C$ values were expressed relative to the Peedee belemite carbonate (PDB) standard in ‰ as

$$\delta^{13}C = (R_{sample}/R_{standard} - 1)*1000$$

where R is the ratio of ^{13}C to ^{12}C atoms of the sample and the standard PDB. Measurement precision was 0.05‰ for $\delta^{13}C$, determined as the average

standard deviation of replicate analyses. Values of $\delta^{13}C$ of the cellulose and the extractives were compared over the radial sequence for each tree.

Quantitative total extractive content of the sapwood and heartwood from each tree was determined on adjacent radial strips of wood (10 mm X 30 mm) comprising the entire sapwood, or the number of rings of heartwood included in the isotope samples, that were ground in a Wiley mill (Arthur Thomas Co, Philadelphia, PA) to pass a 20 mesh screen. Samples of the wood powder (~1.5g) were placed in weighed filter bags, oven-dried at 103°C for 14 hours and reweighed. The bags were extracted according to the ASTM Standard Method for the Preparation of Extractive-free Wood D1105-84 (ASTM 1996), which involves successive extraction steps with toluene/ethanol (2:1), 95% ethanol, and hot water. The sample bags were then oven-dried at 103°C for 24 hours and reweighed. Total extractive content of the heartwood and sapwood of each tree was determined as the mass lost from the sample.

5.5 RESULTS

The trees sampled in this study were relatively uniform in age and sapwood depth but they varied in their growth rates (Table 5.1). Sapwood extractive contents (~6% on average) were approximately half those of the heartwood extractives (~14% on average) in all trees.

The difference between the $\delta^{13}C$ values of the extractives and cellulose was relatively consistent, with the extractives approximately 4-5‰ more

negative than the cellulose (Figure 5.1). The $\delta^{13}C$ values of the cellulose within individual trees varied over a range of about 2‰ over the years represented by the annual rings. The $\delta^{13}C$ values of the extractives varied over time (also over a maximum range of about 2‰ within a tree), and the extractive trends appeared to co-vary in many cases with changes in the cellulose signal (Figure 5.2). Variation among the trees was high in terms of the relationship between cellulose and extractive isotope patterns in a given year (Table 5.2).

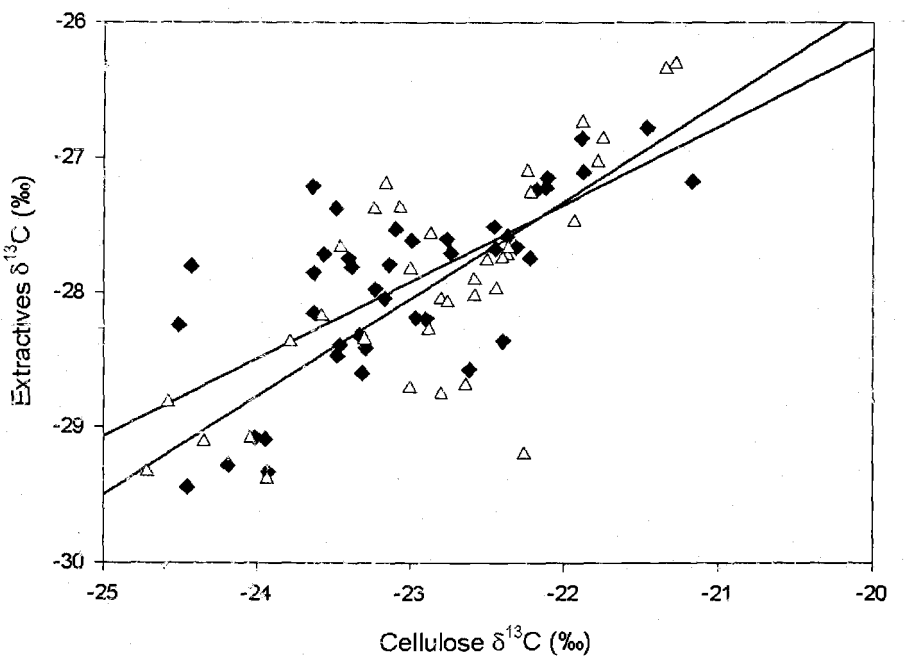

Figure 5.1. Extractive vs. cellulose $\delta^{13}C$ values for the sapwood (Δ) and heartwood (♦) of all six Douglas-fir disks. Note differences axis values. The solid and dashed lines are the regression lines for the sapwood (slope= 0.72, R^2=0.56) and the heartwood (slope = 0.58, R^2= 0.49), respectively.

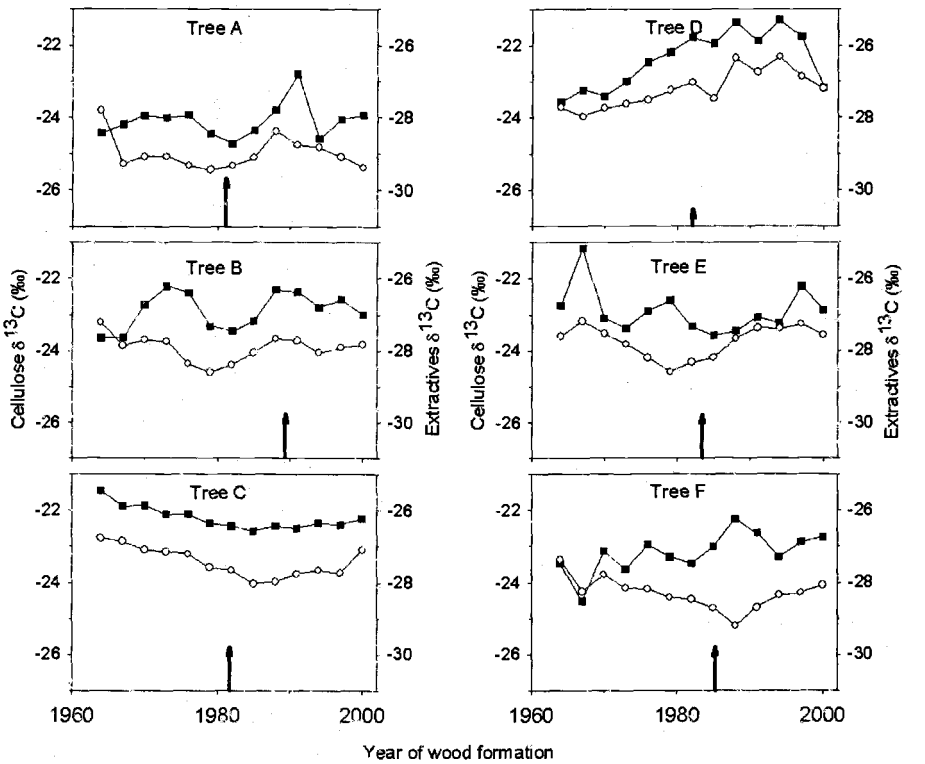

Figure 5.2. Cellulose (-■-) and wood extractive (-○-) $\delta^{13}C$ values versus year of wood formation for each of the six Douglas-fir wood samples. Arrows indicate the location of the heartwood/sapwood boundary at the time of harvest.

Table 5.2. Parameters from simple linear regression models of extractive $\delta^{13}C$ values on cellulose $\delta^{13}C$ values for each tree. An asterisk indicates that the slope of the regression was statistically significant (p-value<0.05).

Tree	Sapwood		Heartwood		Total	
	Slope	R^2	Slope	R^2	Slope	R^2
A	0.2	0.18	-1.0	0.16	0.1	0.01
B	0.2	0.18	0.0	0.00	0.1	0.01
C	2.5*	0.79	0.9*	0.87	1.2*	0.82
D	0.4	0.43	0.4*	0.68	0.6*	0.74
E	0.5	0.51	0.3	0.26	0.3	0.17
F	-0.7	0.39	0.1	0.02	-0.4	0.20
All trees	*0.7**	*0.56*	*0.6**	*0.49*	*0.6**	*0.52*

5.6 DISCUSSION

Previous chemical analyses (Taylor et al. 2003) of extracts of Douglas-fir heartwood using this extraction method have shown that the extract is nearly pure dihydroquercetin (Foo et al. 1992), a biflavonoid that has been shown to be the principle heartwood extractive in Douglas-fir (Dellus et al. 1997). ^{13}C NMR analysis of sapwood extracts obtained in this study confirmed the presence of dihydroquercetin glucoside, procyanidins and pinoresinol (Dellus et al. 1997). It is surprising that the sapwood extracts did not include some of the energy reserve materials (starch, free sugars or lipids) that are thought to exist in sapwood in general (Sjostrom 1993), and that other researchers have observed in Douglas-fir sapwood (Schowalter and Morrell 2002, Pruyn et al. unpublished). It may be that solvents other than the acetone/water combination used here would be more effective at removing such energy reserve materials. It is unlikely that the phenolic sapwood extractives analyzed here composed all of the extractives of the sapwood. Dellus et al. (1997) observed that the phenolic extractives of Douglas-fir sapwood (from one tree) totaled about 0.7% of the dry weight of the wood, whereas we found that the total extractive content of the sapwood was approximately 6% of the wood (Table 5.1). It would be interesting to analyze the isotope patterns of sapwood extractive materials other than the phenolics to see if the patterns of change were consistent.

As expected, there was interannual variation in the $\delta^{13}C$ of the cellulose among annual rings. This variation likely reflects changes in the intrinsic water-use efficiency (the ratio of photosynthesis to stomatal conductance) that occurred over time (Francey and Farquhar 1982). Differences in the stable isotope values among various wood components (i.e. extractives and cellulose in this case) can reflect a) differences in the fractionation that takes place in the biochemical synthesis pathways and/or b) substrate differences over the growing season that reflect climate influences on the intrinsic water-use efficiency of the tree (McCarroll and Loader 2004, Barbour et al. 2001, Gleixner et al. 1993). The fact that there was no significant change in the offset between cellulose and extractives when comparing sapwood to heartwood (p-value=0.15 from two-sample T-test) suggests that little fractionation takes place when sapwood extractives are used as a raw material for the synthesis of heartwood extractives.

The extent of co-variation of the $\delta^{13}C$ in extractives and in cellulose (Figures 5.1 & 5.2) suggests that these sapwood extractives remain in annual rings for many years in Douglas fir. The sapwood of these trees averaged 16 annual rings (Table 5.1). These data suggest that much of the sapwood extractives that were formed at the same time as the annual ring (as indicated by their correlated $\delta^{13}C$ values) remained in that wood ring for as long as it was part of the sapwood.

In general, the sugars, starch and lipids that are often present in sapwood are absent from heartwood, thus extractives in a senescing sapwood ring are believed to be raw materials for the formation of heartwood extractives (Hillis 1987). The sapwood extractives in the Douglas-fir that we analyzed (phenolic glucosides, procyanidins and lignans) are also absent from the heartwood, and are thought to be precursors for the extractives that are found in the heartwood (Dellus et al. 1997). These assumptions are supported by the fact that the $\delta^{13}C$ values of the heartwood extractives in these trees co-varied with the cellulose $\delta^{13}C$ values, and that the sapwood extractive and cellulose $\delta^{13}C$ values were correlated.

In addition, these data suggest that the contribution of extractives in the adjacent sapwood to newly forming heartwood extractives is relatively important. Hillis and Hasegawa (1963) showed that raw materials for heartwood extractive formation could be radially translocated. They injected labeled glucose into the phloem of *Eucalyptus seiberi* trees and detected the label in heartwood extractives a few weeks later. Such "imported" photosynthate, whether from the phloem, outer sapwood or other storage locations, would be expected to have a different stable isotope signature than the reserve compounds in the inner sapwood because it is formed in different years. However, there was a correlation between heartwood extractive and cellulose $\delta^{13}C$ values (Figure 5.1), thus these data suggest that the extractives

in the inner sapwood also play an important role in supplying the heartwood formation process of Douglas-fir.

Heartwood extractive concentrations were greater than sapwood extractives in our wood samples (Table 5.1), indicating that the extractives in the innermost-sapwood ring would be insufficient to supply all of the raw materials for extractive synthesis occurring during the heartwood formation process. Thus, imported sources of photosynthate must also be important in the heartwood extractive formation process. It would be interesting to determine the relative importance of local versus imported photosynthate for heartwood extractive production in species with different ratios of sapwood and heartwood extractive concentrations.

The wood samples used in this study were also part of a separate investigation into the relationship of growth rate and extractive content (Taylor et al. 2003). In that study, heartwood extractive content in a given ring appeared to co-vary with the growth rate that occurred at the cambium *at the same time* that heartwood ring was formed. The finding of that study suggested that heartwood formation in a given year (annual ring) is affected by the physiological status of the tree in that year (as reflected by the width of a different annual ring). In this study, the co-variation of extractives and cellulose within the same annual ring suggests that conditions in the tree as a wood ring is formed will affect the heartwood formation process in that ring many years

later when that ring is converted from sapwood to heartwood. The observations from these two studies on the same wood samples suggest that heartwood extractive properties can be affected by tree ecophysiology, and that such effects may be immediate or delayed.

5.7 CONCLUSION

Stable isotope signatures of cellulose and acetone/water extractives in wood rings show a relatively consistent offset in Douglas-fir. Variations in $\delta^{13}C$ values of the cellulose appeared to coincide with similar shifts in the $\delta^{13}C$ values of extractives located in the same ring, both in the sapwood and heartwood. These data suggest that some sapwood extractives persist within annual rings for many years, and that these substances are sources of heartwood extractives when the sapwood rings are eventually converted to heartwood.

5.8 ACKNOWLEDGEMENTS

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Agency. These experiments complied with the current laws of the country in which they were performed.

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CHAPTER 6. WESTERN REDCEDAR DURABILITY: IS THERE A ROLE FOR THE SILVICULTURIST?

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6.1 ABSTRACT

Understanding how silvicultural treatments and changes in the forest resource will affect wood quality characteristics, including heartwood natural durability, is a critical need for forest managers. Because heartwood properties can be affected by environmental disturbances, including silvicultural practices used to grow trees faster, we need to know if increased growth rates have associated tradeoffs with natural durability. In this study, the effects of thinning and fertilization were studied on 24 western redcedar trees that were part of a silvicultural trial. Growth rate was positively associated with heartwood extractive content within 6 of the 24 trees and was negatively associated with extractive content within 6 trees. The remaining 12 trees showed no association between growth and extractive content. Fertilization and thinning treatments had no significant effect on the average extractive levels of the trees. The physiological state of the tree, as represented by sapwood reserve levels, was related to heartwood extractive concentration (R^2=0.26). Further studies to better understand the relationships between silviculture, heartwood extractives and natural durability are discussed.

6.2 KEYWORDS

Natural durability, heartwood, extractives, silviculture, *Thuja plic*ata

6.3 INTRODUCTION

Heartwood extractives in many tree species play an important role in increasing wood's natural durability to biotic agents. Durability affects the appropriate uses of the wood whereas other properties such as color and chemical composition can affect pulping. The practical significance of heartwood extractives has generated much interest in understanding, and potentially controlling, extractive levels through silvicultural techniques. Surprisingly, there has been little research into the possible connection between environmental manipulations and heartwood properties (Taylor et al. 2002).

Durability is due, in large part, to the presence of non-structural, "extractive" chemicals formed when the oldest portions of the sapwood are converted to heartwood. Western redcedar heartwood contains numerous extractives, many of which have not been identified (Ohira et al. 1994). ß-thujaplicin is one of a number of tropolones found in western redcedar. This extractive chemical has been a focus of much research because it is known to be a powerful antimicrobial agent (MacLean and Gardner 1956, Nault 1987, DeBell et al. 1999).

The heartwood component of the stem varies in proportion and quality, as trees grow older and larger. Sapwood thickness remains relatively constant over time, so the proportion of a tree stem that is heartwood increases in

bigger trees (e.g. Yang and Hazenburg 1991). In addition, extractive concentration has been shown to increase with increasing distance from the pith (DeBell et al. 1999). Thus, the younger, smaller trees that are becoming increasingly important sources of forest products will have less heartwood that also has lower extractive levels than the older, bigger trees that were harvested in the past. This trend is of particular concern in a species like western redcedar, in which the quantity and quality of the heartwood is key to its value in naturally durable wood products. Another part of the current transition to harvesting younger and smaller trees is the use of intensive silvicultural practices to increase the growth rates of crop trees. This raises the question of whether aggressive silvicultural practices will affect heartwood properties (such as decay resistance) of tree species such as western redcedar.

If silvicultural practices affect heartwood extractives, and thus natural durability, then it must do so by changing some aspect of the tree's physiology. We have hypothesized that silviculture influences the energy storage levels in tree stems, and that these energy storage materials are the raw materials that are later used for heartwood extractive formation (Taylor et al. 2003).

Bryant et al. (1983) proposed a link between environmental conditions and the deposition of carbon-based storage and defensive compounds in the

living tissues of small boreal plants. According to their "carbon-nutrient balance hypothesis," carbon reserves either will be accumulated or depleted depending on the balance of nutrient absorption and carbon fixation (photosynthesis). Excess reserve compounds are then converted into carbon-based defensive compounds that make the leaves, buds and twigs less palatable to the herbivores. Previous studies suggest that this carbon-nutrient dynamic may extend to trees and can be affected by silviculture, especially in cases where the "balance" is altered through fertilization (Sundberg et al. 1993, Von Fircks and Sennerby-Forsse, 1998, Viiri et al. 2001, Cheng and Fuchigami 2002, Li et al. 2002).

Because sapwood usually contains free sugar and starch reserves, whereas heartwood does not, it is generally assumed that these reserve materials in the sapwood are consumed in the heartwood formation process (Hillis 1987). This argument is strengthened by evidence that the carbon stable isotope signature in the heartwood extractives and the cellulose of the same ring are often correlated (Taylor et al. submitted). Heartwood extractives also are formed from carbon-based compounds imported from the inner bark (living phloem) (Hillis and Hasegawa. 1963). Thus, it is possible that the levels of carbon-based reserves in the inner bark and sapwood could directly influence the resulting concentrations of carbon-based extractives in the heartwood.

If one applied the model of the carbon-nutrient hypothesis to heartwood extractive formation, treating heartwood extractives as 'carbon-based defensive compounds', then one would predict that silvicultural manipulations that increase nutrient availability more than they increase photosynthate accumulation (e.g. fertilization) will lead to reduced heartwood extractive content. In contrast, if thinning results in increases in water or sunlight to the remaining trees, this would result in increased heartwood extractives if nutrients were constrained. These predictions have not been tested, despite the potential effects of such manipulations on the heartwood quality of naturally durable species.

The objective of this research was to evaluate the impact of thinning and fertilization on the extractive content of western redcedar heartwood.

6.4 MATERIALS AND METHODS

Sample selection

Plant material came from a silvicultural trial of western redcedar near Ozette on the Olympic Peninsula in Washington State, USA (latitude 48°9' N, longitude 124°42'W, 100m elevation). The site was a naturally regenerated stand of western redcedar that established after a clear-cut in 1961. Thinning and fertilization treatments were applied to plots in the stand in a randomized block design. Tree growth was monitored over time (C.A. Harrington, pers. comm.), and the stand was studied by DeBell et al. (1999) as part of an

investigation into the effect of silvicultural treatments on heartwood/sapwood relationships.

We sampled blocks containing each of three treatment combinations: un-thinned, fertilized twice [F2]; thinned, fertilized once [TF1]; and thinned, fertilized twice [TF2] (Table 6.1). Two trees from each of four replicated blocks were randomly selected for sampling. A total of 24 trees were sampled (3 treatment combinations X 4 blocks X 2 trees per block).

Table 6.1. Fertilization and thinning regimes applied to western redcedar trees evaluated in this study.

Sample group	Thinning (Pre-thinning stocking ~5900 stems/ha)	Fertilization (300kg N/ha, 100kg P/ha)	
		1980	1992
F2	No thinning	Yes	Yes
TF1	Thinned to 1100 stems/ha in 1980.	Yes	No
TF2		Yes	Yes

Increment cores (12mm diameter) were taken at breast height through the pith from each tree in September of 2002, wrapped in plastic, transported in a cooler to the lab and then stored at -10°C until they could be analyzed. The cores were conditioned at 65% relative humidity and 20°C for 7 days, to produce a wood moisture content of approximately 12%.

Growth measurements

Fertilization or thinning treatments can affect tree growth, thus growth rate was considered to be an important covariate in this analysis. The width of each growth ring (nearest 0.001 mm) from the pith to the cambium was

measured on each increment core, using a tree-ring measuring device consisting of a dissecting microscope mounted over a moving stage connected to a linear variable differential transformer (LVDT) displacement transducer (Acu-Rite Incorporated, Jamestown NY). The increment cores were not sanded before being measured.

Extractives analysis

The increment cores, including heartwood and sapwood, were divided at each growth ring boundary using a razor blade. In cases where the growth rings were narrow, adjacent ring samples were combined to produce a bulk sample weighing at least 0.35 grams (at 12% moisture content). The samples were ground to powder in a ball-type tissue pulverizer mill (Garcia Manufacturing, Visalia, CA) for 2 minutes.

Samples of the wood powder (0.15-0.20g) were weighed and enclosed in heat-sealable polyester filter bags (mesh size 25 microns, ANKOM Technology, Macedon, NY). Extractives were removed from the samples according to ASTM Standard D1105-84 (ASTM 1996), which involves sequential extraction steps with toluene, ethanol and hot water. The sample bags were then oven-dried at 103°C for 24 hours and reweighed. Total extractive content of the samples was calculated as the mass lost from the sample, with a correction for the original moisture content.

The ß-thujaplicin content of the wood powder was analyzed by gas chromatography, using a technique modified from that described by DeBell et al. (1999). Samples of the wood powder (0.11-0.14g) were weighed into 2ml centrifuge tubes. Acetone (1.0ml) was added to the tubes, which were then capped, shaken, and allowed to stand at room temperature (20-23°C) for 18 hours. The tubes were then centrifuged for 10 minutes at 5000 rpm, and 0.6 ml of the acetone/wood extract solution was pipetted into auto-sampler vials and the vials were capped. The wood extract solutions were analyzed on a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector using He as the carrier gas (1.98 ml/min column flow). 2.0 µL injections were made by auto-sampler onto a capillary column (Rtx-5: 15m long, 0.25mm inner diameter, 0.25µm thick, 5% diphenyl/95% dimethylpolysiloxane coating, Restek, Bellfonte, PA) using a 10:1 split ratio. The oven temperature began at 100°C, then increased at 5°C per minute to 130°C, held at 130°C for 3 minutes, increased by 30°C per minute to 250°C and finally remained at that temperature for 7 minutes. The injector and detector were maintained at 275°C. An external ß-thujaplicin standard (CAS Number 499-44-5, Aldrich) was used to create a calibration curve to quantify the concentration of ß-thujaplicin in each wood extract sample. The concentration of ß-thujaplicin in the wood was then calculated by correcting for the mass of wood powder used in the analysis. Subsequent to the ß-thujaplicin

analysis, standards of γ-thujaplicin, methyl thujate, thujic acid and ß - thujaplicinol were obtained (provided by Dr. R. Daniels, Forintek Canada Corporation). The concentration of each of these compounds in each sample was estimated by using the calibration curves developed for ß-thujaplicin. Correlations ("R" values) among the values of individual extractive components within each sample were calculated using computer software (S-PLUS 6.1 for Windows. Lucent Technologies, Inc. Murray Hill, NJ).

Comparison of growth and extractives trends over time

To assess the relationship between growth patterns and extractive content, we assumed that a new ring of heartwood was formed each year (Nobuchi et al. 1984, Magel 2000). Therefore, the growth increment in a given year was compared graphically with extractive levels from heartwood rings that were assumed to have converted from sapwood to heartwood during the same year. We used the number of rings in the sapwood for a given sample at the time of harvest as the offset for these comparisons (Taylor et al. 2003). When comparing growth rate to total extractive or ß-thujaplicin content in trees over time, five-year moving averages were calculated for both the growth ring width and extractive content to isolate the general trends from background variability.

Whole-tree average values for radial growth rate, total extractive concentration and ß-thujaplicin concentration of the increment cores were

calculated by weighting the sample values by their relative contribution to the total mass of the increment core. Basal area-weighted averages were calculated by weighting the values for each sample by their relative contribution to the area of a circular cross-section, based on the radial distance contained within the growth ring(s) sampled.

6.5 RESULTS AND DISCUSSION

Silvicultural treatments and growth rate

Measurements of the radial annual ring increments in the increment cores indicated that fertilization increased tree growth in the years after treatment (Figure 6.1). Trees that were fertilized twice (groups F2 and TF2) showed two distinct periods of rapid growth, whereas those that were fertilized once had only one growth spike (group TF1).

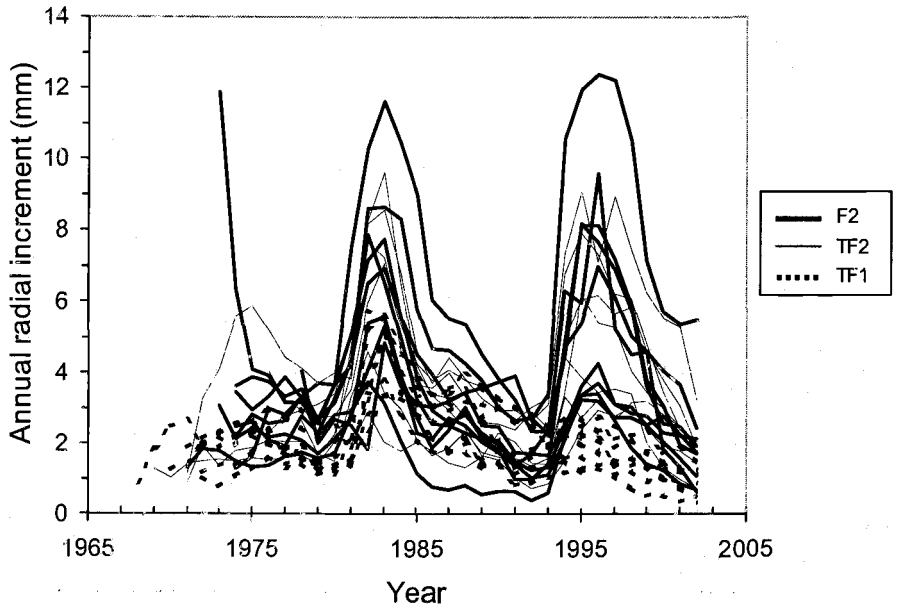

Figure 6.1. Radial growth at breast height over time for western redcedar trees subjected to various thinning and fertilization regimes. Each tree shown individually. Fertilizer treatments were applied in 1980 and 1992.

Thinning appeared to have relatively little effect on the growth of the sampled trees: the thinned-and-fertilized-twice trees (TF2) group did not grow faster on average (mean growth increment = 3.59mm, standard deviation (SD) = 1.11mm) than the un-thinned-and-fertilized-twice group (F2) (mean=3.38mm, SD=1.11mm). Trees in the thinned-and-fertilized-once group (TF1) grew more slowly on average (mean=2.13mm, SD=0.42mm) than the trees in the other two groups, both of which received an additional fertilizer treatment in 1992.

Silvicultural treatments and extractive content

Total extractive content of the heartwood increased with distance from the pith in about half of the trees (Figure 6.2). ß-thujaplicin levels were generally low in all trees, but showed a relatively consistent trend of increasing concentration with distance from the pith (Figure 6.3), similar to that observed by Nault (1987) and DeBell et al. (1999).

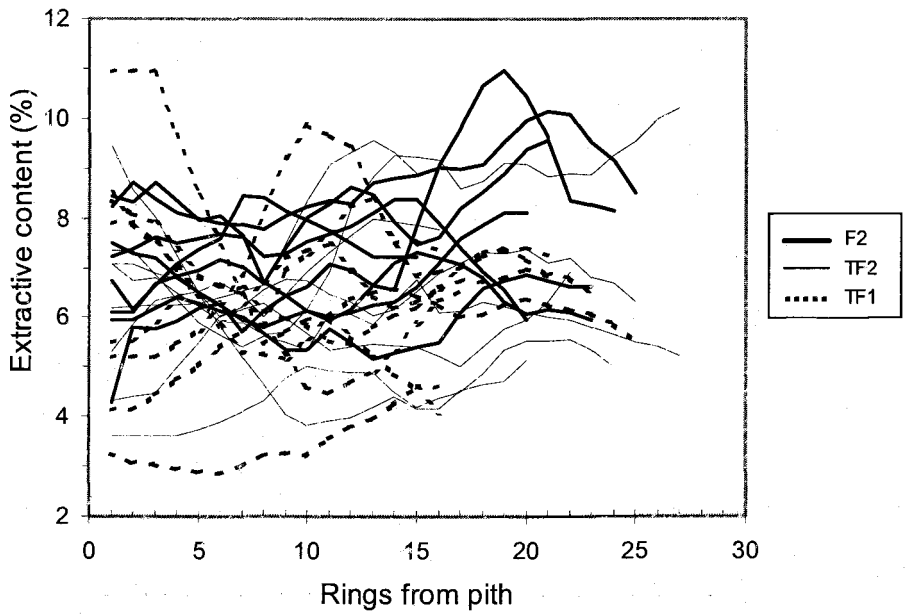

Figure 6.2. Radial changes in heartwood total extractive content at breast height for all trees, with each tree shown individually.

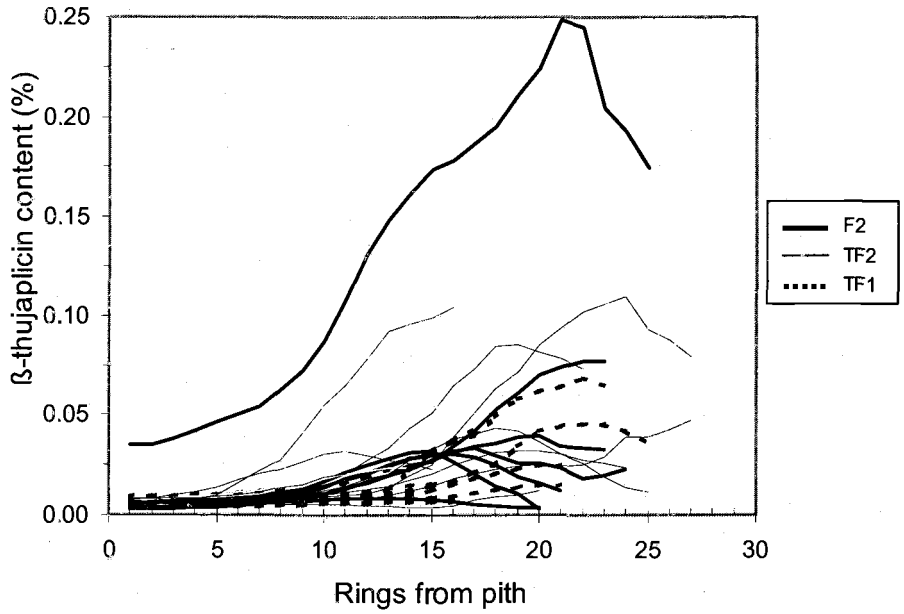

Figure 6.3. Radial changes in heartwood ß-thujaplicin content at breast height for all trees, with each tree shown individually.

The relationship between radial growth rate and heartwood total extractive content within trees was strong in some trees and non-existent in others, as shown by three example trees (Figure 6.4). There appeared to be a positive association between the fertilizer-related spikes in growth rate and total extractive content in 6 of the 24 trees (e.g. Figure 6.4A). This is consistent with trends observed in Douglas-fir (*Pseudotsuga* menziesii) in response to thinning (Taylor et al. 2003). There appeared to be no relationship between fertilization and extractive content in 12 trees (e.g. Figure 6.4B). A negative relationship between fertilizer-induced growth and extractive content had been predicted, but was seen in only 6 of the trees (e.g. Figure 6.4C).

There was no consistent difference between the treatment groups in terms of patterns of growth and total extractive content within the trees.

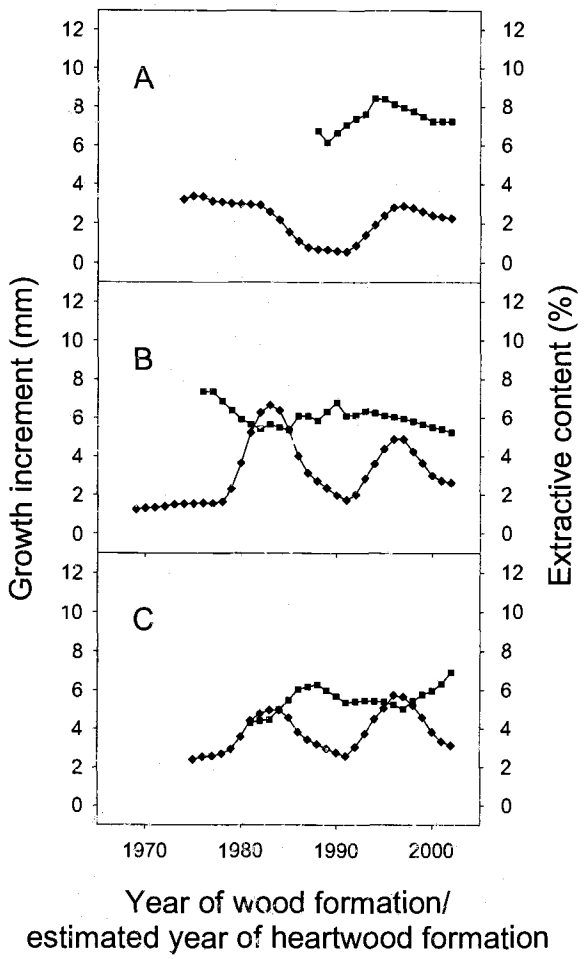

Figure 6.4. Growth rate (-♦-) and heartwood total extractive content (-■-) over time for three trees. Panel A is tree 266 from the F2 treatment, showing a positive association between the fertilizer-related spikes in growth rate and total extractive content. Panel B is tree 25 from the TF2 treatment, showing no association between the fertilizer-related spikes in growth rate and total extractive content. Panel C is tree 32 from the TF2 treatment, showing a negative association between the fertilizer-related spikes in growth rate and total extractive content.

The relationship between growth rate and ß-thujaplicin content within trees was also analyzed. ß-thujaplicin content in all trees showed no consistent association with radial growth rate (data not shown). Overall, growth rate spikes from fertilization did not appear to coincide with consistent changes in total heartwood extractives or ß-thujaplicin content.

There was no significant difference between the treatment groups in whole-tree average extractive content, nor was there any apparent correlation between average growth rate and total extractive content (Figure 6.5). This was true in the case of ß-thujaplicin content as well as total extractives, using both average values or "basal area-weighted" average values (data not shown).

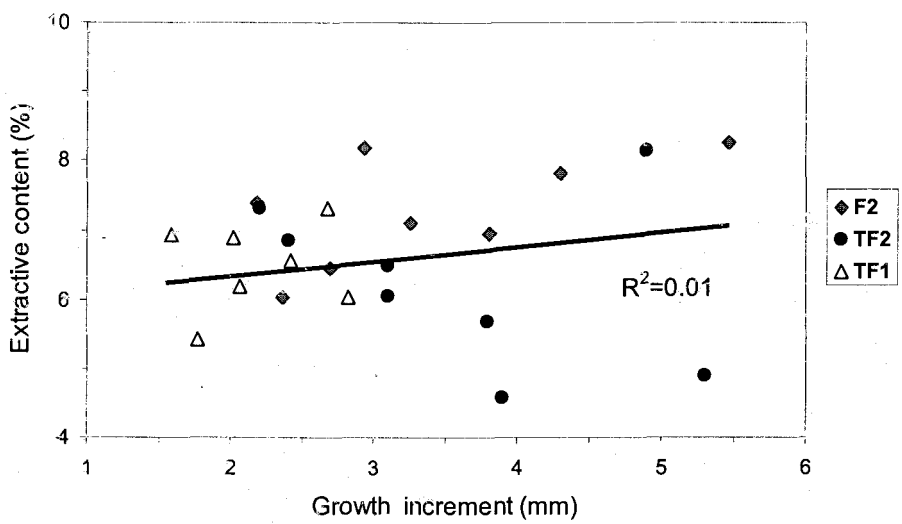

Figure 6.5. Relationship between average growth rate and average heartwood total extractive content for all trees.

There were no consistent effects of silvicultural treatments on total extractives among trees or on the patterns of heartwood extractive accumulation over time within trees. This suggests that there is little relationship between the carbon-nutrient balance dynamic and the accumulation of heartwood extractives in young western redcedar trees. The sample size may have been insufficient to account for natural variability, but the relatively consistent reaction of heartwood extractives to thinning that Taylor et al. (2003) observed in Douglas-fir was absent in these western redcedar trees. The heartwoods of Douglas-fir and western redcedar are quite different in their chemistry and natural durability, which may reflect larger differences in growth strategies. Such ecophysiological differences could also produce different heartwood responses to silvicultural treatments. These differences illustrate the importance of understanding tree response before applying silvicultural regimes from one species to another.

Along with species-specific growth responses, there may also be a "juvenile" period in relation to heartwood formation. Nault (1988) and DeBell et al. (1999) observed that extractive contents in old trees continued to increase with number of years from the pith [or "cambial age"]. Unlike the pattern with other juvenile-to-mature wood property transitions such as cell length (Zobel and Sprague 1998), the extractive content did not level off with number of years from the pith. Such observations suggest that a lower extractive level in

heartwood nearer the pith is not a "juvenile-wood" phenomenon in the normal sense. Still, young trees such as those samples in this study may respond differently to silvicultural manipulation than older trees in terms of heartwood formation.

The silviculturist's role

Our results suggest that increasing growth through thinning or fertilization does not reduce the extractive content of young western redcedar heartwood. It would be valuable to further assess the relationship between silviculture and heartwood properties in other species, and in older redcedar trees growing over a range of environments, to ensure that gains in growth rate do not come at the expense of natural durability.

These data did not support the concept of a relationship between carbon-nutrient balance, environmental manipulation and heartwood extractive levels; however, there was some evidence that higher amounts of reserve materials in the sapwood (presumably starch, free sugars and lipids) were associated with higher total extractive levels in the adjacent heartwood (Figure 6.6.) This provides some support for the hypothesis of a positive relationship between carbon reserves in the living tissues and extractive concentrations in the nearby heartwood. In this case, however, the connection between fertilization and tree response may be more localized to those rings formed during the response, which will not become part of the heartwood for many

years. It would be useful to analyze which sources of carbon contribute the most to the formation of heartwood extractives in western redcedar, because recent work involving carbon stable isotope analysis has suggested that, in Douglas-fir, extractives within senescing sapwood rings are a primary source of carbon for the heartwood extractives that form within that same ring (Taylor et al. submitted).

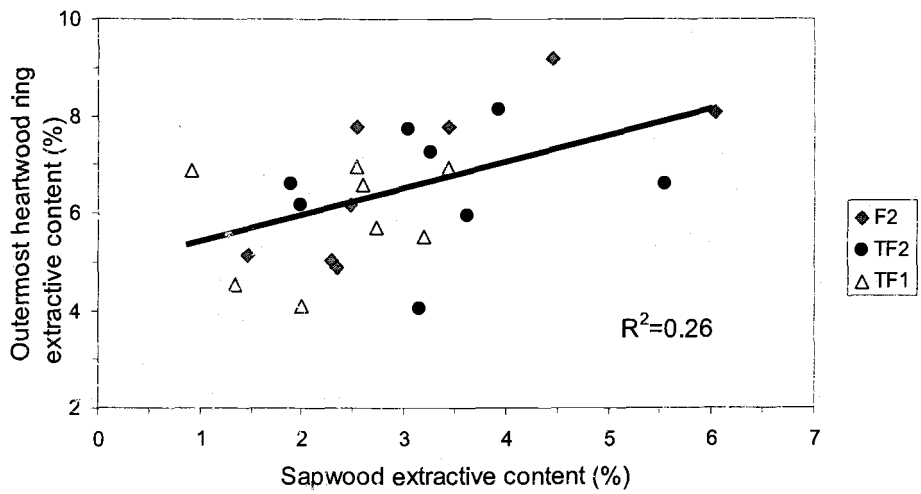

Figure 6.6. Heartwood total extractive content as a function of the concentration of reserve materials in the adjacent sapwood for all trees. Sapwood extractive content is the weighted average of total extractive values from all the sapwood rings in each tree.

The absence of correlations between silvicultural treatments or the resulting growth rate differences and sapwood extractive levels (data not shown), suggests that it would be useful to investigate further the link between silvicultural practices and sapwood extractives in western redcedar. Such connections have been shown in other species (e.g. *Pinus taeda,* Gebauer et

al. 1998), so it would be useful to understand why a similar dynamic was not observed in these trees.

Link between extractives and durability

Total heartwood extractive content and the component compounds analyzed in this study were only very weakly related (Table 6.2), a finding that is consistent with previous studies involving western redcedar (Nault 1988). Other studies examining the natural durability of western redcedar have focused on ß-thujaplicin (MacLean and Gardner 1956, Nault, 1987, DeBell et al. 1999), because is known to be fungitoxic; however, ß-thujaplicin is only a trace component of the extractive mixture and the correlation between ß-thujaplicin and decay resistance is weak (DeBell et al. 1999). These observations, and the lack of toxicity data of many western redcedar extractive compounds, suggest that much research needs to be done to better understand the relationship between extractive variability and natural durability in western redcedar. Statistical analysis procedures (e.g. principal components analysis) that can account for the variation in the many heartwood extractive components, and assess their relationship to durabiiity measures such as weight loss in decay tests, will likely be useful tools in this process.

Table 6.2. Correlation matrix of the concentration of extractive components of heartwood samples of western redcedar. N=269.

Extractive components[a]	*Degree of linear association (R)*[b]					
	ß-in	MT	TA	γ-T	ß-ol	GC
ß-thujaplicin (ß-in)						
Methyl thujate (MT)	0.49					
Thujic acid (TA)	0.41	0.76				
γ-thujaplicin (γ-T)	0.58	0.50	0.53			
ß-thujaplicinol (ß-ol)	0.40	0.41	0.33	0.59		
Total of all standards (GC)	0.63	0.78	0.95	0.72	0.47	
ASTM extractives (ASTM)	0.31	0.24	0.16	0.36	0.38	0.27

[a]Extractive names are abbreviated in the columns to the right. [b]Values (R) range from –1 to 1, where 0 corresponds to no linear association.

6.6 CONCLUSION

Fertilization treatments on western redcedar trees produced no consistent changes in heartwood extractive content. Correlations between sapwood and heartwood extractive concentrations imply that the physiological status of the tree can influence heartwood properties. Increasing the growth rate of young western redcedar trees through silvicultural treatments does not appear to affect natural durability in western redcedar, although more work is required to validate this finding for different tree ages and growing conditions.

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CHAPTER 7. EFFECTS OF HEARTWOOD EXTRACTIVE FRACTIONS OF *THUJA PLICATA* AND *CHAMAECYPARIS NOOTKATENSIS* ON WOOD DEGRADATION BY TERMITES OR FUNGI

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7.1 ABSTRACT

Heartwood of some species has natural resistance to attack by termites and fungi due to the presence of toxic and/or repellent extractives, but the role of individual extractives in durability is poorly understood. The effect of selective removal of extractives on termite or decay resistance was assessed with matched samples of *Thuja plicata* Donn ex D. Don and *Chamaecyparis nootkatensis* (D.Don) Spach heartwood. Samples were extracted using a variety of solvents and then exposed to the subterranean termite, *Coptotermes formosanus* Shiraki in a no-choice feeding test or to the brown-rot fungus, *Postia placenta* (Fr.) M. Larsen & Lombard in a soil bottle test. At the same time, the effect of naturally-occurring variations in heartwood extractives on termite or decay resistance was evaluated by testing samples from the inner and outer heartwood of five trees of each species against *C.formosanus* and *P.placenta* and analyzing matched wood samples for their extractive content. The results suggest that the methanol-soluble extractives in *T.plicata* and *C.nootkatensis* play an important role in heartwood resistance to attack by *C.formosanus* and *P.placenta*. Total methanol-soluble extractive content of the heartwood was positively correlated with both termite and decay resistance; however, there was much unexplained variation and levels of individual extractive components were only weakly correlated with one another. Further

studies are underway to develop a better understanding of the relationships between individual extractives levels and performance.

7.2 KEYWORDS

Western redcedar, Alaska cedar, *Coptotermes formosanus*, *Postia placenta*

7.3 INTRODUCTION

Concerns over the safety and environmental impacts of chemically-treated wood have increased demand for naturally durable wood products. At the same time that demand is increasing, the nature of the forest resource that will be used to meet this demand is changing from older, larger trees, which contain wood with a proven history of durable performance, to younger trees whose wood properties are not as well understood. Changes in the use of wood products in housing applications, and expansion of the areas threatened by termites are putting additional demands on the performance criteria for wood products. Research to better understand the effects of changes in wood properties on resistance to termites and decay fungi could lead to more rational utilization of naturally durable timber.

The heartwood of some tree species is naturally resistant to termite and fungal attack, largely due to the presence of non-structural chemical "extractives" (Hillis 1987, Scheffrahn 1991). For example, the heartwoods of *Chamaecyparis nootkatensis* (D.Don) Spach ("Alaska cedar" or "yellow cedar") and *Thuja plicata* Donn ex D. Don ("western redcedar") have significant resistance to *Coptotermes formosanus* Shiraki and other termite species and against decay fungi (Su and Tamashiro 1986, Grace and Yamamoto 1994, Scheffer and Morrell 1998, Suzuki and Hagio 1999, Morales-Ramos and Rojas 2001). Unfortunately for the users of naturally durable wood products, termite

and decay resistance varies significantly among individual pieces of wood and this variability can be difficult to predict (Barton and MacDonald 1971, Kennedy et al. 1994, Ohtani et al. 1996, DeBell et al. 1999).

Heartwood extractive chemistry is complex and varies significantly within and between trees and tree species (reviewed in Taylor et al. 2002). Lower amounts of extractives have been observed in the heartwood near the pith of a number of species, including *T.plicata* (DeBell et al. 1999). Lower extractive content has been correlated with reduced termite and fungal resistance (Hillis 1987, Hashimoto et al. 1997). Grace and Yamamoto (1994) observed significant variation in termite resistance properties of *C.nootkatensis* wood and suggested that this was a function of heartwood extractive variability.

Heartwood extractives within a piece of wood can range from low molecular weight volatile compounds to large polymers (e.g. Sjostrom 1993), and it appears that not all of these components are equally important in determining natural durability. There is evidence that the volatile fraction is particularly effective in reducing termite attack (Smythe and Carter 1970a & 1970b, Kang et al. 1994, Ohtani et al. 1996 & 1997). In his review of the literature, Scheffrahn (1991) observed that the most commonly identified antitermitic compounds in various wood species were terpenoids. Lower molecular weight terpenoids include the thujaplicins in *T.plicata* and nootkatin

in *C.nootkatensis* (Sjostrom 1993). The thujaplicins are fungitoxic and so have also been a focus of research in durability to fungal attack (MacLean and Gardner 1956, Nault 1987 & 1988, DeBell et al. 1999). However, there are many other compounds in *T.plicata* and *C.nootkatensis* heartwood that make up the extractive mixture in addition to the low molecular weight terpenoids (Barton 1976, Ohira et al. 1994). Many of these compounds have not been identified or examined for their toxicity; furthermore, it is not known how these compounds vary within and between trees. Thus, much remains to be discovered about the relationship between extractives and natural durability in these two species.

Recent work has demonstrated that changes in the tree's environment during growth can alter heartwood extractive content in some species (Taylor et al. 2003). If such variations in heartwood extractives affect the natural resistance of the wood to termites and fungi, then this finding suggests that silvicultural treatments and changes in how forest resources are managed may impact the natural durability of future wood products.

The objectives of this study were 1) to determine which extractive components in *T.plicata* and *C.nootkatensis* most affect attack by the termite *C.formosanus* and the brown-rot fungus *Postia placenta* (Fr.) M. Larsen & Lombard, and 2) to assess the relationship between naturally-occurring variations in heartwood extractive content and resistance to biodeterioration.

7.4 MATERIALS AND METHODS

This study consisted of two parts, each comprising multiple tests. In the extractive fraction test, matched heartwood samples were first subjected to extraction treatments and then tested for termite resistance and decay resistance. For the natural variability test, untreated heartwood samples were assessed for their termite resistance, decay resistance and extractive content.

Wood materials

Fresh, debarked basal cross-sections (approximately 10 cm in the longitudinal direction) from five trees of *T.plicata* and *C.nootkatensis* were provided by a utility pole supplier (Cascade Pole Co, Tacoma, WA). The trees varied in growth rate, age and diameter (Table 1). For each disk, the width of each growth ring (nearest 0.001 mm) from the pith to the cambium was measured along one radius on a dissecting microscope mounted over a moving stage connected to a linear variable differential transformer (LVDT) displacement transducer (Acu-Rite Incorporated, Jamestown NY).

Table 7.1. Growth characteristics of wood cross-sections.

Species	Tree number	Disk age (years)	Disk diameter (cm)	Average growth increment (mm/yr)
Thuja plicata	1	101	13	1.28
	2	40	21	5.34
	3	103	18	1.78
	4	51	33	3.26
	5	78	29	1.86
Chamaecyparis nootkatensis	1	157	26	1.63
	2	166	27	1.65
	3	310	26	0.82
	4	403	51	0.63
	5	283	51	0.91

Sample preparation

For the natural variability test, blocks (30 [R] X 20 [T] X 40mm [L]) were cut from the inner and outer heartwood of each of the five trees of each species. Heartwood was differentiated from sapwood by color. These blocks were cut into three samples 10 mm thick (radial) with a band saw. From each sample, 10 mm (longitudinal) was cut from one end for inclusion in the decay test and 10 mm was cut from the other end for use in the extractive analysis. The remaining 20 mm were used for the termite test (Figure 7.1). After cutting, the samples were stored at room temperature (20-23°C) in the dark in sealed plastic bags.

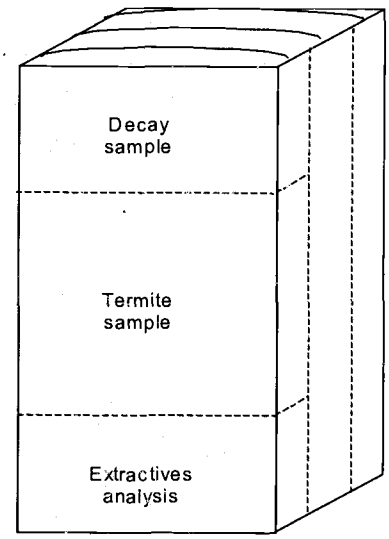

Figure 7.1. Cutting pattern of sample blocks for the natural variation test.

For the extractive fraction test, blocks (40 [R] X 40 [T] X 30mm [L]) were cut from the outer heartwood of each of the five trees of each species. These blocks were cut into (40 X 40 X 2mm thick longitudinal) sub-samples with a band saw (Figure 7.2). Sub-samples were subjected to the following treatments: 1) vacuum-drying at 50±2°C and 13 kPa for 48 hours (to eliminate volatile fractions), 2) vacuum-drying followed by soxhlet extraction with hexane (to remove mostly non-polar extractives), or 3) vacuum-drying, followed by extraction with hexane, followed by soxhlet extraction with methanol (to removing a broad spectrum of extractive components). Additional samples ("fresh sawn") were stored at room temperature (20-23°C) in the dark in sealed plastic bags directly after cutting to serve as non-extracted controls.

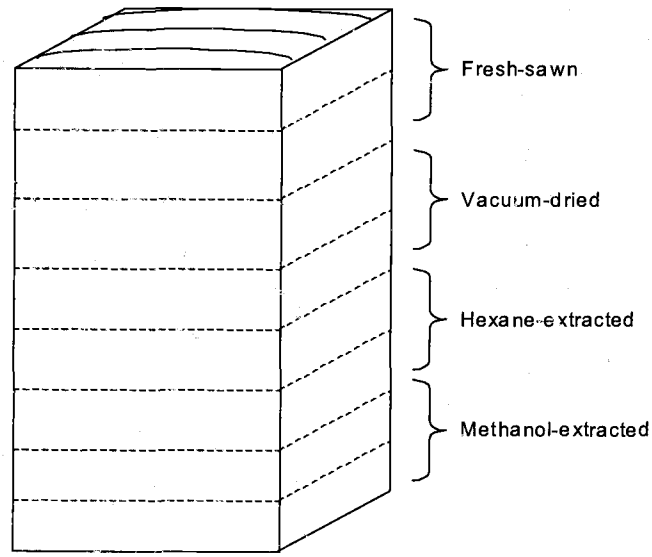

Figure 7.2. Cutting pattern of sample blocks for the extraction test.

No-choice termite tests

Replicates of the extractive fraction test samples (2 from each of 3 trees X 4 treatments = 24) and natural variability test samples (3 each from the inner and outer heartwood of 5 trees = 30) were exposed in a three-week, no-choice feeding test with *C.formosanus* as described in a Japan Wood Preserving Association standard (Tsunoda 1991, Tsunoda and Nishimoto1986). Samples of *Cryptomeria japonica* D. Don ("Sugi") sapwood and heartwood were also included in the study to provide a basis for comparison. *C.japonica* sapwood is not resistant to attack by *C.formosanus*, whereas the heartwood is very resistant (Grace et al. 1996).

Prior to exposure to the termites, replicate wood samples were dried in an oven at 60±2°C for 48 hours and then weighed nearest to 0.001 g. The

moisture content of these samples was used to calculate the original oven-dry mass of the termite-test samples. Wood samples were exposed to termites in acrylic tube sections (90 mm diameter, 60 mm length) that were sealed at one end with 5 mm of plaster of Paris. Two cm square sections of plastic mesh were placed on the bottoms of the containers, and the wood samples were placed on top of the mesh. One hundred fifty workers and 15 soldiers of *C.formosanus* were placed in each container. The containers were held in boxes that were lined with moist cotton wool to provide a water source that could penetrate through the plaster of Paris. The termite tests were maintained in a dark room at 28±2°C and >85% relative humidity for 21 days. The termite test containers were examined daily and the dead termites were removed and tallied. The numbers of living termites in each container were counted after three weeks exposure. At the end of the test period the wood samples were washed, dried at 60±2°C and weighed, then mass loss from the samples was calculated.

Decay testing

Replicates of the extractive fraction test samples (2 from each of 3 trees X 4 treatments = 24) and natural variability test samples (3 from the inner and outer heartwood of 5 trees = 30) were exposed to *Postia placenta* in soil bottle tests according to ASTM D1413-99 (ASTM 1999). *P.placenta* is a brown rot fungus that often attacks wood products in service (Duncan and Lombard

1965). Samples of decay-susceptible *Pinus radiata* D. Don sapwood were included in the test as a control. Each block was cut into two pieces to provide a pseudo-replicate. The average mass lost from the two blocks during the test was used as the (single) decay value.

Extractive fraction relative mass

For analysis of the relative mass of each extractive fraction in the extractive fraction test, fresh-cut wood pieces of each species were ground in a Wiley mill (Arthur Thomas Co, Philadelphia, PA) to pass a 20-mesh screen. Three replicate weighed samples of the wood flour of each species (~2.0g) were enclosed in heat-sealable polyester filter bags (mesh size 25 microns, ANKOM Technology, Macedon. NY). The bags were oven-dried for 12 hours at 50±2°C, reweighed, and then subjected to the vacuum-drying, hexane and methanol extraction steps described above. After each extraction step, the bags were oven-dried and re-weighed. The mass of extractives removed in each step was calculated as the mass lost from the wood samples.

Gas chromatography and extractives quantification

Matched samples from the natural variability test were reduced to powder in a ball-type tissue pulverizer mill (Garcia Manufacturing, Visalia, CA) for 2 minutes. The concentration of known extractive compounds in the wood powder was analyzed by gas chromatography, using a technique modified from that described by DeBell et al. (1999) as follows. Samples of the wood

powder (0.11-0.14g) were weighed into 2 ml centrifuge tubes. 1.0 ml of methanol was added to the tubes, which were then capped, shaken, and allowed to stand at room temperature (20-23°C) for 18 hours. The tubes were then centrifuged for 10 minutes at 5000 rpm, and 0.6 ml of the methanol/wood extract solution was pipetted into auto-sampler vials and the vials were capped. The wood extract solutions were analyzed on a Shimadzu GC-2010 gas chromatograph with a flame ionization detector and He as the carrier gas. 2 µL injections were made by auto-sampler onto a capillary column (Rtx-5: 15m long, 0.25mm inner diameter, 0.25µm thick, 5% diphenyl/95%dimethyl polysiloxane coating, Restek, Bellfonte, PA) using a 10:1 split ratio and 1.98 mL/min of column flow. The temperature program began at 100°C, increasing at 5°C per minute to 130°C, holding at that temperature for 3 minutes, increasing by 30°C per minute to 250°C and remaining at that temperature for 7 minutes. The injector and detector were maintained at 275°C. Available standards were used to make calibration curves to determine the concentrations of known *T.plicata* heartwood extractive components (Frazier 1987) in each extract, including ß-thujaplicin (CAS Number 499-44-5, Aldrich), carvacrol (CAS Number 499-75-2, Aldrich), carvacrol methyl ether (CAS Number 6379-73-3, Aldrich), and γ-thujaplicin, ß-thujaplicinol, thujic acid and methyl thujate (provided by Dr. R.Daniels, Forintek Canada Corporation). Carvacrol (CAS Number: 499-75-2 Aldrich), carvacrol methyl ether (CAS

Number: 6379-73-3, Aldrich) and nootkatone (CAS Number: 4674-50-4, Aldrich) were analyzed in the *C.nootkatensis* extract analysis (Barton 1976). "Total GC" extractive content for each sample was calculated using the sum of the areas of all peaks (excluding the solvent peak) in the chromatogram and using the calibration curve for ß-thujaplicin (for *T.plicata)* and carvacrol (for *C.nootkatensis)*. All gas chromatography extractive values were corrected for the mass of wood powder sampled.

The "methanol-soluble" and "ASTM" extractive content also was determined for the natural variation test samples. Samples of the wood powder (~0.15g) were enclosed in heat-sealable polyester filter bags, oven-dried at 50±2°C and re-weighed, extracted with methanol in a soxhlet apparatus for 6 hours, re-dried and re-weighed. The samples were then subjected to the extraction procedure described in ASTM D1105 (ASTM, 1996): successive soxhlet extractions with a 2:1 toluene/ethanol mixture and 95% ethanol, followed by extraction in a hot water bath, re-drying and re-weighing. Extractive content from the two extraction procedures was calculated as the mass lost from the wood powder compared to the mass of the extractive-free wood.

Data analysis

Group values for all parameters in the extraction fraction test were compared with ANOVA tests using the Tukey-Kramer procedure for multiple

comparisons (S-PLUS 6.1 for Windows. Lucent Technologies, Inc. Murray Hill, NJ). Correlations among the extractive components ("R" values) were calculated for the samples in the natural variation tests. Simple linear regressions were performed with methanol-soluble extractive content as the explanatory variable and termite feeding values and decay mass lost values as the response variables.

7.5 RESULTS AND DISCUSSION

Extractive fraction test

Effect of extraction on T.plicata

The methanol extraction step significantly increased the mass loss from the *T.plicata* samples (Figure 7.3a). The patterns of mass loss for the samples indicate that resistance of *T.plicata* wood to *Coptotermes formosanus* attack is similar to that of *C. japonica* heartwood, and that the vacuum-drying and hexane extractions did not change resistance. Methanol extraction rendered *T.plicata* about as susceptible to attack as the *C.japonica* sapwood.

The pattern of *T.plicata* heartwood resistance to *P.placenta* was similar to that of the termite test results (Figure 7.3b). Although the hexane-extracted wafers had higher average weight loss, only the methanol-extracted wafers showed statistically significantly increased decay levels compared with the unextracted samples.

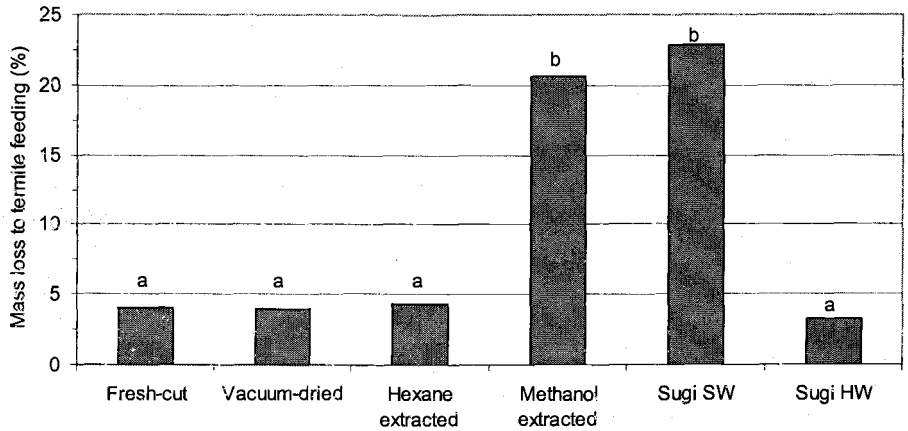

Figure 7.3a. Mass losses after 3 weeks of exposure to *Coptotermes formosanus* of *Thuja plicata* blocks that were left untreated or subjected to various extraction procedures. Sugi sapwood and heartwood are included for the purpose of comparison. Columns with the same letters are not significantly different at α=0.05.

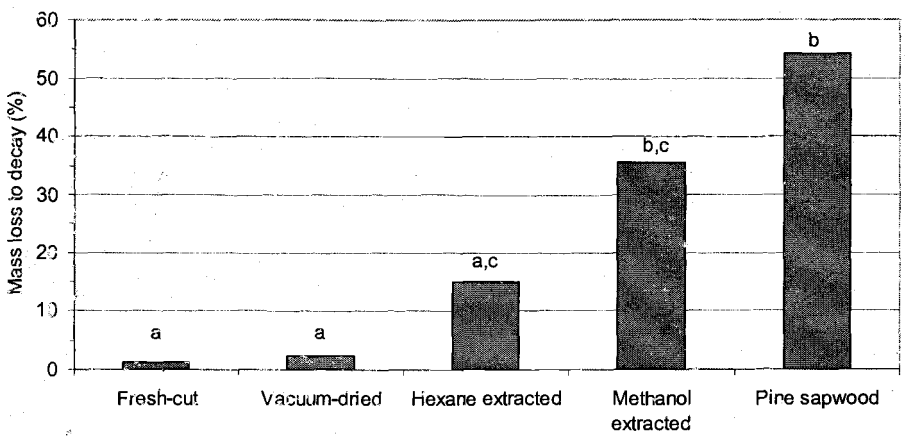

Figure 7.3b. Mass loss of *Thuja plicata* blocks that were left untreated or subjected to various extraction procedures exposed for 12 weeks to the brown-rot fungus *Postia placenta*. Pine sapwood blocks are included for the purpose of comparison. Columns with the same letters are not significantly different at α=0.05

Effect of extraction on C.nootkatensis

C.nootkatensis heartwood samples were very resistant to attack by *C.formosanus*. In some cases, all of the termites exposed to fresh-cut *C.nootkatensis* samples died within 48 hours. This rapid mortality suggests that volatiles from the wood were toxic to the termites. In most cases, however, many of the termites lived until the end of the test period.

Mass losses due to termite feeding in *C.nootkatensis* samples were low (Figure 7.4a). Methanol extraction significantly increased wood susceptibility to feeding by *C.formosanus,* but mass losses never exceeded 10%. The hexane-extracted samples had slightly more mass loss than the vacuum-dried samples, but the differences were not statistically significant.

The pattern of *C.nootkatensis* heartwood resistance to *P.placenta* was similar to that of the termite test results (Figure 7.4b): only the methanol-extracted wafers showed statistically significantly increased decay levels compared with the un-extracted samples.

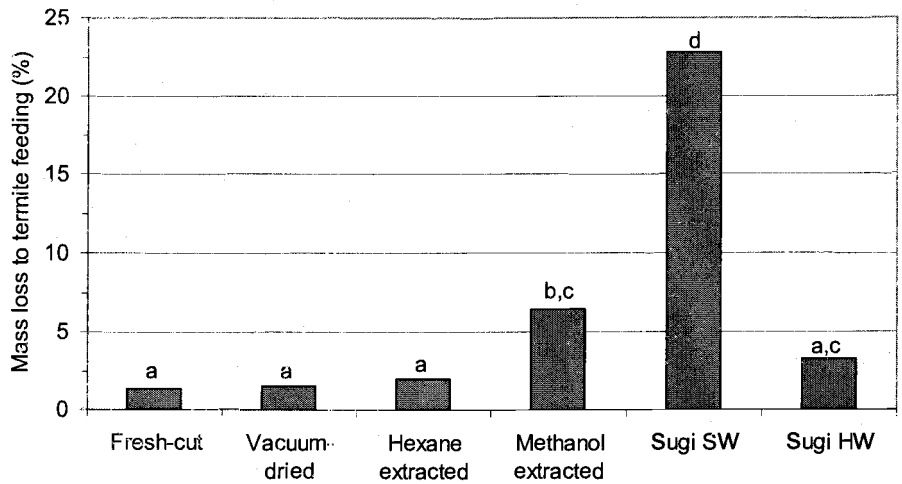

Figure 7.4a. Mass losses after 3 weeks of exposure to *Coptotermes formosanus* of *Chamaecyparis nootkatensis* blocks that were left untreated or subjected to various extraction procedures. Sugi sapwood and heartwood are included the purpose of comparison. Columns with the same letters are not significantly different at α=0.05.

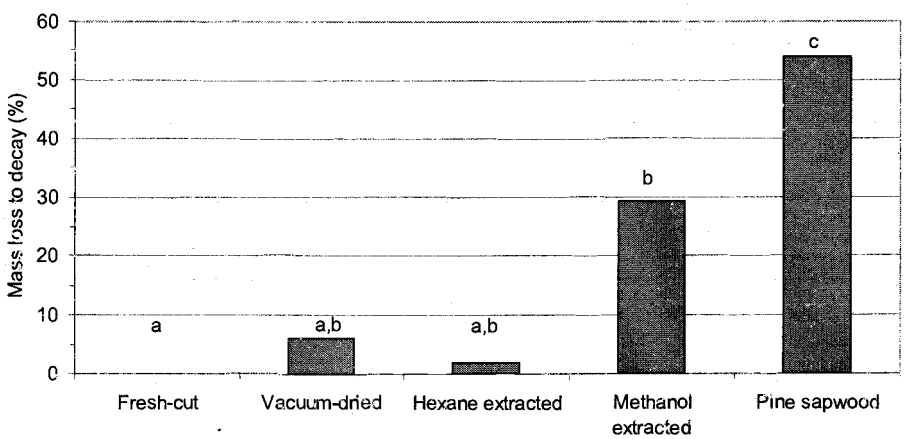

Figure 7.4b. Mass loss of *Chamaecyparis nootkatensis* blocks that were left untreated or subjected to various extraction procedures and exposed for 12 weeks to the brown-rot fungus *Postia placenta*. Pine sapwood blocks are included the purpose of comparison. Columns with the same letters are not significantly different at α=0.05

These data suggest that the more volatile components of the wood may be less important for termite and fungal decay resistance of *T.plicata* and *C.nootkatensis* than the higher molecular weight and more polar compounds. This finding differs from previous work with other species that found the volatile fraction to be especially important (e.*g.* Ohtani et al. 1996 & 1997, Smythe and Carter 1970a & 1970b). Whereas some volatile extractives may have remained in the wood after the vacuum drying and hexane extraction steps, one would expect some differences after those treatments if the volatile fraction were critically important.

The relative importance of the methanol-soluble extracts may be a function of their inherent toxicity and/or their relative abundance. Most of the extractives in the heartwood of the two wood species are methanol-soluble (Figure 7.5), and it was the removal of these extractives that most reduced the durability of the wood pieces in these tests.

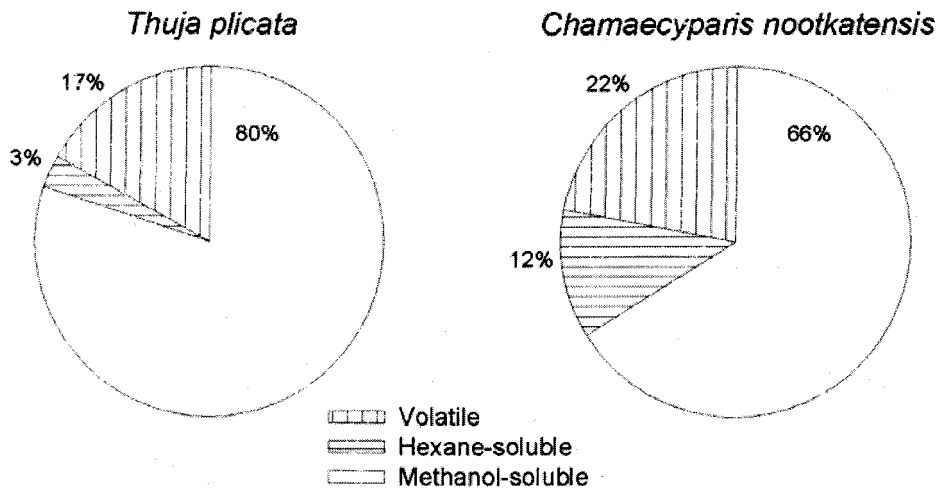

Figure 7.5. Relative abundance of various heartwood extractive fractions by mass.

Natural variability test

In the natural variability samples of both wood species, *C.formosanus* feeding and *P.placenta* decay losses were generally low, with high variability among the samples (Table 7.2). The outer heartwood was more resistant than the inner heartwood to termite attack and decay in many cases. Such high variability in the termite and decay resistance of heartwood within and between trees of the same species is consistent with previous observations of these and other wood species (DeBell et al. 1999, Hillis 1987, Mannesmann 1973, Smythe and Carter 1970a).

Table 7.2. Mass loss due to decay and termite feeding on blocks from natural variability test. For each tree, n=3

Species	Tree number	Average decay (% [standard deviation])		Average termite feeding (% [standard deviation])	
		Inner heartwood	Outer heartwood	Inner heartwood	Outer heartwood
Thuja plicata	1	10.3 (8.3)	-0.1 (2.0)	2.3 (1.0)	2.1 (0.9)
	2	12.7 (20.7)	5.5 (7.3)	3.3 (1.8)	2.1 (1.0)
	3	17.6 (18.2)	2.1 (2.2)	2.9 (0.3)	2.0 (0.6)
	4	17.1 (4.7)	2.7 (2.4)	5.1 (0.6)	2.9 (0.2)
	5	21.2 (13.4)	3.1 (5.9)	11.4 (4.3)	3.2 (0.6)
	All trees	*15.8 (12.8)*	*2.7 (4.3)*	*5.0 (3.9)*	*2.5 (0.8)*
Chamaecyparis nootkatensis	1	7.7 (5.8)	3.3 (0.5)	3.6 (0.3)	3.0 (0.1)
	2	1.6 (1.3)	1.4 (0.5)	2.7 (0.2)	2.4 (0.5)
	3	0.9 (0.8)	2.0 (0.7)	2.3 (0.2)	2.0 (0.1)
	4	1.7 (3.2)	0.7 (1.1)	3.4 (0.4)	2.3 (0.3)
	5	3.6 (3.1)	4.3 (6.3)	2.7 (0.3)	2.4 (0.3)
	All trees	*3.1 (3.8)*	*2.3 (2.8)*	*2.9 (0.6)*	*2.4 (0.4)*

The range of termite feeding values was small in this test, and the susceptibility to decay of a matched sample was positively, but only weakly, correlated to its susceptibility to termite attack over the limited range of values (Figure 7.6). Methanol-soluble extractive concentrations were negatively related to decay mass loss and termite feeding values for the two wood species, but this relationship explained relatively little of the variation (Table 7.3). None of the other extractive measures was a better predictor of the susceptibility of the wood to attack by *C.formosanus* or *P.placenta*. Concentrations of individual components of the heartwood extractives in samples of each wood species were only weakly correlated with one another or with the various measures of total extractive content (Tables 7.4 and 7.5).

These observations suggest that it is not possible to focus on a single heartwood extractive measurement in order to understand the natural durability of the wood, especially if one is considering the resistance of the wood to multiple biodeterioration agents.

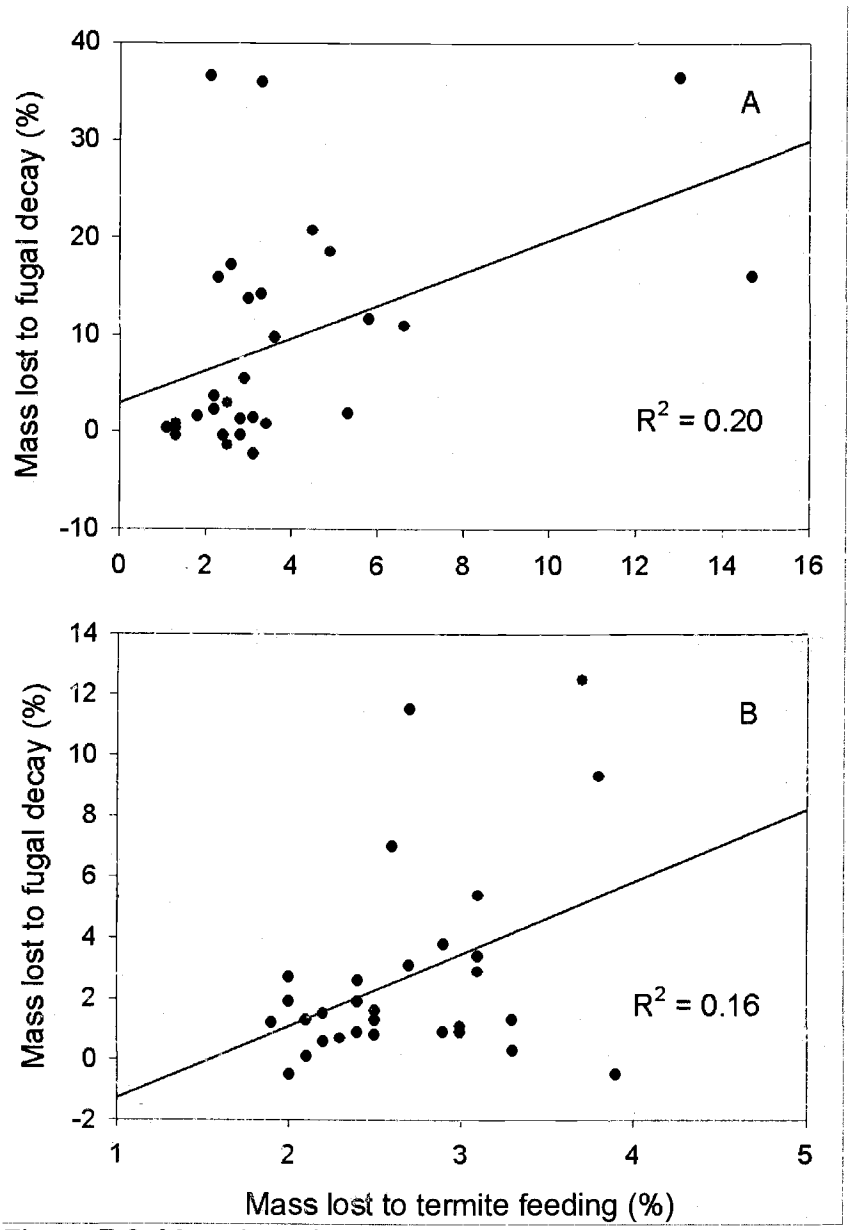

Figure 7.6. Mass loss due to feeding by *Coptotermes formosanus* versus mass loss due to decay by *Postia placenta* of matched heartwood samples of *Thuja plicata* (A) and *Chamaecyparis nootkatensis* (B).

Table 7.3. Results of regression of termite feeding and decay mass loss values on the methanol-soluble extractive content of *Thuja plicata* and *Chamaecyparis nootkatensis*. Low p-values for the regression coefficients indicate that there is a statistically significant linear relationship between the variables. R^2 values measure the fraction of the variation in the response variable explained by explanatory variable.

Species	Decay mass loss			Termite feeding		
	Coefficient	p-value	R^2	Coefficient	p-value	R^2
Thuja plicata	-1.46	0.015	0.20	-0.42	0.007	0.23
Chamaecyparis nootkatensis	-0.93	0.117	0.09	-0.26	0.007	0.23

Table 7.4. Correlation matrix of extractive measurements of samples of *Thuja plicata*. N=30.

Extractive components[a]	*Degree of linear association (R)*[b]								
	CM	CA	MT	TA	ß-in	γ-T	ß-ol	GC	MS
Carvacrol methyl ether (CM)	1								
Carvacrol (CA)	-0.49	1							
Methyl thujate (MT)	-0.04	-0.06	1						
Thujic acid (TA)	-0.61	0.62	0.10	1					
ß-thujaplicin (ß-in)	-0.30	-0.01	0.49	0.06	1				
γ-thujaplicin (γ-T)	-0.39	0.11	0.42	0.36	0.56	1			
ß-thujaplicinol (ß-ol)	-0.33	0.09	0.22	0.36	0.53	0.81	1		
Total extractives by gas chromatography (GC)	-0.33	0.29	0.41	0.17	0.54	0.67	0.47	1	
Methanol-soluble extractive content (MS)	-0.41	0.16	0.27	0.48	0.65	0.82	0.88	0.53	1
ASTM extractive content	-0.40	0.34	0.19	0.66	0.46	0.65	0.66	0.39	0.86

[a]Extractive names are abbreviated in the columns to the right. [b]Values (R) range from –1 to 1, where 0 corresponds to no linear association.

Table 7.5. Correlation matrix of extractive measurements of samples of *Chamaecyparis nootkatensis*. N=30.

Extractive components[a]	*Degree of linear association (R)*[b]				
	CM	CA	NO	GC	MS
Carvacrol methyl ether (CM)	1				
Carvacroi (CA)	0.96	1			
Nootkatone (NO)	0.50	0.42	1		
Total extractives by gas chromatography (GC)	0.48	0.39	0.83	1	
Methanol-soluble extractive content (MS)	0.41	0.29	0.74	0.69	1
ASTM extractive content	0.48	0.45	0.67	0.52	0.66

[a]Extractive names are abbreviated in the columns to the right. [b]Values (R) range from –1 to 1, where 0 corresponds to no linear association.

These data support the general belief that heartwood extractives are responsible for the natural resistance of heartwood against termites and fungi. In particular, *C.nootkantensis and T.plicata* heartwood is resistant to termites and fungi because of the methanol-soluble extractives, which are present in relatively high concentrations. However, the high variability within the heartwood extractive mixtures of each species, and the poor correlations of extractive components with fungal and termite resistance of the wood, suggest that the relationship between heartwood extractives and heartwood durability is complex.

7.6 CONCLUSION

T.plicata and *C.nootkatensis* heartwood showed considerable resistance to *C.formosanus* and *P.placenta* in laboratory tests. Methanol extraction of the wood samples reduced their resistance, whereas vacuum-drying and hexane extraction appeared to have little effect.

Considerable naturally-occurring variability was observed in the extractive components of wood samples taken from different locations within trees and from different trees. Variations in extractive components were only weakly correlated with one another, and explained relatively little of the variation in termite and fungal resistance. These results suggest that the methanol-soluble heartwood extractives of *T.plicata* and *C.nootkatensis* are important factors in the complex relationship between the extractive content of heartwood and its natural durability.

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CHAPTER 8. CONCLUSIONS

This study summarizes and expands on our knowledge of the processes of heartwood formation and the effects of heartwood extractive variation on natural durability.

A comprehensive review of the literature relating to heartwood formation and natural durability (Chapter 2) revealed that the relationship between environmental influences and heartwood extractive variability, and the possible effect of such extractives variation on natural durability, was poorly understood.

In an effort to examine the possible influences of environment on heartwood formation, this study took the novel approach of examining heartwood extractive variation among individual annual rings. As discussed in Chapter 3, this technique, combined with simple estimates of the year of heartwood formation of those rings, allowed for the discovery of a relationship between radial growth rate and heartwood extractive concentration in Douglas-fir. These same methods, combined with more intensive chemical analysis techniques, were applied to western redcedar (WRC) samples. The WRC data suggested that increasing the growth rate of this species did not necessarily require a tradeoff with natural durability in that species (Chapter 6).

A number of experiments showed that heartwood formation is complex (e.g. Chapters 3, 4 and 6), thus novel approaches are required to provide new information about the process. Carbon stable isotopes analysis was used to study heartwood formation (Chapter 5) to help identify the sources of raw materials that contribute to heartwood extractive formation in Douglas-fir.

This study confirmed that the relationship between heartwood extractives and durability is complex (Chapter 7). In western redcedar and Alaska cedar, methanol-soluble extractives were an important factor in determining wood durability; however, the relationship between individual compounds and natural durability was inconsistent. Furthermore, natural durability characteristics varied with the attacking organisms.

8.1 IMPLICATIONS

The global forest resource is changing from relatively old, large trees with good performance characteristics, including natural durability, to smaller, younger trees. The increased use of new tree species will likely change the properties of the wood products made from these logs. It is also known that the age of a tree affects the proportion of the stem that is in (potentially durable) heartwood, with younger trees having less heartwood. In addition, the heartwood tissues formed when a tree is younger often contain lower levels of extractives, the chemicals that are primarily responsible for durability. Thus, younger trees might well be expected to have a smaller proportion of

heartwood that is also less durable. This is a troubling prospect for forest managers who are stuggling to provide increasing quantities of wood products with suitable properties.

In this study, it was shown that environmental influences could affect heartwood formation, in addition to genetic and ontogenetic factors. This is an important finding. because it raises the possibility that we can modify heartwood durability through silviculture. The study of young western redcedar trees suggested that growing trees faster does not necessarily result in reduced heartwood extractives concentration and is reassuring because it indicates that optimizing tree growth rates may not require a tradeoff with natural durability. However, this is only a preliminary study with young trees. Other research indicates that effects may vary among species or trees of different ages. Furthermore, heartwood extractives appear to result from a mixture of carbon sources of different ages, thus the effects of management practices may not be apparent for many years.

This research has focused on extractive concentrations within heartwood, because these substances are responsible for natural durability. However, the relationship between extractives and durability remains poorly understood. This study has shown that not all extractive measures are equally useful as predictors of durability. In addition, relationships between extractives and durability vary with the biodeterioration organism. It is important to

understand this complexity, in addition to the complexity of the heartwood formation process, when attempting to understand the future implications of forest management practices on the natural durability of wood products.

8.2 OPPORTUNITIES FOR FURTHER RESEARCH

This research has provided some exciting insights into the nature of heartwood formation; however, much remains to be discovered about the relationship between the factors that influence heartwood formation in trees of various species and ages. In addition, we need to better understand the effect of heartwood variation on natural durability.

It would be useful to repeat and expand the study of the effect of environmental change on heartwood extractive content of Douglas-fir to include trees of different genotypes and ages, and other treatment variables. It would also be valuable to confirm the findings presented in Chapter 3, and to be able to put those results in context. Unfortunately, there are few controlled genetic trials of species other than Douglas-fir in this region, however, it would also be valuable to do similar expanded studies with species such as western redcedar that have highly durable heartwood. This would provide insights into how ecophysiological differences may affect heartwood properties, and would provide useful information for forest managers.

Stable isotope analysis appears to be a useful tool for the study of heartwood formation. Repeating the study described in Chapter 5 with other

species would likely provide valuable insights into how heartwood formation occurs and how it varies among species. Species included in such a study should include deciduous and evergreen trees, conifers and hardwoods and include species that span a range of sapwood thicknesses, heartwood extractive concentrations and heartwood durabilities.

Clearly, the relationship between heartwood extractives and durability is complex and focussing on a few extractives parameters with one or two biodeterioration organisms is insufficient. The application of scanning techniques that can rapidly generate large amounts of data on many wood samples (e.g. near infrared spectroscopy), combined with computerized statistical techniques that can make use of such huge datasets (e.g. principal components analysis), may be a superior method for helping us to understand the link between the properties and performance of naturally durable wood products.

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