AN ABSTRACT OF THE THESIS OF

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Title: Improving the Durability of Second Growth Timbers of Naturally Durable Species

Abstract approved:

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Jeffrey J.Morrell

While the heartwood of many wood species exhibits excellent resistance to fungal and insect attack, this resistance is sometimes diminished in second-growth material of the same species. The reasons for the reduced durability are unclear, but they may reflect a combination of both higher proportions of sapwood as well as reduced levels of heartwood extractives. Second growth timbers are often faster grown and this accelerated growth may affect the production of toxic extractives present in the heartwood. Reduced durability may affect the reputation, quality, and values of the final products.

One approach for maintaining the quality of naturally durable second-growth timbers is to supplementarily treat the wood with low concentrations of preservatives. In this research, we evaluated the ability of two commercially-available and environmental-friendly preservatives to protect both the sapwood and heartwood of teak, western red cedar, and coastal redwood. Cubes of these timbers and southern pine, a decay susceptible control, were vacuum-pressure treated with varying retentions of didecyldimethylammonium chloride (DDAC), and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI), or alkaline copper quaternary (ACQ).

Preservatives performance was assessed first in a leaching test. Preservative concentrations in the wood changed little after leaching.

Durability improvement was assessed by exposing treated cubes to two decay fungi (*Trametes versicolor* and *Postia placenta*) in a soil block test. The results suggested that DCOI (0.6 kg/m^3) and DDAC (4 kg/m^3) improved sapwood durability of teak, redwood, and western red cedar. While the performance of all treated heartwood was better than the performance of the untreated samples, these differences were too small to delineate differences. These results provided weak evidence that supplemental treatment may enhance heartwood durability.

Finally, we evaluated possible interactions between heartwood extractives and DDAC and DCOI, using a bioassay with *Trametes versicolor* and *Postia placenta* on agar medium. Sawdust of teak, coastal redwood and western red cedar were extracted with solvents of various polarities and impregnated on filter paper disks. The disks were placed on Petri dish surfaces previously inoculated with fungal suspensions. Western red cedar and teak extractives inhibited growth of the fungi, while coastal redwood extractives had no antifungal activity. Combinations of these extractives and chemical preservatives were no more efficient than the individual chemical. The results suggest that supplemental treatments do not synergistically interact with the heartwood extractives.

Improving the Durability of Second Growth Timbers of Naturally Durable Species

by

Yohanna Cabrera Orozco

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APPROVED:

Major Professor, representing Wood Science

Head of the Department of Wood Science and Engineering

Dean of the Graduate School

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

Yohanna Cabrera Orozco, Author

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CONTRIBUTION OF AUTHORS

Dr. Jeffrey J. Morrell assisted with writing the thesis. Dr. Lehong Jin assisted with sample analysis of the leaching test.

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IMPROVING THE DURABILITY OF SECOND GROWTH TIMBERS OF NATURALLY DURABLE SPECIES

CHAPTER 1- INTRODUCTION

The service life of wood is generally limited because it is susceptible to destruction by biological agents (fungi, insects, and mariner borers). These agents use the energy stored in the various constituents of the wood as nutrition and the damage, called biodegradation, negatively affects wood properties.

While the heartwood of many wood species exhibits excellent resistance to biodegradation, the resistance is sometimes diminished in second-growth material of the same species. For example, second growth timber of species such as coastal redwood has reduced heartwood durability (Clark and Scheffer, 1983). The reasons for the reduced durability are unclear, but they may reflect a combination of both higher proportions of sapwood as well as reduced levels of heartwood extractives. Second growth timbers are often faster grown and this more rapid growth may affect the proportion of toxic extractives present in the heartwood.

Ultimately, reduced durability may affect the quality and value of the final products. In fact, in 2003, natural teak, which is appreciated for its quality and durability, had an average price of US$700/$m^3$ for round logs (ITTO, 2003), while second-growth teak had an average price of only US$300/$m^3$ (Akwasi and Amoako, 2004). Sapwood represents an increasing volume of lumber of naturally durable species (Mockus-Lubin *et al*, 1986). Boards containing sapwood are designated as lower grade materials that provide a lower financial net value (USDA, 1999).

One approach for maintaining the quality of naturally durable second-growth timbers is to supplementarily protect the wood with preservative treatments. Protecting the wood with chemicals should result in a more uniform and predictable durability. Ideally, the chemicals used should have several attributes, including:

- High specificity against wood destroying organisms.
- Low mammalian toxicity.
- High water solubility, high stability, and low volatility, all to reduce the risk of negative environmental impacts.
- Colorless.
- Low price.
- Potential resistance to weathering.

For decades, wood preservatives were composed of heavy metals such as chromated copper arsenate (CCA) or were broad spectrum, heavy duty biocides such as pentachlorophenol. The use of these types of chemicals would largely diminish the attraction of using naturally durable woods because the public would not accept the resulting material as natural. The ideal treatment system would be highly targeted towards wood destroying organisms, have little toxicity to non-target organisms and would not significantly alter wood appearance.

A number of attractive biocides have been developed that could be used for such treatments, including isothiazolones and quaternary ammonium compounds. These biocides have been used in a number of formulations, but there is little data on their effectiveness on durable heartwood. In this research, we evaluated the effects of supplemental preservative treatments on durability of teak *(Tectona grandis)*, western red cedar *(Thuja plicata)*, and coastal redwood *(Sequoia sempervirens)*.

OBJECTIVES

- To evaluate the ability of supplemental treatments to enhance the durability of plantation-grown teak, western red cedar, and coastal redwood in laboratory decay tests.
- To assess possible interactions between heartwood extractives and supplemental treatments that could affect durability.

CHAPTER 2- LITERATURE REVIEW

2.1. Natural Durability of Wood

Natural durability is defined as "the degree of resistance of a species of wood to attack by wood-destroying fungi under conditions that favor such attack" (USDA, 1999). Willeitner and Peek (1997) summarized previous investigations on natural durability and pointed out that, while "the inherent resistance of wood to attack by wood destroying organisms" is an acceptable definition for natural durability, each wood has its own natural durability, even if it is very poor. However, the term is used in the lumber industry to designate woods that are resistant to decay (Zabel and Morrell, 1992).

Durability is a property exclusive to the heartwood and not of the sapwood. It depends on many factors including the presence of extractives, lack or inaccessibility of suitable nutrients, and mechanical barriers present on the wood, such as aspirated or encrusted pits or tyloses that reduce moisture uptake (Taylor *et al*, 2003). The presence of toxic extractives appears to be the most common cause for enhanced durability (Taylor *et al*, 2003; Hillis 1987; Scheffer and Cowling 1966). In addition to fungicidal activity, some heartwood extractives have also been shown to possess antioxidant activity that allows them to sequester the free radicals produced by fungi during the decay process. Thus, these extractives inhibit an early step in the degradation process (Schultz and Nicholas, 2000).

Scheffer and Cowling (1966) summarized the role of extractives on wood durability as follows:

- Heartwood extractives from durable woods are much more toxic than sapwood extractives from the same wood.

- Decay resistance of heartwood is reduced by extraction with hot water and/or organic solvents.
- There is a positive correlation between the extractive toxicity and decay resistance of a species.
- The distribution of decay resistance within a tree stem can be correlated with the distribution and nature of extractives.
- There is considerable variation among decay fungi in their tolerance to specific extractives. See Table 2.1.

Table 2.1. Effect of various decay-inhibiting heartwood extractives on wood weight losses. (Scheffer and Cowling, 1966)

Extractives	**Wood weight loss (control loss = 100%)**							
	Assays by Anderson, Scheffer and Duncan (1963)				Assays by Rudman (1963)			
	Trametes versicolor	*Postia placenta*	*Neolentinus lepideus*	*Gloeophyllum trabea*	*Trametes versicolor*	*Postia placenta*	*Neolentinus lepideus*	*Gloeophyllum trabeum*
β-thujaplicins		10	0	4	62		29	
γ-thujaplicins	0	39	3	0	8	0	9	10
α-thujaplicinol		2	3	3				
β – thujaplicinol		13		9	30	0	0	1

Extractives can also act as mechanical barriers to fungal hyphae and may reduce wood wettability (Taylor *et al.*, 2003; Stirling and Morris, 2006). For example, extractives contribute to reduced equilibrium moisture content in western red cedar (WRC) (Stirling and Morris, 2006). In contrast, extractive depletion can result in declining durability. The causes and rates of such depletion remain poorly understood, but have sometimes been attributed to biological detoxification or to simple leaching (Stirling and Morris, 2006). Scheffer and Cowling (1966) stated that "some extractives may be 'locked in' physically, and, thus, may resist loss through leaching".

Microbial degradation of certain decay-resistant-extractives to less active compounds has been observed in WRC (Jin *et al*, 1988). Changes on incense cedar, *Libocedrus decurrens*, and *Eucalyptus marginata* may be attributed to oxidation, catalysis, or polymerization of heartwood polyphenols (Anderson *et al*, 1963; Scheffer and Cowling, 1966).

Zabel and Morrell (1992) reviewed research on the variation in wood durability in a tree. They summarized that:

- Durability in many species is similar from the cambium to the sapwood-heartwood interface.
- Durability increases sharply at the sapwood-heartwood interface and then declines towards the pith. Recently, formed heartwood is presumed to be most toxic. Toxicity tends to decline as heartwood ages.
- Extractive detoxification, oxidation, or polymerization can reduce toxicity. Sherrad and Kurth (1933) observed these tendencies in redwood logs.
- In general, durability decreases with stem height and the most durable wood is near the base of the tree.

It has long been known that some species have higher durability than others (Hillis, 1987), and natural durability has been studied for much of the 20th century (Willeitner and Peek, 1997). However, the nature of heartwood formation and the roles of various extractives in durability remain poorly understood, even in commercially important species such as western red cedar, teak, and coastal redwood. A significant concern about durability is the risk of reduced heartwood activity in second-growth timbers of different species. These timbers may have lower extractive levels or different ratios of more toxic components (Clark and Scheffer, 1983; Mockus-Lubin *et al.*, 1986; DeBell *et al*, 1999).

2.1.1. Natural durability of Western Red Cedar (*Thuja plicata*)

Western red cedar (WRC) is a common tree in the Pacific Northwest Coastal and Interior forests of North America. The heartwood of WRC has long been appreciated for its resistance to fungal and insect attack (Scheffer, 1957). West Coast Native Americans used WRC for baskets, boats, houses, totems and many other applications requiring resistance to fungal attack. Wood of this species is currently used for exterior residential applications in Canada and the United States (Gonzalez, 2004).

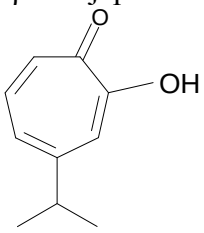
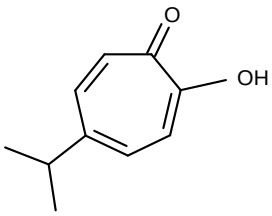
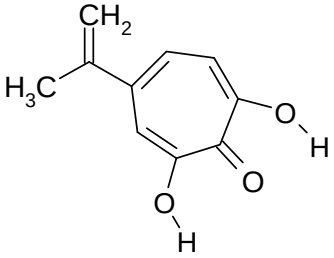
WRC durability can be attributed to the presence of aromatic compounds that inhibit fungal and bacterial growth (Nault, 1988) (Table 2.2). These compounds are produced as the ray parenchyma cells in the sapwood die and become heartwood (Bamber, 1976). Carbohydrate storage compounds in the sapwood undergo a series of complex reactions to produce tropolone compounds including β- and γ-thujaplicin, and β–thujaplicinol, whose fungicidal activity has been compared with pentachlorophenol (Scheffer and Cowling, 1966). The chemistry, relative amounts, and toxicities of these compounds have been studied extensively (Jin *et al.*, 1988, Nault, 1988; DeBell *et al.*, 1999; Chedgy *et al.*, 2005; Clark *et al*., 2004). The decay resistance of the WRC heartwood is believed to be a function of the relative proportion of each compound as well as the microdistribution of each, but there is little definitive data supporting this assertion. Thujaplicin inhibits many basidiomycetes at concentrations between 10 to 20 ppm (Inamori *et al*., 2000; Rennerfelt, 1948; Lim *et al*, 2005) and ascomycetes at concentrations between 8 and 32 ppm (Lim *et al*, 2005). In general, WRC has a broad base of activity against many decay fungi (Scheffer, 1957).

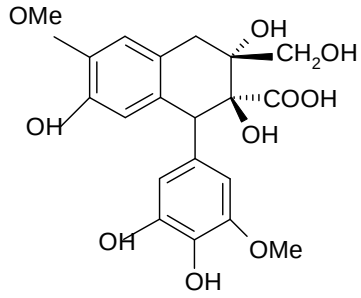
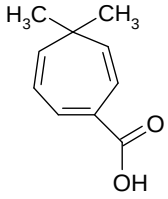
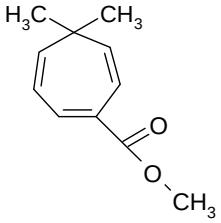
While the fungicidal properties of thujaplicins have been well documented, leaching of these compounds gradually lowers decay resistance in exposed parts of WRC. Thujaplicin in-cold-water extracts from WRC heartwood imparts enough toxicity to inhibit growth of decay fungi, and extractive loss is probably responsible for the gradual decrease in decay resistance in exposed WRC (Scheffer, 1957). For example, western woods in very wet soil did not perform as well as less durable woods

(Englerth and Scheffer, 1955). The poor performance was attributed to leaching because the outermost parts of the stakes below ground were uniformly and heavily decayed, whereas the wood beneath this shell of decay was comparatively sound.

Scheffer (1957) sampled 7 creosote-treated western red cedar poles that had been in service 21 to 29 years, to provide information about the permanence of the decay resistance. Weight losses in heartwood samples removed at the groundline and at six feet above the groundline showed that the outer heartwood was less decay resistant than recently felled heartwood when exposed to five decay fungi.

Table 2.2. Biologically active compounds from the heartwood of *Thuja plicata*.

Extractive	**Properties**	**Source of information**
β-Thujaplicin 	Fungicide	Rennerfelt (1948) Rudman (1963) Jin *et al*, (1988)
	Bactericide	Nault (1988)
γ-Thujaplicin 	Fungicide	Rennerfelt (1948) Rudman (1963) Jin *et al*, (1988)
β-Thujaplicinol 	Fungicide	Chedgy *et al*. (2005)

Plicatic acid 	Fungistatic Reduction of equilibrium moisture content Fungicide	Roff and Atkinson (1954) Stirling and Morris (2006) Stirling and Morris (2006)
Thujic acid 	Unknown	Chedgy *et al*. (2007)
Methyl thujate 	Unknown	Chedgy *et al*. (2007)
2-Acetonaphthone Methoxyhydroquinone	Unknown	Clark *et al*. (2004)

An increasing amount of WRC wood is harvested from second-growth forests. Second-growth WRC heartwood contains lower amounts of the protective extractives and in some cases higher amounts of as yet unknown extractives (Clark *et al*., 2004). Nault (1988) analyzed the ethanol-benzene extractives content of second-growth and old-growth WRC and found 11.4 to 22.8% total extractives in old-growth WRC (1.8% thujaplicins) compared with 6.2 to 9.8% (0.7% thujaplicins) total extractives in second-growth samples.

As noted earlier, natural durability varies with location in the tree, tending to increase from the pith to the heartwood-sapwood boundary as well as with distance from the top of the tree (Zabel and Morrell, 1992; DeBell *et al.*, 1999). The reactions that

produce thujaplicins and other extractives are believed to continue as heartwood ages. DeBell *et al.* (1999) found that tropolone content of samples from 11 second-growth trees was lower near the pith and associated juvenile wood. The reduced tropolone content could reflect reduced extractive content at the time of heartwood formation or it could be the result of continued extractive reactions.

Durability has been thought to be dependant on the geographic origin (Scheffer, 1957; Freitag and Morrell, 2001). However, Scheffer (1957) tested 67 WRC cross sectional disks across the growing region and found no evidence that the variability in decay resistance was related to the elevation of the growing site or growth rate. Freitag and Morrell (2001) performed a similar test and obtained similar results.

Thujaplicins can be metabolized by several organisms. Jin *et al*. (1988) observed microbial colonization in a 420-year-old WRC and correlated this activity with extractive detoxification. They found that *Sporothrix sp*. metabolized γ- and β-thujaplicin to thujin, which showed no toxicity to a common wood decay fungus.

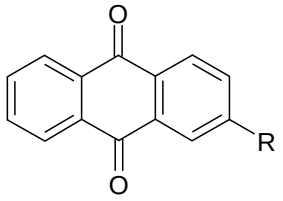
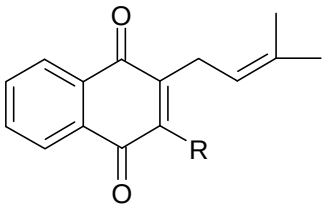
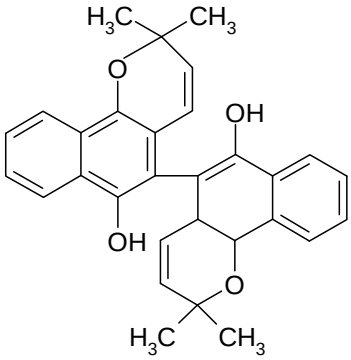
2.1.2. Natural durability of Teak (*Tectona grandis*)

The heartwood of old-growth teak (*Tectona grandis*) is well-known for its natural durability. Teak's natural distribution area covers 27.9 million hectares in Myanmar (16 million ha), India (9 million ha), Thailand (2 million ha) and Laos (20.000 ha) (Ko and Kyaw, 1998). Excellent mechanical properties and high durability teak have made teak the third most widely planted tropical species in the world, and the most widely used species for luxury applications (Behaghel, 1999).

Teak heartwood contains a number of toxic extractives (Table 2.3) (Rudman and Dacosta, 1959; Hillis, 1987; Thevenon *et al*. 2001). Tectoquinone, a potent insecticide extractive found in teak, is highly repellent to the dry-wood termite *Cryptotermes brevis* (Wolcott, 1955) and to the subterranean-termite *Reticulitermes flavipes* (Sandermann and Dietrichs, 1957). Other extractives including tectoquinone showed

little activity against the fungi *Coniophora puteana* or *Trametes versicolor* or against the subterranean-termite *Coptotermes lacteus* (Rudman and Dacosta, 1958). Interactions between extractives, rather than the activity of tectoquinone alone, was postulated to be the cause of teak durablilty.

Table 2.3. Biologically active compounds from the heartwood of *Tectona grandis*.

Extractive	Properties	Source of information
Tectoquinone  R= CH3 2-Methylanthraquinone R= CH2OH 2-Hydroxymethylanthraquinone	Insecticide	Sanderman and Dietrich, 1957
Lapachol, deoxylapachol  R= OH = Lapachol R= H = Deoxylapachol	Fungicide	Sanderman and Dietrich , 1957
Tectol 	Unknown	Rudman, 1961
Rubber Dehydrotectol	Unknown	Rudman, 1961

Second-growth teak apparently has lower durability, possible as a result of lower extractive content or differential distribution in comparison with old-growth wood (Haup *et al*., 2003). Resistance to two test fungi did not normally begin until five to ten growth rings from the pith suggesting that heartwood in the juvenile wood zone was less durable.

Leithoff *et al*. (2001) compared the durability of second-growth teak from Panama with old-growth timber from Myanmar. While the second-growth specimens showed mass losses between 32% and 43%, the old-growth samples experienced only 2.3% to 12.3% mass losses. Haupt *et al.* (2003) found that total extractives content in plantation teak was 9.4% weight/weight (wt/wt), while the old growth had 14.1%

Haupt *et al.* (2003) also evaluated the role of tectoquinone and other extractives in relation to durability of the same Panama and Myanmar woods. Tectoquinone yield was high while deoxylapachol content was low in specimens with high natural durability. They suggested that deoxylapachol was a precursor of tectoquinone and attributed reduced durability to a lower biosynthetic efficiency in conversion of deoxylapachol into tectoquinone.

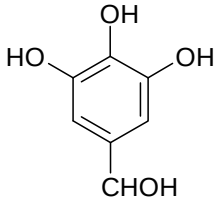
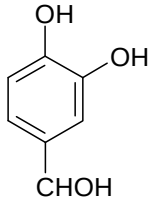
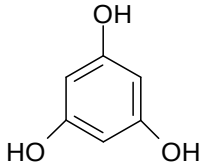
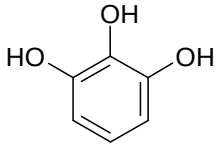
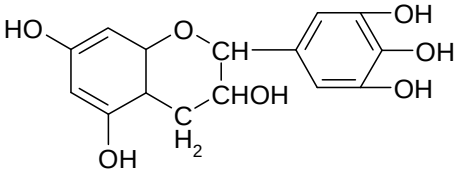
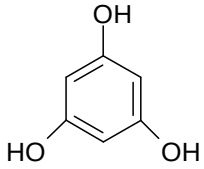
2.1.3. Natural durability of Coast Redwood (*Sequoia sempervirens*)

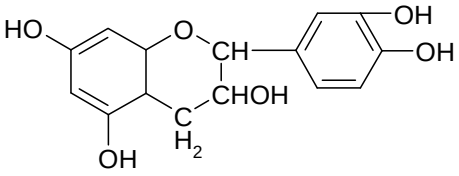
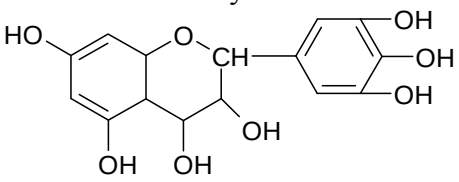
California or coast redwood is found along the northern California coast and a few kilometers into Oregon. Redwoods are especially noted for their immense height and great age. The wood is highly valued for its dura`bility, low shrinkage, and texture (Anderson, 1961).

The composition and amounts of extractives in redwood have been well studied (Table 2.4) (Balogh and Anderson, 1965 and 1966; Luxford and Markwardt, 1932; Sherrard and Kurth, 1933). Extractives are responsible for the color and odor of redwood and play important roles in heartwood durability (Anderson, 1961).

Redwood extractive content decreases with height in the tree and towards the center of the lower trunk; distribution becomes more uniform with increasing tree height (Sherrard and Kurth, 1933; Resch and Arganbright, 1968).

Table 2.4. Biologically active compounds from the heartwood of *Sequoia sempervirens*.

Extractive	Properties	Source of information
Gallid acid 	Antifungal and antiviral	Anderson, 1961
Protocatechuic acid 	Antioxidant	Anderson, 1961
Phloroglucinol 	Fungicide	Anderson, 1961
Pyrogallol 	Reducing agent	Anderson, 1961
L-Gallocatechin 	Antioxidant	Anderson, 1961
Phloroglucinol 	Fungicide	Anderson, 1961

Cathechin 	Antioxidant	Anderson, 1961
Leucocyanidin 	Antioxidant	Anderson, 1961

Sherrard and Kurth (1933) demonstrated that the water soluble extractives from the outer part of some old-growth heartwood sections inhibited the wood-destroying fungus *Heterobasidium annosum* while extracts from inner-sections of the same trees did not. Anderson (1961) calculated that extractives content in redwood heartwood range from 15 to 30% of the wood.

Clark and Scheffer (1983) tested the durability of both second-growth (84 specimens), and old-growth redwood logs (83 specimens) using soil block tests. All the old-growth specimens were rated as "highly resistant"[1], while less than a half of the second-growth samples were rated as "resistant" or "highly resistant".

Hillis (1987) found that the heartwood contained 9.9% water soluble extractives and 1.1% in ether soluble extractives, while sapwood contained neither of these materials.

Resch and Arganbright (1968) found that old-growth extractive content was 11.7%, while second growth extractives was 9.0%. The differences were statistically significant; however, extractive distributions were more uniform in second growth trees than in old-growth.

While high durability is desirable, predictability or uniform performance are also desirable because they allow the end users to predict performance of a certain wood

[1] Tested according to the ASTM D 2017.

product for a given use. Natural durability varies widely between species but also can be variable in a single piece of wood. This variation can cause problems particularly in engineered applications. Treating wood with supplemental chemicals might help improve durability while enhancing the uniformity of the product.

2.2. Treatability of Natural Durable Wood

There is no direct relationship between the natural durability and treatability of species. Hwang *et al.* (2007), suggested that heartwood provided enhanced protection against biodeterioration, despite the limited uptake of preservatives in heartwood compared with sapwood. The limited uptake could be due to high extractive content, aspirated pits, tyloses, or smaller pore sizes (Wang and DeGroot, 1996)

Mockus-Lubin *et al*., (1986) treated second-growth redwood lumber with chromated copper arsenate (2% solution) using a full-cell pressure treating process. They concluded that the heartwood lumber was easily treated with CCA, as long as it had been kiln-dried before treatment.

Newbill and Morrell (1990) evaluated 30 chemical formulations for their ability to prevent decay of western red cedar sapwood during a 2 year test fence exposure. Four formulations provided protection equivalent to pentachlorophenol, including a substituted isothiazolone. The results suggest that supplemental organic preservatives can enhance the performance of durable heartwood.

DeGroot (1995) pointed out that WRC shakes and shingles often contain non-durable sapwood that requires protection. Longitudinal penetration in the products is generally limited (12.7 to 19.1 mm) but this degree of treatment may be acceptable because this initial attack tends to began on the butt ends. Coincidentally, this is also where the highest moisture levels develop.

2.3. Supplemental Treatments

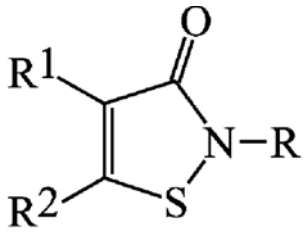
There are a variety of newer generation organic biocides that may be suitable for enhancing heartwood durability. Among these systems are quaternary ammonium compounds and substituted isothiazolones.

2.3.1. Substituted isothiazolones

Isothiazolones are biologically active compounds used as bactericides or fungicides where moisture is present. Morley *et al*, (2005) reported the early history of isothiazolones: *"the first commercial biocide, 4,5-benzo-3-isothiazolone, was synthesized in 1923, but its anti-microbial properties were reported and patented in 1957 by Katz and Schroeder. The synthesis of N-methyl-3-isothiazolone and the N-ethyl derivative were first described by Crow and Leonard in 1965, but their biological properties were not discussed. In 1973, the synthesis and biological properties of a large number of related 3-isothiazolones were described by Lewis, Miller and Law, with some showing a broad spectrum of antibacterial and antifungal activity at extremely low concentrations".*

The general structure of 3-isothiazolones (Figure 2.1) includes a heterocyclic ring containing activated N-S bonds and three substituents (Paulus, 2005).

Figure 2.1. General structure of 3-isothiazolones



The biological efficacy of individual isothiazolones is highly dependent on the nature and position of the substituents attached to the isothiazolone ring. For example,

Morley *et al*., (2005) reported that while 4-methyl-N-methyl-3-isothiazolone and N-(2-hydroxethyl)-3-isothiazolone had similar biological activity against the bacteria *Escherichia coli* and *Staphylococcus aureus* and the fungus *Aspergillus niger*, another compound 5-chloro-N-vinyl-3-isothiazolone was several orders of magnitude more active against these same organisms.

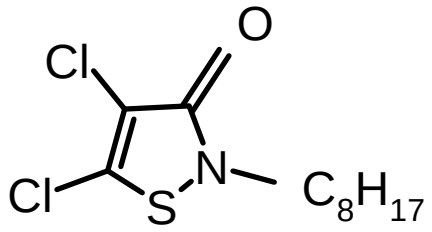
2.3.1.1. Reaction Mechanisms and Mode of action

Isothiazolones are thought to diffuse through the cell membranes or walls and then react with sulfur containing proteins. The mechanism has not been fully elucidated, but the mode of action involves nucleophilic attack by the sulfur atom of glutathione at the sulfur atom of the 3- isothiazolone, leading to the cleavage of the S–N bond to produce a ring opened amidodisulfide that can react further with the same nucleophile to yield β-mercaptoacrylamide. This process can result in the cell death (Morley *et al*., 2005). Lethal effects include the production of highly reactive intermediates and free radicals, inhibition of respiration, rapid growth inhibition, and loss of viability (Diehl and Chapman, 2000; Chapman *et al*, 1998; Collier *et al*., 1990)

The isothiazolone derivative 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI) has demonstrated excellent potential as a wood preservative and has performed well in laboratory and field tests both in ground contact and above ground (Hegarty *et al*., 1997; Nicholas *et al*., 1984; Leightley and Nicholas, 1990).

DCOI in a water microemulsion tends to penetrate more deeply to wood than either emulsion- and solvent- based formulations (Yu and Leightley, 1993).

Figure 2.2. Structure of DCOI (4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one).



2.3.1.2. DCOI Performance in Above Ground Applications

Laboratory decay tests carried out with DCOI showed antifungal effects against three brown-rot fungi, with toxic thresholds ranged from 0.37 to 0.50 kg/m³. Toxic thresholds were 0.42 and 2.3 kg/m³, respectively, for treated pine and sweetgum when exposed to the white-rot fungus *Trametes versicolor*. A toxic threshold value of 0.48 kg/m³ was obtained in a non sterile soil soft rot test, which indicates a high degree of effectiveness against these fungi (Nicholas *et al*, 1984).

Exposure of Scots pine blocks treated with different DCOI microemulsions to target retentions of 0.3 kg/m^3 to 2.0 kg/m^3 indicates that thresholds for brown and white fungi were 0.33 kg/m^3 and showed no significant differences between leached and unleached samples (Hegarty *et al.*, 1997)

A variety of small scale laboratory tests (AWPA Standard E10, European standard EN-113, and Japanese Wood Preservers' Association JWPA-1) have suggested that 0.37 to 0.50 kg/m^3 of DCOI inhibited growth of various fungi on pine blocks, suggesting a threshold value of 0.50 kg/m^3 (Greenley, 1986).

An above ground test showed no decay on L-joint samples treated with DCOI after two years exposure (Greenley, 1986). Performance of DCOI treated samples after 39 months was still comparable with those treated with pentachlorophenol and significantly better than untreated samples (Greenley and Hegarty, 1988)

In another DCOI above ground decay test in Mississippi all test units treated to levels of 0.41 kg/m^3 and higher had no deterioration after 45 months of exposure. DCOI appeared to be 10 times more effective than pentachlorophenol against decay fungi. Analytical results from test units exposed for 45 months indicated that DCOI was stable and was not depleted from the wood (Nicholas *et al*, 1989).

DCOI treated Scots pine L-joints exposed in Hawaii were largely free of decay after 5 years of exposure and there was a strong positive dose-response (Preston *et al.*, 1996)

2.3.1.3. DCOI Performance in Ground Contact Applications

In a 5 year stake test, samples treated with 2 kg/m^3 of DCOI in toluene or petroleum oil performed similarly to samples treated with 5 kg/m^3 of pentachlorophenol in soil contact (Greenley, 1986; Leightley and Nicholas, 1990).

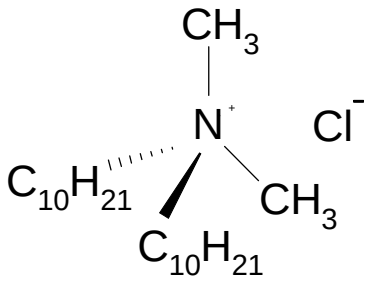
In two stakes tests, samples treated with DCOI in toluene at 4.6 kg/m^3 were still sound after 48 months with slight decay attack and no termite attack, while untreated samples were destroyed by decay or by termites (Greenley and Hegarty, 1988).

2.3.2. Quaternary Ammonium Compounds or Alkylammonium Compounds

Alkylammonium compounds (AACs) have been used for over 30 years as wood preservatives. AACs are derived from ammonia by replacement of hydrogen by alkyl chains of various lengths (Doyle, 1995). The hydrophobic chain length has profound effects on performance and absorption. Initial research into the use of AACs as wood preservatives gave promising results in controlling basidiomycetes, however, this system performs poorly in ground contact applications. As a consequence of this poor performance, AAC's must be used in combination with other biocides in most applications.

AAC's are attractive preservatives because they are broadly effective against a range of fungi, have limited volatility, the ability to be soluble in organic and aqueous solvents, and are inexpensive. In addition, AACs are colorless, have low toxicity to non-target organisms and their cationic nature allows them to react with anionic wood to resist leaching. These characteristics have resulted in widespread use of AAC's as biocides.

Figure 2.3. Structure of DDAC (Didecyldimethylammonium chloride)



2.3.2.1. Reaction Mechanisms of AACs

AAC's can react with wood through a number of mechanisms that affect fixation, distribution, and leachability.

Fixation: AAC fixation in wood can occur by ion pair or cation exchange mechanisms (Jin and Preston, 1991; Loubinoux *et al*, 1992). Ion exchange was suggested because of the influence of treating solution pH on fixation. Protons compete with the AAC cation for binding sites in the wood under slightly acidic treating solution conditions (Butcher and Drysdale, 1978). Alkaline treating solutions create more negative binding sites that increase the fixation rate. Increased treating-solution-pH increases didecyldimethylammonium chloride (DDAC) fixation in the wood (Jin and Preston, 1991; Doyle and Ruddick, 1994)

Fixation can also occur by cation exchange on the carboxyl and phenolic hydroxyl groups on the lignin (Jin and Preston, 1991). Cation exchange involves the removal of the ammonium from the solution by negative sites present on the lignin, and by replacement of wood protons by ammonium (Loubinoux *et al*, 1992). AAC chemical structure, wood type and extractive content influence the quantity of fixation. AAC fixation also is higher and more rapid at higher temperatures (Vinden, 1984).

Distribution: Poor distribution of AACs in wood has led to performance issues. Vinden (1984) first reported poor distribution of AACs in *Pinus radiata* during treatment trials. Butcher and Preston (personal communication) had earlier observed

excellent AAC distribution in *Pinus radiata*, but somewhat poorer distribution in *Pinus nigra* and very poor distribution in *Pinus ponderosa*, even when solution uptakes were excellent. Ruddick and Sam (1982) found that AAC distribution in four Canadian softwoods did not appear to be particularly uneven and could not be blamed for any failure of AAC-treated wood. Analysis of the AAC distribution in L-joints suggested that end grain penetration was excellent, but lateral penetration was very poor (Doyle, 1995, from Morris and Ingram, 1988). Later studies found that earlywood retained more AAC than latewood (Nicholas *et al*, 1991). This uneven AAC distribution could negatively influence the field performance.

Leachability: Laboratory trials suggest that AACs do not leach excessively from wood; however, the leaching rates depend on the initial retention of AACs (Doyle, 1995). Nicholas *et al,* (2000) observed increased water sorption rate on AAC treated southern pine.

2.3.2.2. Mode of action

AAC compounds are thought to affect the semi-permeable membrane of fungi causing leaking of cell constituents. AAC are also believed to inhibit respiratory activity (Xiao and Kreber, 1999; Eaton and Hale, 1993).

2.3.2.3. AAC performance in Above Ground Applications

Butcher and Greaves (1982) reviewed the Australian and New Zealand experience with AAC's and reported toxic thresholds for above ground applications between 1.12 and 3.6 kg/m^3.

DDAC treated Southern yellow pine shakes showed excellent performance in above ground field trials after 28 months of exposure (Barnes *et al*, 1985). No decay fungi were isolated from AAC-treated L-joints field trials after five years of exposure, while untreated controls contained viable decay fungi (Morris and Ingram, 1988). *Pinus radiata* shingles treated with 1.39 kg/m^3 alkyl (C_{12}, C_{14}, C_{16})

dimethylbenzylammonium chloride showed no sign of decay after a seven years in the field (Plackett *et al*. 1984). Later studies found that western hemlock, Pacific silver fir and western white pine treated with 4.8 kg/m^3 of DDAC were highly durable (DeGroot *et al.*, 1992). Both laboratory and field trials have generally indicated that AAC's performed well, especially in above ground commodities such as decking.

While the effectiveness of DDAC as a wood preservative has generally been satisfactory, some failures have occurred. Butcher (1985) reviewed the reasons for poor performance of AACs including substandard treatment, misuse in service, poor preservative distribution, depletion of the active ingredient during long term storage, and *Coniophora spp*. resistance to DDAC. Norton (2002) explained that alkyldimethylbenzyl ammonium chloride (benzalkonium chloride) (BAC) and alkyldimethylamine acetates were approved by the Timber Preservation Authority of New Zealand in 1978 for the protection of *Pinus radiata*, but continued failures resulted in cessation of use in the mid 1980's. However, a year later, results from a ground field exposure trial of BAC treated plywood at higher retentions than those used in New Zealand indicated that the system can perform well.

2.3.2.4. AAC performance in Ground Contact Application

Preliminary trials of various quaternary ammonium compounds in *Pinus radiata* against three fungi (*Postia placenta*, *Fomes gilvus* and *Chaetomium globosum*) generated toxic thresholds between 1.6 and 6.4 kg/m^3 (Table 2.5). Dimethylaurylamine and alkyltrimethylammonium halide generated toxic thresholds between 1.6 and 3.2 kg/m^3 (Butcher *et al*, 1977a). Further laboratory work with AACs in different woods species against soft rot showed better performance compared to the CCA treated wood and slightly less effective performance against basidiomycetes (Butcher *et al*, 1977b).

Table 2.5. Examples of toxic thresholds in selected AAC's tests

AAC	Test	Threshold kg/m^3	Reference
DDAC and Benzalkonium-chloride	Sapwood stakes of radiata pine and silver birch (Betula alba) exposed for 12 months in a fungus cellar and up to 30 months in field tests	1-2	(Butcher and Drysdale, 1978)
Dialkyldimethyl-ammonium-chloride and other 3 AAC's	Laboratory decay and termite test (ASTM Standard 1413)	1.6-6.4	Preston and Nicholas, 1982
DDAC and other eight formulations of AAC	Laboratory decay test (Japanese Industrial Standard JIS A 9302)	2 - 4.6	Tsunoda and Nishimoto, 1983
Dialkyldimethylammonium chloride	Laboratory decay test (ASTM Standard 1413)	2.5-6.4	Butcher and Drysdale, 1978
DDAC, different alkyl lengths	Soil block test (C10/C12 chain were the most effective)		Preston, 1983
DDAC and others	Simulated above ground decay test. C16/C18 were most effective	<1-2	Preston and Chittenden, 1982

While AAC's performed well in laboratory tests, they performed poorly in field trials. Non-modified AAC's did not perform as well as CCA after 30 months field exposure (Butcher *et al*, 1979); and showed signs of surface degradation soon after being placed in the field. Tillott and Coggins (1981) detected decay and failure of stakes treated with dialkyldimethyl amine chloride (14.6 kg/m^3) after one year in the field. Ruddick (1983) found that DDAC-treated stakes (3.2 kg/m^3) failed after two years in the field. AAC-treated wood (<11 kg/m^3) had noticeable decay after six years (Ruddick, 1987; Ruddick and Ingram, 1987; Morris and Ingram, 1988). Drysdale (1983) also found that DDAC (6.2 kg/m^3) and benzalkonium chloride (10.6 kg/m^3) treated *P. radiata* stakes were colonized and attacked by brown, soft and white rot fungi after four years in the field.

AAC-treated wood performance is variable and 6.4 kg/m^3 of AAC was required to provide adequate protection in three soil jar evaluations (Butcher, 1979). Pre-exposing AAC-treated wood blocks to staining fungi increased threshold values from 0.7-5 kg/m^3 to 5-10 kg/m^3 for the same decay fungi (Ruddick, 1986). Later studies suggested that 6-8 kg/m^3 benzalkonium chloride was required to protect the wood in a laboratory decay tests (Preston *et al.* 1987). DDAC treated *Cryptomeria japonica* (2.5 kg/m^3) was decayed by *Tyromyces palustris* and *Trametes versicolor* (Tsunoda and Nishimoto, 1987). DDAC treated wood (4 kg/m^3) also performed poorly against seven decay fungi in an Australian test (Greaves *et al*, 1988).

2.3.2.5. Insecticide Application

Benzalkonium chloride (6.2 kg/m^3) was shown to be effective against various insects including *Mastotermes darwinienses*, *Nasutitermes exitiosus* and *Coptotermes acinoaciformes* in *Pinus radiata* (Butcher and Greaves, 1982; Howick *et al*, 1983). Preston and Nicholas (1982) found that dialkyl (C_{12},C_{14}) dimethylammonium chloride (1.6 kg/m^3) prevented attack by *Reticulitermis flavipes* in southern yellow pine when tested in the laboratory test ASTM Standard D3345. Laboratory trials in Japan showed that DDAC (2.2 kg/m^3) controlled attack by *Coptotermes formosanus* in *Tsuga heterophylla and Pinus densiflora* (Tsunoda and Nishimoto, 1983).

Later studies found that DDAC (1.7 kg/m^3) did not prevent *Lyctus brunneus* attack in *Quercus serrata* (Tsunoda and Nishimoto, 1987), possibly because of the low retention tested. Termite tests showed that 4 kg/m^3 of DDAC protected wood, but did not cause complete mortality of *C. formosanus* (Tsunoda and Nishimoto, 1983). Overall, unmodified AAC's appeared to have good potential as insecticides. Preston *et al*. (1985) exposed DDAC treated southern yellow pine samples in Florida and Hawaii. After 16 months of exposure at Florida and two years of exposure at Hawaii, DDAC (3.4 kg/m^3) completely resisted attack by *R. flavipes* and *C. formosanus*, respectively. A laboratory bioassay showed that DDAC provided protection to *Pinus radiata* against *M. darwiniensis* and *C. acinaciformis* at 2 kg/m^3 (Creffield, 1995)

2.3.2.6. Secondary Biocides to improve AACs

As a result of the poor field performance of AACs, focus turned to formulating AACs with others biocides. Addition of acidic copper salts to the AAC improved the effectiveness in both laboratory and field trials (Butcher *et al.*, 1979; Drysdale, 1983). Tsunoda and Nishimoto (1987) found that copper amended DDAC (1 kg/m^3) had an improved effectiveness against *T. palustris* and *T. versicolor*. Tillott and Coggins (1981) found that acidic copper used in the various formulations did not undergo any apparent chemical fixation in the wood. In order to fix the copper in the wood, Sundman (1984) found that ammoniacal copper oxide gave better protection than the acid copper modified AAC's. Addition of ammoniacal copper oxide (3.2 kg/m^3) to octyldecyldimethylammonium chloride (8 kg/m^3) resulted in superior protection in a severe fungal cellar exposure when compared to CCA (24 kg/m^3) or ammoniacal copper arsenate (10 kg/m^3) (Sundman, 1984; Wallace, 1986). Hedley *et al*. (1982) found increasing degrees of effectiveness from unmodified AACs, ammonium hydroxide amended AAC's, copper-modified AAC's and the ammoniacal-copper-AAC formula. A five year field trial showed that ammoniacal copper oxide and alkyl (C_8, C_{10}) dimethylbenzylammonium chloride (4.8 kg/m^3) amended with tributyltin chloride was the most effective system after six years in the field (Ruddick, 1983; Morris and Ingram, 1988). Ruddick (1987) found that sodium tribromophenate and an AAC (10 kg/m^3) gave reasonable protection in a four year field exposure. While unmodified AAC's appeared to have little potential as wood preservatives, formulation of AACs with co-biocides has great potential. Field research has suggested that ammoniacal copper/AAC systems can perform as well as CCA-treated wood (Preston *et al.*, 1986; Jin *et al.* 1992). As a consequence of improved field performance; the ammoniacal copper modified AACs such as ammonium copper quaternary (ACQ) have been approved for use in many countries including Scandinavia countries, Japan and USA (Jin and Preston, 1992).

CHAPTER 3- METHODS AND MATERIALS

3.1. Wood Treatment

Heartwood and sapwood lumber of teak (*Tectona grandis*), coastal redwood (*Sequoia sempervirens*), western red cedar (*Thuja plicata*) and Southern pine (likely *Pinus taeda*) were obtained from various sources.

- Teak sapwood: Ecuador (20 year old plantation)
- Teak heartwood: Colombia (30 year old plantation)
- Redwood sapwood and heartwood: California, USA
- Western red cedar: Oregon, USA
- Southern pine: USA

Cubes (19 mm) cut from the boards were oven dried (103ºC / 24 hours) and weighed. The blocks were then conditioned (70% relative humidity / 20ºC) prior to shipment to Charlotte, NC for treatment with 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI), didecyldimethyl ammonium chloride (DDAC), or alkaline copper quaternary (ACQ), as reference preservative, to the following retentions:

- DCOI: 0.1, 0.3, 0.6 kg/m^3
- DDAC: 1,2, 4 kg/m^3
- ACQ: 4 kg/m^3

Forty blocks were treated with each chemical combination. The treatment began with an initial vacuum of 25 mm of Hg followed by 1034 KPa of pressure for 10 minutes. The blocks were weighed as a treatment group and net weight gains were used to calculate chemical retentions. This treatment produced nearly 100% weight gain in southern pine, but was slightly less effective for some other species.

The blocks were then oven dried (103ºC/24 hours) and weighted individually prior to the following tests.

3.2. Leach Resistance Test

The twelve blocks from each species/treatment combination that were closest to the average retention were selected. Six samples from each of these groups were subjected to a leaching treatment for 336 hours according to AWPA Standard E11-97 (American Wood Preservers' Association, 2006). The other six samples were kept in a standard room (70% relative humidity / 20ºC).

The leaching procedure consisted of submerging the 19 mm cubes in 300 ml of deionized water. The samples were subjected to a pressure of 96 KPa for 45 minutes. The leaching water was replaced and collected at 6, 24, 48, 96, 144, 192, 240, 288, and 336 hours. After the treatment, the leached and unleached samples were ground to pass a 40 mesh screen and the ground material was analyzed for chemical retention using the methods described below. Leachate water and ground wood samples were then shipped to Charlotte, NC for chemical analysis using the following methods.

- Samples treated with DDAC were extracted sonication in methanol for three hours (1 gram of oven-dried sawdust). The extract was allowed to settle overnight, and then analyzed by titration according to AWPA Standard A17-03 (American Wood Preservers' Association, 2004).
- Samples treated with DCOI were also extracted with methanol using an ultrasonic bath. The filtered extract was analyzed using a high performance liquid chromatography (HPLC) equipped with a UV detector according to AWPA Standard A30-00 (American Wood Preservers' Association, 2004).
- Samples treated with ACQ-D, which contains 66.7% copper oxide and 33.3% quat as DDAC, were analyzed for both DDAC and copper. DDAC was analyzed as described for DDAC treatment alone. Copper was analyzed by X-ray fluorescence

spectroscopy according to AWPA Standard A9 (American Wood Preservers' Association, 2004).

Retention levels were expressed in parts per million (ppm), then converted to kg/m^3, using the reported density of each species at 12% moisture content (USDA, 1999). The chemical analysis was performed on the mixture of two ground experimental cubes to produce a single value. This process was replicated three times; the result is the average of three single values. Given the low replication, no statistical analysis was performed.

3.3. Soil Block Test

Preservative efficacy of the leached and unleached blocks was tested under laboratory conditions.

Twelve leached and twelve non-leached blocks were randomly selected from each species - treatment combination. The leached blocks were subjected to the same water immersion described in Section 3.2. The cubes were oven-dried (103ºC / 24 hours), weighed (nearest 0.01 g) and then soaked with water prior to being placed in plastic bags and sterilized by exposure to 2.5 mrad of ionizing radiation from a cobalt 60 source.

Test chambers were prepared by half-filling 454 ml French squares bottles with moist forest loam and placing a western hemlock (*Tsuga heterophylla*) (for brown rot fungi) or red alder (*Alnus rubra*) (for white rot fungus) feeder strip on the soil surface. Sufficient water was added to the loam to achieve 42 to 45% moisture content. The bottles were then loosely capped and autoclaved for 45 minutes at 121ºC. After cooling, the bottles were inoculated with 3 mm diameter malt agar disks cut from the actively growing edges of cultures of the test fungus previously grown on malt-agar, in Petri dishes. The fungi used were *Postia placenta* (Fr.) Larsen and Lombard (Isolate # Madison 689), a brown rot fungus, and *Trametes versicolor* (L. ex Fr.) Pilat (Isolate # Madison 107R), a white rot fungus. The agar plugs were placed on the

edges of the wood feeder strips, then the jars were loosely capped (to allow air exchange), and incubated until the feeder strip was thoroughly covered with fungal mycelium. The sterile test blocks were then placed on the surfaces of the feeder strips, and the bottles were loosely capped and incubated at 28ºC for 12 weeks for the brown rot fungus and 16 weeks for the white rot fungus. Additional bottles were prepared without the decay fungus to determine weight losses caused by leaching of either extractives or preservatives.

At the end of the incubation period, the blocks were removed, scraped clean of adhering mycelium, and weighed to determine wet weight. The blocks were then oven-dried (103ºC / 24 hours) and weighed. The difference between initial and final oven-dry weight was used as a measure of the decay resistance of each material. The greater the weight loss, the more extensive the decay, and therefore the lower the decay resistance.

After assessment of the weight losses, each set of species - treatment combinations was compared with the weight loss values of the untreated control of the same group in a t-test using S+® (Enterprise Developer Version 8.0.4 for Microsoft Windows: 2007)

3.4. Synergic Behavior of Heartwood Extractives and Supplemental Wood Preservatives on Fungi Resistance

Possible interactions between heartwood extractives (from the same second-growth timber of the previous experiments) and DCOI and DDAC were examined in this experiment.

Fungal Inoculum: *Postia placenta* (Fr.) Larsen and Lombard (Isolate # Madison 689) and *Trametes versicolor* (L. ex Fr.) Pilat (Isolate # Madison 107R) were cultured in 1.5% malt agar medium (Merck, Germany). A sterilized spatula was used to collect an agar disk of each fungus which was blended in 500 ml of prior-sterilized distilled

water to make a liquid suspension. The suspension was blended and refrigerated until it was used.

Test Compounds: For the extraction, solvents of different polarities were employed, including hexane, dichloromethane, and ethanol (all solvents were HPLC reagent grade with the exception of distilled water and 95% ethanol). During the development of the bioassay, however, the biological activity of some extractives was lost because of high extraction temperatures and/or exposure to air and/or light. Thus, we tried three different extraction methods before obtaining a procedure that maintained biological activity.

Method 1: Teak, western red cedar, and redwood heartwood were ground to pass a 30 mesh screen. Seventy grams ground material was added to a 1000 ml flask together with 600 ml of hexane, dichloromethane, or water. A condenser was attached to the flask, which was heated at 70ºC for 3 hours. The solvent with the extractives was filtered and then evaporated on a rotavapor at 70º, 40º, and 90ºC for hexane, dichloromethane, or water, respectively. These temperatures were extremely high due to a problem with either the apparatus or the vacuum system.

Concentrated extractives were dissolved in acetone to produce solutions containing: 4000, 8000, or 12000 ppm of the extract. Filter paper discs (2.4 cm Whatman International Ltda) were impregnated with the 200 µL of each solution and then allowed to air dry for one hour to disperse the organic solvent.

Method 2: After oven drying, 900 grams of flour from each of the experimental woods were extracted in a 3L Soxhlet apparatus during a nine hour period. Temperatures used were slightly above the boiling point of each solvent (69ºC for hexane and 39ºC for dichloromethane). The resulting solution was filtered and the solvent was evaporated on a rotavapor at 30 ºC.

The concentrated extractives were dissolved in the same extraction solvents to produce concentrations of 4000, 8000, and 12000 ppm. Filter paper discs (2.4 cm Whatman

International Ltda) were impregnated with 200 μL of each extractive and then air dried to disperse the organic solvent. Western red cedar extracts removed using dichloromethanol (8000 and 12000 ppm) showed biological activity using this method.

Method 3: High temperatures were believed to be reducing the biological activity of the extracts. To avoid this problem, a cold extraction method was tested. Instead of water, ethanol was used as the most polar solvent. Nine hundred g of ground teak, western red cedar, or redwood heartwood were added to 2L flasks along with 1000 ml of dichloromethane or ethanol, or 1500 ml of hexane. The nine flasks (three solvents multiplied by three woods) were capped, wrapped with aluminum foil to exclude light, and placed in a dark room for 20, 25, or 30 days for ethanol, dichloromethane, or hexane extracts, respectively. The extracts were filtered and the solvent was evaporated on a rotavapor at 30ºC. The limited amounts of extractives obtained with hexane led us to seek alternative extraction methods.

Bioassay: Small filter paper discs (13 mm BBL^{TM}) were treated with the 200 μL aliquots of the following solutions and then air dried for 20 minutes.

For *Postia placenta* bioassay:

- Wood extractives alone at 8000, 16000, or 24000 ppm
- DCOI at 40, 60, or 80 ppm
- Wood extractives at each concentration mixed with each of the DCOI solutions
- DDAC at 4000, 8000, or 1200 ppm
- Wood extractives at each concentration mixed with each of the DDAC solutions

For *Trametes versicolor* bioassay:

- Wood extractives alone at 8000, 16000, or 24000 ppm
- DCOI at 10, 20, or 40 ppm

- Wood extractives at each concentration mixed with each of the DCOI solutions
- DDAC at 1000, 2000, or 400 ppm
- Wood extractives at each concentration mixed with each of the DDAC solutions

In addition, solvents alone were used as negative controls.

The filter paper disks were then exposed to the test fungi. Petri dishes were inoculated with 1000 µL suspensions of *P. placenta* or *T. versicolor* and the suspension was uniformly distributed on the agar plates. Three treated filter paper discs were placed on the agar surface of each dish. The fungi were incubated at 28ºC. After incubation for 4 days, the halos formed around the filter paper were measured (nearest 0.1 mm) and the zone of inhibition was calculated by averaging nine filter papers (three disks in three Petri dishes).

Table 3.1. Combinations of heartwood extracts and biocides tested to determine if extractives interacted with biocides to affect fungal growth.

Chemical	**Concentration (ppm)**	**Wood Extract Concentration (ppm)**							
		0		8000		16000		24000	
		P. placenta	*T. versicolor*	*P. placenta*	*T. versicolor*	*P. placenta*	*T. versicolor*	*P. placenta*	*T. versicolor*
None	-	X	X	X	X	X	X	X	X
DCOI	10		X		X		X		X
	20		X		X		X		X
	40	X	X	X	X	X	X	X	X
	60	X		X		X		X	
	80	X		X		X		X	
DDAC	400		X		X		X		X
	1000		X		X		X		X
	1200	X		X		X		X	
	2000		X		X		X		X
	4000	X		X		X		X	
	8000	X		X		X		X	

CHAPTER 4- RESULTS AND DISCUSSION

4.1. Wood Treatment

The target values were achieved for all woods except for the teak, which reflects the lower permeability of both heartwood and sapwood of this species (Table 4.1) (Chudnoff, 1987). It is important to note that the WRC sapwood retained slightly more preservative than the redwood sapwood. Conversely, the heartwood of both species had very similar retention values. These differences correlate with the differences in specific gravity of both woods: 0.33 for WRC and 0.37 for redwood (USDA, 1999).

As expected, heartwood retentions were lower than the sapwood, however these differences were less pronounced for DCOI. Considering that the target concentrations were lower for this preservative, it can be concluded that the materials were less saturated.

Table 4.1. Target vs. actual chemical retentions of selected wood species treated with three different preservatives. (WRC: Western red cedar)

Wood Species	Retentions (Kg/m^3)							
	Target	DCOI			DDAC			ACQ
		0.1	0.3	0.6	1	2	4	4
Teak sapwood	Actual	0.06	0.20	0.36	0.54	1.08	2.10	1.89
Teak heartwood		0.04	0.15	0.27	0.35	0.50	0.58	0.71
Redwood sapwood		0.11	0.32	0.66	1.07	1.79	3.78	4.08
Redwood heartwood		0.09	0.29	0.59	0.94	1.66	3.36	3.81
WRC sapwood		0.12	0.37	0.73	1.21	2.09	4.12	4.58
WRC heartwood		0.09	0.28	0.58	0.88	1.63	3.17	2.92
Pine sapwood		0.09	0.30	0.61	0.95	1.67	3.35	3.82

In comparing the retentions of the different sapwoods, the following trend could be established: WRC>Redwood>Pine>Teak. The heartwood trend is WRC≈Redwood>Teak.

4.2. Leach Resistance

Preservatives levels in the leachate water were below the detection limits of the methods used (Section 3.2). The detection limit of the HPLC method employed was 20 ppb. At the target retentions employed, loss of all DCOI in the wood into 300 mL of water would produce concentrations between 2500 ppb to 13000 ppb. Similarly, DDAC has complete solubility in water, and the detection limit of the titration method employed is 100 ppb. If all DCOI was dissolved in 300 mL of the leaching water, concentrations in the leaching water would be between 21000 and 90000 ppb. Thus, if DCOI and DDAC were not well fixed onto the wood, leaching would have been likely to occur and be detected by the method employed, even at very low leaching rates.

Tables 4.2, 4.3, 4.4, and 4.5 show the target and actual retentions of the different wood-treatment combinations. There were no apparent differences in chemical retentions between leached and non-leached wood treated with DCOI, DDAC, or ACQ. The chemical analyses were performed on the mixture of two ground experimental cubes to produce a single data. This process was replicated three times and the average value is reported. No statistical analyses were made because of the low number of replicates.

Preservative concentrations in the wood after leaching were generally close to the initial values (Tables 4.2, 4.3, 4.4, and 4.5). This may reflect the low solution concentrations employed. As a result, wood binding sites were available to react with the preservatives (Lebow, 1996). For example, DDAC is thought to react with the wood by cation exchange mechanisms that involve the removal of the ammonium groups from the solution by negative binding sites present on the lignin, and by replacement of wood protons by ammonium functionality (Loubinoux *et al*, 1992).

Reaction mechanisms of DCOI are based on electrophilic interactions, and the presence of these reactive sites are directly correlated with fixation (Diehl and Chapman, 2000)

We observed evidence of extractive leaching from teak, redwood, and western red cedar heartwood, which is in agreement with the literature data about extractive solubility in water. Although there was no apparent preservative leaching in our tests, extractive solubility may affect treatability of this species when more concentrated preservatives solutions are used.

The data in table 4.2 shows that leaching losses of the samples treated with DCOI ranged from 6 to 20% in sapwood samples. These differences, while generally small, suggest that wood species and initial loadings had relatively little effect on leaching losses.

Leaching losses in heartwood samples were independent of preservative retention. As with the sapwood, the losses were relative small.

Hegarty *et al.* (1997) performed a leaching test of DCOI in different microemulsion formulations and found percentages of DCOI leached between 0.5 and 4%. They assumed that these values correlated with the low solubility of DCOI in water (20 ppm). This value is much lower than the leaching losses and is in agreement with the lack of preservative in the leachates.

Table 4.2. Target vs. actual retentions in sapwood or heartwood blocks treated with DCOI, and analyzed directly or after being subjected to leach treatment.

Target Retentions	Species	Sapwood			Heartwood		
		Actual Retention		Difference**	Actual Retention		Difference **
		Leached	Unleached		Leached	Unleached	
		Kg/m^3	Kg/m^3	%	Kg/m^3	Kg/m^3	%
0.1 kg/m^3	Teak	0.06	0.07	14	0.03	0.05	40
	RW	0.09	0.11	18	0.10	0.10	0
	WRC	0.11	0.13	15	0.09	0.11	18
	Pine	0.08	0.09	11			
0.3 kg/m^3	Teak	0.12	0.13	8	0.12	0.12	0
	RW	0.23	0.27	15	0.28	0.25	-12
	WRC	0.32	0.34	6	0.26	0.29	10
	Pine	0.23	0.28	18			
0.6 kg/m^3	Teak	0.25	0.29	14	0.18	0.23	22
	RW	0.47	0.61	23	0.57	0.53	-8
	WRC	0.62	0.66	6	0.52	0.60	13
	Pine	0.45	0.51	12			

**Difference is the percentage difference between leached and unleached samples

The data in table 4.3 show that the DDAC retention levels in leached samples were equal to or higher than those from unleached samples. Higher values may reflect treatment variability in these woods. However, the values are essentially similar and near to the target retention. The results suggested that DDAC was fixed in the wood at these retention levels and resisted leaching during exposure to distilled water. Losses in sapwood at the higher preservative concentrations, here the experimental error and the variability should be the lowest, showed that relative losses followed the trend: Teak>RW>WRC>Pine.

Previous studies suggest that DDAC did not leach from Southern yellow pine treated at 32 kg/m^3 (Ruddick and Sam, 1982). Nicholas *et al*. (1991) also observed little leaching in samples from the same species treated at retention levels between 3.2 and

5.6 kg/m^3. All these results support our conclusion that DDAC is tightly fixed to the different woods tested.

Table 4.3. Target vs. actual retentions in sapwood or heartwood blocks treated with DDAC, and analyzed directly or after being subjected to leach treatment.

Target Retentions	Species	Sapwood			Heartwood		
		Actual Retention		Difference	Actual Retention		Difference
		Leached	Unleached	**	Leached	Unleached	**
		Kg/m^3	Kg/m^3	%	Kg/m^3	Kg/m^3	%
2,7 kg/m^3	Teak	1.09	1.50	27	0.36	0.33	-9
	RW	2.27	2.65	14	2.24	2.00	-12
	WRC	2.72	2.94	7	1.99	2.20	10
	Pine	2.08	2.56	19			
**Difference is the percentage difference between leached and unleached samples							

Mitsuhashi (2007) obtained 19% CuO loss and 3.2% DDAC loss in a leaching test of Southern pine treated with ACQ at 4 kg/m^3, which is in complete agreement with the results of this test (Table 4.4 and 4.5).

Copper losses were much higher than DDAC losses. DDAC appears to compete with the complexed copper for the ion exchange sites in the wood and may reduce the proportion of copper fixed in this manner (Lebow, 1996).

Table 4.4. Target vs. actual retentions in sapwood or heartwood blocks treated with ACQ and analyzed as cooper oxide directly or after being subjected to leach treatment.

Target Retentions	Species	Sapwood			Heartwood		
		Actual Retention		Difference	Actual Retention		Difference
		Leached	Unleached	**	Leached	Unleached	**
		Kg/m^3	Kg/m^3	%	Kg/m^3	Kg/m^3	%
2,7 kg/m^3	Teak	1.09	1.50	27	0.36	0.33	-9
	RW	2.27	2.65	14	2.24	2.00	-12
	WRC	2.72	2.94	7	1.99	2.20	10
	Pine	2.08	2.56	19			
**Difference is the percentage difference between leached and unleached samples							

Table 4.5. Target vs. actual retentions in sapwood or heartwood blocks treated with ACQ, and analyzed as DDAC directly or after being subjected to leach treatment.

Target Retentions	Species	Sapwood			Heartwood		
		Actual Retention		Diference**	Actual Retention		Diference**
		Leached	Unleached		Leached	Unleached	
		Kg/m^3	Kg/m^3	%	Kg/m^3	Kg/m^3	%
1,3 kg/m^3	Teak	1.13	1.29	12	0.78	0.79	1
	RW	1.52	1.65	8	1.54	1.45	-6
	WRC	1.94	1.81	-7	1.47	1.70	14
	Pine	1.56	1.63	4			

**Diference is the percentage difference between leached and unleached samples

4.3. Soil Block Test

The weight-losses produced in the soil block test for the various species-treatment combinations are shown in Tables 4.6 - 4.9.

Weight losses of the controls and validity of the experiment

The validity of the experiment is discussed as a function of the weight losses recorded in the decay susceptible control. The brown rot fungus, *Postia placenta*, produced weight losses on untreated southern pine control blocks averaging 53.62% (unleached) and 45.32% (leached). These results indicated that the conditions were suitable for aggressive fungal attack (Table 4.9). Weight losses for the white rot fungus, *Trametes versicolor*, on the southern pine control blocks averaged 15.98% (unleached) and 4.62% (leached). These values were low, and may indicate that the conditions were not suitable for aggressive fungal attack. However, weight losses on WRC sapwood controls averaged 37.89% (unleached) and 32.23% (leached) (Table 4.8). WRC sapwood is very prone to decay, and this indicated that the weight losses of the white rot fungus were indeed low, but still sufficient to make statistical comparisons between the treatments.

Moisture contents after treatment were relatively low for the white rot blocks when compared to the brown rot blocks (Tables 4.10- 4.13), which may help explain the low weight losses. Moisture contents of the southern pine control blocks averaged 183% (unleached) and 180% (leached) when exposed to *P. placenta*. Moisture contents of the WRC sapwood controls averaged 70% (unleached) and 64% (leached) when exposed to *T. versicolor*. All chambers were prepared on the same manner, thus this difference was attributable to the decay process.

Weight losses of the wood/treatment combinations

Surprisingly, leaching according to AWPA E11 prior to fungal exposure did not produce significant increases in mass loss in any of the wood/treatment combinations. On the contrary, most samples subjected to leaching had lower mass losses than unleached samples. We do not know the exact reason for these results, but contributing factors might include the decomposition of the extractives in unleached samples, leaching of extractives and/or preservatives, and redistribution of the preservatives. Tables 4.18, 4.19, 4.20, and 4.21 showed weight losses obtained in soil block bottles prepared without the fungi ranged between 0.01 and 3.53%. These losses were attributed to leaching and might also contribute to the increase in mass loss.

The statistical comparisons between treated and untreated sample weight losses of are shown in Tables 4.14 - 4.17. Untreated teak, redwood, and western red cedar heartwood samples had mass losses of less than 3%. Since the soil block test induces some weight losses as a result of wetting and drying, any weight losses in this range must be viewed cautiously.

Samples treated with low concentrations of DCOI and, to a lesser extent, low concentrations of DDAC, experienced increased mass losses, compared with untreated ones (see underlined values in Tables 4.14, 4.15, 4.16, and 4.17). Most fungicidal treatments produce positive biocidal effects at higher doses, but some produce

beneficial biological effects on the target fungus at low doses. This effect is known as hormesis (Calabrese *et al*, 1999).

- **Teak**

Teak retentions were much lower than the target retentions (Table 4.1.). Neither DCOI nor DDAC were able to protect teak sapwood from decay (Table 4.6). However, there was a dose effect, suggesting that higher doses might produce protection. The reference preservative (ACQ) improved sapwood performance, indicating that the sapwood was treatable, despite the low permeability of this species. DCOI at 0.6 kg/m^3 (0.12 kg/m^3) and DDAC at concentrations higher than 2.0 kg/m^3 (0.83 kg/m^3) produced slight improvements on teak durability. Similar behavior was observed for sapwood and heartwood for the brown rot, while only the highest preservative concentrations improved performance against the white rot. Samples treated with the lowest preservative concentrations experienced more decay than the untreated samples.

Finally, it is important to note the large differences in sapwood durability of leached (22.9%) and unleached samples (8.2%) for the brown rot. The highest doses of DCOI and DDAC reduced the weight losses to 4.93% and 3.00%, respectively, which was more effective than the leached blocks. In the case of the heartwood, the highest preservative concentrations did not perform at levels comparable to ACQ treated sapwood, but did improve durability. Nevertheless, it is important to consider that the high variability in weight losses for the heartwood samples made it difficult to detect differences between treated and untreated samples.

Table 4.6. Wood weight losses for teak blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test.

					Wood Weight Loss (%)							
Wood	Chemical	Target Retention (Kg/m^3)	Actual Retention		Brown Rot				White Rot			
			Unleached	Leached	Unleached		Leached		Unleached		Leached	
			Kg/m^3	Kg/m^3	X	(SD)	X	(SD)	X	(SD)	Media	(SD)
Sapwood	DCOI	0.1	0.07	0.06	**29.5**	(8.7)	**23.3**	(3.8)	**30.5**	(10.7)	**32.5**	(9.2)
		0.3	0.13	0.12	**9.0**	(6.0)	**27.1**	(5.2)	**35.7**	(10.2)	**26.8**	(9.5)
		0.6	0.29	0.25	**5.9**	(2.3)	**4.9**	(3.6)	**19.5**	(8.4)	**13.7**	(6.9)
	DDAC	1.0	0.91	1.02	**19.0**	(3.6)	**17.8**	(4.5)	**33.8**	(11.9)	**30.5**	(10.3)
		2.0	2.10	1.81	**3.0**	(2.7)	**12.3**	(4.2)	**33.7**	(6.4)	**27.4**	(9.9)
		4.0	3.13	2.76	**3.4**	(2.7)	**3.0**	(1.8)	**17.5**	(13.9)	**18.6**	(4.0)
	ACQ DDAC	2.7	1.50	1.09	**0.7**	(0.4)	**0.7**	(0.3)	**0.9**	(0.3)	**0.4**	(0.1)
	ACQ CuO	1.3	1.29	1.13								
	Control	0.0			**8.2**	(4.3)	**22.9**	(3.3)	**27.0**	(4.7)	**28.0**	(4.8)
Heartwood	DCOI	0.1	0.05	0.03	**1.5**	(0.6)	**4.2**	(2.1)	**2.0**	(0.7)	**0.9**	(0.3)
		0.3	0.12	0.12	**1.1**	(1.0)	**3.2**	(1.9)	**3.2**	(5.5)	**0.4**	(0.3)
		0.6	0.23	0.18	**0.6**	(0.4)	**0.4**	(0.1)	**4.1**	(5.0)	**0.5**	(0.3)
	DDAC	1.0	0.59	0.98	**1.8**	(2.4)	**1.6**	(1.8)	**5.1**	(10.8)	**0.6**	(0.2)
		2.0	0.83	0.99	**0.7**	(0.4)	**0.5**	(0.3)	**6.8**	(13.6)	**1.9**	(1.1)
		4.0	1.15	0.96	**0.6**	(0.1)	**0.3**	(0.2)	**0.9**	(0.4)	**0.6**	(0.5)
	ACQ DDAC	2.7	0.33	0.36	**0.3**	(0.1)	**0.3**	(0.2)	**0.4**	(0.4)	**0.0**	(0.0)
	ACQ CuO	1.3	0.79	0.78								
	Control	0.0			**1.2**	(1.0)	**1.8**	(0.8)	**2.1**	(1.2)	**1.8**	(1.4)

White rot attack tends to be greater in hardwoods (Zabel and Morrell, 1992). In our test, weight losses of teak by *T. versicolor* were higher than those caused by *P. placenta*. In practical terms, thresholds for hardwoods should be developed against white rot fungi because the woods are more susceptible to this type of decay. Kamden *et al*. (1995) treated two hardwoods with different copper formulations and found a protection threshold of 3.00 kg/m^3 for *P. placenta* and a much lower threshold of 1.8 kg/m^3 for *T. versicolor*. These thresholds reflect, however, the copper tolerance of *P. placenta*.

- **Redwood, Western Red Cedar and Pine**

Both DCOI (0.6 kg/m^3) and DDAC (4 kg/m^3) were adequate to control decay in redwood and western red cedar sapwood (Table 4.7 and 4.8). Generally, degradation by *P. placenta* is greater than attack by *T. versicolor* in softwoods. In this test, we observed the same tendency: attack by brown rot fungi was greater in redwood, western red cedar, and southern pine, than in teak. This behavior is reasonable considering the lower preservatives retention in teak.

Wood decay in the untreated sapwood of redwood and western red cedar resulted in small differences between leached and unleached samples. The highest and the middle doses of DCOI and DDAC reduced weight losses. As in teak, these preservative levels were more effective that the untreated leached heartwood.

Heartwood redwood durability was improved with DDAC and was even more effective than ACQ against the white rot. The results with DCOI were not as pronounced and only the highest concentration (0.6 kg/m^3) was better than ACQ.

The situation for WRC was similar to redwood. The performance of the highest concentration of both preservatives in sapwood was equal to or better than ACQ. A reasonable durability was achieved for DCOI even at 0.3 kg/m^3. It is important to note that the differences between leached and unleached samples were greater than for teak and redwood.

There were no differences between the untreated controls and the samples treated with ACQ, especially in the unleached WRC heartwood. Weight losses were slightly lower in DDAC treated samples than ACQ and the controls. The weight losses of the samples treated with DCOI tended to be slightly higher.

Table 4.7. Wood weight losses for redwood blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test.

					Wood Weight Loss (%)							
Wood	Chemical	Target Retention (Kg/m^3)	Actual Retention		Brown Rot				White Rot			
			Unleached	Leached	Unleached		Leached		Unleached		Leached	
			Kg/m^3	Kg/m^3	X	(SD)	X	(SD)	X	(SD)	Media	(SD)
Sapwood	DCOI	0.1	0.11	0.09	**38.5**	(12.3)	**32.9**	(6.8)	**8.7**	(5.8)	**5.1**	(6.0)
		0.3	0.27	0.23	**2.5**	(1.9)	**2.6**	(1.9)	**2.8**	(3.0)	**0.7**	(1.0)
		0.6	0.61	0.47	**0.4**	(0.2)	**0.5**	(0.4)	**1.4**	(1.1)	**0.1**	(0.2)
	DDAC	1.0	1.41	1.43	**29.6**	(9.7)	**16.9**	(11.0)	**21.4**	(5.4)	**7.4**	(5.0)
		2.0	2.88	2.82	**11.4**	(7.3)	**7.5**	(3.1)	**11.9**	(7.9)	**2.0**	(1.9)
		4.0	4.00	3.75	**2.1**	(0.8)	**2.4**	(1.1)	**0.7**	(1.3)	**0.1**	(0.4)
	ACQ DDAC	2.7	2.65	2.27	**0.7**	(0.2)	**0.3**	(0.1)	**0.9**	(0.3)	**0.3**	(0.1)
	ACQ CuO	1.3	1.65	1.52								
	Control	0.0			**28.7**	(5.2)	**31.7**	(3.9)	**20.8**	(5.5)	**11.6**	(3.0)
Heartwood	DCOI	0.1	0.10	0.10	**3.1**	(1.3)	**0.1**	(0.1)	**2.3**	(0.6)	**0.2**	(0.2)
		0.3	0.25	0.28	**3.2**	(3.7)	**0.1**	(0.1)	**2.0**	(0.7)	**0.2**	(0.1)
		0.6	0.53	0.57	**0.8**	(0.3)	**0.0**	(0.1)	**0.8**	(0.4)	**0.3**	(0.1)
	DDAC	1.0	1.21	1.45	**1.6**	(0.7)	**0.3**	(0.2)	**1.2**	(0.7)	**0.2**	(0.1)
		2.0	2.03	2.38	**1.6**	(0.5)	**0.5**	(0.1)	**1.1**	(0.3)	**0.2**	(0.1)
		4.0	3.49	3.78	**1.7**	(0.3)	**0.2**	(0.0)	**1.5**	(0.4)	**0.0**	(0.1)
	ACQ DDAC	2.7	2.00	2.24	**1.4**	(0.6)	**0.6**	(0.1)	**1.7**	(0.5)	**0.5**	(0.1)
	ACQ CuO	1.3	1.45	1.54								
	Control	0.0			**2.3**	(0.5)	**2.2**	(1.3)	**1.3**	(0.3)	**0.4**	(0.1)

Table 4.8. Wood weight losses for western red cedar blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leach procedure prior to exposure to a white or brown rot in an AWPA E10 soil block test.

					Wood Weight Loss (%)							
Wood	Chemical	Target Retention (Kg/m^3)	Actual Retention		Brown Rot				White Rot			
			Unleached	Leached	Unleached		Leached		Unleached		Leached	
			Kg/m^3	Kg/m^3	X	(SD)	X	(SD)	X	(SD)	Media	(SD)
Sapwood	DCOI	0.1	0.13	0.11	**35.8**	(13.0)	**36.8**	(5.7)	**35.7**	(10.0)	**13.5**	(10.9)
		0.3	0.34	0.32	**12.3**	(8.7)	**1.8**	(1.3)	**3.9**	(3.0)	**-0.2**	(0.3)
		0.6	0.66	0.62	**0.7**	(0.6)	**0.3**	(0.4)	**0.5**	(0.1)	**0.0**	(0.2)
	DDAC	1.0	1.50	1.55	**15.7**	(9.5)	**10.3**	(9.3)	**26.0**	(8.5)	**21.9**	(6.3)
		2.0	2.88	2.82	**5.3**	(4.4)	**2.4**	(0.6)	**16.6**	(5.5)	**9.5**	(6.3)
		4.0	5.35	5.18	**6.3**	(2.6)	**1.4**	(0.5)	**6.1**	(3.0)	**0.2**	(1.0)
	ACQ DDAC	2.7	2.94	2.72	**1.4**	(0.3)	**0.1**	(0.1)	**1.7**	(0.6)	**0.5**	(0.1)
	ACQ CuO	1.3	1.81	1.94								
	Control	0.0			**35.2**	(5.3)	**36.5**	(3.9)	**37.9**	(13.7)	**32.2**	(6.2)
Heartwood	DCOI	0.1	0.11	0.09	**1.1**	(0.4)	**0.9**	(0.4)	**1.4**	(0.2)	**0.8**	(0.4)
		0.3	0.29	0.26	**1.0**	(0.2)	**0.8**	(0.1)	**0.8**	(0.2)	**0.4**	(0.1)
		0.6	0.60	0.52	**1.0**	(0.1)	**0.6**	(0.1)	**1.0**	(0.2)	**0.4**	(0.2)
	DDAC	1.0	1.35	1.35	**0.9**	(0.2)	**0.5**	(0.1)	**1.2**	(0.7)	**0.5**	(0.1)
		2.0	2.48	2.65	**0.9**	(0.2)	**0.5**	(0.2)	**0.8**	(0.2)	**0.6**	(0.1)
		4.0	4.22	4.34	**0.7**	(0.8)	**0.6**	(0.1)	**1.0**	(0.4)	**0.4**	(0.1)
	ACQ DDAC	2.7	2.20	1.99	**1.0**	(0.2)	**0.5**	(0.1)	**1.0**	(0.4)	**0.3**	(0.1)
	ACQ CuO	1.3	1.70	1.47								
	Control	0.0			**1.1**	(0.1)	**2.5**	(2.1)	**1.0**	(0.3)	**0.2**	(0.2)

In other studies, the toxic thresholds for treated southern yellow pine obtained with different white rot fungi were approximately 5 kg/m^3 for DDAC (Nicholas *et al*, 1991), and 0.3 kg/m^3 for DCOI (Hegarty *et al*, 1997). Similar thresholds were found in this test. Toxic thresholds for treated southern yellow pine obtained with different brown rot fungi were less than 3.2 kg/m^3 for DDAC (Preston and Nicholas, 1982), and 0.3 kg/m^3 for DCOI (Hegarty *et al*, 1997). However, these doses were unable to protect wood against *P. placenta* in this test.

Leaching of untreated pine samples did not result in increased mass loss as compared to WRC, redwood, and teak.

Increasing chemical retentions produced lower weight losses in sapwood samples of all species with less variability. Thus, wood treatment increased predictability.

Table 4.9. Wood weight losses for southern pine blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test.

					Wood Weight Loss (%)							
Wood	**Chemical**	**Target Retention (Kg/m^3)**	**Actual Retention**		**Brown Rot**				**White Rot**			
			Unleached	**Leached**	**Unleached**		**Leached**		**Unleached**		**Leached**	
			Kg/m^3	Kg/m^3	X	(SD)	X	(SD)	X	(SD)	Media	(SD)
Sapwood	DCOI	0.1	0.09	0.08	**31.6**	(6.2)	**24.7**	(3.3)	**14.9**	(4.4)	**16.2**	(8.4)
		0.3	0.28	0.23	**36.9**	(7.1)	**31.6**	(7.6)	**8.6**	(2.8)	**1.5**	(2.1)
		0.6	0.51	0.45	**16.8**	(3.8)	**12.4**	(6.6)	**2.0**	(1.4)	**-0.3**	(0.5)
	DDAC	1.0	1.30	1.36	**27.2**	(6.3)	**15.3**	(8.8)	**19.5**	(11.9)	**6.4**	(4.9)
		2.0	2.36	2.49	**22.0**	(9.8)	**6.6**	(5.4)	**8.0**	(4.3)	**0.7**	(0.2)
		4.0	3.94	4.01	**11.9**	(6.1)	**9.9**	(4.0)	**8.5**	(4.0)	**0.3**	(0.3)
	ACQ DDAC	2.7	2.56	2.08	**2.2**	(0.4)	**1.5**	(0.9)	**1.7**	(0.3)	**0.2**	(5.7)
	ACQ CuO	1.3	1.63	1.56								
	Control	0.0			**53.6**	(10.2)	**45.3**	(17.6)	**16.0**	(8.3)	**4.6**	(2.4)

Table 4.10. Moisture contents for teak blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values represent mean and standard deviations of 6 blocks.

			Wood Moisture Content (%)							
Wood	Treatment		Brown Rot				White Rot			
	Chemical	Target Retention	Unleached		Leached		Unleached		Leached	
		Kg/m^3	Media	(SD)	Media	(SD)	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	53	(17.8)	38	(4.2)	49	(7.8)	49	(17.9)
		0.3	43	(9.4)	42	(3.9)	54	(18.5)	44	(14.0)
		0.6	33	(5.7)	42	(8.2)	46	(9.2)	33	(2.8)
	DDAC	1.0	50	(5.5)	40	(5.2)	75	(35.9)	47	(13.0)
		2.0	52	(19.1)	41	(2.8)	43	(7.3)	45	(11.0)
		4.0	38	(11.0)	30	(2.6)	33	(21.1)	35	(3.0)
	ACQ	4.0	38	(4.1)	27	(1.2)	45	(7.0)	29	(2.2)
	Untreated	0.0	53	(18.5)	48	(10.8)	56	(12.5)	51	(11.0)
Heartwood	DCOI	0.1	46	(4.6)	29	(1.5)	49	(15.8)	35	(3.7)
		0.3	38	(4.7)	29	(7.1)	40	(8.3)	32	(3.0)
		0.6	42	(6.2)	36	(5.3)	43	(10.7)	31	(8.7)
	DDAC	1.0	39	(9.0)	41	(7.3)	52	(8.5)	32	(7.2)
		2.0	35	(8.3)	28	(6.4)	35	(2.6)	31	(6.9)
		4.0	56	(9.8)	32	(3.6)	50	(7.1)	28	(1.1)
	ACQ	4.0	40	(9.2)	28	(2.8)	51	(15.7)	38	(6.9)
	Untreated	0.0	50	(10.1)	31	(3.1)	33	(4.7)	30	(3.5)

Table 4.11. Moisture contents for redwood blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values represent mean and standard deviations of 6 blocks.

			Wood Moisture Content (%)							
Wood	Treatment		Brown Rot				White Rot			
	Chemical	Target Retention	Unleached		Leached		Unleached		Leached	
		Kg/m^3	Media	(SD)	Media	(SD)	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	62	(11.4)	56	(4.2)	35	(3.4)	33	(3.0)
		0.3	30	(0.8)	29	(1.4)	30	(0.7)	29	(1.1)
		0.6	29	(0.7)	29	(0.8)	31	(1.1)	29	(1.2)
	DDAC	1.0	55	(12.6)	41	(6.2)	46	(18.2)	31	(1.4)
		2.0	39	(6.7)	36	(4.0)	36	(4.9)	30	(1.0)
		4.0	29	(1.9)	30	(1.5)	30	(1.1)	29	(0.9)
	ACQ	4.0	38	(9.5)	29	(0.5)	34	(2.1)	31	(1.5)
	Untreated	0.0	51	(10.4)	51	(4.8)	37	(3.5)	31	(1.2)
Heartwood	DCOI	0.1	43	(9.1)	24	(0.8)	38	(15.2)	38	(20.8)
		0.3	47	(7.5)	24	(0.9)	46	(6.1)	26	(1.4)
		0.6	50	(14.4)	26	(4.3)	63	(21.1)	25	(0.9)
	DDAC	1.0	37	(7.8)	25	(0.4)	53	(19.0)	24	(1.4)
		2.0	32	(6.3)	24	(0.5)	69	(12.6)	26	(1.2)
		4.0	42	(9.1)	23	(1.1)	53	(11.4)	24	(1.1)
	ACQ	4.0	43	(7.9)	26	(1.6)	79	(22.4)	26	(1.9)
	Untreated	0.0	46	(10.2)	26	(2.5)	54	(18.2)	25	(1.1)

Table 4.12. Moisture contents for western red cedar blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values represent mean and standard deviations of 6 blocks.

			Wood Moisture Content (%)							
Wood	Treatment		Brown Rot				White Rot			
	Chemical	Target Retention	Unleached		Leached		Unleached		Leached	
		Kg/m^3	Media	(SD)	Media	(SD)	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	57	(11.9)	68	(22.9)	55	(11.7)	38	(5.4)
		0.3	36	(4.7)	28	(0.9)	34	(4.5)	29	(1.2)
		0.6	33	(4.5)	51	(34.4)	31	(1.5)	29	(1.3)
	DDAC	1.0	45	(12.9)	40	(10.1)	49	(10.6)	42	(6.3)
		2.0	37	(8.1)	31	(2.0)	37	(4.7)	37	(10.0)
		4.0	43	(8.1)	31	(2.6)	32	(2.5)	28	(0.5)
	ACQ	4.0	38	(2.7)	31	(1.0)	38	(4.3)	31	(1.1)
	Untreated	0.0	51	(7.3)	54	(3.4)	71	(26.6)	64	(20.8)
Heartwood	DCOI	0.1	46	(24.8)	28	(0.8)	30	(1.1)	28	(1.7)
		0.3	27	(0.9)	27	(0.4)	29	(1.1)	28	(0.6)
		0.6	27	(0.8)	27	(0.9)	29	(0.8)	28	(0.7)
	DDAC	1.0	28	(2.4)	27	(0.3)	31	(4.9)	27	(0.8)
		2.0	27	(1.4)	26	(1.0)	28	(0.7)	28	(0.9)
		4.0	54	(45.0)	26	(0.5)	30	(3.2)	27	(1.0)
	ACQ	4.0	35	(5.0)	28	(1.7)	42	(10.1)	30	(1.1)
	Untreated	0.0	26	(0.9)	28	(2.5)	27	(0.5)	28	(1.8)

Table 4.13. Moisture contents for southern pine blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values represent mean and standard deviations of 6 blocks.

			Wood Moisture Content (%)							
Wood	Treatment		Brown Rot				White Rot			
	Chemical	Target Retention	Unleached		Leached		Unleached		Leached	
		Kg/m^3	Media	(SD)	Media	(SD)	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	53	(8.6)	44	(2.6)	32	(1.7)	32	(1.6)
		0.3	59	(11.6)	60	(13.2)	30	(0.6)	29	(1.2)
		0.6	39	(2.2)	36	(5.4)	30	(1.7)	27	(0.9)
	DDAC	1.0	52	(8.2)	40	(10.3)	34	(4.0)	30	(1.8)
		2.0	46	(11.4)	34	(6.9)	30	(0.8)	28	(0.7)
		4.0	38	(8.2)	129	(14.2)	30	(0.9)	28	(0.4)
	ACQ	4.0	106	(10.4)	79	(38.0)	35	(2.4)	31	(0.5)
	Untreated	0.0	184	(66.6)	180	(110.3)	32	(3.6)	29	(1.2)

Improvements on natural durability

The p-values recorded in the statistical comparisons between treated and untreated weight losses are shown in Tables 4.14 - 4.17. As noted, heartwood weight losses were generally low and variable. Significant weight loss differences were found in the following wood – treatment combinations where the performance of the treated wood was better than that of the untreated woods (See bold values of Tables 4.14- 4.17).

- All ACQ treated samples.
- Redwood sapwood treated with 0.3 and 0.6 kg/m^3 of DCOI and 2 and 4 kg/m^3 of DDAC.
- Western red cedar sapwood treated with 0.3 and 0.6 kg/m^3 of DCOI and 2 and 4 kg/m^3 of DDAC.
- Southern pine sapwood treated with 0.6 kg/m^3 of DCOI.

Leaching produced significantly higher weight losses in a number of species – treatment combinations, including.

- Teak sapwood treated with 0.6 kg/m^3 of DCOI and 4 kg/m^3 of DDAC.
- Teak heartwood treated with 0.6 kg/m^3 of DCOI.
- Redwood sapwood treated with 1 kg/m^3 of DDAC.
- Redwood heartwood treated with 1 kg/m^3 of DDAC.

Positive significant differences were found also in unleached samples of redwood heartwood treated with 0.6 kg/m^3 of DDAC.

Table 4.14. P-values comparing weight losses of untreated teak blocks with teak blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values from a student's-t test (for each treatment n=6).

Wood	Treatment		Brown Rot		White Rot	
	Chemical	Target Retention	Unleached	Leached	Unleached	Leached
		Kg/m^3	p-value			
Sapwood	DCOI	0.1	<0.001	0.85	0.47	0.32
		0.3	0.78	0.13	0.08	0.79
		0.6	0.29	**<0.001**	0.09	**<0.001**
	DDAC	1.0	0.00	**0.05**	0.22	0.61
		2.0	**0.03**	**<0.001**	0.06	0.90
		4.0	**0.05**	**<0.001**	0.14	**<0.001**
	ACQ	4.0	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Heartwood	DCOI	0.1	0.51	0.03	0.88	0.13
		0.3	0.83	0.12	0.63	**0.04**
		0.6	0.21	**<0.001**	0.37	**0.04**
	DDAC	1.0	0.62	0.76	0.51	0.07
		2.0	0.28	**<0.001**	0.42	0.88
		4.0	0.16	**<0.001**	**0.05**	0.07
	ACQ	4.0	**0.04**	**<0.001**	**<0.001**	**<0.001**

Numbers in bold differ significantly from the untreated control.

Table 4.15. P-values comparing weight losses of untreated redwood blocks with redwood blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values from a student's-t test (for each treatment n=6).

Wood	Treatment		Brown Rot		White Rot	
	Chemical	Target Retention	Unleached	Leached	Unleached	Leached
		Kg/m^3	p-value			
Sapwood	DCOI	0.1	0.10	0.72	**<0.001**	**0.04**
		0.3	**0.04**	**<0.001**	**<0.001**	**<0.001**
		0.6	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	DDAC	1.0	0.86	**0.01**	0.85	**0.11**
		2.0	**<0.001**	**<0.001**	**0.05**	**<0.001**
		4.0	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	ACQ	4.0	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Heartwood	DCOI	0.1	0.21	**<0.001**	≤0.001	**0.06**
		0.3	0.56	**<0.001**	0.04	**0.01**
		0.6	**<0.001**	**<0.001**	**0.03**	0.45
	DDAC	1.0	**0.09**	**<0.001**	0.90	**0.01**
		2.0	**0.03**	**<0.001**	0.39	**0.02**
		4.0	**0.03**	**<0.001**	0.34	**<0.001**
	ACQ	4.0	**0.02**	**<0.001**	**0.08**	**0.09**

Numbers in bold differ significantly from the untreated control.

Table 4.16. P-values comparing weight losses of untreated western red cedar blocks with western red cedar blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values from a student's-t test (for each treatment n=6).

Wood	Treatment		Brown Rot		White Rot	
	Chemical	Target Retention	Unleached	Leached	Unleached	Leached
		Kg/m^3	p-value			
Sapwood	DCOI	0.1	0.93	0.91	0.76	**<0.001**
		0.3	**<0.001**	**<0.001**	**<0.001**	**<0.001**
		0.6	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	DDAC	1.0	**<0.001**	**<0.001**	0.10	0.02
		2.0	**<0.001**	**<0.001**	**0.01**	**<0.001**
		4.0	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	ACQ	4.0	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Heartwood	DCOI	0.1	0.71	0.12	0.05	0.02
		0.3	0.79	0.08	0.15	0.17
		0.6	0.67	**0.06**	0.79	**0.08**
	DDAC	1.0	0.07	**0.05**	0.57	**0.02**
		2.0	0.22	**0.04**	0.24	**0.00**
		4.0	0.31	**0.05**	0.90	**0.08**
	ACQ	4.0	0.84	**0.05**	1.00	0.37

Numbers in bold differ significantly from the untreated control.

Table 4.17. P-values comparing weight losses of untreated southern pine blocks with southern pine blocks treated with selected biocides and left unleached or subjected to an AWPA E11 standard leaching procedure prior to exposure to a white or brown rot fungus in an AWPA E10 soil block test. Values from a student's-t test (for each treatment n=6).

Wood	Treatment		Brown Rot		White Rot	
	Chemical	Target Retention	Unleached	Leached	Unleached	Leached
		Kg/m^3	p-value			
Sapwood	DCOI	0.1	**<0.001**	**0.02**	0.79	**0.01**
		0.3	**0.01**	0.11	**0.07**	**0.04**
		0.6	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	DDAC	1.0	**<0.001**	**<0.001**	0.57	0.43
		2.0	**<0.001**	**<0.001**	0.06	**<0.001**
		4.0	**<0.001**	**<0.001**	0.08	**<0.001**
	ACQ	4.0	**<0.001**	**<0.001**	**<0.001**	**0.01**

Numbers in bold differ significantly from the untreated control.

Table 4.18. Moisture contents and wood weight losses for teak blocks treated with selected biocides and left unexposed to a fungus as a negative control in a soil block test.

Wood	Treatment		Wood Moisture Content (%)		Wood Weight Loss (%)	
	Chemical	Target Retention				
		Kg/m^3	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	34	(2.8)	1.16	(0.0)
		0.3	27	(2.3)	0.55	(0.2)
		0.6	29	(4.7)	0.38	(0.2)
	DDAC	1.0	23	(0.3)	0.58	(0.0)
		2.0	23	(0.4)	0.67	(0.4)
		4.0	25	(3.5)	0.83	(0.6)
	ACQ	4.0	22	(0.4)	0.71	(0.2)
	Untreated	0.0	24	(0.3)	2.92	(0.0)
Heartwood	DCOI	0.1	26	(0.6)	0.59	(0.1)
		0.3	21	(0.1)	0.41	(0.1)
		0.6	27	(0.2)	0.47	(0.3)
	DDAC	1.0	20	(1.3)	0.31	(0.1)
		2.0	18	(0.1)	0.69	(0.2)
		4.0	33	(0.5)	0.67	(0.0)
	ACQ	4.0	32	(3.2)	0.53	(0.2)
	Untreated	0.0	27	(2.5)	1.00	(0.1)

Table 4.19. Moisture contents and wood weight losses for redwood blocks treated with selected biocides and left unexposed to a fungus as a negative control in a soil block test.

Wood	Treatment		Wood Moisture Content (%)		Wood Weight Loss (%)	
	Chemical	Target Retention Kg/m^3	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	23	(0.1)	0.20	(0.1)
		0.3	24	(0.6)	0.38	(0.2)
		0.6	24	(0.0)	0.06	(0.1)
	DDAC	1.0	38	(1.6)	0.14	(0.1)
		2.0	26	(0.6)	0.53	(0.2)
		4.0	23	(0.6)	0.15	(0.1)
	ACQ	4.0	45	(10.0)	1.14	(0.0)
	Untreated	0.0	32	(9.5)	0.01	(0.1)
Heartwood	DCOI	0.1	24	(0.0)	2.82	(1.1)
		0.3	22	(1.9)	1.11	(0.1)
		0.6	31	(7.5)	1.73	(0.1)
	DDAC	1.0	28	(9.3)	0.78	(0.2)
		2.0	21	(1.7)	2.22	(1.4)
		4.0	21	(2.4)	2.64	(0.1)
	ACQ	4.0	28	(1.8)	-2.26	(4.6)
	Untreated	0.0	32	(0.0)	2.42	(0.6)

Table 4.20. Moisture contents and wood weight losses for western red cedar blocks treated with selected biocides and left unexposed to a fungus as a negative control in a soil block test.

Wood	Treatment		Wood Moisture Content (%)		Wood Weight Loss (%)	
	Chemical	Target Retention Kg/m^3	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	25	(0.3)	1.39	(1.1)
		0.3	25	(0.7)	0.26	(0.3)
		0.6	25	(0.5)	0.45	(0.0)
	DDAC	1.0	26	(0.2)	0.45	(0.5)
		2.0	32	(3.5)	0.29	(0.6)
		4.0	26	(0.1)	1.42	(0.3)
	ACQ	4.0	29	(1.9)	1.89	(0.1)
	Untreated	0.0	46	(5.5)	1.24	(0.6)
Heartwood	DCOI	0.1	22	(0.0)	1.05	(0.2)
		0.3	22	(0.3)	1.21	(0.2)
		0.6	27	(0.9)	0.39	(0.1)
	DDAC	1.0	22	(0.5)	0.80	(0.1)
		2.0	21	(0.2)	1.05	(0.3)
		4.0	24	(0.8)	0.55	(0.2)
	ACQ	4.0	25	(0.2)	0.72	(0.2)
	Untreated	0.0	21	(0.3)	1.07	(0.4)

Table 4.21. Moisture contents and wood weight losses for southern pine blocks treated with selected biocides and left unexposed to a fungus as a negative control in a soil block test.

Wood	Treatment		Wood Moisture Content (%)		Wood Weight Loss (%)	
	Chemical	Target Retention				
		Kg/m^3	Media	(SD)	Media	(SD)
Sapwood	DCOI	0.1	37	(0.9)	1.41	(0.3)
		0.3	23	(0.0)	0.50	(0.0)
		0.6	24	(0.4)	0.53	(0.0)
	DDAC	1.0	24	(0.4)	0.75	(0.2)
		2.0	23	(0.1)	1.88	(0.7)
		4.0	23	(0.6)	1.61	(0.2)
	ACQ	4.0	31	(0.9)	3.53	(0.1)
	Untreated	0.0	32	(1.8)	1.27	(0.5)

The American Society for Testing and Materials Standard D-2017 lists the criteria for various decay resistance classes from weight loss results in a soil-block test as follows (ASTM, 2001):

- 0-10 % Highly resistant
- 11-24% Resistant
- 25-44 % Moderately resistant
- >45 % Slightly or non-resistant

This classification would place the untreated heartwood of teak, redwood, and western red cedar as highly resistant, as well as the treated heartwood of all treatments. Thus, there was no improvement on natural durability in these tests. One possible reason for this lack of effect was that the exposure times were too short to detect differences. In contrast, untreated sapwood of all species would be classified as moderately resistant. However, the treated sapwood of the following combinations would be classified as highly resistant:

- 4.0 kg/m^3 of ACQ in:
  - Teak, western red cedar and redwood against *P. placenta* and *T. versicolor*.
- 4.0 kg/m^3 of DDAC in:
  - Teak against *P. placenta*.
  - Western red cedar and redwood against *P. placenta* and *T. versicolor*.
  - Southern pine against *T. versicolor*.
- 2.0 kg/m^3 of DDAC in:
  - Western red cedar against *P. placenta* and *T. versicolor*.
  - Southern pine against *T. versicolor*
- 0.6 kg/m^3 of DCOI in:
  - Teak treated with against *P. placenta*.
  - Western red cedar and redwood against *P. placenta* and *T. versicolor*.
  - Southern pine against *T. versicolor*.
- 0.3 kg/m^3 of DCOI in:
  - Western red cedar and redwood against *P. placenta* and *T. versicolor*.
  - Southern pine against *T. versicolor*.

4.4. Synergic Behavior of Heartwood Extractives and Supplemental Wood Preservatives on Fungi Resistance

Extraction Methods

The extractive yields of the methods employed for wood extraction can be seen on Table 4.22. Soxhlet extraction produced higher yields than cold extraction, but biological activity was lower. Higher temperatures used in Soxhlet extraction (above the boiling point of each solvent) or the long cycle duration (9 hours) may have diminished extractive activity. WRC extractives were the only samples that exhibited biological activity using Soxhlet extraction.

Table 4.22. Extractive yield by solvent and extraction method for heartwood of three naturally durable woods

Wood	Solvent	Extractive Yield by Solvent (%)		
		Method 1	**Method 2**	**Method 3**
		Flask extraction	**Soxhlet extraction**	**Cold extraction**
Teak	Hexane	0.1	1.0	1.0
	Dicholoromethane	0.5	6.2	2.1
	Ethanol	Not tested	8.3	4.2
Redwood	Hexane	0.1	0.3	0.1
	Dicholoromethane	Not tested	1.8	2.0
	Ethanol	Not tested	5.0	3.1
WRC	Hexane	0.2	0.2	0.1
	Dicholoromethane	Not tested	2.2	1.2
	Ethanol	Not tested	4.1	1.8

Clark *et al*, 2004, obtained similar yields with Soxhlet extraction of WRC using the same solvents. They analyzed extractives by HPLC and found that ethanol yielded more extractives which contained mostly plicatic acid. Hexane extractives were richer in γ- and β- thujaplicins, which are the most antifungal compounds of WRC, than the dichloromethane extract. Clark's analysis suggest that WRC hexane extractives should exhibit higher antifungal activity, but dichloromethane and ethanol extraction resulted in better activity in our bioassay (Table 4.23). These results, coupled with low extractives yields obtained with hexane, led us to use alternative extraction methods for the remainder of the trial.

Table 4.23. Zones of inhibition formed around filter paper disks treated with different levels of western red cedar extractives and exposed to *Postia placenta*

Extractive concentration (ppm)	Zone of Inhibition (mm)					
	Hexane		DCM		Ethanol	
	Mean	SD	Mean	SD	Mean	SD
1200	5.2	(4.0)	10.0	(0.0)	7.7	(3.0)
8000	1.0	(0.4)	6.6	(3.4)	6.7	(2.2)
4000	1.2	(2.6)	1.1	(3.3)	2.5	(1.1)
No disk	0.0	(0.0)				
Sterile disk	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Negative control	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)

Bioassay of interaction between heartwood extractives and preservatives

Inhibition zones of the various extractive-preservative combinations are reported in Tables 4.22 and 4.23.

Extracts alone: The ethanol extract of western red cedar produced a dose response at the concentrations tested. The same wood extracted with dichloromethane was extremely antifungal, even at the lowest concentration tested. Teak produced a clear dose response effect with extractives from ethanol, but this effect was mixed in the case of extractives from dichloromethane. Redwood extractives showed no biological activity, which is in agreement with previously reports (Anderson, 1961). Redwood fungicidal components apparently lose their fungicidal activity, if any, during the extraction and concentration stages.

Extract – preservative mixtures: Preservative concentrations tested were based on a pre-test and the thresholds reported in the literature (Table 4.24). Clearly, DCOI concentrations were too high, while DDAC concentrations produced the desired dose response.

Table 4.24. Zones of inhibition formed by filter paper disks treated with different biocides and exposed to *Postia placenta.*

Treatment	Concentration (ppm)	Zone of inhibition (mm)
Control	0	0
DCOI	200	10
DCOI	400	10
DCOI	600	10
DDAC	1000	4
DDAC	2000	8
DDAC	4000	9

However, in the case of DCOI, even though we reduced the concentrations in the final tests and the preservative was dissolved into the medium, the inhibition rates of the controls were at their maximum for all concentrations tested. A negative concentration effect was observed with DDAC. (Tables 4.25 and 4.26)

Teak extracts had a slight effect on *P. placenta* compared to *T. versicolor*. The effects of the western red cedar extracts were stronger on *P. placenta* than on *T. versicolor*

Synergistic antifungal activity exists when the combination of two or more antifungal compounds results in greater inhibitory activity than the use of either compound acting alone. Inhibition zones around mixtures of extractives and preservatives were smaller than zones for the preservatives alone for all treatment combinations. These results suggest that the extractives had a detrimental effect on the preservative performance. However, it is possible that the extractives-preservative combinations interacted with each other, and were less able to diffuse into the medium to inhibit fungal growth. Further tests to assess changes in biocide or extractive chemistry would be required to better understand these possible interactions.

Table 4.25. Zones of inhibition formed around filter paper disks treated with different combinations of extractives and preservatives and exposed to *Postia placenta* (DCM = dichloromethane).

		Zone of Inhibition (mm)											
Extractive	**Preservative concentration (ppm)**	**Teak**				**Redwood**				**Western red cedar**			
		Ethanol		**DCM**		**Ethanol**		**DCM**		**Ethanol**		**DCM**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
24000 ppm	--	12.0	(7.8)	5.3	(4.2)	0.0	(0.0)	0.0	(0.0)	15.4	(4.1)	18.3	(2.5)
	DCOI (40)	8.4	(7.3)	7.0	(5.0)	17.8	(2.6)	0.0	(0.0)	18.0	(4.1)	19.4	(1.7)
	DCOI (60)	9.2	(6.6)	7.2	(2.9)	17.8	(2.6)	7.1	(6.4)	16.9	(6.0)	19.4	(1.7)
	DCOI (80)	11.6	(7.5)	4.3	(1.7)	18.9	(2.2)	13.8	(5.6)	17.2	(2.8)	19.4	(1.7)
	DDAC (4000)	4.7	(2.1)	7.2	(3.0)	0.2	(0.4)	1.0	(0.0)	17.3	(4.2)	20.0	(0.0)
	DDAC (8000)	5.3	(1.5)	5.3	(2.2)	0.0	(0.0)	4.3	(1.1)	18.3	(2.5)	18.3	(2.5)
	DDAC (12000)	4.6	(1.7)	10.9	(2.5)	2.7	(1.1)	6.2	(0.4)	17.2	(2.6)	18.9	(2.2)
16000 ppm	--	5.1	(1.9)	6.1	(4.1)	0.0	(0.0)	0.0	(0.0)	13.4	(4.4)	20.0	(0.0)
	DCOI (40)	5.9	(2.3)	4.2	(1.6)	17.8	(2.6)	2.0	(1.3)	14.1	(2.8)	20.0	(0.0)
	DCOI (60)	11.3	(7.4)	8.1	(1.7)	18.9	(2.2)	3.9	(2.9)	15.7	(3.0)	20.0	(0.0)
	DCOI (80)	10.7	(3.2)	13.1	(5.0)	19.9	(0.3)	8.7	(3.5)	13.3	(3.6)	20.0	(0.0)
	DDAC (4000)	5.9	(1.9)	9.4	(3.2)	0.0	(0.0)	8.0	(4.3)	14.4	(3.3)	20.0	(0.0)
	DDAC (8000)	4.7	(1.7)	7.3	(1.9)	1.9	(1.3)	8.9	(4.5)	9.1	(3.4)	20.0	(0.0)
	DDAC (12000)	5.3	(1.6)	8.9	(2.9)	2.9	(1.1)	11.1	(3.3)	12.8	(3.6)	20.0	(0.0)
8000 ppm	--	3.6	(1.3)	5.8	(3.3)	0.3	(0.5)	0.0	(0.0)	5.0	(2.1)	20.0	(0.0)
	DCOI (40)	9.1	(8.9)	6.2	(6.8)	19.8	(0.4)	4.0	(3.2)	14.6	(3.1)	20.0	(0.0)
	DCOI (60)	11.8	(8.3)	7.3	(3.4)	18.9	(2.2)	14.0	(5.0)	10.2	(3.7)	20.0	(0.0)
	DCOI (80)	14.3	(6.8)	4.1	(1.6)	20.0	(0.0)	10.4	(3.4)	19.4	(1.7)	20.0	(0.0)
	DDAC (4000)	4.4	(1.7)	6.9	(2.7)	1.0	(0.0)	3.9	(1.5)	8.7	(3.7)	20.0	(0.0)
	DDAC (8000)	5.1	(2.7)	9.1	(3.2)	1.8	(0.4)	9.9	(3.8)	6.6	(3.1)	20.0	(0.0)
	DDAC (12000)	4.9	(1.8)	11.2	(4.1)	4.8	(0.4)	7.1	(2.0)	5.2	(1.2)	20.0	(0.0)
	No disk	0.0	(0.0)										
	Sterile disk	0.0	(0.0)	0.0	(0.0)								
	Negative control	0.0	(0.0)	0.0	(0.0)								
	DCOI (40)	18.1	(3.8)										
	DCOI (60)	20.0	(0.0)										
	DCOI (80)	20.0	(0.0)										
	DDAC (4000)	7.1	(8.0)										
	DDAC (8000)	3.2	(1.1)										
	DDAC (12000)	4.6	(1.1)										

Table 4.26. Zones of inhibition formed around filter paper disks treated with different combinations of extractives and preservatives and exposed to *Trametes versicolor* (DCM = dichloromethane)

		Zone of Inhibition (mm)											
Extractive	**Preservative concentration (ppm)**	**Teak**				**Redwood**				**Western red cedar**			
		Ethanol		**DCM**		**Ethanol**		**DCM**		**Ethanol**		**DCM**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
24000 ppm	--	3.2	(2.0)	5.9	(3.1)	0.0	(0.0)	0.0	(0.0)	18.9	(2.2)	20.0	(0.0)
	DCOI (10)	8.7	(2.8)	7.0	(4.2)	5.8	(6.6)	17.0	(5.7)	17.2	(4.4)	20.0	(0.0)
	DCOI (20)	4.3	(2.8)	9.1	(5.2)	2.8	(2.2)	14.8	(6.9)	18.9	(2.2)	20.0	(0.0)
	DCOI (40)	5.0	(2.7)	12.3	(6.4)	4.1	(3.7)	7.7	(7.5)	14.4	(6.4)	20.0	(0.0)
	DDAC (1000)	2.1	(0.6)	5.0	(2.2)	0.0	(0.0)	8.9	(8.5)	17.8	(2.6)	20.0	(0.0)
	DDAC (2000)	2.7	(1.2)	3.9	(1.8)	0.0	(0.0)	3.3	(1.2)	14.6	(5.6)	20.0	(0.0)
	DDAC (4000)	3.9	(1.5)	8.0	(5.4)	0.0	(0.0)	8.1	(2.0)	19.4	(2.5)	20.0	(0.0)
16000 ppm	--	2.7	(1.3)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	13.8	(5.4)	7.9	(0.0)
	DCOI (10)	5.1	(3.4)	5.8	(6.3)	3.2	(4.3)	0.0	(0.0)	18.9	(0.9)	17.8	(2.2)
	DCOI (20)	6.4	(3.0)	9.9	(6.8)	5.7	(2.8)	5.1	(4.0)	2.8	(5.3)	13.2	(0.0)
	DCOI (40)	11.1	(3.3)	0.0	(0.0)	7.4	(4.5)	17.4	(4.5)	20.0	(0.0)	14.9	(4.6)
	DDAC (1000)	2.4	(1.1)	8.2	(5.0)	0.0	(0.0)	5.0	(5.9)	5.3	(4.0)	20.0	(0.0)
	DDAC (2000)	3.1	(0.8)	8.0	(2.3)	1.9	(0.6)	11.8	(4.1)	9.4	(5.3)	20.0	(0.0)
	DDAC (4000)	3.8	(2.3)	10.8	(6.2)	1.7	(0.5)	7.1	(2.0)	7.9	(5.2)	14.4	(5.3)
8000 ppm	--	1.9	(1.5)	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)	3.8	(6.6)	18.3	(2.5)
	DCOI (10)	5.6	(3.3)	18.3	(2.5)	14.9	(8.0)	18.9	(2.2)	19.4	(1.7)	8.6	(7.7)
	DCOI (20)	5.9	(3.6)	10.4	(5.6)	0.0	(0.0)	15.3	(5.8)	13.8	(7.4)	7.9	(7.0)
	DCOI (40)	4.0	(3.1)	9.0	(7.2)	4.9	(3.7)	18.9	(2.2)	18.9	(3.3)	17.8	(4.4)
	DDAC (1000)	2.6	(0.7)	9.2	(4.2)	1.0	(0.0)	4.1	(1.6)	2.8	(2.0)	13.2	(5.9)
	DDAC (2000)	4.1	(1.3)	9.3	(3.7)	2.4	(2.2)	16.1	(4.2)	6.3	(5.2)	11.3	(5.7)
	DDAC (4000)	4.4	(1.6)	5.1	(0.8)	2.4	(1.1)	11.0	(3.1)	4.8	(1.0)	17.9	(3.4)
Controls	No disk	0.0	(0.0)										
	Sterile disk	0.0	(0.0)	0.0	(0.0)								
	Negative control	0.0	(0.0)	0.0	(0.0)								
	DCOI (10)	16.9	(4.0)										
	DCOI (20)	17.7	(3.7)										
	DCOI (40)	13.8	(4.2)										
	DDAC (1000)	4.2	(1.3)										
	DDAC (2000)	4.8	(1.1)										
	DDAC (4000)	5.4	(0.9)										

CHAPTER 5- CONCLUSIONS

5.1. Wood Treatment

Second-growth timber of teak, redwood, and western red cedar can be treated, and the treatments may result in improved durability, even if only low retentions are obtained. In such cases, retention may be limited but the degree of treatment may be acceptable. However, the high impermeability of teak prevented proper penetration under the conditions employed in this experiment. More rigorous treatment conditions may be needed for treatment of both teak sapwood and heartwood.

5.2. Leach Resistance Test

No significant leaching was noted, even at the highest preservative concentrations used in this experiment. In future research, higher doses should be evaluated, although this may lead to increased leaching in some treatments. Future research will be needed to determine the doses at which leaching becomes a negative aspect of the treatment.

5.3. Soil Block Test

Supplemental treatments with DCOI at 0.6 kg/m^3 and DDAC at 4 kg/m^3 improved the durability of most sapwood samples, according to the ASTM standard D-2017 classification. However, longer incubation times would be needed to evaluate improvements in durability of the heartwood samples. Results obtained in the soil block test were inconclusive because mass losses in the untreated heartwood materials were less than 3%, which was within the error limits estimated for the soil block test. These mass losses could be caused by extractive or preservative leaching rather than fungal activity. Nonetheless, treatment with higher chemical retentions resulted in lower mass losses and reduced variability, and it provided weak evidence that durability and predictability of the heartwood may be improved with supplemental treatments.

Leaching has no significant negative effect on performance of treated samples. Leaching of untreated samples resulted in higher weight losses than the unleached samples. DCOI at 0.6 kg/m^3 and DDAC at 4 kg/m^3 provide better protection than the untreated leached heartwood.

DCOI (0.1 and 0.3 kg/m^3) and DDAC (1 and 2 kg/m^3) treated blocks both experienced greater weight losses than the controls. The hormesis effect may have positive implications in the risk assessment of these preservatives.

5.4. Synergic Behavior of Heartwood Extractives and Supplemental Wood Preservatives on Fungi Resistance

- Cold extraction resulted in higher biological activity than flash and Soxhlet extractions. The latter two procedures seemed to reduce the biological activity at the temperatures and extraction times used. However, the yields with Soxhlet extractions were substantially higher than those from the cold extraction.
- The method proved an inefficient way to test synergetic interactions between extractives and preservatives.
- Because it is impossible to measure the amount of the test substance diffusing into the agar medium, the apparent negative synergistic effect may simply be due to lack of solubility of the mixtures.

Implications and Future Research

Wood remains one of the most important structural materials. Wood products are increasingly challenged by the use of alternate materials produced from other resources such as steel, concrete, glass and plastic. However, as we move increasingly towards a future based on sustainable practices for the planet, renewable forestry-derived wood products provide considerable potential to fulfill vital needs for structural materials in a highly sustainable manner. In that regard, a major goal of the wood industry is to maximize the competitiveness of wood-based materials, and controlling biodegradability is an important step for remaining competitive.

In the past, the use of natural durable wood species was highly important for the efficient use of wood resources as building materials. Species derived from old-growth forests possessed natural resistance to biodegradability, however decay resistance in the heartwood of trees grown in plantation forests is variable and difficult to predict. More importantly, second and third growth timber has a substantially higher proportion of non-durable sapwood and this material is frequently utilized in structural applications where it is presumed to be equivalent in durability.

This project evaluated the ability of supplemental preservatives to protect the sapwood and heartwood of second-growth timbers of naturally durable species. The results showed that supplemental treatments may enhance sapwood durability. However, longer tests are needed to further evaluate this enhancement of both sapwood and heartwood durability.

Field trials have been established to evaluate the performance of candidate supplemental treatments under extreme decay hazards. These trials will help develop a better understanding of the microorganisms associated with various preservative treatments in different wood species. These results will be used to determine the interactions between non-decay microorganisms and the heartwood extractives. These data can then be used to study interactions that result in detoxification of introduced preservatives, and how these processes impact long term performance of such treatments. Furthermore, studies into the chemistry and mode of action of extractives in the presence of introduced preservatives would determine whether synergies exist that will allow relatively benign preservative treatments to provide appropriate protection to plantation derived naturally durable species such as cedar.

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