AN ABSTRACT OF THE DISSERTATION OF

Yohanna Cabrera Orozco for the degree of Doctor of Philosophy in Wood Science presented on July 16, 2010.
Title: Effects of Biocide Treatment on Durability and Fungal Colonization of Teak, Western Redcedar, and Redwood

Abstract approved:

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Jeffrey J. Morrell

Natural durability is generally lower in wood coming from second-growth or plantation-grown material compared to that coming from old-growth forests. Higher proportions of non-durable sapwood and reduced levels of heartwood extractives may account for reduced durability. Additionally, natural durability varies widely among lumber of the same species. This variation can cause problems in engineered applications due to prematurely decaying wood and lack of predictability of the performance of the product in service. Heartwood boards with included sapwood are commonly sold in the markets. Sapwood has little resistance to decay and can open the way to fungal colonization of the heartwood.

Treating durable, second-growth or plantation-grown material with supplemental chemicals could improve durability while enhancing the uniformity of the products. To test this hypothesis, the effect of two candidate biocide treatments on the durability of teak, redwood, and western redcedar was assessed in a ground proximity field test established in Hawaii. The objective was to determine if 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI) or didecyldimethylammoniumchloride (DDAC) provided supplemental protection to either sapwood or heartwood of the three wood species. DDAC (4 kg/m^3) improved sapwood durability of teak, redwood, and western redcedar, while DCOI (0.6 kg/m^3) improved sapwood durability of teak and western

redcedar after 36 months of sample exposure. Western redcedar heartwood durability was improved with 4 kg/m^3 of DDAC. There was a slight improvement in decay resistance for redwood heartwood samples treated with DDAC or DCOI; however, further exposure will be required to test the effects of biocide treatment on the heartwood of this species. Conversely, teak heartwood durability appeared to be reduced by biocide treatments.

Biocides retentions were assessed after 6, 12, 18, and 24 months of field exposure. All samples except teak heartwood had acceptable biocide retentions after 24 months of exposure, but there was evidence of biocide leaching. Teak heartwood samples had very low and poorly distributed biocide loadings. There was no evidence of natural extractives depletion due to biocide treatment.

Fungal diversity and community development were monitored at six month intervals over a 24 month period from wood samples in the same field test. Fungi were isolated and identified by coupling microscopic techniques with the comparisons of the internal transcribed spacer (ITS) regions of the fungal rDNA with sequences available on the National Center for Bioscience Informatics (NCBI) website. 2361 isolates were obtained, representing 241 unique ITS sequences that were grouped into 81 morphotaxa. The most represented groups were: 63 ascomycetes (15 molds, 26 soft rot fungi, 2 stain fungi, and 21 genera with unknown capabilities), 16 basidiomycetes (11 white rot fungi and 5 genera with unknown capabilities), and 3 zygomycetes. Non-metric multidimensional scaling ordination revealed that the strongest differences in fungal community composition were related to time. Multi-response permutation procedures did not indicate a change in fungal communities as a result of biocide treatment, wood species, or wood type (sapwood/heartwood). Fungal communities in all wood types contained taxa with similar decay capabilities. This similarity in fungal capabilities suggested that the observed decay rates in samples of the same wood type were due to biocide treatments and not due to changes in fungal communities. Further studies are underway to better characterize the decay capability of the isolated fungi.

Effects of Biocide Treatment on Durability and Fungal Colonization of Teak, Western Redcedar, and Redwood

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

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CONTRIBUTION OF AUTHORS

Dr. Jeffrey J. Morrell assisted with writing the dissertation. Dr. Lehong Jin assisted with wood treatment and biocide chemical analysis. Camille Freitag helped with the design and data collection for Chapter 4 and 5.

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EFFECTS OF BIOCIDE TREATMENT ON DURABILITY AND FUNGAL COLONIZATION OF TEAK, WESTERN REDCEDAR, AND REDWOOD

CHAPTER 1- INTRODUCTION

1.1. Background

Beauty, strength, abundance, and versatility give wood a high value as a construction material. Some woods have an additional property that increases their value, i.e. outstanding resistance to biodeterioration. The resistance of certain woods to attack by wood destroying organisms, or natural durability (USDA, 1999), is a recognized, very useful and appreciated wood property. For example, the heartwoods of teak (*Tectona grandis*), western redcedar (*Thuja plicata*), and redwood (*Sequoia sempervirens*) are famous for their natural durability (Willeitner and Peek, 1997).

However, natural durability is sometimes lower in wood coming from second-growth or plantation grown material compared to that coming from old-growth forests (Zabel and Morrell, 1992). Combinations of higher proportions of non-durable sapwood as well as reduced levels of heartwood extractives may account for reduced durability. Second growth timbers are often faster grown and the rapid growth may affect the concentration of heartwood extractives. For some second-growth species there is a correlation between the amounts of known protective chemicals and durability, while the correlation is poor for other species, possibly due to the presence of unknown bioactive compounds (Clark *et al.*, 2004).

Natural durability varies widely, not only between wood from different species, but also between wood from different trees of the same species. It can even be variable in a single piece of wood (Zabel and Morrell, 1992). This variation can cause problems, particularly in engineered applications (Clark *et al.*, 2004). Moreover, heartwood boards with included sapwood are commonly sold in the markets. The sapwood zone has little resistance to decay.

Treating differentially durable woods with supplemental chemicals might help improve durability while enhancing the uniformity of the products. 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. These attributes would reduce the risk of negative environmental impacts. The preservatives should be also colorless to maintain the aesthetic value of the wood, be economical, and resistant to weathering.

Previous laboratory studies showed that two chemicals, 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI) and didecyldimethylammonium chloride (DDAC) met these requirements, and that low loadings could potentially improve the durability of western redcedar, teak, and redwood (Cabrera, 2008). Leaching losses were below 10% and sapwood durability improved when treated cubes of sapwood and heartwood of the three wood species were evaluated both in an AWPA E-10 soil block test (AWPA, 1999) and an AWPA E-11 leaching test (AWPA, 2006b). In addition, the aesthetic value of the wood was maintained. However, heartwood was highly durable without treatment and the results only provided weak support of improved durability.

As a result of the promising but inconclusive laboratory tests, a ground proximity field test (AWPA, 2006) was established in Hilo, Hawaii to assess the ability of DCOI and DDAC to protect sapwood and heartwood of teak, western redcedar and redwood. The test was established under tropical conditions because the combination of high rainfall and temperature produces excellent conditions for wood decay (Scheffer, 1971). The abundance and diversity of microorganisms in tropical conditions may also contribute to rapid decay.

The main objective of the field test was to further assess the hypothesis that supplemental treatment with DCOI and DDAC increases the decay resistance of both sapwood and heartwood of teak, western redcedar and redwood. Decay resistance was evaluated by exposing wood samples treated to varying retentions with the

preservatives of interest. Untreated samples and samples treated with a conventional biocide were included for reference. All samples were exposed in a high decay hazard test site in Hawaii following AWPA Standard E18 (AWPA, 2006a). Samples were visually examined for extent of fungal attack at six month intervals. Preservative losses from the wood were also evaluated.

Differences in wood decay rates can be caused by differences in fungal communities (Chen *et al.*, 2002). Wood from the same tree species may, under similar environmental conditions, decompose at different rates depending upon the fungal species present (Ritschkoff, 1996; Vandegrift, 2002). One way to validate field test results is to monitor and compare fungal taxa present in each of the wood treatment combinations over time.

The fungal flora of sapwood and heartwood samples exposed in the ground proximity test was monitored by periodically collecting wood samples from each wood and treatment combination. Wood samples were surface sterilized and sawn into small cubes that were flame-sterilized and placed into petri dishes with malt extract agar. Fungi growing from the wood were placed on fresh malt extract plates until pure cultures were obtained. Fungal taxa were identified using a combination of morphological and molecular techniques to obtain the identity of the species. DNA comparisons of specific fungal gene sequences isolated from pure fungal cultures were used to identify subsets of species richness, and to monitor changes in fungal communities growing in decaying wood (Rodriguez *et al.*, 2004; Artz *et al.*, 2007).

1.2. Objectives

- To assess the ability of DDAC and DCOI to limit decay of sapwood and heartwood of teak, redwood, and western redcedar in a ground proximity test.
- To characterize fungal community development in decaying heartwood and sapwood of various naturally durable second-growth timbers.
- To analyze extractive and preservative depletion in these wood samples over time.

1.3. Thesis Organization

This dissertation describes the results of a ground proximity test established in Hawaii to assess the ability of some supplemental treatments to limit decay of sapwood and heartwood of teak, redwood, and western redcedar. The dissertation has a comprehensive literature review, Chapter 2, followed by chapters describing individual study questions. Chapter 3 describes the effect of preservative treatments on durability of teak, western redcedar and redwood, and contains the results of an observational study on biocide and extractive depletion from samples used on the field test. Chapter 4 describes the fungal diversity found at the field test. Chapter 5 describes the effect of preservative treatment on fungal communities. These chapters are followed by an overall conclusion in Chapter 6.

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CHAPTER 2- LITERATURE REVIEW

2.1. Natural Durability of Wood

Natural durability, the resistance of certain woods to attack by wood destroying organisms, is a property exclusive to the heartwood of certain tree species. Sapwood is generally not considered to be durable. Durability depends on many factors including the presence of extractives, lack or inaccessibility of suitable nutrients for wood destroying organisms, and mechanical barriers present in the wood cells, such as aspirated or encrusted pits, or tyloses that reduce moisture uptake (Taylor, 2003). The presence of toxic extractives appears to be the most common cause for enhanced durability (Scheffer and Cowling, 1966; Hillis, 1987; Taylor, 2003). In addition to fungicidal properties, 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, extractives may inhibit an early step in the degradation process (Schultz and Nicholas, 2000).

Scheffer and Cowling (1966) summarized the role of extractives in 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 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.

Extractives can also act as mechanical barriers to fungal hyphae and may reduce wood wettability (Taylor, 2003; Stirling and Morris, 2006). For example, extractives contribute to reduced equilibrium moisture content in western redcedar (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 (Ibidem). 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 in durability of incense cedar, (*Libocedrus decurrens*), and jarrah (*Eucalyptus marginata*) have been attributed to oxidation, catalysis, or polymerization of heartwood polyphenols (Anderson *et al.*, 1963; Scheffer and Cowling, 1966).

Zabel and Morrell (1992) summarized research on the variation in wood durability in a tree as follows:

- 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, and toxicity tends to decline as heartwood ages.
- Extractive detoxification, oxidation, or polymerization can reduce toxicity. These tendencies have been observed in redwood logs (Sherrard and Kurth, 1933).
- Durability decreases with stem height. The most durable wood is near the stem base.

The roles of various extractives in durability remain poorly understood, even in commercially important species such as western redcedar, teak, and redwood. A significant concern about durability is the risk of reduced heartwood activity in second-

growth timbers. These naturally durable second-growth timbers may have lower extractive levels or different ratios of more toxic components that affect durability (Clark and Scheffer, 1983; Mockus-Lubin *et al.*, 1986; DeBell *et al.*, 1999).

2.1.1. Natural Durability of Western Redcedar (*Thuja plicata*)

Western redcedar (WRC) is a common tree in coastal and interior forests of the Pacific Northwest of North America. WRC heartwood 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 (Gonzalez, 2004). Because of its high durability, WRC has been used in Canada and the United States for exterior residential applications, shingles, poles, posts, and products that are subjected to conditions conducive to decay (Ibidem).

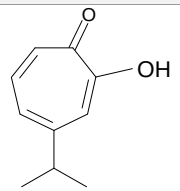
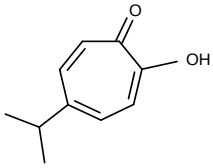
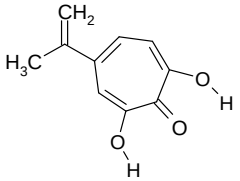
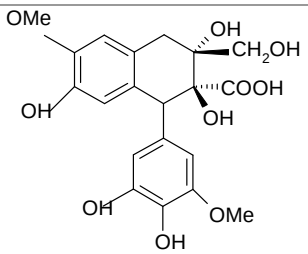
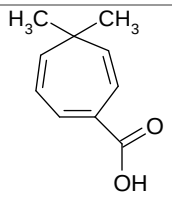
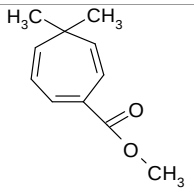
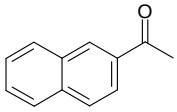
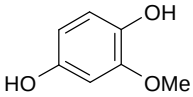
WRC durability can be attributed to the presence of aromatic compounds that inhibit fungal and bacterial growth (Nault, 1988) (Table 2.1). These compounds are produced as the ray parenchyma cells in the sapwood die and become heartwood (Bamber, 1976). Carbohydrate storage compounds in the sapwood undergo a series of 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; Clark *et al.*, 2004; Chedgy *et al.*, 2007). The decay resistance of WRC heartwood is believed to be a function of the relative proportions 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 (Rennerfelt, 1948; Lim *et al.*, 2005; Inamori *et al.*, 2000) and ascomycetes at concentrations between 8 and 32 ppm (Lim *et al.*, 2005). In general, WRC extractives are broadly active against many decay fungi (Scheffer, 1957).

Leaching of thujaplicins gradually lowers decay resistance of exposed wood. Thujaplicin in-cold-water extracts from WRC heartwood inhibits growth of decay fungi, and extractive loss is probably responsible for the gradual decrease in decay resistance in exteriorly exposed WRC (Scheffer, 1957). For example, WRC stakes performed like less durable woods when exposed in very wet soil (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.

Extractive leaching in treated WRC poles is minimal because of the protection afforded by the layer of preservative treated sapwood (Scheffer, 1957). To test this hypothesis, Scheffer (1957) sampled seven creosote-treated western redcedar poles in service for 21 to 29 years at the groundline and at six feet above the groundline. Heartwood samples from these poles were removed and exposed to five different decay fungi under laboratory conditions. Weight losses showed that the outer heartwood of the poles was less decay resistant than recently felled heartwood that was used as a control. The resistance of the groundline and of the higher portions of the poles was practically the same, so the author concluded that leaching did not affect durability but extractive detoxification may have affected decay resistance.

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 persist as heartwood ages. Tropolone content of samples from 11 second-growth trees was lower near the pith and associated juvenile wood (DeBell *et al.*, 1999). Reduced tropolone content could reflect lower extractive content at the time of heartwood formation or continued extractive reactions to produce less protection compounds.

Table 2.1. Biologically active compounds from the heartwood of *Thuja plicata*.

Extractive	Chemical Structure	Activity	Source
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.*, 2007)
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		Bactericide	(Clark *et al.*, 2004)
Methoxy-hydroquinone		Free radical inhibitor	(Teng, 2008)

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

Durability has also been thought to be dependent on wood 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) obtained similar results on a broader geographical sample.

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

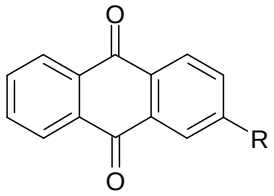
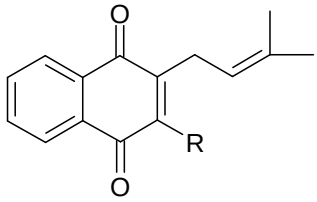
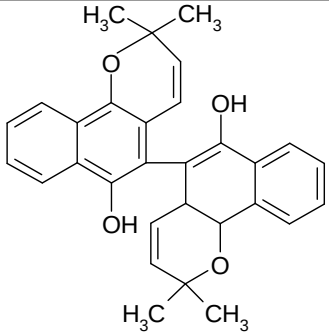
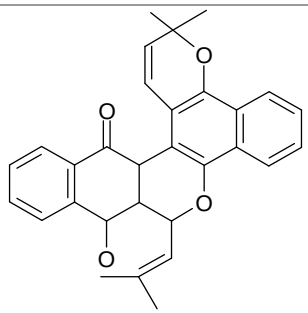
2.1.2. Natural Durability of Teak (*Tectona grandis*)

The heartwood of old-growth teak (*Tectona grandis*) is well-known for its natural durability. Teak's natural distribution covers an area of 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 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 2.2). Tectoquinone is highly repellent to the dry-wood termite *Cryptotermes brevis* (Wolcott, 1955) and to the subterranean-termite *Reticulitermes flavipes* (Sandermann and Dietrichs, 1957).

However, tectoquinone along with other extractives showed little activity against the fungi *Coniophora puteana* and *Trametes versicolor* and against the termite *Coptotermes lacteus* (Rudman, 1959). Interactions between extractives, rather than the activity of tectoquinone alone, could to be the cause of teak durability (Ibidem).

Table 2.2. Biologically active compounds from the heartwood of *Tectona grandis*.

Extractive	Chemical Structure	Activity	Source
Tectoquinone R= CH3: 2-Methylanthraquinone R= CH2OH: 2-Hydroxymethyl-anthraquinone		Insecticide	(Sandermann and Dietrichs, 1957)
Lapachol, deoxylapachol R= OH: Lapachol; R= H: Deoxylapachol		Fungicide	(Sandermann and Dietrichs, 1957)
Tectol		Unknown	(Rudman, 1961)
Rubber	Undetermined	Unknown	(Rudman, 1961)
Dehydrotectol		Unknown	(Khanna *et al.*, 1987)

Second-growth teak apparently has lower durability, possible as a result of lower extractive content or differential distribution in comparison with old-growth wood (Haupt *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 (Ibidem).

Leithoff *et al. (*2001) compared durability of second growth teak from Panama and old-growth teak from Myanmar. He found mass losses between 32% and 43% for second-growth material, while old-growth timber only experienced mass losses between 2.3% to 12.3%. Haupt *et al.* (2003) found total extractives content of the same plantation teak to be 9.4% weight/weight (wt/wt), while the old growth material had 14.1% extractives. The role of tectoquinone and other extractives was evaluated in relation to durability of the same Panama and Myanmar woods (Ibidem). Tectoquinone yield was high while deoxylapachol content was low in specimens with high natural durability. It was suggested that deoxylapachol was a precursor of tectoquinone and the reduced durability was attributed to a lower biosynthetic efficiency in conversion of deoxylapachol into tectoquinone.

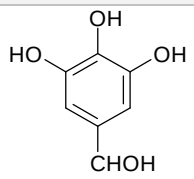
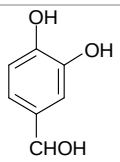
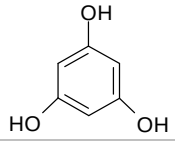
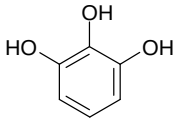
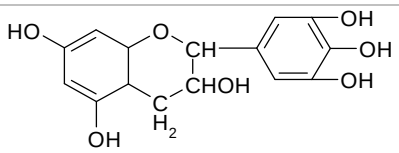
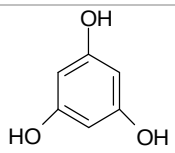
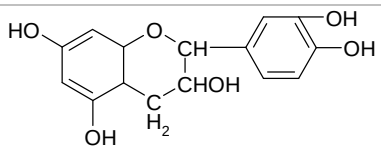
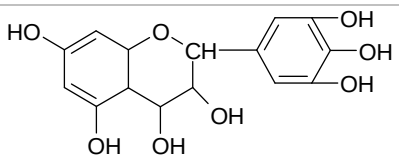
2.1.3. Natural Durability of Coast Redwood (*Sequoia sempervirens*)

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

The composition and amounts of extractives in redwood have been well studied (Table 2.3) (Luxford and Markwardt, 1932; Sherrard and Kurth, 1933; Balogh and Anderson, 1966). Extractives are responsible for the color and odor of redwood and play important roles in heartwood durability (Anderson, 1961). As with WRC, extractive content in redwood 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 2.3. Biologically active compounds from the heartwood of *Sequoia sempervirens.*

Extractive	Chemical Structure	Activity
Gallid acid		Antifungal and antiviral
Protocatechuic acid		Antioxidant
Phloroglucinol		Fungicide
Pyrogallol		Reducing agent
L-Gallocatechin		Antioxidant
Phloroglucinol		Fungicide
Cathechin		Antioxidant
Leucocyanidin		Antioxidant
*Source: (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. Extractives content in redwood heartwood was calculated to range from 15 to 30% of the wood (Anderson, 1961). The heartwood contains 9.9% water soluble extractives and 1.1% in ether soluble extractives, while sapwood contained neither of these materials (Hillis, 1987). Resch and Arganbright (1968) found that old-growth extractive content was 11.7%, while second growth extractives represented 9.0% of the wood mass. The differences were statistically significant; however, extractive distributions were more uniform in second growth trees than in old-growth.

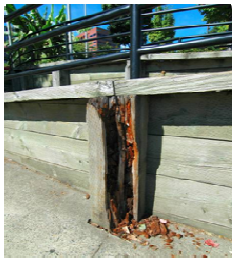
The durability of both second-growth (84 specimens) and old-growth redwood logs (83 specimens) was estimated using soil block tests (Clark and Scheffer, 1983). According to the ASTM D2017 (ASTM, 2001), all old-growth specimens were rated as “highly resistant”, while less than half of the second-growth samples were rated as “resistant” or “highly resistant”.

2.2. Testing Natural Durability with Ground Proximity Tests

Natural durability and the preventive treatments against wood decay are normally evaluated using standardized laboratory and field tests. In laboratory tests, parameters such as wood moisture content, temperature, and microorganisms, are well controlled and provide rapid results. In field tests, treated and untreated wood samples are exposed in the open and evaluated periodically in order to establish how long they remain serviceable despite decay and other deterioration hazards (Fougerousse, 1976). In field tests, environmental parameters are not controlled, in hope that the test exposures mimic the real situation of wood in service. The main disadvantage of field testing is the long time required to perform the test. The relevance of laboratory and field trials have been previously reviewed (Scheffer and Morrell, 1993; Nicholas and Militz, 2008).

Field test methods to evaluate natural durability are standardized by the American Wood Protection Association (AWPA). If the final wood product is intended to be used in exterior applications, the standards make a distinction between “in ground” and “above ground” applications, because wood generally deteriorates much faster in-ground than above ground applications (Table 2.4).

Table 2.4. Examples of different wood exposures and its testing methodology.

	In ground applications	**Above ground**
Real Life Example		
Testing Methodoly		

Field test methods to quantitatively evaluate wood durability exposed above ground do not exist (DeGroot, 1992). The evaluation methodology then relies on visual observations to detect decay, on ratings to quantify the extent of decay, and on comparisons made between the treated wood under study and untreated samples, or between durable and non durable woods. This methodology is subjective and does not detect early decay (Nicholas and Militz, 2008). However, the methodology is conducive to rapid decay, exhibits minimal variation between replicates in decay response, and the samples are not destroyed during the evaluation (DeGroot, 1992).

One field test methodology is the ground proximity test which is standardized in AWPA Standard-E18 (AWPA, 2006a). The method consists of exposing small wood samples (2 by 5 by 12 cm) on arrays of concrete and covered with a shade cloth that allows rain to strike the wood but slows wood drying. Samples are visually inspected periodically, but at least once every year, following an ordinal rating scheme provided by the standard.

The ordinal rating scheme provides a simple and convenient way to distinguish between decay severities. The primary characteristic of the scale representing the severities is that the numbers assigned to successive categories represent a "*greater than*" or "*less than*" severity. For example, the rating scale provided by AWPA Standard-E18 (AWPA, 2006a) goes from 10 to 0 and each number is used to assess severity of decay with scale categories such as sound (10), moderate decay (7), advanced decay (4), and broken or failed (0). The numbers ascribed to the severity of decay represent increased severity in the sense that "*advanced decay*" is more severe than "*moderate decay'*. The numerical rating given to the "advanced" case does not imply that "advanced" is three times as severe as "*moderate*", only that the severity of decay for samples in the "*advanc*e" category is greater than the severity of decay for those samples on the "*moderate*" category.

Although ordinal outcomes can be simple and significant, the statistical analysis remains challenging (O'Connell, 2006). As explained by O'Connell (2006), researchers have relied on diverse approaches for the analysis of ordinal outcomes. Some researchers have applied parametric models, such as multiple linear regressions, while other have treated the ordinal variables as strictly categorical and applied non-linear or log-linear approaches to understand the data, and other researchers apply logistic regression.

Where to locate the test? Deterioration rates of wood in service will vary according to the environment. Wood durability is dependent both on the material properties and the

environmental conditions to which the wood is exposed. For example, tropical environments, where the rainfall and the temperature are highly elevated, have excellent conditions for wood decay (Scheffer, 1971; Freitag *et al.*, 1995). The abundance and diversity of microorganisms may also contribute to rapid decay. The long exposure times typically required in field tests can be minimized by establishing the tests in tropical environments.

The fact that wood deteriorates faster in warm and wet climates than in cold or dry climates led to the development of climate indexes (Lebow and Highley, 2008). One index is the Scheffer index which uses a correlation between temperature and rainfall to estimate the decay risk of wood exposed out of ground contact (Scheffer, 1971). Field trials to evaluate biocide effectiveness or natural durability are often established where the index values are high, when rapid evaluation time is required, i.e., less than five years (Table 2.5).

Table 2.5. Example of Scheffer indices (annual) for various U.S. locations..

Location	Index	Location	Index	Location	Index
Hilo, HA	312	Atlanta, GA	67	Denver, CO	33
Miami, FL	131	Boston, MA	51	Phoenix, AZ	7
Mobile, AL	99	Seattle, WA	50	Long Beach, CA	4
Houston, TX	77	Chicago, IL	46	Yuma, AZ	0

$$SchefferIndex = \frac{\sum_{Jan}^{Dec}(T-35)(D-3)}{30}$$

T=mean monthly temperature (°F); D=number of days in month with at least 0.01 in of precipitation.

Based on Scheffer index estimations, a field test site was installed in Hilo, Hawaii in 1987 to evaluate wood preservatives. The site has a Scheffer index over 300 (Table 2.6) indicating an extreme above ground hazard. The field test site has an average annual temperature and precipitation of approximately 23°C and 3000 mm, respectively, and a Scheffer index of 312. Winter months tend to be wetter than summer months.

2.2.1. Previous Research at the Hilo Site Related to Microbial Wood Colonization

Wood inhabiting fungi and bacteria were sampled on treated and untreated lap-joints made from southern yellow pine and exposed for 12, 18, and 24 months (Molnar *et al.*, 1997). Bacteria, yeasts, and molds were the most predominant groups after 24 months of exposure, but only weak differences were observed in fungal communities between treated and untreated samples.

Freitag *et al,* (1995) sampled wood inhabiting fungi in treated and untreated ponderosa pine and southern pine lap-joints that were exposed between 3 and 18 months. Some fungi were specific for treatments, e.g., *Antrodia sinuosa* was exclusively isolated from samples treated with a triazole biocide. However, no distinctions were observed between fungal communities growing in treated and untreated wood. The study suggested that seasonal variations may affect isolation patterns, i.e., a nine month dry period inhibited colonization by basidiomycetes.

2.3. Treatability of Natural Durable Wood

Uniform performance of naturally durable woods is desirable because it allows the end users to predict the performance of a certain product for a given use. Natural durability varies widely between species, but also can be variable in a single piece of wood (Zabel and Morrell, 1992). 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.

Treated heartwood may provide enhanced protection against decay, despite the limited uptake of preservatives in heartwood compared with sapwood (Hwang *et al.*, 2007). The limited uptake could be due to high extractive content, aspirated pits, tyloses, or smaller pore sizes (Wang and DeGroot, 1996). Second-growth redwood lumber was easily treated with chromated copper arsenate (2% solution), using a full-cell pressure treating process, as long as it had been kiln-dried before treatment (Mockus-Lubin *et*

al., 1986). Thirty chemical formulations were evaluated for their ability to prevent decay of WRC sapwood during a two year test fence exposure (Newbill and Morrell, 1990). Four formulations provided protection equivalent to pentachlorophenol, including a substituted isothiazolone. The results suggest that supplemental organic preservatives can enhance WRC performance. WRC shakes and shingles often contain non-durable sapwood that requires protection (DeGroot, 1995). Longitudinal penetration of preservatives is generally limited (13 to 19 mm) but this degree of treatment may be acceptable because the initial attack tends to begin on the butt ends where the exposed end grain traps moisture.

2.4. Supplemental Treatments to Enhance Natural Durability

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.

2.4.1. Substituted Isothiazolones

Isothiazolones are biologically active compounds used as biocides where moisture is present. The general structure of 3-isothiazolones (Figure 2.1) includes a heterocyclic ring containing activated N-S bonds and three substituents (Paulus, 2005).

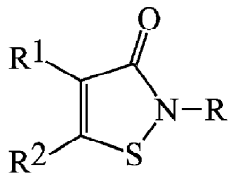


Figure 2.1. General structure of 3-isothiazolones

The biological efficacy of individual isothiazolones is dependent on the nature and position of the substituents attached to the ring. For example, 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 (Morley *et al.*, 2005).

2.4.1.1. Reaction Mechanisms and Mode of Action

Isothiazolones are thought to diffuse through the cell membranes or walls and then react with sulfur containing proteins (Paulus, 2005). 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 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 (Collier, 1990; Chapman *et al.*, 1998; Diehl and Chapman, 2000).

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

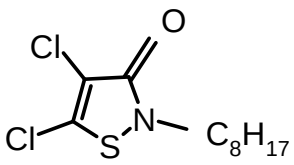


Figure 2.2. Structure of DCOI (4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one)

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

2.4.1.2. DCOI Performance in Above Ground Applications

Laboratory decay tests carried out with DCOI showed antifungal effects against three brown-rot fungi species, with toxic thresholds ranging from 0.4 to 0.5 kg/m³. Toxic thresholds were 0.4 and 2.3 kg/m³, respectively, for treated pine and sweetgum when exposed to the white-rot fungus *Trametes versicolor*. A threshold value of 0.5 kg/m³ was obtained in a 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 indicated that thresholds for brown and white rot fungi were 0.3 kg/m^3 and showed no significant performance 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 0.4 to 0.5 kg/m^3 of DCOI inhibited growth of various fungi on pine blocks, suggesting a threshold value of 0.5 kg/m^3 (Greenley, 1986). An above ground test showed no decay on L-joint samples treated with DCOI after two years exposure (Ibidem). 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, test units treated to levels of 0.4 kg/m^3 and higher had no deterioration after 45 months of exposure. DCOI appeared to be ten 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 decay free after five years of exposure and there was a strong positive dose-response (Preston *et al.*, 1996).

2.4.1.3. DCOI Performance in Ground Contact Applications

In five year stake tests at two test sites in Mississippi, 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).

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

2.4.2. 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. Initial research into the use of AACs as wood preservatives gave promising results in controlling basidiomycetes; however, these systems performed poorly in ground contact applications. As a consequence of this poor performance, AAC's must be used in combination with other biocides.

AAC's used with co-biocides are attractive preservatives because they are effective against fungi, have limited volatility, are 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.

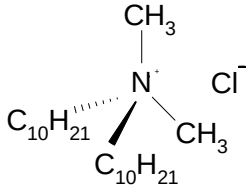


Figure 2.3. Structure of DDAC (Didecyldimethylammonium chloride)

2.4.2.1. 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 (Loubinoux, 1992). Ion exchange was suggested because of the influence of the treatment 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, 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. Preston (personal communication, 2007) 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. 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 (Ruddick and Sam, 1982). Analysis of the AAC distribution in L-joints suggested that end grain penetration was excellent, but lateral penetration was very poor (Doyle, 1995). Later studies found that

earlywood retained more AAC than latewood (Nicholas *et al.*, 1991). This uneven AAC distribution may negatively influence field performance.

Leachability: Laboratory trials suggest that AACs do not leach excessively from wood; but the leaching rates depend on the initial retention of AACs (Doyle, 1995). Water sorption rates tend to increase on AAC treated southern pine (Nicholas *et al.*, 2000).

2.4.2.2. Mode of Action

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

2.4.2.3. AAC Performance in Above Ground Applications

Both laboratory and field trials have generally indicated that AAC's performed well, especially in above ground commodities such as decking. Data from Australia and New Zealand indicate that AAC's toxic thresholds for above ground applications are between 1.1 and 3.6 kg/m^3 (Butcher and Greaves, 1982). 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 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).

While the effectiveness of DDAC as a wood preservative has generally been satisfactory, some failures have occurred. The reasons for poor performance include substandard treatment, misuse in service, poor preservative distribution, depletion of the

active ingredient during long term storage, and *Coniophora spp*. resistance to DDAC (Butcher, 1985). Alkyldimethylbenzyl ammonium chloride (benzalkonium chloride) (BAC) and alkyldimethylamine acetate 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 (Norton, 2002). However, 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.

2.4.2.4. AAC Performance in Ground Contact Application

While AAC's performed well in laboratory tests, they performed poorly in field trials. Preliminary trials of various quaternary ammonium compounds in *Pinus radiata* against three fungi (*Postia placenta*, *Phellinus gilvus*, and *Chaetomium globosum*) gave toxic thresholds between 1.6 and 6.4 kg/m^3 (Table 2.6). Dimethylaurylamine and alkyltrimethylammonium halide generated toxic thresholds between 1.6 and 3.2 kg/m^3 (Butcher *et al.*, 1977b). Further laboratory work with AACs in different wood species against soft rot fungi showed better performance compared to the CCA treated wood and slightly less effective performance against basidiomycetes (Butcher *et al.*, 1977a).

Non-modified AAC's did not perform as well as CCA after 30 months field exposure (Butcher, 1979) and showed signs of surface degradation soon after being placed in the field. Decay and stake failures were detected in dialkyldimethyl amine chloride (14.6 kg/m^3) treated wood after one year in the field (Tillott and Coggins, 1981). DDAC-treated stakes (3.2 kg/m^3) failed after two years in the field (Ruddick, 1983). AAC-treated wood (<11 kg/m^3) had noticeable decay after six years (Ruddick, 1987; Ruddick and Ingram, 1987; Morris and Ingram, 1988). DDAC (6.2 kg/m^3) and benzalkonium chloride (10.6 kg/m^3) treated *P. radiata* stakes were attacked by decay fungi after four years in the field (Drysdale, 1983).

Table 2.6. 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 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 formulations	Laboratory decay test (ASTM Standard 1413), and a filter paper disk termite test	1.6-6.4	(Preston and Nicholas, 1982)
DDAC and other 8 formulations	Laboratory decay test (Japanese Industrial Standard JIS A 9302)	2 - 4.6	(Tsunoda and Nishimoto, 1987)
Dialkyldimethyl-ammonium chloride	Laboratory decay test (ASTM Standard 1413)	2.5-6.4	(Butcher *et al.*, 1978)
DDAC, different alkyl lengths	Soil block test (C10/C12 chains 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)

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 wood in 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).

2.4.2.5. Insecticide Application

Overall, unmodified AAC's appeared to have good potential as insecticides. Benzalkonium chloride (6.2 kg/m^3) was shown to be effective against various insects including *Mastotermes darwinienses*, *Nasutitermes exitiosus* and *Coptotermes*

acinaciformes in *Pinus radiata* (Butcher and Greaves, 1982; Howick *et al.*, 1983). 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 (Preston and Nicholas, 1982). 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 or variable distribution since oaks tend to treat unevenly. 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). After 16 months of exposure at Florida and two years of exposure at Hawaii, DDAC (3.4 kg/m^3) treated southern yellow pine samples resisted attack by *R. flavipes* and *C. formosanus* (Preston *et al.*, 1985). A laboratory bioassay showed that DDAC provided protection to *Pinus radiata* against *M. darwiniensis* and *C. acinaciformis* at 2 kg/m^3 (Creffield, 1995).

2.4.2.6. 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 effectiveness in both laboratory and field trials (Butcher *et al.*, 1979; Drysdale, 1983). Copper amended DDAC (1 kg/m^3) had an improved effectiveness against *T. palustris* and *T. versicolor* (Tsunoda and Nishimoto, 1987). Acidic copper used in the various formulations did not undergo any apparent chemical fixation in the wood (Tillott and Coggins, 1981). In order to fix the copper in the wood, ammoniacal copper oxide gave better protection than the acid copper modified AAC's (Sundman, 1984). 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). 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 (Ruddick, 1987). While unmodified AAC's appeared to have little potential as wood preservatives in soil contact, 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). As a consequence of improved field performance; the ammoniacal copper modified AACs such as ammonium copper quaternary (ACQ) have been approved for use around the world (Jin and Preston, 1991).

2.5. Studies on Microbial Ecology on Decomposing Wood

A key component in understanding wood decay and the methods that we use to prevent it is to comprehend the impact of microbial species diversity on decay (Zabel *et al.*, 1982). Microbial communities develop and change as wood decay progresses. Microbial colonization of wood is generally considered to progress from bacteria, to ascomycetes to the principal decomposers which are basidiomycetes (Rayner and Boddy, 1988). Bacteria and some ascomycetes are generally more tolerant to biocides than basidiomycetes (Nicholas and Crawford, 2003). While some fungi may be responsible for decay, many non-decay fungi may also be present in the wood. Some fungi can consume non-structural wood components, while others can consume decay fungi or their metabolic products (Rayner and Boddy, 1988). Although a large number of patterns and organisms have been identified, the population structure and turnover rates have been poorly resolved (Raberg *et al.*, 2009).

Understanding the fungal communities inhabiting exposed samples, including information on the identity and role of the species isolated, is necessary to determine if field test results obtained at one location can be extrapolated to other environments. For

example, early colonizers such as non-decay fungi or bacteria may be present only at one field test and may detoxify wood preservatives and/or extractives. In western redcedar, certain fungal species can detoxify fungicidal extractives (Jin *et al.*, 1988; Lim *et al.*, 2005). A variety of microorganisms are able to detoxify biocides. For example, the organic fungicide tebuconazole was metabolized under laboratory conditions in 21 days by *Meruliporia incrassata, Chaetomium globosum, Trichoderma harzianum*, and *Pseudomonas fluorescens* into a form that may be less toxic (Obanda and Shupe, 2009).

There are numerous studies on the effects of microbial diversity on degradation of wood, its extractives, or the biocides used to protect wood (Molnar *et al.*, 1997; Jasalavich *et al.*, 1998; Jellison *et al.*, 2003; Raberg *et al.*, 2009). However, there is little consensus in identifying patterns of species appearance. This is in part due to the difficulties in identifying fungal species growing in wood. There are also difficulties in measuring species diversity, some reasons for this include: 1) scale variability that produces a species richness underestimate when sampling an area/volume of any size, 2) dependence on skill of field or lab personnel, 3) inconsistent taxonomic resolution, and 4) difficulty in sampling (Stohlgren, 2006).

Until recently, species diversity measurements relied only upon culture-based methods. Rodriguez *et al.*, (2004) highlighted the limitations of that approach. Improved research techniques and a growing understanding of microbial biochemistry and genetics provided higher resolution methods to study microbial communities (Jasalavich *et al.*, 1998; Jellison *et al.*, 2003; Raberg *et al.*, 2009). Improved mathematical and computational methods also allow us to examine how species richness and composition may interact with different environmental or experimental variables (McCune, 2009). The first step for describing and comparing microbial communities, however, is to be able to identify and distinguish fungal communities from each other (Rodriguez *et al.*, 2004). One method commonly used in mycology to identify species is called the ITS-BLAST technique, which is described in the next section.

2.6. The Use of Fungal ITS Genes in Taxonomy

Taxonomy is the science of hierarchically classifying organisms into ranks. Preferably, the classification should reflect the evolutionary history of the organisms. Traditional taxonomic ranks include phylum, class, order, family, genus, and species. Ideally, all organisms would be classified to the species level.

Our current understanding of fungal biology and fungal taxonomy make it clear that morphological, anatomical, and physiological characters may not be enough to classify fungi. The problems in species identification include the intraspecific morphological and physiological variation (Rodriguez *et al.,* 2004), a high phenotypic plasticity, and pleomorphism, the separation in space and time of sexual and asexual fungal phases which are associated with morphological differences between phases (Burnett, 2003). In addition, there are few trained taxonomists to identify fungi and there numerous undescribed species (Seifert, 2008). The comparisons between rDNA, and other genes, which are present in all fungal species but with variations between individual species, holds great promise for simplifying and standardizing species identification (Nilsson *et al.*, 2006).

A gene cluster in DNA codes for the production of ribosomal RNA. The gene cluster is conserved in all eukaryotes and consists of three major coding genes separated by the ITS regions (Internal Transcribed Spacers). The ITS regions evolves more rapidly than the coding units and different species normally have different ITS sequences. After DNA extraction and polymerase chain reaction (PCR) amplification of the ITS region of a fungus, the amplified ITS fragment can be sequenced. The sequence is compared to sequences in a database belonging to the National Center for Biotechnology Information (NCBI), although there are other databases available, with a tool called Basic Local Alignment Search Tool (BLAST). If the species sequence exists in the database, the fungus was previously identified, and if the statistical significance of the match is high, the species identity is revealed (Rodriguez *et al.,* 2004).

ITS-BLAST relies on DNA sequence comparison for species identification. The following assumptions have to be made when using this technique: 1) that the NCBI database contains a satisfactory taxonomic sampling of sequences, 2) that the sequences in the reference database are correctly identified, and 3) that the process of translating the comparison into species names is standardized (Nilsson *et al*., 2006).

None of these criteria is completely met with fungi. Discussions on the issues were provided by Nilsson *et al* (2006) and Seifert (2008). The taxon sampling of fungi is far from complete; in 2006, it was estimated about 20% of the entries in the International Nucleotide Sequence Database may be incorrectly identified to species level, and the majority of entries lacked descriptive and up-to-date annotations (Nilsson *et al.*, 2006). However, the ITS-BLAST technique has been used successfully to identify subsets of species richness (Vandenkoornhuyse *et al.*, 2002), and has provided insights into the ecological processes that affect the structure and diversity of fungal communities (Rodriguez *et al.*, 2004; Artz *et al.*, 2007).

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CHAPTER 3- EFFECTS OF PRESERVATIVE TREATMENT ON DURABILITY OF TEAK, REDWOOD, AND WESTERN REDCEDAR

Abstract

The effect of two biocide treatments on durability of teak, redwood, and western redcedar was assessed in a ground proximity field test established in Hawaii. The objective was to determine if didecyldimethylammonium chloride (DDAC) or 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI) provided supplemental protection to either sapwood or heartwood of the three wood species. The results showed that after three years of sample exposure, DDAC (4 kg/m^3) improved sapwood durability of teak, redwood, and western redcedar, and DCOI (0.6 kg/m^3) improved sapwood durability of teak and western redcedar. Western redcedar heartwood durability was improved with 4 kg/m^3 DDAC. Although the performance of treated redwood heartwood seemed to be better than the performance of the untreated samples the improved performance was not statistically significant. Some untreated controls had not decayed completely and a longer exposure time will be needed to evaluate the long term performance of the treatments in redwood heartwood samples. Increased biocide loadings decreased biodeterioration rates in all wood types tested except in teak heartwood. The decay resistance of teak heartwood was not improved by any of the treatments. On the contrary, biocide treatment appeared to be detrimental for durability.

Additionally, an observational study was undertaken to examine extractive and biocide depletion as a function of time from samples removed from the ground proximity field test. Biocides retentions were assessed after 6, 12, 18, and 24 months of field exposure. Extractive content was determined for heartwood samples at the same time intervals. All samples except teak heartwood had acceptable biocide retentions after 24 months of exposure, but there was evidence of biocide leaching. Teak heartwood samples had very low and poorly distributed biocide loadings. There was no evidence of natural extractives depletion due to biocide treatment.

Keywords: Ground proximity test, natural durability, DCOI, DDAC, ACQ, teak, western redcedar, redwood.

3.1. Introduction

The natural durability, or increased resistance of certain woods to attack by wood destroying organisms (USDA, 1999), is a recognized, useful and appreciated wood property. For example, the heartwood of teak (*Tectona grandis*), western redcedar (*Thuja plicata*), and redwood (*Sequoia sempervirens*) are famous for their high natural durability (Zabel and Morrell, 1992). However, natural durability is sometimes lower in wood coming from second-growth or plantation grown material compared to that from old-growth forests (Ibidem). A combination of higher proportions of non-durable sapwood and reduced levels of heartwood extractives may account for reduced durability. Second growth timbers are often faster grown and the rapid growth may affect the concentration of heartwood extractives. For some second-growth trees, there is a correlation between the amounts of known protective extractives and durability, while the correlation is poor for other species, possibly due to the presence of unknown bioactive compounds (Clark *et al.*, 2004).

Fig 3.1. Logs of teak coming from a plantation in Ecuador showing the high proportion of sapwood.

Natural durability varies widely between species but can also vary in a single piece of wood (Zabel and Morrell, 1992). This variation can cause problems, particularly in engineered applications due to the presence of prematurely decaying wood and lack of predictability of the performance of the products in service (Clark *et al.*, 2004).

Moreover, heartwood boards with included sapwood are commonly sold on the market. Sapwood zone has little resistance to decay and can open the way to fungal colonization of the heartwood.

Treating differentially durable woods with supplemental chemicals might help improve durability while enhancing the uniformity of the products. Ideally, the chemicals used should be highly specific against wood destroying organisms and have low mammalian toxicity. Chemicals that react with the wood and have high stability and low volatility can be advantageous because these properties reduce the risk of biocide leaching during the service life of the products. It is equally important that the biocide be biodegradable so that leached biocides do not contaminate the surrounding environment. In addition, the preservatives should be colorless to maintain the aesthetic value of the wood, be economical, and resistant to weathering. Two organic preservatives that meet these requirements are didecyldimethylammonium chloride (DDAC) and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI).

Previous laboratory studies showed that low loadings of DDAC and DCOI could potentially improve the durability of western redcedar, teak, and redwood (Cabrera, 2008). Leaching losses were below 10% and sapwood durability improved when treated cubes of sapwood and heartwood of the three wood species were evaluated in AWPA E-10 soil block tests (AWPA, 1999) and AWPA E-11 leaching tests (AWPA, 2006b). In addition, the aesthetic value of the wood was maintained. However, the results only provided weak support for improved durability of heartwood because of the high variability in the results. This indicated the need for longer and more severe decay tests to further evaluate enhancement of both sapwood and heartwood durability of these wood species. One way to stricter evaluate wood preservative performance is through the use of field tests. In this tests, treated and untreated wood samples are exposed in the open and evaluated periodically in order to establish how long they remain serviceable despite decay and other deterioration hazards (Fougerousse, 1976). In field tests

environmental parameters are not controlled, in hope that the test exposures mimic the real situation of wood in service.

Wood deterioration rates vary according to the environment. Wood durability depends on both the material properties and the environmental conditions to which the wood is exposed. For example, wood extractives can be leached or detoxified in rainy locations, which may lead to a reduction in durability, but not in dry environments. Likewise, biocides can be leached from the wood before they provide the expected protective effect. Thus, when wood preservatives are field tested, it is important to quantify extractive and preservative depletion, to assure that during the exposure time biocides and extractives remain in the wood and the decay rates observed are due to its effects.

The purpose of this study was to further test the hypothesis that DCOI and DDAC could provide supplemental protect to the sapwood and heartwood of teak, western redcedar, and redwood using an AWPA Standard E18 ground proximity field test (AWPA, 2006a). The test was established under tropical conditions, in Hilo, Hawaii, because the combination of high rainfall and temperature produces excellent conditions for wood decay (Scheffer, 1971). The abundance and diversity of microorganisms in tropical conditions may also contribute to rapid decay. In addition, total extractive content and biocide retention were assessed in samples as a function of field exposure time to test the hypothesis that treatments produced variable effects in durability because of biocide and/or extractive depletion from wood.

3.2. Methods

Experimental Design: Recently cut lumber of teak, redwood, and western redcedar (WRC) was obtained from various sources (Table 3.1). Additionally, southern pine (likely *Pinus taeda*) was obtained to serve as a decay susceptible control. For each wood type, 240 wood samples were cut (19 by 50 by 125 mm long) and conditioned at 23ºC and 70% relative humidity for one month. No attempt was made to segregate

samples from a given board into various treatments. Preservative treatments were assigned to eight 30-sample-groups using a list of random numbers generated in S-plus (*v8*). The wood was vacuum-pressure treated at Viance LLC (Charlotte, NC) with solutions of DCOI to target retentions of 0.1, 0.3, or 0.6 kg/m^3, or DDAC to retentions of 1, 2, or 4 kg/m^3 in southern pine. Samples from each wood type were left untreated or treated with ACQ (alkaline copper quaternary) at 4 kg/m^3, to serve as a reference preservative. Wood treatment began with an initial vacuum of 3.3 kPa for 10 minutes, followed by 1034 kPa of pressure for 30minutes, and a final vacuum for 5 minutes. The treatment conditions were applied to achieve the target retentions in southern pine. For practical reasons, all wood species were treated with the same treatment conditions despite the refractory nature of the other wood species. Gauge retentions were calculated based on biocide total weight retained and the total volume of the wood treated. Ten samples of each species by treatment combination were retained as negative controls, while 20 samples of each combination were placed in the field. Each sample was tagged with a barcode.

Table 3.1. Wood sources.

Experimental Wood Type	Source
Teak sapwood	Ecuador (20 year old plantation)
Redwood sapwood	California, USA
Western redcedar sapwood	Oregon, USA
Teak heartwood	Colombia (30 year old plantation)
Redwood heartwood	California, USA
Western redcedar heartwood	Oregon, USA

Following AWPA Standard-E18 (AWPA, 2006a), 16 arrays of concrete blocks were placed in the open at the Hawaiian field test and covered with shade cloth (Fig 3.2A-B) that allowed rain into the arrays but slowed wood drying. Samples were placed on the arrays (60 samples/ array) without consideration for the position of each individual sample. Samples were visually inspected (Fig 3.2-C), and the degree of decay was scored on a scale from zero to ten (Table 3.2). The inspection was performed every six months for three years. The complete set of data can be seen in Appendices 1 and 2.

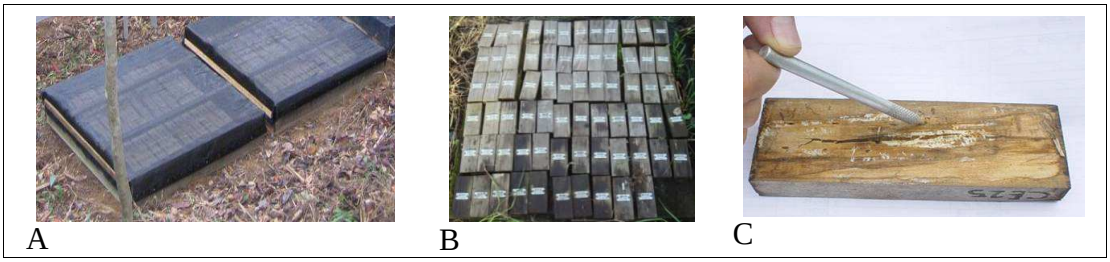
The data analyzed here correspond to the decay scores collected after 36 months of sample exposure. Other arrays were adjacent to the arrays used in these trials, so we did not consider possible border effects.

Three samples from each wood-treatment combination were collected from the field every six months. The selected sub-set of samples was randomly selected previous to the evaluation using the barcode number. These samples were removed and returned to Oregon State University (Corvallis, OR) to monitor preservative depletion, extractive depletion, and to isolate fungal species growing in the wood. At month 36, the number of samples left in each wood-treatment combination was between 7 and 14 (see frequency values in tables 3.5, 3.8 and 3.11). The variation reflects the loss of specimens in some treatment groups due to biological failure.

Table 3.2. Evaluation system used to score the degree of decay in a field trial in Hawaii. Adapted from AWPA Standard E18 (AWPA, 2006a).

Score	Description	Description
10	Sound	No signs or evidence of decay, wood softening, or discoloration caused by microorganism attack.
7	Severe Attack	Sample has between 20 and 40% of its cross-sectional area weakened to the point it can be damaged by a dull probe.
4	Very Severe Attack	Greater than 40% of sample cross-section compromised and severely softened, but does not meet the requirements for failure.
0	Failure	Sample has functionally failed. It can either be broken by hand due to decay or the evaluation probe can penetrate through the sample.

Sources of variation in this experiment included wood species, preservative treatments, and individual wood samples. Variation also may exist between arrays due to different fungal communities or microclimatic conditions. Because southern pine is the reference species in many wood preservation trials, the patterns of decay of treated and untreated samples of this species are known (Cabrera and Preston, 2008), and were used as an indicator of trial validity.



A B C

Figure 3.2. A ground proximity test AWPA E-18 (A) wood samples are located over concrete blocks (B) and visually assessed for decay (C).

Biocide Analysis: Chemical analysis was performed on a composite sample from three ground samples to produce a single value. Given the lack of replication, no statistical analysis was performed. The samples were analyzed by Viance LLC (Charlotte, NC) for biocide retention using the following methods.

- One gram of oven-dried sawdust from samples treated with DDAC was extracted in methanol for three hours using a sonicator. The extract was allowed to settle overnight, and then analyzed for DDAC content by titration according to AWPA Standard A17-03 (AWPA, 2004a).
- Samples treated with DCOI were also extracted with methanol using an ultrasonic bath. The filtered extract was analyzed according to AWPA Standard A30-00 (AWPA, 2004b) using a high performance liquid chromatograph (HPLC) equipped with a UV detector.

Retention levels were expressed in parts per million and converted to kg/m^3 using the reported density of each species at 12% moisture content (USDA, 1999).

Extractive Depletion Analysis: A subset of ground wood samples corresponding to the heartwood of teak, western redcedar, or redwood (treated and untreated samples), was air dried and wrapped in 6 by 8 cm extraction bags made from ANKOM^{TM} filter paper, pore size 30 (± 15) microns. The sealed extraction bags were oven-dried overnight at 80ºC and weighed.

For extraction of hydrophobic extractives, teak extraction bags were submerged in a 2:1 toluene/ethanol solution for seven weeks. The solution was drained and replaced three times, on the first, third, and fifth week. This procedure was not followed for redwood or western redcedar samples because most of their toxic extractives are water soluble (Anderson, 1961; Chedgy *et al.*, 2007). Water extraction was performed in each of the three wood types by boiling the extraction bags for 12 hours in distilled water with water changes every two hours. After extraction, each bag was oven-dried (80ºC) and re-weighed. Extractive content was calculated as the percent mass lost from the sample. Samples from some treatments were missing because of the difficulty of finding them in the field, and some bags were damaged during extraction. Thus, some wood-treatment combinations lack complete replication.

Statistical Analysis: The primary characteristic of the scale representing the severity of decay is that the numbers assigned to successive categories represent a "*greater than*" or "*less than*" severity (Table 3.2). The severity of decay was assessed using the following scale categories: sound (10), moderate decay (7), advanced decay (4), and broken (0). The numbers ascribed to the severity of decay represented increased severity in the sense that "advanced decay" is more critical than "moderate decay'. The numerical rating given to the "advanced" case does not imply that "advanced" is three times as critical as "moderate", only that the severity of decay for samples in the "advanced" category is greater than the severity of decay for those samples on the "moderate" category.

Given that the response variable of the trial (decay score) was categorical with four different outcomes, logistic analysis was used to model the odds of decay occurrence as a function of preservative treatment as described by O'Connell (2006). The odds of decay occurrence is a proportion that compares the probability that decay occurs, *P(decay)*, to the probability that it does not occur, *1-P(decay)*. Decay occurrence can be defined in different ways depending on how the data are viewed. For example, decay occurrence may be defined for samples that scored 7 or below, samples scoring 4 or

below or only samples scoring 0. Thus, a *cumulative odds model* was used that made three predictions. The three predictions were: category 0 vs. all above, category 0 and 4 combined versus all above, categories 0, 4, and 7 versus category 10.

To model the odds of decay occurrence, the proportion of P(decay)/1-P(decay) was calculated for each wood treatment combination and for each of the possible predictions. The odds were computed from the data by dividing the probability of each possible outcome (coded as Y=1) by the probability of scoring 10, as displayed in equation 1. This probability produces values between 0 and 1. The logistic or logit function was used to transform the range of the data to between -∞ and ∞.

$$\text{logit}(p) = \log\left(\frac{p(Y=1)}{1-p(Y=0)}\right) = \log\left(\frac{\frac{Score}{10}+0.005}{\frac{10-Score}{10}+0.005}\right) \qquad \textbf{Equation 1}$$

The odds are greater than 1.0 when the probability of decay occurrence is greater than the probability of non-decay occurrence. The odds are 1.0 if the two outcomes are equally likely and less than 1.0 if the probability of non-decay occurrence is greater than the probability of occurrence. To examine effects of preservative treatment on each wood type, odds ratios (OR) were calculated. An OR compares the odds for decay scores of non treated samples (coded x=1) with the odds for decay scores of the treated sample (coded x=0), by computing the following ratio (equation 2):

$$OR = \left(\frac{\frac{P(Y=1/x=1)}{1-P(Y=1/x=1)}}{\frac{P(Y=1/x=0)}{1-P(Y=1/x=0)}}\right) \qquad \textbf{Equation 2}$$

The advantages of logit transformation were discussed by O'Connell (2006). After transformation, the relationship between probabilities of decay (P) as a function of preservative treatment could be modeled in a manner that is similar to a simple linear regression, as stated in equation 3. However, the distribution of this model is binomial.

$$\text{logit}(p) = \alpha + \beta x, \text{ where, } x = \text{preservative treatment} \qquad \textbf{Equation 3}$$

The coefficients α and β were estimated using maximum likelihood. Wald χ^2 statistics were used to test the significance of individual OR in the model. Each Wald statistic was compared with a χ^2 distribution with 1 degree of freedom to obtain an estimated cumulative probability of OR's. Estimated cumulative probabilities were compared with actual cumulative probabilities obtained from the data. Computations were performed using PROC LOGISTIC in SAS (v9.2) (SAS Institute, Cary, NC).

3.3. Results and Discussion

3.3.1. Target retentions

Target retentions were achieved for sapwood of WRC and redwood, but not for the heartwood of either species (Table 3.3). Gauge retentions for teak were very low, which reflects the low permeability of both heartwood and sapwood of this species (Chudnoff, 1984). As expected, heartwood retentions were lower than sapwood retentions.

Table 3.3. Target vs. gauge chemical retentions of selected wood species treated with three different preservatives

Wood type / **Target retention**	**Retention (Kg/m^3)**					
	DCOI			**DDAC**		
	0.1	**0.3**	**0.6**	**1**	**2**	**4**
Teak sapwood	0.04	0.16	0.30	0.54	0.96	1.80
Teak heartwood	0.02	0.05	0.08	0.20	0.27	0.56
Redwood sapwood	**0.10**	**0.32**	**0.57**	**1.07**	**1.81**	**3.49**
Redwood heartwood	0.07	0.17	0.33	0.83	1.35	2.82
WRC sapwood	**0.09**	**0.29**	**0.45**	**0.97**	**1.64**	**3.26**
WRC heartwood	0.03	0.09	0.16	0.46	0.96	2.09

3.3.2. Sample Performance in the Ground Proximity Test

A cumulative odds model was used to predict the *odds of being at or above* a particular category. Because there were four different decay categories, 10, 7, 4, and 0, the model made three predictions, each corresponding to the accumulation of probabilities across successive categories. The predictions were: category 0 vs. all above, category 0 and 4 combined vs. all above, categories 0, 4, and 7 vs. category 10. The effect of preservative treatment for all wood types was not statistically significant across the three predictions, i.e., the proportional odds assumption was met (Table 3.4). This assumption implied that preservative treatment had the same effects on the odds regardless of the different distribution of the data. As a result, the coefficients describing the relationship between category 0 vs. all above are the same as those that describe the relationship between category 0 and 4 combined versus all above. This assumption was confirmed with a Wald χ^2 test. The practical implication of the proportional odds assumptions was that a common odds ratio (C_{OR}) could be used to summarize the effect of wood preservative on decay score, as opposed to using different models for different split of the data.

Table 3.4. Score test for the proportional odds assumptions of 36 month scoring data from samples of three wood species exposed to decay in Hawaii.

Wood Type	**Degrees of freedom**	**Wald χ^2**	**P> χ^2**
Redwood sapwood	12	14.26	0.28
Redwood heartwood	6	2.65	0.85
WRC sapwood	12	19.12	0.08
WRC heartwood	6	10.91	0.09
Teak sapwood	14	14.47	0.41
Teak heartwood	No calculated	No calculated	No calculated

It is important to note, that each of the seven wood types (Table 3.1) came from different boards at single locations. Thus statistical inferences can only be applied to the samples from that site or source. However, because we had three different naturally durable species, we believe that the conclusions drawn from these trials are indicative of

the ability of preservatives to prolong the service life of naturally durable second-growth timbers.

3.3.2.1. Western Redcedar

Table 3.5 displays the frequency (f), and cumulative proportion (cp) of each of the four decay categories for WRC samples. It also contains the cumulative odds (co) for each preservative treatment, the associated odds ratio OR (untreated:treated), the approximate common odds ratio (C_{OR}) for each treatment and 95% Wald confidence intervals (95% CI). *The C_{OR}'s are the most relevant values in the table.* For example, if the estimated C_{OR} comparing untreated-sapwood-samples with DCOI-(0.1 kg/m^3)-treated-samples was 13 (95%CI: 2, 69), this C_{OR} value indicated that the probability of decay occurrence for untreated samples was 13 times higher than the probability of decay occurrence in the treated samples. However, C_{OR}'s are only approximate values and must be used with caution. Data sets contained many cases in which none of the samples scored a particular rating category, and this meant that rating category had a frequency of zero. Zero was replaced by 0.005 to allow for a computational solution.

Yet if the value 0.005 is modified, C_{OR}'s will change accordingly, but they will always be greater than 1.0 if the probability of decay occurrence is greater than the probability of non-decay occurrence, 1.0 if the two outcomes are equally likely, and less than 1.0 if the probability of non-decay occurrence is greater than probability of decay occurrence.

Table 3.5. Frequency (f), cumulative proportion (cp), cumulative odds (co), odds ratio OR, common odds ratio and 95% confidence intervals (C_{OR}: (CI)) that quantify decay damage of western redcedar samples exposed in Hawaii.

Decay categories*		Sapwood					Heartwood			
		0	**4**	**7**	**10**	**Sum**	**4**	**7**	**10**	**Sum**
None	F	5	3	2	2	12	1	6	2	9
	Cp%	42	67	83	100		11	78		
	Co	**0.71**	**2.00**	**5.00**			**0.12**	**3.50**		
ACQ 4 kg/m^3	F	0.005	0.005	0.005	8	8	0.005	0.005	9	9
	Cp%	0	0	0	100		0	0		
	Co	**0.01**	**0.01**	**0.01**			**0.01**	**0.01**		
	OR**	∞***	∞	∞	**c_{OR}: N/A**		∞	∞	**c_{OR}: N/A**	
DCOI 0.1 kg/m^3	F	0.005	1	7	2	10	1	7	2	10
	Cp	0	10	8	100		10	80		
	Co	**0.01**	**0.13**	**4.80**			**0.10**	**3.60**		
	OR	∞	**15**	**1**	**$c_{OR:}$ 13 (2, 69)**		**1**	**1**	**$c_{OR:}$ 1 (0.2, 6)**	
DCOI 0.3 kg/m^3	F	0.005	2	4	6	12	3	5	2	10
	cp%	0	17	50	100		30	80	100	
	Co	**0.00**	**0.20**	**1.00**			**0.39**	**3.60**		
	OR	∞	**10**	**5**	**$c_{OR:}$ 27 (5, 146)**		**0**	**1**	**$c_{OR:}$ 0.4 (0.1, 3)**	
DCOI 0.6 kg/m^3	F	0.005	0.005	3	7	10	1	6	3	10
	Cp%	0	0	30	100		10	70	100	
	Co	**0.01**	**0.01**	**0.51**			**0.10**	**2.10**		
	OR	∞	∞	**10**	**$c_{OR:}$77 (11, 523)**		**1**	**2**	**$c_{OR:}$ 1 (0.2, 6)**	
DDAC 1 kg/m^3	F	0.005	8	6	0.005	14	3	6	1	10
	Cp%	0	57	100			30	90	100	
	Co	**0.00**	**1.14**	**2399**			**0.39**	**8**		
	OR	∞	**2**	**0**	**$c_{OR:}$ 3 (0.6, 12)**		**0**	**0**	**$c_{OR:}$ 0.3 (0.1, 2)**	
DDAC 2 kg/m^3	F	1	1	4	4	10	0.005	5	5	10
	Cp%	10	20	60	100		0	50	100	
	Co	**0.13**	**0.30**	**1.80**			**0.00**	**0.90**		
	OR	**5**	**7**	**3**	**$c_{OR:}$ 17 (3, 94)**		∞	**4**	**$c_{OR:}$ 3.3 (0.5, 20)**	
DDAC 4 kg/m^3	F	0.005	0.005	2	8	10	1	1	8	10
	Cp%	0	0	20	100		10	20	100	
	Co	**0.01**	**0.01**	**0.30**			**0.100**	**0.225**		
	OR	∞	∞	**17**	**$c_{OR:}$ 129 (16, ∞)**		**1**	**16**	**$c_{OR:}$ 11 (1, 80)**	

*Categories: sound sample (10), moderate decay (7), advanced decay (4), and broken sample (0).
**OR= (odds for non treated samples /odds for treated samples).
***When there were zero samples scoring for a given category, ORs tented to ∞. To allow computational solution, zero was replaced by 0.005.

C_{OR}'s showed that most of the biocide treatments reduced the risk of decay occurrence in WRC *sapwood* samples. Likewise, the percentage of samples with some degree of decay (i.e., number samples rated 0, 4, and 7 over the total number of samples) for each treatment indicated increased durability (Fig 3.3-A). Note that the lower 95%CI of DDAC at 1 kg/m^3 is below 1, indicating a probability of equal performance between this treatment and untreated samples.

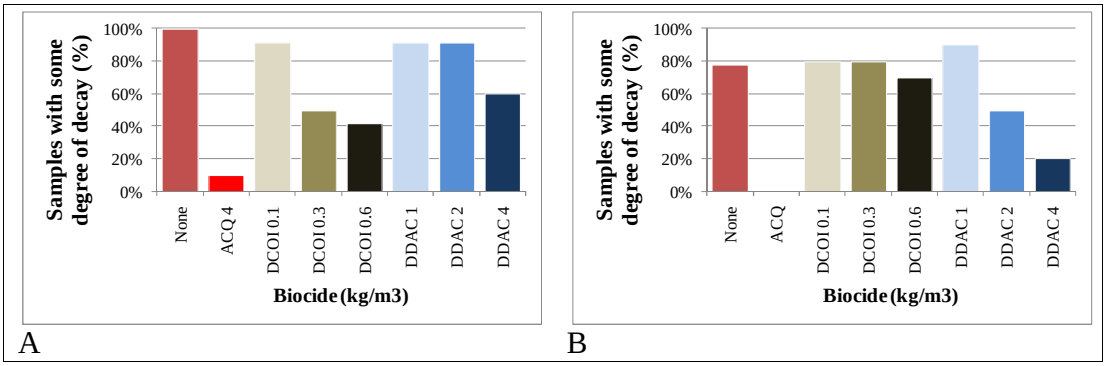


Figure 3.3. Percentage of western redcedar sapwood (A) and heartwood (B) samples treated to selected target retentions that exhibited some degree of decay after 36 months of exposure in a ground proximity test.

None of the treated or untreated heartwood samples scored 0, reflecting the high durability of WRC heartwood. Heartwood samples treated with DDAC at 4 kg/m^3 *significantly* increased decay resistance, although DDAC at 2 kg/m^3 also appeared to have superior performance (Fig 3.3-B). In contrast, DCOI treated samples had decay damage comparable with the untreated samples. Analysis of actual preservative retentions showed that DCOI retention was very low, i.e., most of the heartwood-WRC-DCOI-treated-samples retained less than 0.1 kg/m^3 of the biocide (see Section 3.4.1). Reported thresholds of efficacy in above ground applications for DCOI range from 0.3 to 0.5 kg/m^3 (see Chapter 2). Thus, actual biocide loadings of the DCOI tested samples were likely too low to provide protection against decay. Low biocide doses were applied to the samples intentionally, since it was expected that combinations of the fungicides with the wood extractives would act synergistically to enhance efficacy at lower concentrations.

Cumulative odds ratios C_{OR} of WRC sapwood samples (Table 3.5) indicated that treated samples had a slower deterioration rate than untreated samples and that increased biocide loading reduced decay rates. For example, the OR of being at or below category seven increased from 1 to 10 as retention increased from 0.1 kg/m^3 to 0.6 kg/m^3. A similar tendency was exhibited by DDAC treated samples. There were more untreated samples at category 0 than in any of the treatments, and therefore, the cumulative odds ratio was positive. Equally, there were more untreated samples at categories 0 and 4 together than in any of the treatments and also more untreated samples at categories 0, 4 and 7 together than treated samples (Fig 3.4),.

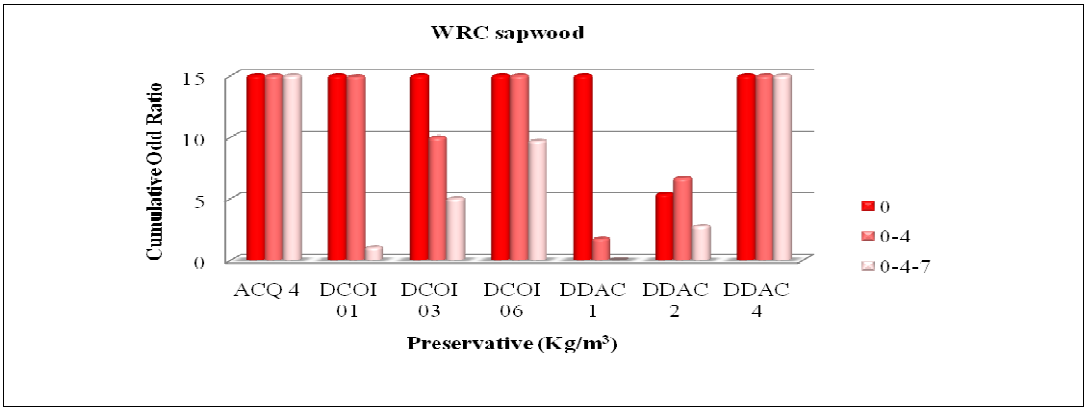


Figure 3.4. Cumulative odds ratios plotted against preservative treatment for western redcedar sapwood samples exposed in a ground proximity test in Hawaii. Note that the maximum cumulative OR is not 15, but a number above 15 and towards ∞.

Maximum likelihood estimates of the C_{OR}'s indicated that only DCOI at 0.6 kg/m^3 and DDAC at 4 kg/m^3 contributed significantly to improved *sapwood* decay resistance (Table 3.6). The difference was also significant for DDAC at 1 kg/m^3, but the estimate was below 1, suggesting that DDAC treatment at this low loading increased decay risk. Only DDAC at 4 kg/m^3 improved *heartwood* decay resistance significantly. The difference was also significant for DDAC at 1 kg/m^3, but the estimate was below 1. Because most of the samples treated with ACQ scored 10 with no variability (Table

3.5), we excluded this treatment from statistical analysis although it clearly improved decay resistance.

Table 3.6. Analysis of maximum likelihood estimates for the cumulative odds ratio of western redcedar samples developing decay when exposed for 36 months in Hawaii. SE = standard error. Values in bold are statistically significant.

Treatments		WRC sapwood				WRC heartwood			
		Estimate	SE	χ^2	Pr> χ^2	Estimate	SE	χ^2	Pr> χ^2
DCOI	0.1 kg/m^3	0.86	0.55	0.07	0.78	0.71	0.58	0.34	0.55
	0.3 kg/m^3	1.83	0.53	1.28	0.26	0.37	0.60	2.80	0.09
	0.6 kg/m^3	5.19	0.64	6.43	**0.01**	0.99	0.58	0.00	0.98
DDAC	1 kg/m^3	0.18	0.51	10.58	**0.00**	0.27	0.60	4.58	**0.03**
	2 kg/m^3	1.15	0.56	0.06	0.80	0.41	0.59	2.28	0.13
	4 kg/m^3	8.66	0.72	8.74	**0.00**	7.95	0.70	8.61	**0.00**

Maximum likelihood estimates of C_{OR}'s are different than the actual C_{OR}'s (Table 3.7). With the actual C_{OR}'s, it is assumed that if the C_{OR} and the lower 95% CI is above 1, then the treatment have superior performance. With the C_{OR}'s estimates, superior performance is assumed if the p-value is below 0.05. The computational reasons for obtaining different results in those estimates are explained by O'Connell (2006).

Table 3.7. Effect of preservative treatment on decay resistance of western redcedar samples exposed in Hawaii as shown by significant estimated and actual cumulative probabilities. Treatments with X were found to have superior performance.

Treatments		Sapwood		Heartwood	
		Estimated	Actual	Estimated	Actual
DCOI	0.1 kg/m^3		X		
	0.3 kg/m^3		X		
	0.6 kg/m^3	X	X		
DDAC	1 kg/m^3				
	2 kg/m^3		X		
	4 kg/m^3	X	X	X	X

It is important to compare results presented in Table 3.7 with Fig 3.3 and Table 3.5. The actual data indicated that most of the treatments increased decay resistance of the samples; however these effects were not significant. The lack of significance may be due to the small sample sizes and the high variability among replicates.

3.3.2.2. Redwood

None of the heartwood samples including the untreated ones scored 0 or 4, which reflects the excellent durability of this species (Table 3.8).

The percentage of samples with some degree of decay (i.e., number samples rated 0, 4, and 7 over the total number of samples) for each treatment showed little evidence of biocide dose response effects (Fig 3.5). This most likely reflects the overall low decay rate of this species. C_{OR}'s showed that, with exception of DDAC at 1 kg/m^3, preservative treatment reduced decay risk in sapwood samples (Table 3.8, Fig 3.5-A). However, the 95%CI for most treatments included values below 1. This indicates that there was a probability of equal performance between those treatments and untreated samples. The only treatment in which the lower 95%CI was above 1 was DDAC at 4 kg/m^3. None of the treatments appeared to produce superior performance on redwood heartwood samples. However, samples treated with DCOI at 0.1 kg/m^3, DDAC at 2 and 4 kg/m^3 did not develop any decay, which may be interpreted as superior performance even if statistical inference could not be calculated (Fig 3.5-B).

Table 3.8. Frequency (f), cumulative proportion (cp), cumulative odds (co), odds ratio OR, common odds ratio and 95% confidence intervals (C_{OR}: (CI)) to quantify decay damage of treated and untreated redwood samples exposed in Hawaii.

Decay categories*		Sapwood					Heartwood		
		0	**4**	**7**	**10**	**Sum**	**7**	**10**	**Sum**
None	F	1	3	4	1	9	3	5	8
	Cp%	11	44	89	100				
	Co	**0.125**	**0.800**	**8.000**			**0.600**		
ACQ 4 kg/m^3	F	0.005	0.005	0.005	8	8	0.005	7	7
	Cp%	0	0	0	100				
	Co	**0.001**	**0.001**	**0.002**			**0.001**		
	OR**	∞***	∞	∞	**c_{OR}: N/A**		∞	**N/A**	
DCOI 0.1 kg/m^3	F	1	2	3	4	9	0.005	10	10
	Cp%	10	30	60	100				
	Co	**0.125**	**0.385**	**1.350**			**0.001**		
	OR	**1**	**2**	**6**	**c_{OR}: 3 (0.5, 16)**		∞	**N/A**	
DCOI 0.3 kg/m^3	F	0.005	3	3	5	11	1	9	10
	Cp%	0	27	55	100				
	Co	**0.000**	**0.307**	**0.982**			**0.111**		
	OR	∞	**3**	**8**	**c_{OR}: 4 (0.7, 22)**		**5.4 (0.43, 67)**		
DCOI 0.6 kg/m^3	F	0.005	1	5	4	10	2	9	11
	Cp%	0	10	60	100				
	Co	**0.000**	**0.100**	**1.350**			**0.222**		
	OR	∞	**8**	**6**	**c_{OR}: 5 (0.8, 27)**		**2.7 (0.3, 22)**		
DDAC 1 kg/m^3	F	1	1	7	0.005	9	1	9	10
	Cp%	11	22	100	100				
	Co	**0.125**	**0.285**	**1799**			**0**		
	OR	**1**	**3**	**0**	**c_{OR}: 1 (0.2, 7)**		**5.4 (0.4, 67)**		
DDAC 2 kg/m^3	F	1	2	2	4	9	0.005	10	10
	Cp%	11	33	56	100				
	Co	**0.125**	**0.500**	**1.250**			**0.001**		
	OR	**1**	**2**	**6**	**c_{OR}: 3 (0.5, 18)**		∞	**N/A**	
DDAC 4 kg/m^3	F	0.005	0.005	6	4	10	0.005	9	9
	Cp%	0	0	60	10				
	Co	**0.000**	**0.001**	**1.351**			**0.001**		
	OR	∞	∞	**6**	**c_{OR} : 6 (1, 33)**		∞	**N/A**	

*Categories: sound sample (10), moderate decay (7), advanced decay (4), and broken sample (0).
**OR= (odds for non treated samples /odds for treated samples).
***When there were zero samples scoring for a given category, ORs tented to ∞. To allow computational solution, zero was replaced by 0.005.

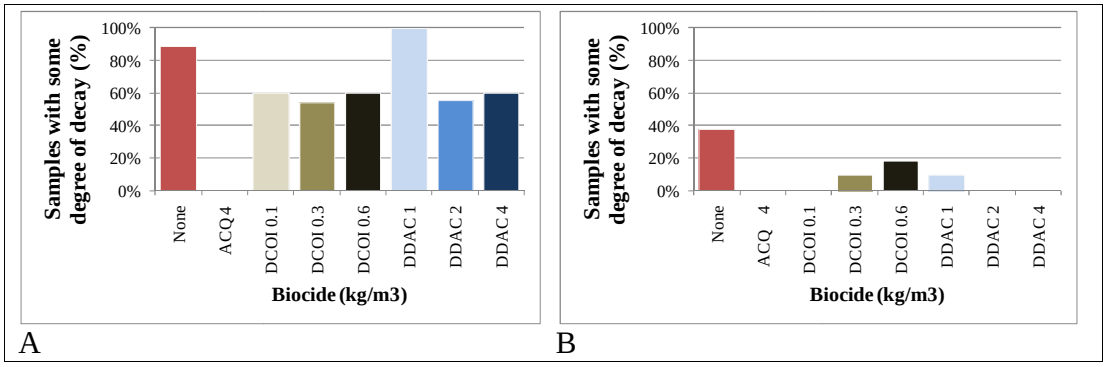


Figure 3.5. Percentage of redwood sapwood (A) and heartwood (B) samples treated to selected target retentions that exhibit some degree of decay after 36 months of exposure in a ground proximity test.

Analysis of actual retentions showed that redwood heartwood samples still retained significant levels of DDAC and DCOI after 24 months of field exposure and there was little evidence of leaching (see section 3.4.2). Likewise, it is important to note that most of the untreated samples were not decayed after 36 months of exposure. Thus, a longer time may be needed to evaluate the performance of redwood heartwood treatments.

Similarly to WRC, cumulative odds ratios of redwood sapwood samples (Table 3.8, Fig 3.6) indicated that increased biocide loading reduced decay rates of sapwood samples.

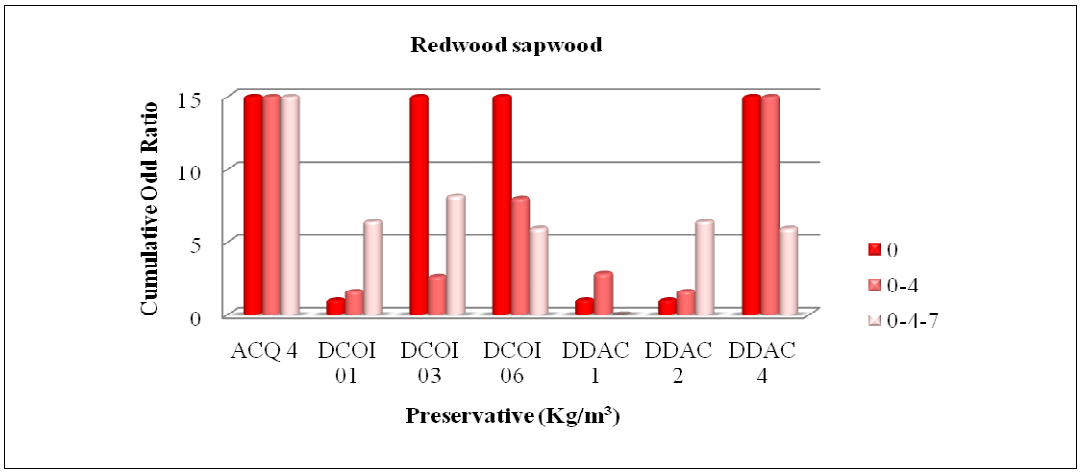


Figure 3.6. Cumulative odds ratios plotted against preservative treatment for redwood sapwood samples exposed in a ground proximity test in Hawaii. Note that the maximum cumulative OR is not 15, but a number above 15 and towards ∞.

Table 3.9. Analysis of maximum likelihood estimates for the cumulative odds ratio of redwood samples. SE = standard error. Values in bold are statistically significant.

Parameter		Redwood sapwood				Redwood heartwood			
		Estimate	SE	$\chi 2$	Pr> $\chi 2$	Estimate	SE	$\chi 2$	Pr> $\chi 2$
DCOI	0.1 kg/m^3	0.71	0.58	0.34	0.55	812.4	219.7	0.00	0.97
	0.3 kg/m^3	0.37	0.60	2.80	0.09	0.01	62.7	0.00	0.94
	0.6 kg/m^3	0.99	0.58	0.00	0.98	0.01	62.7	0.00	0.93
DDAC	1 kg/m^3	0.27	0.60	4.58	**0.03**	0.01	62.7	0.00	0.94
	2 kg/m^3	0.41	0.59	2.28	0.13	812.4	219.7	0.00	0.97
	4 kg/m^3	7.95	0.70	8.61	**0.00**	812.4	230.6	0.00	0.76

Table 3.10. Effect of preservative treatment on decay resistance of redwood samples exposed in Hawaii as shown by significant estimated and actual cumulative probabilities. Treatments with X were found to have superior performance.

Treatments		Sapwood		Heartwood	
		Estimated	Actual	Estimated	Actual
DCOI	0.1 kg/m^3				
	0.3 kg/m^3				
	0.6 kg/m^3				
DDAC	1 kg/m^3				
	2 kg/m^3				
	4 kg/m^3	X	X		

Estimated and actual cumulative probabilities indicated that DDAC at 4 kg/m^3 was the only treatment that *significantly* reduced the risk of redwood sapwood decay (Tables 3.9 and 3.10). However, all treatments, except redwood sapwood treated with DDAC at 1 kg/m^3, reduced decay rates. As discussed with WRC, the statistical results could be the result of the small sample sizes and the high variability of the response variable in each treatment combination.

3.3.2.3. Teak

All of the untreated heartwood samples scored 10, i.e., the samples were sound after 36 months of exposure (Table 3.11). Because treatment performance is evaluated in reference to the untreated samples, statistical analysis for teak heartwood samples

provided little insight to possible effects of treatments. Surprisingly, some treated samples experienced some degree of decay, suggesting that treatment increased decay risk. Analysis of actual retentions (see section 3.4.1) showed that teak heartwood samples had very low and poorly distributed retentions probably due to the low permeability of this species. Low biocide loading could increase wood hygroscopicity without producing a significant biocidal effect. It also suggests the possibility of hormesis effects: most fungicidal treatments produce positive biocidal effects at higher doses, but some produce beneficial biological effects at low concentrations (Calabrese, 1999). Further research would be required to test that possibility.

Both scientific and anecdotal evidence indicated reduced decay resistance for naturally durable second-growth timbers (Cabrera, 2008). However, natural durability is variable. For example, in a lab trial with decay fungi, second-growth teak from Panama showed mass losses between 32% and 43% while old-growth timber from Myanmar experienced only 2.3% to 12.3% mass losses (Leithoff *et al.*, 2001). It is likely that wood from different plantations in the same geographical region also has different decay resistance. Thus, it is possible that teak heartwood used for these trials was inheritably durable. Future trials should use wood from plantations reported to have lower durability, like the teak plantation in Panama and/or different sources of material to reduce the potential influence of variable durability.

Table 3.11. Frequency (f), cumulative proportion (cp), cumulative odds (co), odds ratio OR, common odds ratio and 95% confidence intervals (C_{OR}: (CI)) to quantify decay damage of treated and untreated teak samples exposed in Hawaii.

Decay categories*		Sapwood					Heartwood			
		0	**4**	**7**	**10**	**Sum**	**4**	**7**	**10**	**Sum**
None	F	5	3	1	0.005	9	0	0	8	8
	Cp%	56	89	100					100	
	Co	1.248	7.960	1800						
ACQ 4 kg/m^3	F	0.005	0.005	1	9	10	0.005	1	9	10
	Cp%	0	0	10	100		0	10	100	
	Co	0.000	0.001	0.101						
	OR**	∞	∞	∞	**N/A**					
DCOI 0.1 kg/m^3	F	6	3	2	1	12	3	2	1	6
	Cp%	50	75	92	100		50	83	100	
	Co	0.750	2.251	8.255						
	OR	2	4	3	**C_{OR}: 2 (0.2, 8)**					
DCOI 0.3 kg/m^3	F	2	2	1	5	10	2	1	5	8
	Cp%	20	40	50	100		25	38	100	
	Co	0.225	0.600	0.901						
	OR	6	13	∞	**C_{OR}: 13 (2, 75)**					
DCOI 0.6 kg/m^3	F	1	1	3	7	12	1	3	7	11
	Cp%	8	17	42	100		9	36	100	
	Co	0.068	0.150	0.536						
	OR	18	53	∞	**C_{OR}: 28 (4, 170)**					
DDAC 1 kg/m^3	F	4	2	5	1	12	2	5	1	8
	Cp%	33	50	92	100		25	88	100	
	Co	0.375	0.750	8.255						
	OR	3	11	0	**C_{OR}: 3 (0.6, 18)**					
DDAC 2 kg/m^3	F	1	3	7	1	12	3	7	1	11
	Cp%	8	33	92	100		27	91	100	
	Co	0.068	0.375	8.255						
	OR	18	21	0	**C_{OR}: 7 (2, 35)**					
DDAC 4 kg/m^3	F	1	3	2	4	10	3	2	4	9
	Cp%	10	40	60	100		33	56	100	
	Co	0.100	0.600	1.351						
	OR	12	13	0	**C_{OR}: 10 (2, 63)**					

*Categories: sound sample (10), moderate decay (7), advanced decay (4), and broken sample (0).
**OR= (odds for non treated samples /odds for treated samples).
***When there were zero samples scoring for a given category, ORs tented to ∞. To allow computational solution, zero was replaced by 0.005.**

The percentage of samples with some degree of decay (i.e., number samples scored with 0, 4, and 7 over the total number of samples) for each treatment showed that increased levels of biocide tended to improve performance for teak sapwood, but not heartwood (Fig 3.7). C_{OR}'s for sapwood samples showed that DCOI at 0.3 and 0.6 kg/m^3 and DDAC at 2 and 4 kg/m^3 reduced the decay risk in sapwood samples and had 95%CI above 1, which is consistent with the observations (Fig 3.7).

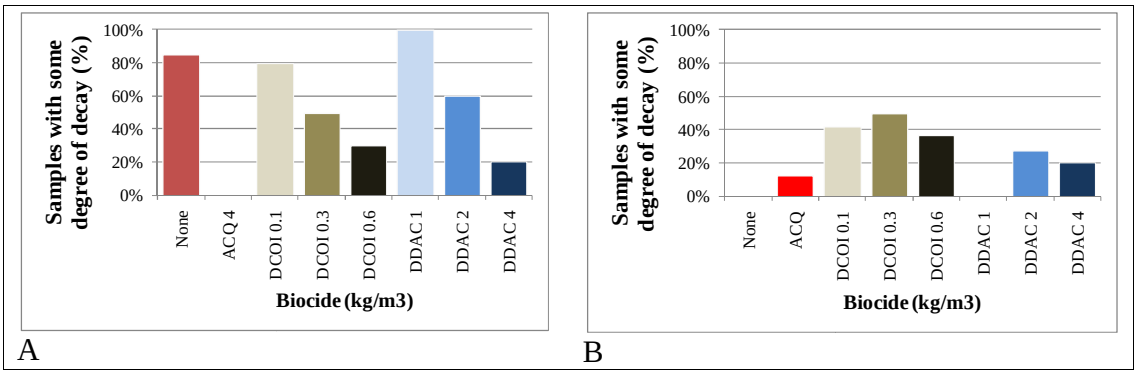


Figure 3.7. Percentage of teak sapwood (A) and heartwood (B) samples treated to selected target retentions that exhibit some degree of decay after 36 months of exposure in a ground proximity test.

As with WRC, cumulative odds ratios of teak sapwood samples (Table 3.11 and Fig 3.8) indicated that increased biocide loading reduced decay rate.

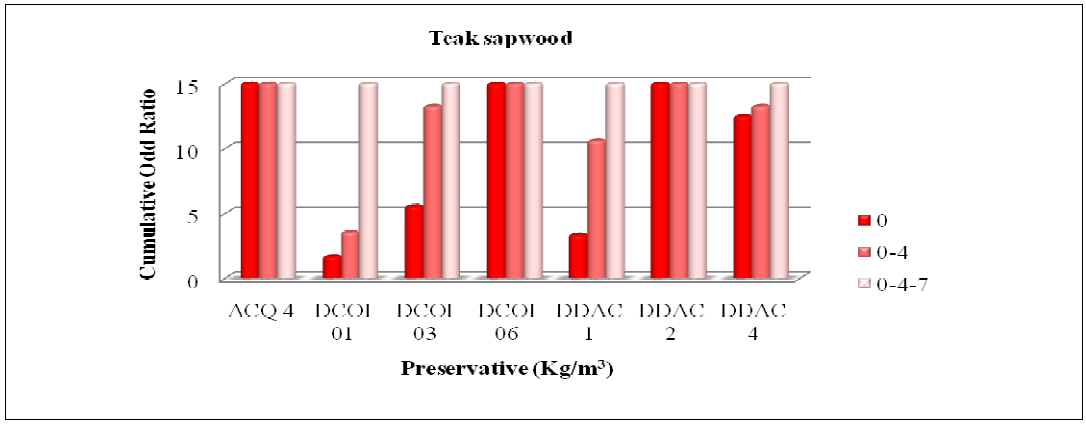


Figure 3.8. Cumulative odds ratios plotted against preservative treatment for teak sapwood samples exposed in a ground proximity test in Hawaii. Note that the maximum cumulative OR is not 15, but a number above 15 and towards ∞.

Maximum likelihood estimates of the C_{OR}'s and actual C_{OR}'s indicated that 0.6 kg/m^3 of DCOI was the only treatment that significantly improved sapwood decay resistance (Tables 3.12 and 3.13). The difference was also significant for DDAC at 1 kg/m^3, but the estimate was below 1, suggesting that DDAC treatment at this low biocide loading increased decay risk.

Table 3.12. Analysis of maximum likelihood estimates for the cumulative odds ratio of teak samples. SE = standard error. Values in bold are statistically significant.

		Teak sapwood			
Parameter		Estimate	SE	$\chi 2$	Pr> $\chi 2$
DCOI	0.1 kg/m^3	0.18	0.54	10.21	0.38
	0.3 kg/m^3	1.50	0.55	0.53	0.46
	0.6 kg/m^3	3.35	0.55	4.79	**0.03**
DDAC	1 kg/m^3	0.41	0.51	3.09	0.07
	2 kg/m^3	0.78	0.50	0.23	0.63
	4 kg/m^3	1.27	0.55	0.19	0.66

Table 3.13. Effect of preservative treatment on decay resistance of teak samples exposed in Hawaii as shown by significant estimated and actual cumulative probabilities. Treatments with X were found to have superior performance.

Treatments		**Sapwood**		**Heartwood**	
		Estimated	**Actual**	**Estimated**	**Actual**
DCOI	0.1 kg/m^3				
	0.3 kg/m^3		X		
	0.6 kg/m^3	X	X		
DDAC	1 kg/m^3				
	2 kg/m^3		X		
	4 kg/m^3		X		

3.3.3. Southern Pine (SYP)

The validity of the experiment must be viewed as a function of the decay rates exhibited in a decay susceptible control. Because SYP decay rates in tropical environments are well known (Cabrera and Preston, 2008), and because SYP is an easy-to-treat wood

species with low natural durability, its performance in the field is a good indicator of the effectiveness of the wood treatment process, the toxicity of the biocides tested, and the propensity to decay of samples exposed in the field. Decay scores for the SYP samples estimated for the 36 month period for different treatments are shown in Table 3.14.

Table 3.14. Average, standard deviation (SD), number of samples (N), and 95% confidence intervals of decay ratings of southern pine samples treated with different preservative systems and exposed for 36 months in Hawaii.

Chemical	**None**	**ACQ**	**DCOI**			**DDAC**		
Retention (kg/m^3)	**0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
Mean	0	10	4.9	6.2	6.3	5.0	5.1	6.2
SD	(0)	(0)	(3.2)	(3.1)	(1.7)	(3.2)	(3.1)	(1.4)
N	7	8	8	9	10	9	9	8
95% CI	N/A	N/A	2.2-7.5	3.8-8.6	5.1-7.5	2.5-7.5	2.7- 7.5	5.1-7.4

The results indicated that the conditions were suitable for aggressive fungal attack. Seven of eight untreated SYP samples had failed while the remaining sample had a score of seven. Eight ACQ treated samples all scored ten indicating that wood treatment was effective. As expected, all the DDAC and DCOI treated samples provided some protection, but the performance was variable. This indicated that the DDAC and DCOI biocide formulations employed on the trials were able to limit fungal attack (Table 3.14, Fig 3.9). Since toxicity thresholds for DDAC and DCIO are already known for SYP, and the loadings employed for this trial were below those limits, it is not of scientific value to compare the effect of these treatments in SYP, so no further statistical analysis was performed. Results from the SYP data validated the experiment since (1) conditions were conducive to decay, (2) the formulations employed had toxicity and, (3) wood treatment was effective.

3.3.4. Biocide Depletion Analysis

Analyzed preservative levels tended to be much lower than the target retentions for most wood-treatment combinations, reflecting the refractory nature of some wood species (Fig 3.9-3.11). The results must be viewed with some caution since they represent single analyses and since the analyses were performed on different blocks removed at each time point. Thus, the inherent variability associated with both wood and treatment could make it difficult to reach conclusions regarding overall trends.

Western Redcedar: DDAC retentions in sapwood and heartwood tended to decline over time, although there was some variability (Fig 3.9 A-B). Sapwood samples treated to 1 or 2 kg/m^3 experienced the sharpest declines in DDAC, while the levels in the 4 kg/m^3 treatment level declined more slowly.

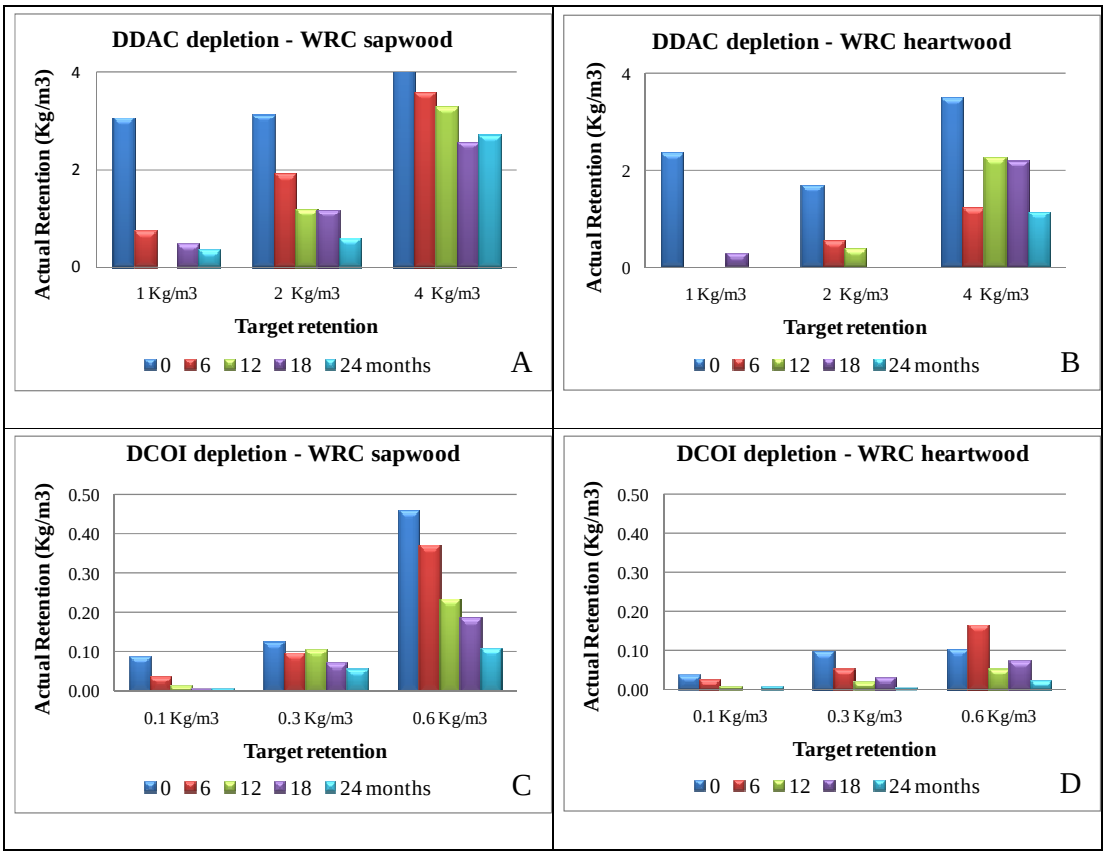


Figure 3.9. DDAC or DCOI retentions in western redcedar sapwood and heartwood samples exposed for 0 to 24 months in a ground proximity test in Hawaii.

The trend for the highest DDAC retention to experience less leaching than the lower retentions may indicate that high DDAC loadings may be needed to produce a strong interaction between DDAC and the wood. The high retention effect was also noticeable in the heartwood, but the reduction in DDAC loss rate was less distinguishable than in the sapwood. Sapwood retentions of DCOI (Fig 3.9 C-D) followed similar trends, but increasing retention did not appear to affect leaching loss. As with DDAC, DCOI losses from WRC heartwood were more variable, reflecting the poorer initial treatment results.

Redwood: DDAC retentions in sapwood and heartwood tended to decline over time, following the same tendencies as WRC (Fig 3.10 A-B).

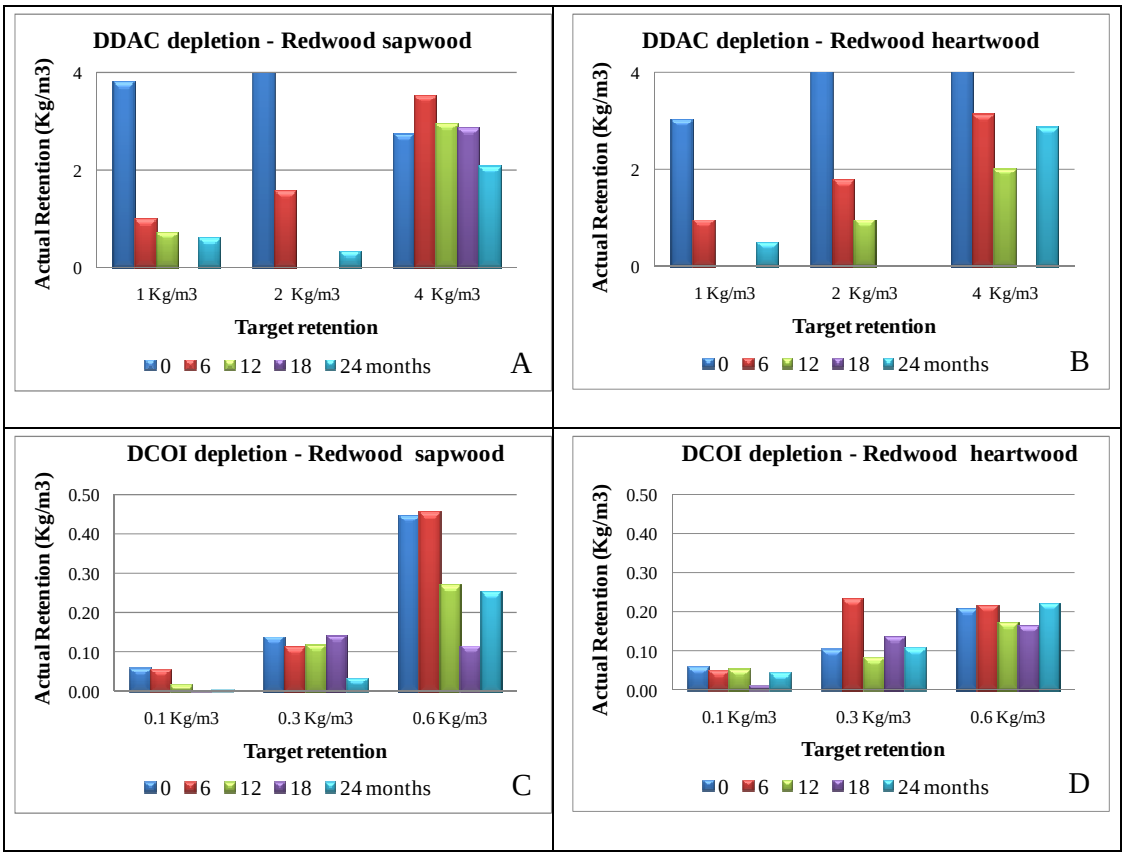


Figure 3.10. DDAC or DCOI retentions in redwood sapwood and heartwood samples exposed for 0 to 24 months in a ground proximity test in Hawaii.

DCOI retentions were all below the initial target retention (Fig 3-10 C-D), but they appeared to decline very little over the test period in either redwood sapwood or heartwood. DCOI should have relatively little interaction with the wood, while DDAC should be more strongly fixed to the wood. The lack of DCOI loss suggests that the redwood interacted to limit DCOI losses to a much greater extent than what was found with the WRC samples treated with the same chemical.

Teak: Initial DDAC retentions in sapwood were above the 1 and 2 kg/m^3 target levels and met the 4 kg/m^3 target. Retentions declined sharply over the 24 month exposure period for all three retentions (Fig 3.11 A-B).

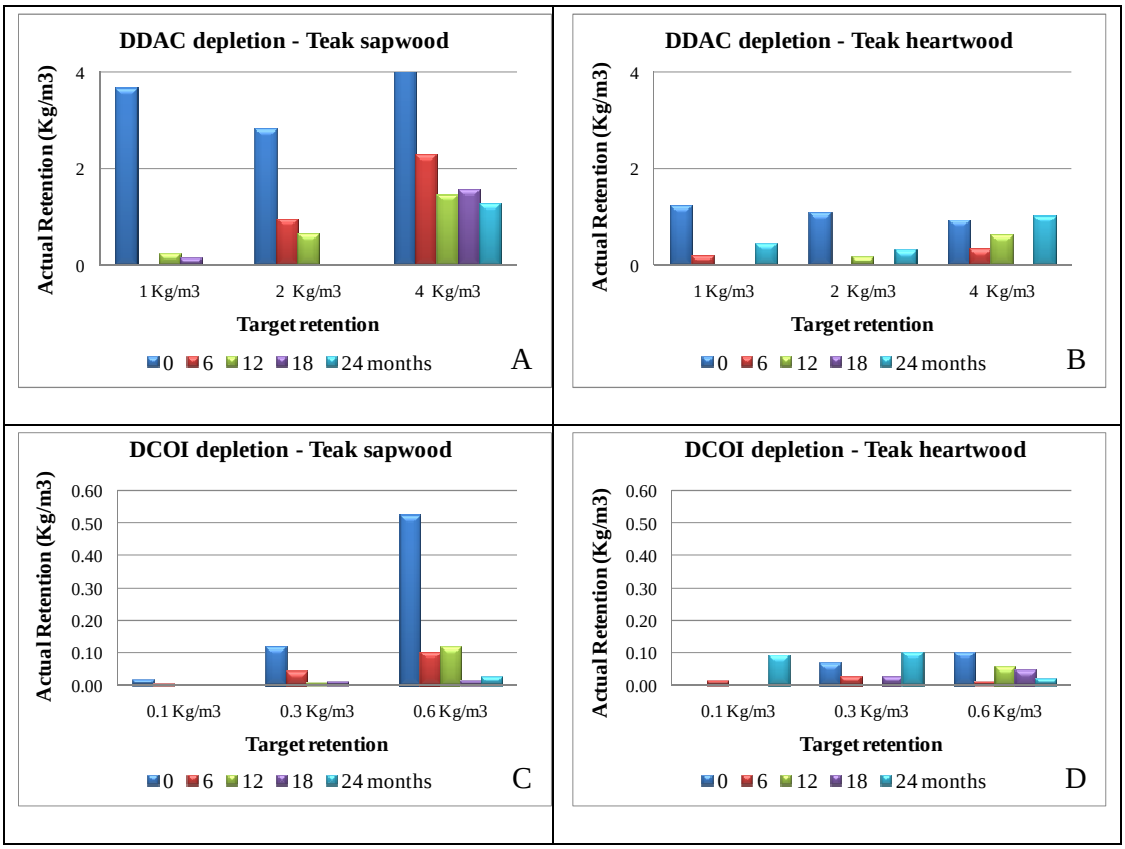


Figure 3.11. DDAC or DCOI retentions in teak sapwood and heartwood samples exposed for 0 to 24 months in a ground proximity test in Hawaii.

DDAC retentions in the heartwood were all well below the targets for the 2 and 4 kg/m^3 treatments. Retentions declined sharply in blocks treated to the 1 or 2 kg/m^3 targets, but tended to change little in the 4 kg/m^3 treatment. The loss rates for the teak sapwood at the higher retention differed from those found for redwood and WRC and may reflect subtle differences in wood-DDAC interactions that merit further study. DCOI retentions were also below the targets in both sapwood and heartwood samples (Fig 3.11 C-D). Retentions showed sharp declines in sapwood samples treated to the two higher retentions, while there was no discernable pattern in heartwood blocks treated with the same chemical.

The relationship between performance and residual chemical loadings is difficult to discern because of the overall low rate of decay in some samples, the poor treatment results experienced with some species, and some evidence that low levels of some treatments actually experienced more decay than untreated samples of the same species (Fig 3.3, 3.5, 3.7).

Given the potential for interactions between the heartwood and the chemical treatment, it is probably best to limit comparisons to the sapwood samples. In some cases, there appeared to be a relationship between reduced leaching and performance. For example, WRC sapwood treated to 4 kg/m^3 with DDAC performed very well and experienced little or no leaching, while samples treated to the two lower retentions experienced more leaching and more decay. However, DDAC treated redwood sapwood samples experienced similar low leaching losses at the highest retention, but the performance was similar to the lower retention that experienced much higher leaching losses.

One weakness of these comparisons is the limited replication as well as the low incidence of failures in any treatment group. Thus, reaching definitive conclusions may require further exposure to allow decay to become more visible among the various treatments.

3.3.5. Analysis of Heartwood Extractive Depletion

Heartwood extractives play a critical role in natural durability and long term exposure in the ground proximity test under the high rainfall conditions at the test site should result in substantial losses of these chemicals. One hope of preservative treatment is that the biocide might interact with the extractives to slow chemical losses over time, thereby prolonging the useful life of the product.

Western Redcedar: Pre- and post exposure extractive analysis of WRC heartwood samples with or without treatment showed that all of the treatment groups experienced extractives losses in the first six months of exposure, but there appeared to be little or no effect of supplemental preservative treatment on corresponding leaching loss (Table 3.15, Fig 3.12). Extractives levels at the time of installation did vary widely, between 7 to 13%, which is in agreement with previously reported values (6 to 10%) for ethanol-benzene extractive content of untreated second growth WRC (Nault, 1988). Our total extractive contents (weight/weight) probably included some of the preservative. However, this would account for no more than 0.9% of the total extractives level. Extractives losses stabilized after the first 6 months, suggesting that any water soluble, mobile extractive components were rapidly lost from the wood.

Table 3.15. Mean extractive content (%) and standard deviation of western redcedar heartwood samples treated with different preservative systems and exposed in a field test in Hawaii for 6-24 months.

Treatment	Exposure time (months)				
	0	6	12	18	24
ACQ	9.52 (0.8)	5.35 (1.3)	8.01 (0.5)	7.29 (0.2)	7.23 (1.2)
Untreated	7.60 (0.2)	5.80 (0.7)	7.77 (1.2)	6.37 (1.1)	6.77 (1.1)
DCOI 0.1 kg/m^3	15.40 (1.9)	6.08 (1.2)	7.86 (1.2)	7.56 (0.4)	8.01(0.7)
DCOI 0.3 kg/m^3	9.36 (0.7)	7.91 (3.9)	8.39 (2.1)	7.13 (1.2)	7.31 (0.9)
DCOI 0.6 kg/m^3	13.62 (0.4)	6.75 (1.3)	8.84 (0.5)	8.80 (1.3)	6.90 (0.8)
DDAC 1 kg/m^3	13.37 (0.2)	6.62 (1.3)	6.73 (N/A)	11.30 (3.8)	6.47 (0.9)
DDAC 2 kg/m^3	10.40 (1.0)	6.42 (1.3)	6.87 (1.5)	7.33 (1.4)	7.55 (1.3)
DDAC 4 kg/m^3	8.01 (0.4)	4.81 (1.2)	8.57 (1.1)	7.28 (1.5)	6.76 (1.4)

Redwood: There was little evidence that preservative treatments affected extractive losses. Water soluble extractives levels in heartwood samples were somewhat lower than those in WRC. However, post-exposure extractive analysis of redwood samples with or without treatment also showed that most of the treatment groups experienced extractives losses in the first 6 months of exposure. Water soluble extractive content on redwood varied between 8 to 12 % prior to exposure (Fig 3.13, Table 3.16). Extractives content in untreated redwood heartwood was reported to range from 15 to 30% of the wood (Anderson, 1961). Second growth redwood extractives represented 9 to 10% of the wood mass (Resch and Arganbright, 1968; Hillis, 1987).

Table 3.16. Mean extractive content (%) and standard deviation of redwood heartwood samples treated with different preservative systems and exposed in a field test in Hawaii for 6-24 months.

Treatment	Exposure time (months)				
	0	6	12	18	24
ACQ	10.73 (0.6)	6.01 (0.7)	6.94 (0.7)	8.10 (1.1)	7.27 (0.4)
Untreated	9.95 (0.2)	7.54 (0.1)	7.05 (0.8)	7.86 (0.3)	7.86 (0.5)
DCOI 0.1 kg/m^3	9.62 (0.0)	10.6 (2.1)	8.53 (2.6)	9.44 (1.0)	7.92 (0.9)
DCOI 0.3 kg/m^3	8.49 (0.4)	10.7 (3.8)	13.01 (8.2)	9.40 (1.5)	9.07 (1.6)
DCOI 0.6 kg/m^3	11.75 (0.8)	9.98 (1.4)	8.68 (1.7)	9.32 (2.0)	9.69 (0.1)
DDAC 1 kg/m^3	10.60 (0.1)	8.66 (1.4)	7.65 (1.3)	8.27 (0.2)	7.21 (1.2)
DDAC 2 kg/m^3	9.88 (0.4)	6.98 (0.2)	8.67 (0.4)	9.68 (1.2)	8.26 (1.2)
DDAC 4 kg/m^3	10.27 (0.4)	8.47 (3.4)	9.70 (1.1)	10.68 (0.8)	7.90 (1.4

Teak: There was no evidence that preservative treatment affected extractive losses. Total extractive content varied from 5 to 10% prior to sample exposure (Fig 3.14, Table 3.17). Total extractive content of the heartwood of a teak plantation in Panama was reported to be 9.4% (Haupt *et al.*, 2003). Post-exposure extractive analysis of teak samples with or without treatment also showed that most of the treatment groups experienced small extractives losses during exposure.

Table 3.17. Mean extractive content (%) and standard deviation of teak heartwood samples treated with different preservative systems and exposed in a field test in Hawaii for 6-24 months.

Treatment	Exposure time (months)				
	0	**6**	**12**	**18**	**24**
ACQ	10.05 (0.3)	6.27 (1.12)	6.07 (1.41)	5.65 (0.85)	5.19 (0.27)
Untreated	7.77 (0.02)	6.65 (1.22)	5.35 (0.33)	6.12 (0.75)	5.56 (0.78)
DCOI 0.1 kg/m^3	5.45 (0.17)	5.13 (0.72)	6.20 (1.70)	6.23 (N/A)	6.19 (1.03)
DCOI 0.3 kg/m^3	6.23 (0.05)	5.71 (0.37)	6.71 (1.18)	6.05 (0.29)	6.80 (1.90)
DCOI 0.6 kg/m^3	5.93(0.19)	7.05 (0.94)	5.96 (1.02)	11.11(6.52)	6.70 (1.72)
DDAC 1 kg/m^3	9.76 (0.30)	5.56 (1.09)	6.05 (0.68)	3.90 (0.20)	6.07 (1.49)
DDAC 2 kg/m^3	9.40 (0.14)	5.15 (0.42)	5.01 (0.04)	5.69 (2.68)	6.39 (1.10)
DDAC 4 kg/m^3	8.18 (0.20)	7.42 (0.96)	6.75 (1.25)	4.68 (0.53)	5.32 (1.02)

In general, extractives did not appear to interact with the supplemental preservatives in any of the species tested. Chemical systems may run the risk of actually reducing the inherent durability if they detoxify naturally toxic compounds in the impregnation process. Removal of extractives during the impregnation process may also result in solution stability problems as the extractives accumulate in the treating solution and begin to react with solution components to form sludges.

3.4. Conclusions

Treatments produced variable effects in durability. Sapwood durability of the three species was increased with DCOI at 0.6 kg/m^3 and/or DDAC at 4 kg/m^3. There were indications that the lower concentrations of DCOI (0.3 kg/m^3) or DDAC (2 kg/m^3) helped to improve sapwood durability. However, the observed improvements at lower treatment levels were not statistically significant, probably due to the low number of samples and the high variability among replicates. Increased biocide loadings reduced variability among replicates, and reduced the decay rate of most treatments except teak heartwood.

WRC heartwood durability was improved with 4 kg/m^3 DDAC. Likewise, observations suggested slight improvement in decay resistance for redwood heartwood samples

treated with DDAC or DCOI, however, longer exposure time will be required to test the effects of biocide treatment on this wood type. Although durability improvements in heartwood could be insignificant, increased sapwood durability would make it worth treating WRC and redwood lumber containing high proportions of sapwood.

Teak heartwood durability appeared to be reduced by biocide treatment. This reduction could be caused by disruption of the extractives distribution and/or composition during the treatment process or very low biocides uptakes. However, lumber containing high proportions of sapwood could benefit by treatment with DCOI at 0.6 kg/m^3 or DDAC at 4 kg/m^3.

The field data are still too limited to make definitive conclusions about the relationship between preservative leaching losses and performance. However, DDAC appeared to be more resistant to leaching at the higher loading retained than a lower doses. While extractives content declined in heartwood samples both as a result of initial treatment and over time in the field, there did not appear to be any relationship between specific treatments and loss rate. This suggests that care must be taken to limit the potential for extractive loss in treatment, but there appeared to be little interaction between the chemicals naturally present in the heartwood and those that are artificially delivered into the wood.

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CHAPTER 4- FUNGAL DIVERSITY IN DECAYING SAMPLES OF TEAK, WESTERN REDCEDAR, REDWOOD, AND SOUTHERN PINE

ABSTRACT

Fungal diversity and community development play important roles in wood decay, but little is known about how these factors vary with wood species or how they are affected by biocide treatments. The goal of this study was to examine fungal diversity in teak, western redcedar, redwood, and southern pine at various stages of decay. The study included both sapwood and heartwood samples which were treated with DCOI (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), DDAC (didecyldimethylammonium chloride), ACQ (alkaline copper quaternary), or left untreated. Samples were exposed in an above ground field test in Hawaii for 24 months. Fungi were isolated from the wood samples at six month intervals and identified by coupling microscopic techniques with the comparisons of the internal transcribed spacer (ITS) regions of the fungal rDNA with sequences available on the National Center for Bioscience Informatics (NCBI) website. More than 2100 isolates were obtained, representing 241 unique ITS sequences that were grouped into 81 morphotaxa. The most represented groups were: 63 ascomycetes (15 discoloration and/or stain fungi, 26 soft rot fungi, 2 stain fungi, and 21 species with unknown capabilities), 16 basidiomycetes (11 white rot fungi and 5 species with unknown capabilities), and 3 zygomycetes. Most morphotaxa were either rare or low in abundance. Ascomycetes and basidiomycetes appeared over time in all samples regardless of wood treatment and wood type.

Keywords: Fungal diversity, Hawaii, ground proximity test, DCOI, DDAC, ACQ, teak, western redcedar, redwood, wood decay, fungal community.

4.1. Introduction

Freshly cut lumber is a rich source of nutrients for microorganisms with wood decay capabilities, including many fungi. Fungi can degrade wood by the use of oxidative or

hydrolytic enzymes coupled with other fungal metabolic products (Zabel and Morrell, 1992). Variations in the ability of different decay fungi to express degrading enzymes and the observed change of fungal assemblages during wood decay suggest that the diversity of the fungal communities may be important for wood decay. Surprisingly little is known about how this diversity affects the decay process. Wood is usually protected from decay by the application of broad spectrum biocides. Understanding fungal diversity and community development in wood during decay could be used to develop biocides with more specific toxicity (Kang *et al.*, 2009).

Determining the succession of microbial communities attacking wood requires the identification of clusters of indicator species active during different stages of decay. In general, studies of fungal succession have established similar patterns with time, i.e. early, intermediate, or late colonizers (Jones and Hyde, 2002). Wood microbial colonization is generally considered to progress from bacteria to ascomycetes to the principal decomposers, primarily basidiomycetes (Rayner and Boddy, 1988). Bacteria and some ascomycetes are generally more tolerant to biocides than basidiomycetes (Nicholas and Crawford, 2003). While some fungi may be responsible for decay, many non-decay fungi may also be present in the wood. Some of these fungi can consume non-structural wood components, while others can consume decay fungi or their metabolic products (Rayner and Boddy, 1988). Soft rot is considered to be the first decay type found in a general sequence of wood colonization (Raberg *et al.*, 2009).

Although a large number of wood inhabiting organisms have been identified, and the wood degrading capabilities of many organisms have been investigated, many questions remain concerning the decay process (Raberg *et al.*, 2009). For example, microbial communities that affect decomposition are continuously changing over time (Rayner and Boddy, 1988). There is little data on what triggers this turnover and its rate. There are also questions about whether early colonizers condition wood for attack of decay fungi and if this shift can be detectable in the succession process. If so, inhibiting early colonizers could postpone the initiation of decay, limiting the need for broad spectrum

biocides. There are only a limited number of systematic studies of decaying wood that reveal the range of organisms present in microbial communities. Problems in studying fungal succession were reviewed by Jones and Hide (2002). They noted that microbial communities contained many organisms that cannot be identified because techniques do not exist that can recognize them (e.g., non-culturable species, dormant, or senescing species). It is also very difficult to determine the contribution of a single species to the fungal population in wood decay, and its interaction with other species. A growing understanding of microbial biochemistry and genetics is providing higher resolution methods to study microbial communities, but these techniques are only just beginning to be used to study wood decay.

The main objectives of this research were to identify subsets of fungi inhabiting teak, redwood, western redcedar, and southern pine exposed in a ground proximity field test in Hilo, Hawaii for 6, 12, 18, or 24 months, and to gain a better understanding of fungal abundance and capabilities. The study included both sapwood and heartwood samples treated with DCOI (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), DDAC (didecyldimethylammonium chloride), ACQ (alkaline copper quaternary), or left untreated (see Chapter 3).

The Study Area: The Hawaiian field test site has an average annual temperature and precipitation of approximately 23°C and 3300 mm, respectively. Winter months tend to be wetter than summer months. Soil formation processes has been influenced by volcanic activity. The surrounding areas contain different crops and natural forests that may increase microbial diversity (Fig 4.1).

Freitag *et al,* (1995) sampled wood inhabiting fungi in treated and untreated ponderosa pine and southern pine L-joints exposed for 3 to 18 months in this field test site. Some fungi were specific for treatments, e.g., *Antrodia sinuosa* was exclusively isolated from samples treated with a triazole biocide. However, no distinction could be made between fungal communities in treated and untreated wood. The study suggested that seasonal

variations may affect isolation patterns. A nine month dry period during this test may have inhibited basidiomycete colonization. Another study at the same site examined wood inhabiting fungi and bacteria growing on treated and untreated southern pine L-joints exposed for 12 to 24 months. It was found that bacteria, yeasts, and molds were the major groups after 24 months of exposure (Molnar *et al.*, 1997). Only weak differences in fungal communities were found between treated and untreated samples.

A.

B.

C.

Figure 4.1. Google Earth images of the field test site. **A**. Hawaii. **B**. Field test site near Hilo. Note the volcano. The field sites are in an area called a kīpuka. This is where the lava flow separates and provides an area where soil develops over time. **C**. Close view of the field test. Note the agricultural crops and natural forest around.

4.2. Material and Methods

Wood Treatment and Exposure: Teak, redwood, western redcedar (WRC), or southern pine lumber was cut into 19 by 50 by 125 mm long samples and conditioned at 23ºC and 70% relative humidity. Preservative treatments were assigned to groups using a list of random numbers. The wood was vacuum-pressure treated at Viance LLC (Charlotte, NC) with solutions of DCOI to target retentions of 0.1, 0.3, 0.6 kg/m^3, DDAC to retentions of 1, 2, or 4 kg/m^3, or ACQ to retention of 4 kg/m^3. Samples from each wood type were left untreated. The wood samples were exposed according to AWPA Standard E18 (AWPA, 2006). Sixteen arrays of concrete blocks were placed in the open at the Hawaiian field test site. Samples were placed randomly on the arrays.

Every six months, three pre-identified samples from each wood-treatment combination were collected from the field and returned to Oregon State University (Corvallis, OR).

Fungal Culturing and Identification: Upon arrival at Oregon State University, the samples were surface sterilized with a dilute bleach solution (5% sodium hypochlorite) and then sawn to obtain sixteen 10-mm-cubes per sample. The cubes were flame-sterilized and placed into petri-dishes with 1.5% malt extract agar (MEA). Any fungi growing from the wood were isolated on fresh MEA plates until pure cultures were obtained. Pure cultures were grouped into *morphotaxa* based on macroscopic and microscopic characteristics. Representatives of each morphotaxon were grown in potato dextrose broth until a sufficient amount of tissue was available for DNA extraction.

DNA from 885 cultures was extracted and purified according to previously published methods (Pomraning *et al.*, 2009), a DNeasy Plant Mini Kit (Qiagen Sciences, Valencia, CA, USA), or a DNA extraction kit (Epoch Biolabs, Inc., Sugar Land, TX, USA). The ITS region of the DNA was amplified using the polymerase chain reaction (PCR) with the ITS1-F (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) sequences as a primers (White *et al*, 1990). The resulting amplified DNA was further purified by agarose gel electrophoresis (12 g agarose and 1 mg ethidium bromide/L in TAE buffer). The bands were excised and the DNA extracted with a QIAquick gel extraction kit (Qiagen Inc., Valencia, California). The DNA was sequenced at the Center for Genome Research and Biocomputing at Oregon State University using the ITS1F primer. Sequences were compared with sequences available on the National Center of Bioscience Informatics (NCBI) website using the BLAST tool.

The retrieved sequences were aligned with the CLUSTALW default alignment followed by maximum parsimony methods of phylogenetic inference using the software MEGA 4.0.(Tamura *et al*., 2007). Morphotaxa with rDNA sequences with more than 95% sequence similarity were lumped together, since experimental evidence indicated that

90-99% sequence similarity cutoffs have been used for the ITS rDNA region to define molecular operational taxonomic units (O'Brien et al., 2005). Wood decay capabilities of the taxa were classified as discoloration/stain fungi, stain fungi, stain/soft rot fungi, white rot fungi, or uncertain on the basis of previous reports. In some cases, it was necessary to assign a capability to a given isolate based upon the capability of other species in that genus. Further studies will be required to analyze the decay capability of these fungi.

4.3. Results

A total of 2161 fungi were isolated from the various wood-treatment combinations, and 241 unique ITS sequences were recovered which were assigned to 81 unique morphotaxa (Table 4.1). Note that caution must be used in assigning a particular species name using sequence matches from BLAST searches because there are sequences in the NCBI database are not correctly identified. To address this issue, the genera of some sequenced cultures were microscopically confirmed by Dr. Jeff Stone at Oregon State University. Not all cultures could be confirmed either because they did not sporulate in culture or because of time limitations. A database with confirmed fungal genera, their ITS-sequence, photos of the cultures and microscopic photos of the reexamined fungi is under construction.

The isolates had the following characteristics:

- There were 62 ascomycetes, 16 basidiomycetes, and 3 zygomycetes.
- 52 morphotaxa (66%) were isolated between 1 and 10 times, 12% of the morphotaxa were isolated between 11-20 times, and 9% between 21-40 times.
- Five morphotaxa were isolated between 50 and 85 times: *Phoma, Nigrospora, Cochliobolus-Curvularia, Exophiala-Rhinocladiella, Arthrographis-Cosmospora*. Members of these genera can produce soft rot decay.

- *Pilidiella,* a genus that includes plant pathogens (Van Niekerk *et al* 2004), was isolated 152 times. Microscopic identification revealed that isolates named *Pilidiella* can also belong to the genus *Harknessia.* Distinction between the two genera can be made by growing the cultures in potato dextrose agar instead of MEA.
- *Fusarium* (411 times), *Paecilomyces* (278 times), and *Trichoderma* (280 times) were the dominant taxa. These three genera are common mold fungi and are not associated with substantial wood decay. Their presence may be related to the proximity of the test configuration to the soil. Some species in these genera can produce antibiotic compounds, while other species are preservative tolerant and may condition the wood for more substantial wood decay.

Table 4.1. Fungal morphotaxa and number of isolates identified from untreated or treated wood samples exposed in a ground proximity test in Hawaii for 6 to 24 months. Taxa are classified according to their possible role in the wood decay process.

Morphotaxa	**Months**				**Literature source for possible wood decay type**
	6	**12**	**18**	**24**	
	Number of isolates				
Fungicolous					
Microsphaeropsis arundinis	1			1	(Hawksworth, 1981)
Mariannaea camptospora		2			(Hawksworth, 1981)
Discoloration and/or stain fungi					
*Fusarium***	186	88	67	70	(Molnar et al., 1997)
*Paecilomyces***	117	65	47	58	(Molnar et al., 1997)
Zygomycete	6	2	12	10	
Cladophialophora	2	2	11	12	(Isenberg, 1992)
*Penicillium***	8	3	1	5	(Molnar et al., 1997)
Leptosphaeria	4			2	(Radford *et al.*, 1997)
*Ochroconis***	2	3		1	(Radford *et al.*, 1997)
*Leptosphaerulina***	3	1			(Hugues and Nicole, 1994)
Stemphylium	1		1	1	(Isenberg, 1992)
*Cladosporium tenuissimum***		2			(Molnar et al., 1997)
Periconia macrospinosa	1				(Kuhn and Ghannoum, 2003)
Lecanicillium lecanii	1				(Zabielska-Matejuk and Czaczyk, 2006)
Saccharicola bicolor		1			(Radford *et al.*, 1997)
Mold/ Soft rot					
Trichoderma	95	31	75	79	(Osono and Takeda, 2002)
*Kabatiella***	4	1		3	(Nilsson, 1973)
*Bionectria ochroleuca***		1			(Nilsson, 1973)
Soft rot					
*Arthrographis-Cosmospora***	3	21	51	56	(Molnar et al., 1997)
*Exophiala - Rhinocladiella***	25	25	14	21	(Wang and Zabel, 1990)
*Cochliobolus-Curvularia***	44	18	6	11	(Mandels and Reese, 1965)
*Nigrospora***	9	15	23	15	(Osono and Takeda, 2002)
*Phoma***	20	17	11	5	(Rayner and Boddy, 1988)
*Pestalotiopsis vismiae***	8	11	12	14	(Osono and Takeda, 2002)
*Phaeoacremonium***	2	7	1	8	(Aroca *et al.*, 2008)
Phialemonium	1	7		6	(Worrall *et al.*, 1997)

**Morphotaxa that contain isolates from which microscopic identification was performed.

Table 4.1. Fungal morphotaxa and number of isolates identified from untreated or treated wood samples exposed in a ground proximity test in Hawaii for 6 to 24 months. Taxa are classified according to their possible role in the wood decay process. **(Continued).**

Morphotaxa	**Months**				**Literature source for possible wood decay type**
	6	**12**	**18**	**24**	
	Number of isolates				
*Chaetomella***	1		3	7	(Eriksson *et al.*, 1990)
*Colletotrichum-Glomerella***	3	2	1	2	(Acosta-Rodriguez *et al.*, 2005)
Arthrinium	7				(Wang and Zabel, 1990)
*Chaetomium-Trichocladium***	5	2			(Nilsson, 1973)
*Phialophora***	4			1	(Eriksson *et al.*, 1990)
*Acremonium***	2	2			(Nilsson, 1973)
Phaeosphaeria sp.			1	3	(Bucher *et al.*, 2004b)
Aspergillus	3				(Nilsson, 1973)
*Lecythophora***	1	2			(Bugos *et al.*, 1988)
*Bispora***	2				(Wang and Zabel, 1990)
Aporospora terricola		2			(Wang and Zabel, 1990)
Alternaria	1				(Molnar et al., 1997)
Diaporthe		1			(Bucher et al., 2004a)
Cylindrocarpon olidum			1		(Bucher *et al.*, 2004a)
Stain					
Botryosphaeria - Lasiodiplodia	7	18	25	28	(Rayner and Boddy, 1988)
*Aureobasidium pullulans***	26	3		1	(Molnar *et al.*, 1997)
Uncertain					
*Pilidiella** /Harknessia sp**.*	4	61	40	47	
Montagnulaceae	2	8	16	7	
Hypocreales sp.	5	7	8	11	
Uncultured Ascomycete	2	12	2	8	
Simplicillium lamellicola		1	1	14	
Glionectria tenuis	1	1	5	8	
Valsa myrtagena	2	2	3	6	
Fungal sp		4		3	
Xylaria sp.	1	1	1	3	
Uncultured Xylariales clone		2			
Fungal sp.		4		3	
Xylaria sp.	1	1	1	3	

**Morphotaxa that contain isolates from which microscopic identification was performed.

Table 4.1. Fungal morphotaxa and number of isolates identified from untreated or treated wood samples exposed in a ground proximity test in Hawaii for 6 to 24 months. Taxa are classified according to their possible role in the wood decay process **(Continued).**

Morphotaxa	**Months**				**Literature source for possible wood decay type**
	6	**12**	**18**	**24**	
	Number of isolates				
Basidiomycete sp.		1	3	1	
Atractiellales sp			1	3	
Pleurostomophora		3	1		
Sordariales sp.		3			
Codinaeopsis sp.		2		1	
Basidiomycete sp	2				
Fungal sp.	2				
Foliar endophyte	1			1	
Truncatella angustata		2			
Discostroma tricellulare		2			
Carpoligna pleurothecii	1	1			
Uncultured fungus				1	
Uncultured basidiomycete		1			
Leaf litter ascomycete		1			
White rot					
Resinicium friabile			2	31	(Nakasone, 2007)
Phanerochaete	7	1	4		(Rayner and Boddy, 1988)
Sphaerobolus sp	2	3	2	3	(Tanesaka *et al.*, 1993)
Polyporus arcularius	1	1	3	2	(Tanesaka *et al.*, 1993)
Coriolopsis caperata	4	1		1	(Bergemann *et al.*, 2009)
Phlebia	1		1	2	(Rayner and Boddy, 1988)
Trametes versicolor	1	2	1		(Rayner and Boddy, 1988)
Coprinellus disseminatus	3				(Oliver, 2006)
Schizophyllum commune	1				(Tanesaka *et al.*, 1993)
Stereum hirsutum			1		(Rayner and Boddy, 1988)
Oxyporus latemarginatus	1				(Fackler *et al.*, 2006)

**Morphotaxa that contain isolates from which microscopic identification was performed.

Eleven white rot decay fungi were isolated at different time points. The most commonly isolated basidiomycete was *Resinicium friabile* which was the predominant white rot fungus in the samples exposed for 24 months. Contaminating molds, especially

Trichoderma species, made decay fungi isolations very difficult. *Resinicium friabile* appeared to be better able to compete with molds than other decay fungi. One issue with all isolation studies is determining how isolation conditions (e.g., media, temperature) affect the ability to isolate a given fungus. Thus, isolation of a specific fungus does not always mean that this species is dominant or even active in the actual wood. The BLAST identification of *Resinicium friabile* was not confirmed microscopically. No brown rot decay fungi were isolated, even though brown rot decay was observed in some wood samples, possibly because of the rapid growth of molds.

Whittaker's beta diversity estimates indicated that diversity was high at each time point sampled (Table 4.2). Fungal diversity was similar after 6 and 12 months of exposure. Fungal diversity after 18 or 24 months of exposure was similar, but lower than the diversity at the previous time points. This suggests that early colonizers, which are typically rapidly growing species that utilize readily accessible nutrients, had begun to decline. However, Shannon`s diversity indices at each time point indicated that the diversity remain essentially unchanged, with an small increase at the 24 month period, where the number of unique species was lower but species evenness was most constant.

Table 4.2. Number of fungal isolations, species richness, Whittaker's β-diversity (βd), and Shannon`s diversity indices from fungal isolates collected from decaying wood exposed in a ground proximity test in Hawaii for 6 to 24 months. βd is a measure of the heterogeneity in species composition of the data set (βd = (whole sample diversity) / (average diversity in wood blocks of a specific group)). As a rule: βd <1 is low; 1< βd < 5 is medium; βd > 5 is high (McCune and Grace, 2002).

	6 months	**12 months**	**18 months**	**24 months**
Number of isolates	649	478	459	574
Species richness	54	53	38	45
Bd	17.2	18.3	13.0	13.6
Shannon`s diversity index	1.27	1.34	1.32	1.61

Two thirds of morphotaxa that were classified microscopically corresponded to a single ITS genotype (Fig 4.2). However, one third of the morphotaxa contained multiple ITS genotypes, reflecting the phenotypic plasticity of the fungi or the fact that different

species may have the same ITS sequence (Arnold *et al.*, 2007). Inconsistent taxonomic resolution is possible (i.e, that the 241 ITS genotypes correspond to more than 81 morphotaxa) and could lead to underestimation of species richness.

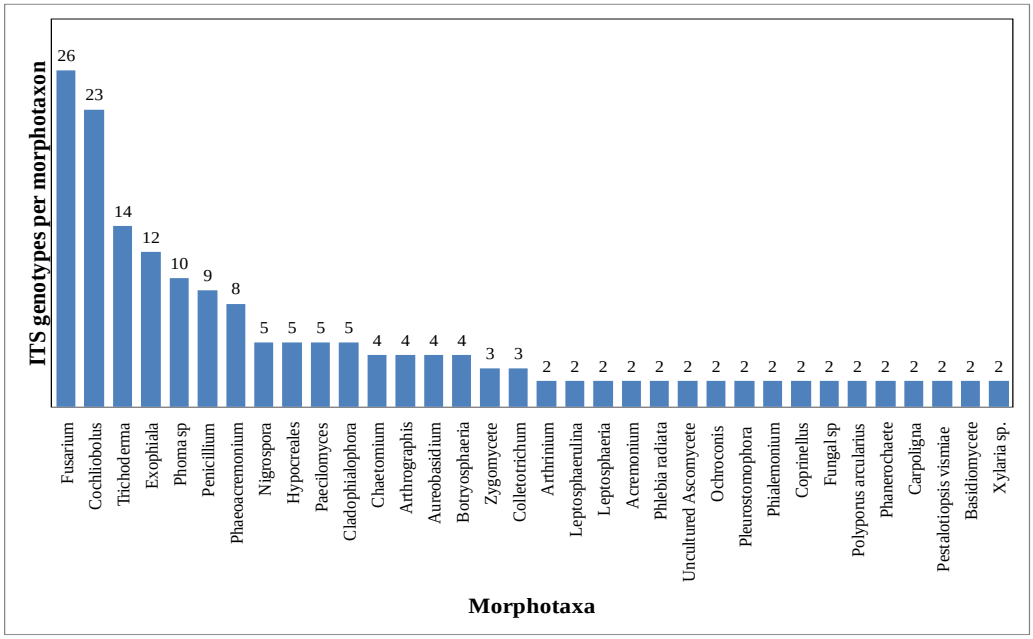
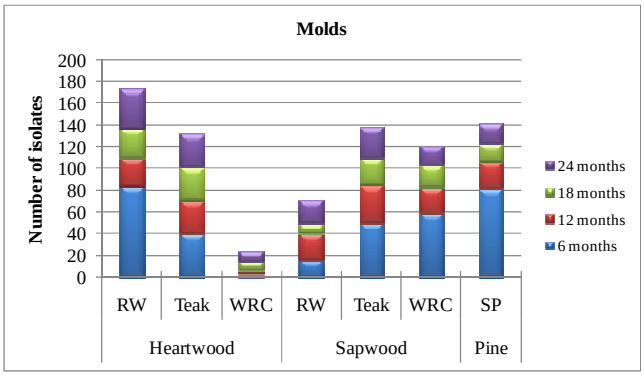


Figure 4.2. Correspondence between fungal morphotaxa and ITS genotypes based on 95% ITS sequence similarity. Bars represent the frequency distribution indicating the occurrence of multiple ITS genotypes within morphotaxa.

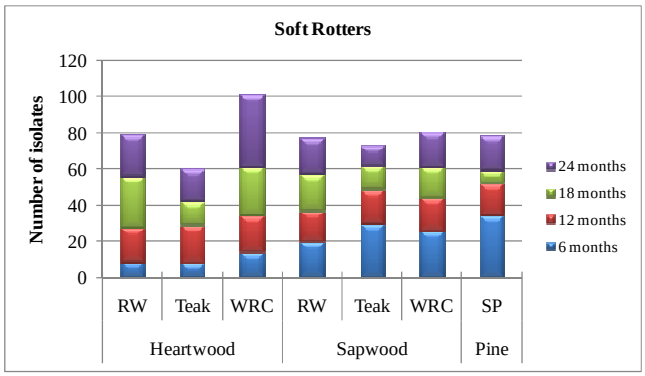
Fungal Decay Capabilities and Wood Species: The species identified in this study were limited to those growing on MEA and it is a given that the fungi observed were not the only species active in fungal succession. However, some familiar patterns were recognized (Fig 4.3, Appendix 3). For example, molds were widely isolated in all wood types and at time points except in WRC (Fig 4.3 -A). Compounds from WRC tended to diffuse into the MEA and appeared to inhibit mold growth.

More soft rot decay fungi were isolated from sapwood samples than from heartwood samples after 6 months of exposure (Fig 4.3-B). Sapwood should be much more susceptible to soft rot attack than heartwood. However, soft rot isolations per wood type

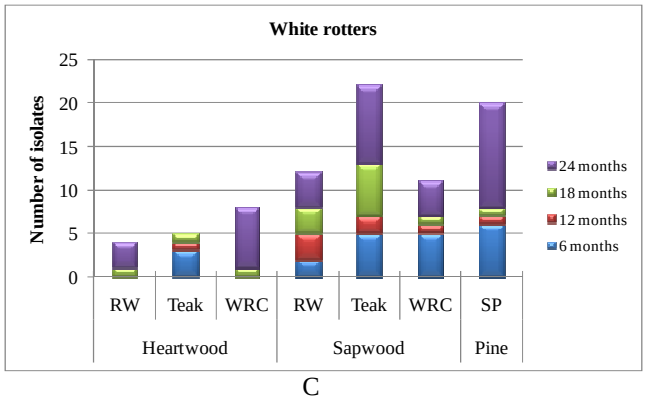
were almost equal for both wood types after the initial sampling. White rot decay fungi were more abundant in sapwood samples than in heartwood samples.



A



B



C

Figure 4.3. Fungal frequency from treated and untreated teak, redwood (RW), western redcedar (WRC) or southern pine (SP) samples exposed in a field test in Hawaii for 6 to 24 months, classified according to three decay damage capabilities: A) molds, B) soft rot decay fungi, and C) white rot decay fungi.

It is important to note that species diversity is not the same as functional diversity. A fungal community with high functional overlap among species has a lower functional diversity than a fungal community with low functional overlap (Ricotta and Burrascano, 2009). For example, different mold species may be fulfilling similar ecological roles; hence, the functional diversity may be low despite the high number of species.

Assessment of Sampling Effort: The number of taxa recovered from untreated samples increased with each additional wood block sampled (Fig 4.4). Many of the treated samples followed a similar pattern. Taxa continue to accumulate with increased sampling, suggesting the need for more replication. The replication in this test was limited by the ability to manage a higher number of fungal isolates at each time point.

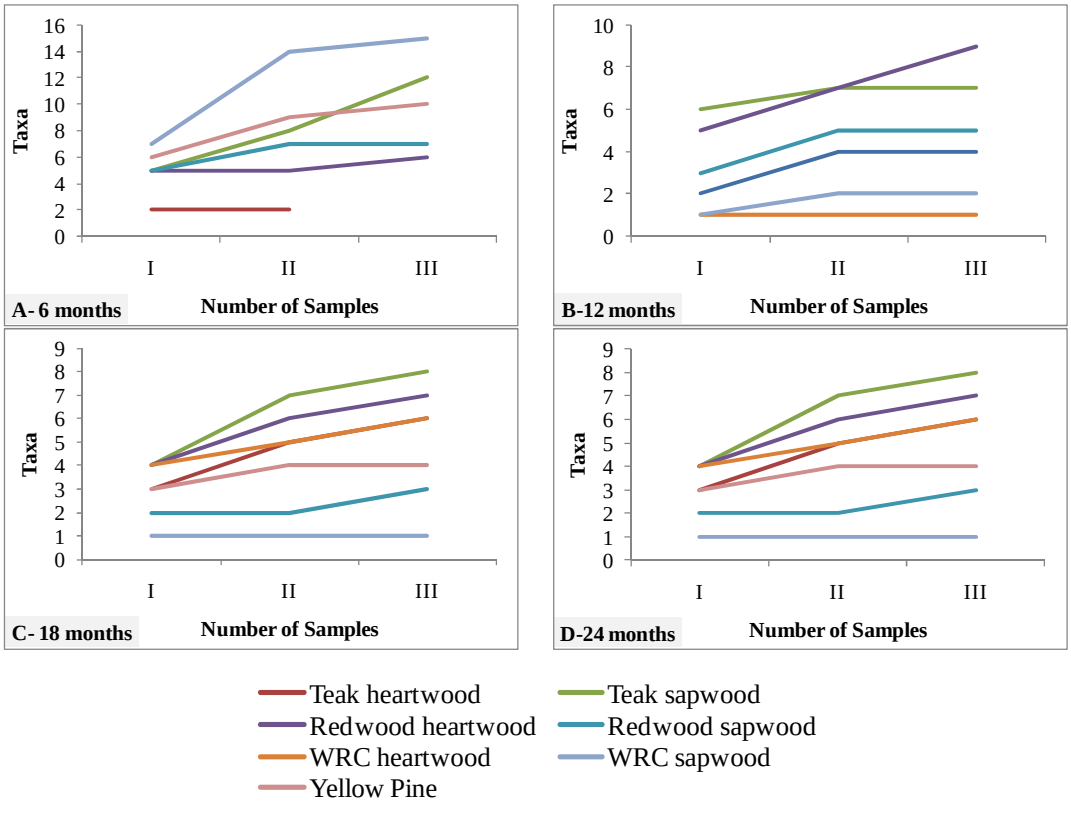


Figure 4.4. Taxa accumulation curves for fungi isolated from I, II, or III different untreated wood blocks. Wood blocks were exposed in Hawaii for 6 to 24 months and 48 one-mm cubes were sawn per block before isolation.

The observed number of species is often not a good estimator of true species richness because not all species are recoverable with the methods available (McCune and Grace, 2002). Non-parametric Jackknife 1st and 2nd order estimators were calculated using PC-ORD v. 5.18 (McCune and Mefford, 1999) to estimate how many taxa were missed by sampling. The estimators indicated that at least 26 - 34% of the taxa were missing (from 81 morphotaxa, 1st order jackknife estimate =93 taxa and 2nd order jackknife estimate =110 taxa). However, because taxa were lumped together, these estimators are likely lower than the actual number of species missing. For example, if each of the 209 unique ITS sequence represented a species, the 1st order jackknife estimate would be 272 species while the 2nd order jackknife estimate would be 315 species.

4.4. Discussion

The material examined in this study supported a diverse mycoflora (Table 4.1) whose abundance differed with wood species and between sapwood and heartwood (Fig 4.3). Beta diversity, a measure of the heterogeneity of the species composition in different wood types, was high in the four time points sampled and the diversity remain basically unchanged (Table 4.2). However, Jackknife estimators indicated an underestimation of species richness. Possible reasons for this underestimation include.

- The limitations that culturing impose, i.e., many species do not grow in culture, or are outgrown by coexisting rapid growing species in the culture medium. The aim of fungal isolations in decaying wood is *not* to recover every single species inhabiting the wood. The goal is rather to isolate species clusters that indicate a new stage in the biodegradation process, and that include the species expressing enzymes responsible for wood decay. Many soft rot decay fungi were recovered in this study, but only a few white rot fungi and no brown rot fungi were isolated. Brown and white rot fungi are generally the main wood decomposers and the isolation levels should be higher in decaying samples than in non-decaying samples.

- The high diversity and insufficient sampling intensity made it difficult to recover all the species (Fig 4.4). Increasing taxa recovery with each additional wood block sampled, suggests the need for more intensive sampling on individual samples or more samples.

The occurrence of fungi in most of the wood blocks could reflect the relative uniformity of the samples (regardless of chemical treatment). It also could reflect a low functional diversity of the species isolated, where species of molds and soft rot fungi were the most commonly wood decay types isolated.

A variety of rapid growing organisms were isolated from the wood after only 6 months of sample exposure. This rapid colonization could be due to the proximity of the field trial to the soil, which has higher microbial populations. Initial colonization of a vacant resource depends on early arrival of the species and usually does not involve competition between organisms. Success is determined by effective dispersal mechanisms, spore germination, mycelium extension rates, possession of suitable enzymes, and tolerance to adverse conditions. Ruderal and stress tolerant fungi are typically favored at this early colonization stage. Later colonizers rely more on combative competition that leads to secondary resource capture (replacement) or to successful defense of the resource (Rayner and Boddy, 1988; Freitag *et al.*, 1991). Species diversity should decline towards the end of the colonization process. While there was a slightly decline in diversity with time, the differences were small and suggest that most of the materials are still in the early stage of colonization.

Rapid growing species are not only isolated because of the proximity of the test to the soil as suggested by the long persistence of molds over time (Fig 4.3-A), these organisms may be consuming non-structural wood components, decay fungi, or their metabolic products, or may be detoxifying biocides. For example, the genus *Trichoderma* contains a number of species that are capable of very fast growth, antibiosis, and/or mycoparasitism. Incidentally, these traits could affect the ability to isolate other fungi from the samples.

We expected the frequency of basidiomycetes to increase with time, as other studies at the same site had shown (Freitag et al, 1995; Molnar, 1997), but the results were not consistent across material. These previous studies, however, used different sample units that were exposed much further from the soil. This distance from the soil may have reduced colonization by fast growing competitors, thereby improving the chances for basidiomycete recovery.

An interesting observation from this study was that WRC heartwood compounds appeared to inhibit molds and this was associated with isolations of more soft rot and white rot fungi than in teak and redwood heartwood samples. Even more successful soft rot fungi isolations were made in WRC heartwood samples that in sapwood samples, which are more susceptible to soft rot. That could be an applicable observation for laboratory practice in decay fungi isolations. Molds are common contaminants in agar plates when attempting to isolate decay fungi from rotten wood (Richter *et al.*, 2008). Inhibition of these species could increase isolation of decay fungi

Freitag *et al*, (1995) and Molnar *et al*, (1997) used classic culturing and microscopic techniques to isolate fungi from the same Hawaiian test site. We used similar techniques coupled with ITS-BLAST to identify the taxa and recovered approximately 60 additional genera. We believe that many taxa are still unrecovered. While the ITS-BLAST approach is useful for rapid identification, more effort is still required to characterize species richness in the test site.

Arnold *et al* (2007) noted that there is no threshold value of sequence similarity that is universally useful for distinguishing fungal species. ITS genotypes are employed as a proxy for estimating species boundaries, but different biological species can have identical ITS sequences.

4.5. Conclusion

Fungal diversity and abundance at the Hawaiian field site was high but underestimated. Eighty one unique morphotaxa were identified of which 63 were ascomycetes, 16 basidiomycetes, and 3 zygomycetes. While a high number of fungi were identified, few of these were white or brown rot fungi even though white rot or brown rot damage was visible in many samples. Inability of the basidiomycetes to compete with the mold fungi in the growth medium may have prevented their isolation in the laboratory. Placing the samples further from the ground might decrease the colonization by mold fungi.

High diversity and abundance implied that the ground proximity test allowed rapid colonization of wood blocks regardless of biocide treatment, which is a positive characteristic of the site for testing the performance of biocide treatments. In contrast, underestimation of species richness limits our understanding of the functional consequences of fungal succession during wood decay and suggests that future evaluations should consider more replication and perhaps utilize a number of different exposure configurations to create different environmental conditions.

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CHAPTER 5- EFFECT OF PRESERVATIVE TREATMENT, WOOD TYPE, AND EXPOSURE TIME ON WOOD FUNGAL COLONIZATION IN A FIELD TEST IN HAWAII

ABSTRACT

Wood decay is influenced by the specific assemblage of organisms that colonize wood. Differences in fungal communities could explain observed differences in decomposition rates among different wood samples or even in wood with different biocide treatments. The main goal of this study was to compare fungal communities growing in wood samples exposed in a field test in Hawaii over a 24 month period. Wood samples included both sapwood and heartwood of teak, western redcedar and redwood, and southern pine sapwood. The samples were treated with DCOI (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), DDAC (didecyldimethylammonium chloride), ACQ (alkaline copper quaternary), or left untreated. Fungi were isolated from the wood samples after 6, 12, 18, or 24 months of sample exposure and identified by coupling microscopic techniques with the comparison of the internal transcribed spacer (ITS) regions of the fungal rDNA with sequences available on the National Center for Bioscience Informatics (NCBI) website. The impact of preservative treatment, wood species and type (sapwood/heartwood), and exposure time on fungal communities was assessed using multivariate statistical analyses. Non-metric multidimensional scaling ordination revealed that the strongest differences in fungal community composition were related to time. Multi-response permutation procedures did not indicate a change in fungal communities as a result of biocide treatment or wood type. Fungal communities in all wood types contained taxa with similar decay capabilities. This similarity in fungal capabilities suggested that the observed decay rates in samples of the same wood type were due to biocide treatments and not due to changes in fungal communities.

Keywords: Fungal diversity, fungal communities, Hawaii, ground proximity test, DCOI, DDAC, ACQ, teak, western redcedar, redwood.

5.1. Introduction

Wood exposed under adverse environmental conditions is invaded by a variety of organisms, some of which will produce wood decay. Invasion does not generally start with the principal decomposers which are mostly basidiomycetes. Observations of fungal colonization in decaying wood have indicated that species abundance and composition of microbial communities change as decay advances (Rayner and Boddy, 1988; Vandegrift, 2002; Jellison *et al.*, 2003; Raberg *et al.*, 2009). This change can be defined as fungal succession (Hyde and Jones, 2002). Functional species replace one another as communities change in space and time, and each species is adapted to the occupation of particular niches (Egerton-Warburton and Jumpponen, 2005).

Changes in fungal communities occur as a result of substrate changes (e.g., moisture content change, nutrient depletion, etc), and/or interactions with other species (e.g., commensalism, mutualism, or competition) (Hyde and Jones, 2002). The fact that fungal communities differ with time does not necessarily denote succession, it could be the result of pure chance, or changes in climatic conditions. Likewise, the appearance of a fungal community in a treated piece of wood and another in an untreated piece does not necessarily imply differences in community composition. Hence, to understand the impact of fungal diversity and fungal succession on wood decay processes, it is necessary to 1) identify fungal communities over time, 2) monitor substrate changes (e.g., wood moisture content, nutrient depletion, biocide treatment), and 3) model the relationship between species (e.g., presence-absence, abundance, physiological rates) and substrate changes (McCune, 2009).

Much of the effort to understand how fungal diversity and community development impact wood decay has been directed to identifying individual species, determining their decay capabilities, and identifying patterns of species appearance. The model of early, intermediate and late colonizer appearance as a function of environmental stress, incidence of competitors, and disturbance, described by Rayner and Boddy (1988) is

widely accepted. This model resembles fungal succession processes in other substrates, and in plant communities (Jones and Hyde, 2002), but it is not the only model. For example, soft rot fungi are often the first decay type found in a general sequence of wood colonization (Raberg *et al.*, 2009).

Observed patterns of wood fungal colonization vary considerably with wood species, wood type (sapwood/heartwood), and the environment in which decay is occurring. However, there is little consensus regarding the extent to which the development and succession of fungal communities affects wood decay, even under similar exposure conditions.

Failure to gain consensus has been due, in part, to the difficulties in identifying fungal species growing on wood (Chapter 4), but also reflects difficulties in understanding species' response functions resulting from the limitations of standard statistical models to represent these functions (McCune, 2009). Improved research techniques and a growing understanding of microbial biochemistry and genetics provide higher resolution methods for identifying microbial communities (Jasalavich *et al.*, 1998; Jellison *et al.*, 2003; Raberg *et al.*, 2009). Improved mathematical and computational methods also allow examination of how species richness (number of taxa) and composition may interact with different environmental or experimental variables (McCune, 2009).

In the previous chapter, the taxonomic richness found in decaying wood exposed in a ground proximity field test in Hilo, Hawaii for 6, 12, 18, or 24 months was described. Wood samples included both sapwood and heartwood of teak, western redcedar and redwood, as well as sapwood of southern pine. The samples were treated with 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOI), didecyldimethylammonium chloride (DDAC), alkaline copper quaternary (ACQ), or left untreated. Fungal diversity and abundance at the field test site was found to be high but probably underestimated since some species could be not be identified due to the limitations of the isolation

techniques. The goal of this study was to evaluate the impact of preservative treatment, wood type (sapwood/heartwood), and exposure time on fungal communities growing in the same samples.

5.2. Methods

Wood Treatment and Exposure: Teak, redwood, western redcedar (WRC), or southern pine lumber was cut into 19 by 50 by 125 mm long samples and conditioned at 23ºC and 70% relative humidity. Preservative treatments were assigned to groups using a list of random numbers. The wood was vacuum-pressure treated at Viance LLC (Charlotte, NC) with solutions of DCOI to target retentions of 0.1, 0.3, 0.6 kg/m^3, DDAC to retentions of 1, 2, or 4 kg/m^3, or ACQ to a retention of 4 kg/m^3. Samples from each wood type were also left untreated. The wood samples were exposed in a ground proximity test according to AWPA Standard-E18 (AWPA, 2006). Sixteen arrays of concrete blocks were placed in the open at the Hawaiian field test site. Samples were placed randomly on the arrays and were inspected at six month intervals. The degree of decay was scored visually on a scale from 0 to 10 (Table 5.1). At the same time three pre-identified samples from each wood-treatment combination were collected from the field and returned to Oregon State University (Corvallis, OR) to assess fungal colonization.

Table 5.1. Evaluation system used to score the degree of decay in a field trial in Hawaii. Adapted from AWPA Standard E18 (AWPA, 2006).

Score	Description	Description
10	Sound	No signs or evidence of decay, wood softening, or discoloration caused by microorganism attack.
7	Severe Attack	Sample has between 20 and 40% of its cross-sectional area weakened to the point it can be damaged by a dull probe..
4	Very Severe Attack	Greater than 40% of sample cross-section compromised and severely softened, but does not meet the requirements for failure.
0	Failure	Sample has functionally failed. It can either be broken by hand due to decay or the evaluation probe can penetrate through the sample.

It was assumed that there was no spatial implication in the position of the samples. Other arrays were adjacent to the arrays used in these trials, so we did not consider possible border effects.

Fungal Culturing and Identification: Upon arrival at Oregon State University, the three samples removed at each time point were surface sterilized with a dilute bleach solution (5% sodium hypochlorite) and then sawn to obtain sixteen 10-mm-cubes per sample. The cubes were flame-sterilized and placed into petri-dishes with 1.5% malt extract agar (MEA). Fungi growing from the wood were isolated on fresh MEA plates until pure cultures were obtained. Pure cultures were grouped into *morphotaxa* based on macroscopic and microscopic characteristics. Representatives of each morphotaxon were grown in potato dextrose broth until a sufficient amount of tissue was available for DNA extraction.

DNA from 885 cultures was extracted and purified according to previously published methods (Pomraning *et al.*, 2009), a DNeasy Plant Mini Kit (Qiagen Sciences, Valencia, CA, USA), or a DNA extraction kit (Epoch Biolabs, Inc., Sugar Land, TX, USA). The ITS region of the DNA was amplified using the polymerase chain reaction (PCR) with the ITS1-F (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) sequences as a primers (White *et al*, 1990). The resulting amplified DNA was further purified by agarose gel electrophoresis (12 g agarose and 1 mg ethidium bromide/L in TAE buffer). The bands were excised and the DNA extracted with a QIAquick gel extraction kit (Qiagen Inc., Valencia, California). The DNA was sequenced at the Center for Genome Research and Biocomputing at Oregon State University using the ITS1F primer. Sequences were compared with sequences available on the National Center of Bioscience Informatics (NCBI) website using the BLAST tool and assigned to the nearest taxonomic group.

Species with rDNA sequences with more than 95% sequence similarity were lumped together, since experimental evidence indicated that 90-99% sequence similarity cutoffs

have been used for the ITS rDNA region to define molecular operational taxonomic units (O'Brien *et al.*, 2005). Wood decay capabilities of the taxa were classified as mold, stain fungi, mold/soft rot fungi, white rot fungi, or uncertain on the basis of previous reports (Chapter 5). In some cases, it was necessary to assign a capability to a given isolate based upon the capability of other species in that genus. This approach entails some risk since the capabilities of individual species in the genus can vary.

Statistical Analyses: Multivariate statistical analyses were performed using PC-ORD *v.5.18* (McCune and Mefford, 1999). Multi-response permutation procedures (MRPP) using Euclidean distances were used to test the hypothesis of no differences between two or more communities (McCune and Grace, 2002). The null hypotheses tested through MRPP were: (1) fungal communities at different time points were not different, and (2) fungal communities found in different wood species were not different.

A ***compiled data matrix*** was created including wood blocks sampled (rows) at each of the time points (6, 12, 18, and 24 months) and the 81 fungal taxa (columns). A binary system was used to indicate the presence (1) or absence (0) of a morphotaxa in a wood block. The data matrix had an associated environmental matrix that contained quantitative (biocide retention and decay visual assessment) and categorical (wood species, sapwood/heartwood, and biocide treatment) variables. Smaller matrices were created to explore different aspects of the communities as follows:

- Time points: four different matrices for each of the time points.
- Seven wood type matrices (teak sapwood, teak heartwood, WRC sapwood, WRC heartwood, redwood sapwood, redwood heartwood, and southern pine) were created to evaluate the effect of preservative treatment in each group.
- Eight matrices were created to assess the effect of biocide treatment. Fungal assemblages isolated from DDAC and DCOI treated material were analyzed independently. However, fungal assemblages isolated from ACQ-treated-samples and untreated samples were left in each group for comparison.

Similarity patterns in species composition between the fungal communities were explored using non-metric multidimensional scaling (NMS) ordination. NMS is an iterative ordination technique that displays n-sample units (in this study, wood blocks) on k-dimensions (ordination axes) of an abstract space in which species were represented based on their patterns of occurrence. NMS seeks to minimize the *stress* of the k-dimensional configuration. This value is a measure of departure from monotonicity in the relationship between the dissimilarity (distance) in the original p-dimensional space and distance in the reduced k-dimensional ordination (McCune and Grace, 2002).

The wood blocks were represented in a fungal species space using Euclidean distance measures (Krustal, 1964). Different ordinations were run for each of the matrices created using fifty iterations from random starting coordinates. Most of the NMS ordinations had a three dimensional solution, although some of them had a two dimensional solution. For consistency, all ordinations were performed for three dimensional solutions. The coefficient of determination (r^2) was calculated to evaluate how well the distances in the ordination space represented the distances in the original space. This value is a measure of the quality of data reduction, along with an assessment of how the variance is distributed among the primary axes. Monte Carlo tests were conducted using 256 randomized runs and a stability criterion of a maximum of 0.00001 to evaluate whether NMS extracted stronger axes than expected by chance. Mantel tests were used to test for association between wood attributes and ordination scores (McCune and Grace, 2002).

5.3. Results

After exclusion of the ubiquitous molds (*Fusarium*, *Paecilomyces*, and *Trichoderma* species) from the matrices, morphotaxa were recovered from 544 of 624 wood blocks sampled. Each morphotaxa was given an ordination code (Table 5.2) from which the ***compiled data matrix*** was created with wood blocks as rows and fungal taxa as

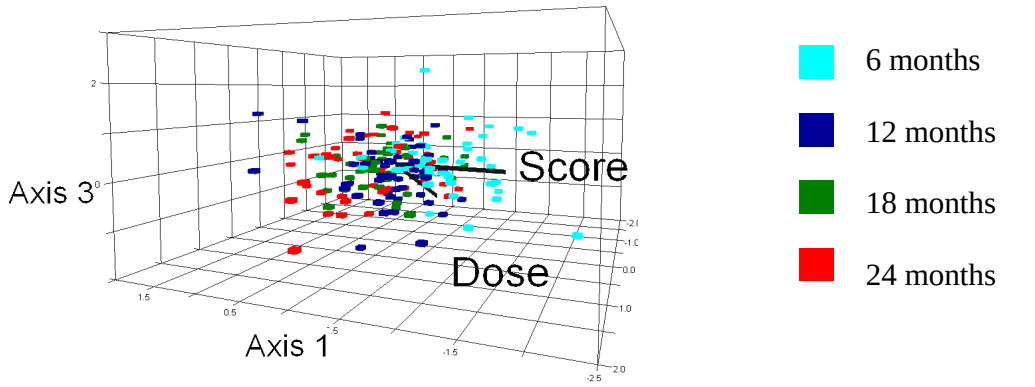
columns. A NMS ordination of the compiled data matrix showed that the proportion of variance represented (r^2) by axis 1 was 15%, the r^2 of axis 2 represented an additional 14% (cumulative r^2 = 29%), and the r^2 of axis 3 represented an additional 12% (cumulative r^2 = 31%). One possible explanation for this very low cumulative r^2 of 31% is that only few morphotaxa were isolated from each wood block. To overcome this issue, the morphotaxa isolated at each wood-treatment-time point combination were merged into a single block, to create a data matrix with 212 rows, but no repetition.

The cumulative r^2 within this ordination was 73% (axis 1= 24%; axis 2=21%; axis 3=28%). According to McCune and Grace (2002), a cumulative r^2 of more than 50% for data sets with more than 20 taxa is an indicator of good quality of the ordination results. The cumulative r^2 also indicated that the morphotaxa represented in this ordination were somehow correlated, i.e., that some species co-occurred several times in specific fungal assemblages. The visual decay score was correlated weakly with the axis of the ordination (axis 1 R^2=0.04; axis 2 R^2=0.1), and very weakly to biocide dose (axis 1 R^2=0.05) (Fig 5.1). The lowest stresses for this ordination, and for all ordinations presented in this study, were between 0.17 and 0.20. P–values in the Monte Carlo tests were a maximum of 0.0040 which indicated that the NMS extracted stronger axes than expected by chance.

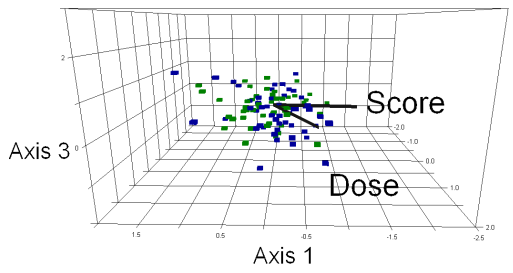
Time Effects: The effects of exposure time were evaluated with the reduced compiled matrix. A MRPP test showed that fungal communities at the four time points were broadly overlapping (A=0.026; p=0.00) (Table 5.2). The agreement statistic A describes within-group homogeneity; the groups are different if A=1; while the groups are identical if A=0 (McCune and Grace, 2002). In community ecology, values are often below 0.1, and differences are assumed if the A statistic $\geq$ 0.1 (Ibidem). Pair-wise comparisons showed that the A values were below 0.05 for the four time points, indicating that the communities were similar. However, the groups started to differ more with increasing exposure time and this drift was not due to chance (p values < 0.00) (Table 5.2, Fig 5.1).

Table 5.2. Summary statistics for MRPP comparing the similarity of fungal communities isolated at different time points (6, 12, 18, 24 months) in a ground proximity test in Hawaii. The pair-wise comparisons were also made with MRPP.

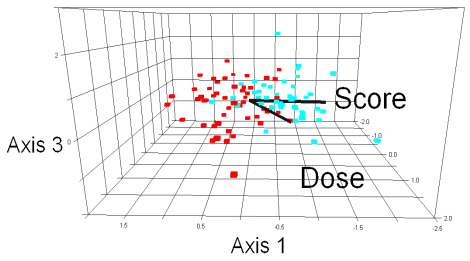
	A-statistics	**P-value**
Total	0.02	0.00
Multiple comparisons		
6 vs. 12 months	0.016	0.00
6 vs. 18	0.029	0.00
6 vs. 24	0.035	0.00
12 vs. 18	0.084	0.00
12 vs. 24	0.013	0.00
18 vs. 24	0.005	0.00



A



B



C

Figure 5.1. Three dimensional NMS ordination of wood blocks on fungal species space. Each point in the ordination represents a wood block, and each color the time point at which the blocks were sampled. A = all 4 points represented. B = 12 and 18 months. C = 6 and 24 months. Joint plots show weak correlations of decay score and preservative dose with fungal composition. Vectors lengths correspond to the strengh of the correlation.

Because there was evidence of differences in fungal communities with time, succession vectors were overlaid in the ordination to trace the movement of sample units through species space as a function of time (Figure 5.2). This was done by connecting the sample units with the same wood-treatment combination over the 4 time periods with a linear vector. The vectors were moved to the origin to observe the directionality and the rate of change. None of the vectors occupied the same region of the species space when wood species, wood type, or biocide treatment were superimposed (Figure 5.2 only depicts wood species but results were similar for wood type and biocide treatment). The points did not converge in the same area of the species space. This indicated that communities could change over time, but they did not change towards specific fungal assemblages, and that the change was independent of wood species, wood type, or biocide treatment.

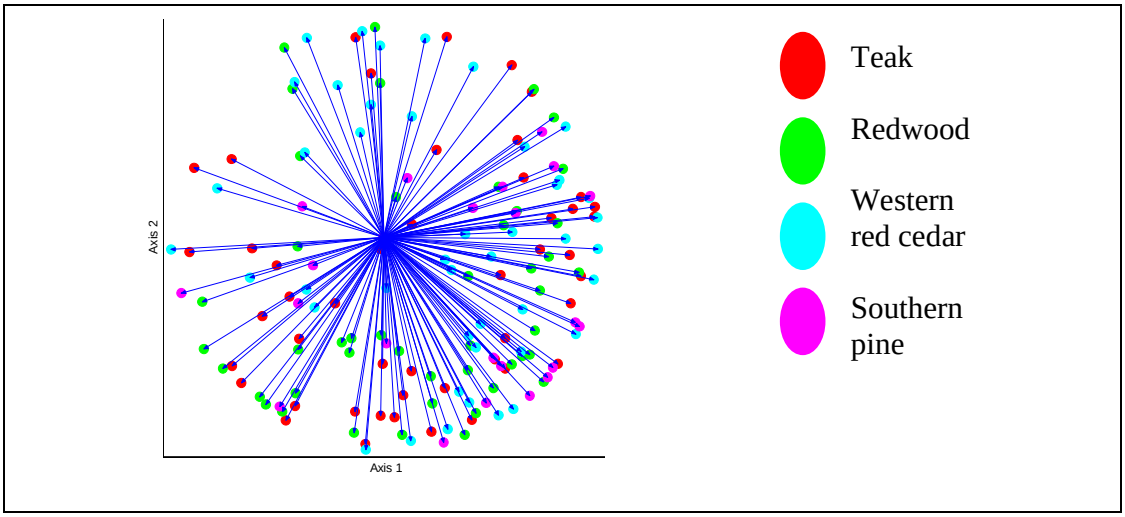


Figure 5.2. Succession vectors superimposed in an ordination of wood samples on a species space.

Fungal morphotaxa identified from untreated or treated wood samples were classified according to their phylum and codes were assigned for NMS ordination (Table 5.3).

Observed changes in time were, in part, due to the influence of species that were only isolated once or twice (Fig 5.3). Many species were isolated for the first and only time

after six months of sample exposure. More consistent patterns of species association and isolation were found after 12, 18 and 24 months. At 24 months, most fungal assemblages contained the species putatively identified as *Resinicium friabile*. This species clustered most of the 24 month data together (Fig 5.3).

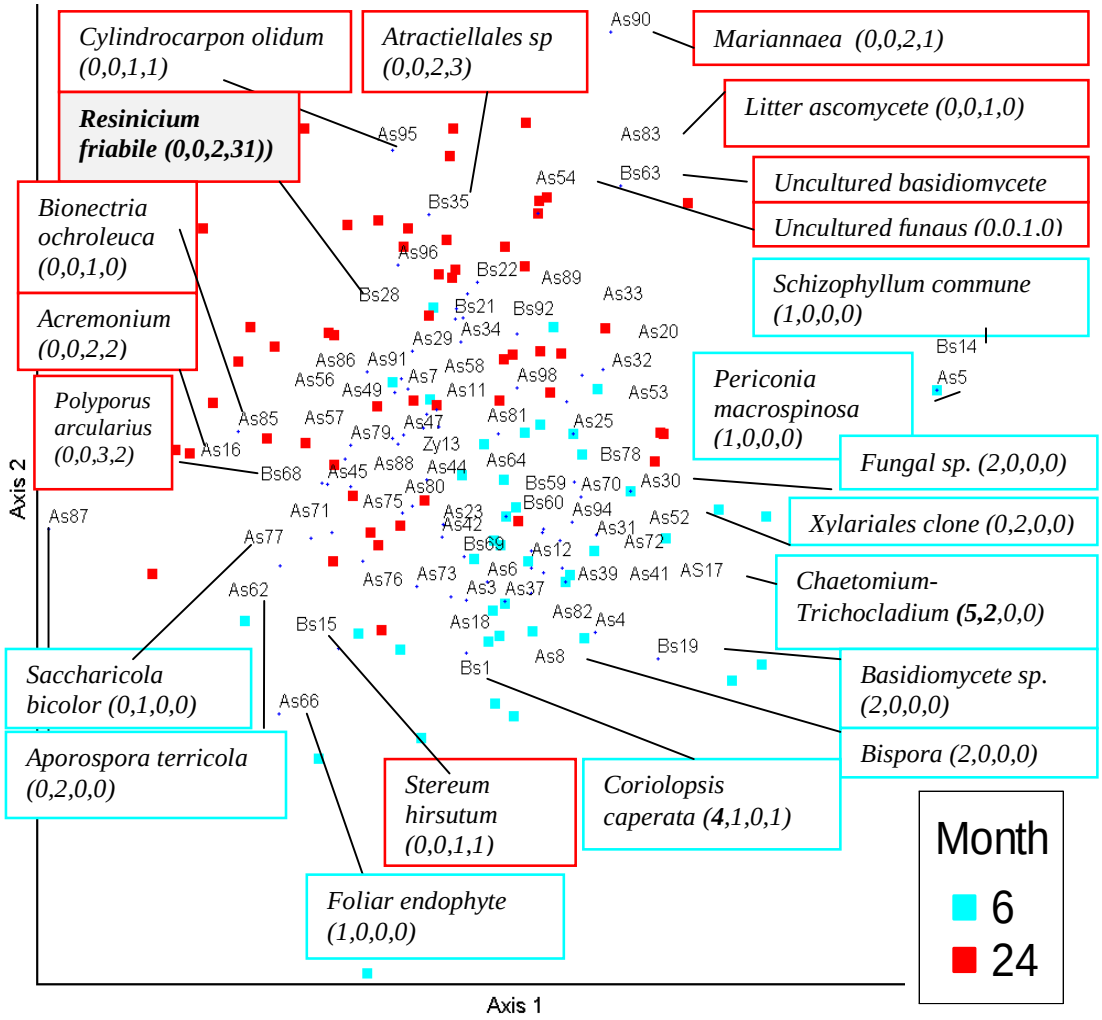


Figure 5.3. Two dimensional NMS ordination of wood blocks on fungal species space. Each point in the ordination represents a wood block, and each color the time point at which the blocks were sampled. Species were superimposed and coded according to Table 5.3. Text boxes contain examples of ITS-BAST morphotaxa names and number of isolations at time points (6,12,18, 24 months). The color of the textbox matches the color of the time point indicating that the morphotaxa had more influence in that time point.

Table 5.3. Fungal morphotaxa identified from untreated or treated wood samples exposed in a ground proximity test in Hawaii for 6 to 24 months. Taxa were classified according to their phylum and codes were assigned for NMS ordination.

Morphotaxa	Code	Morphotaxa	Code
Basidiomycetes		**Ascomycetes**	
Coriolopsis caperata	Bs1	*Lecythophora*	As41
Schizophyllum commune	Bs14	*Phoma*	As42
Stereum hirsutum	Bs15	*Pleurostomophora*	As44
Basidiomycete sp	Bs19	*Botryosphaeria-Lasiodiplodia*	As45
Sphaerobolus sp	Bs21	*Montagnulaceae*	As47
Phlebia	Bs22	*Kabatiella*	As49
Resinicium friabile	Bs28	*Uncultured Xylariales clone*	As52
Atractiellales sp	Bs35	*Exophiala / Rhinocladiella*	As53
Kurtzmanomyces tardus	Bs50	*Uncultured fungus*	As54
Oxyporus latemarginatus	Bs59	*Phaeosphaeria sp.*	As56
Coprinellus disseminatus	Bs60	*Phialemonium*	As57
Uncultured basidiomycete	Bs63	*Chaetomella*	As58
Polyporus arcularius	Bs68	*Aporospora terricola*	As62
Phanerochaete	Bs69	*Fungal sp*	As64
Trametes versicolor	Bs78	*Foliar endophyte*	As66
Basidiomycete sp.	Bs92	*Stemphylium sp.*	As70
		Valsa myrtagena	As71
Ascomycetes		*Sordariales sp.*	As72
Arthrinium	As3	*Cladosporium tenuissimum*	As73
Leptosphaerulina	As4	*Fungal endophyte*	As74
Periconia macrospinosa	As5	*Truncatella angustata*	As75
Phialophora	As6	*Codinaeopsis sp.*	As76
Phaeoacremonium	As7	*Discostroma tricellulare*	As77
Bispora	As8	*Diaporthe*	As79
Nigrospora	As11	*Penicillium*	As81
Leptosphaeria	As12	*Carpoligna pleurothecii*	As82
Acremonium	As16	*Leaf litter ascomycete*	As83
Chaetomium-Trichocladium	AS17	*Bionectria ochroleuca*	As85
Colletotrichum-Glomerella	As18	*Pestalotiopsis vismiae*	As86
Microsphaeropsis arundinis	As20	*Saccharicola bicolor*	As87
Cochliobolus-Curvularia	As23	*Glionectria tenuis*	As88
Lecanicillium lecanii	As25	*Cladophialophora sp*	As89
Uncultured Ascomycete	As29	*Mariannaea camptospora*	As90
Fungal sp	As30	*Pilidiella /Harknessia sp.*	As91
Aspergillus	As31	*Rhinocladiella sp.*	As94
Ochroconis	As32	*Cylindrocarpon olidum*	As95
Arthrographis-Cosmospora	As33	*Simplicillium lamellicola*	As96
Hypocreales sp.	As34	*Xylaria sp.*	As98
Aureobasidium pullulans	As37		
Alternaria	As39	**Zygomycete**	**Zy13**

Effects of Biocide Treatments: Tables 5.4 to 5.7 display the fungal communities isolated after 6, 12, 18 and 24 months exposure for each wood-treatment combination. As a reference, graphs of the proportion of samples decayed after 36 months were given in Fig 3.3, 3.5-3.7 (Chapter 3). There were no apparent patterns of differences in fungal composition between different wood-treatment combinations, i.e., samples with higher proportions of decay contained very similar communities as samples that were less decayed, suggesting that the fungal communities *isolated* had little effect on decay.

Table 5.4. Morphotaxa isolated from untreated, or ACQ-, DDAC- or DCOI-treated southern pine samples, after 24 months exposure in Hawaii. Each number represents a morphotaxon coded in Table 5.3. A: Ascomycete; B: Basidiomycete; Z: Zygomycete.

Southern Pine Sapwood																															
Phylum	None				ACQ				DCOI												DDAC											
	0				4 kg/m^3				0.1 kg/m^3				0.3 kg/m^3				0.6 kg/m^3				1 kg/m^3				2 kg/m^3				4 kg/m^3			
	Exposure time (months)																															
	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
B.		21		28					21		69	22				28				28					69			22				
												28																28				
A.		11	29	23	11	41		91	3	7	33	29	23	29	91	33		33	89	33	30	23		29	12	91	33	33	23	81		33
		53	47	29	37	52			17	11	34	33	29	33	33	81			91		33	91		34	23		47	34	37	91		34
				58	39	88			31	23		58	86	34	53	91					42	98			37			58	42			64
				91	49				42	29		86	89	53							53				42			91	53			91
									53	32		91	91	89							81				53							
										33				91											81							
										53															86							
										81																						
										86																						
										90																						
Z.																	13															13

A

Table 5.5. Morphotaxa isolated from untreated, or ACQ-, DDAC- or DCOI-treated **(A)** sapwood or **(B)** heartwood teak samples, after 24 months exposure in Hawaii. Each number represents a morphotaxon coded in Table 5.3. A: Ascomycete; B: Basidiomycete; Z: Zygomycete.

Teak Sapwood

Phylum	None				ACQ				DCOI												DDAC											
	0				4 kg/m^3				0.1 kg/m^3				0.3 kg/m^3				0.6 kg/m^3				1 kg/m^3				2 kg/m^3				4 kg/m^3			
	Exposure time (months)																															
	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
B.	78	1		28					69						69	68		69	68	28	1		68	28			22	68				
																					21		28					28				
		14																	69				78									
	4	18	23	33	37	17	45		3	42	33	33	7	11	33	11	3	11	91	11	23	45	45	11	3	7	11	23	3	29	45	45
	5	29	33	34	81	42			23	45	45	45	23	29	45	45	23	91		37	45		91	88	8	23	29	45	16	42	70	
	11	45	47			47			45	86	86	86	42	42		66	42			53				91	23	29	33		37	45		
	12	57	53			62			71	91	91	88	47	45		89				86				98		45	45		45	47		
A.	53	72				64			86			91	53	47		91				89						49	91		53	57		
	81	86				91								91												77			91			
	98																									87						
																										89						
																										91						
Z.	13								13			13			13														13			13

A

Teak Heartwood

Phylum	None				ACQ				DCOI												DDAC											
	0				4 kg/m^3				0.1 kg/m^3				0.3 kg/m^3				0.6 kg/m^3				1 kg/m^3				2 kg/m^3				4 kg/m^3			
	Exposure time (months)																															
	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
B.									60						28														60	68		
		23	11	42		37	91	7	12	47	42	33	4	44	11	7	37	23	45	23	8	7	33	34	37	23	58	45	31	11	33	33
		37	13	45		45		45		62	45	42	23	45	42	11	49	91	91	33	17	11	86	45	81	33	86	47		23	42	45
		42	42	91		91		91		42	91	45	37	91	45	33	91			45	49	23	91	86		42	91	57		29	44	86
		45	45							91		81	42		47	42				89		41		88				58		47	45	88
A.		91	91												89	45				71		42		91				91		86	89	96
																56				47		91						96		91	91	91
																71														96		
																88																
																89																
Z.											13	13	13		13				13				13				13				13	

B

Table 5.6. Morphotaxa isolated from untreated, or ACQ-, DDAC- or DCOI-treated **(A)** sapwood or **(B)** heartwood western redcedar samples, after 24 months exposure in Hawaii. Each number represents a morphotaxon coded in Table 5.3. A: Ascomycete; B: Basidiomycete; Z: Zygomycete.

Western Red Cedar Sapwood

Phylum	None	ACQ	DCOI			DDAC		
	0	4 kg/m^3	0.1 kg/m^3	0.3 kg/m^3	0.6 kg/m^3	1 kg/m^3	2 kg/m^3	4 kg/m^3
	Exposure time (months)							
	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24
B.	1		28		78 28 28	59	21	28
	4 91	44 23 45	33 33 33 33	33 33 33	7 11 7	18 4 86 33	18 18 11 11	17 1 33 33
	11	47	34 34 34 86	91 91	33 33 11	86 72 89 96	23 34 88 33	18 11 53 53
	23	56	81 91 47 88	96	89 33		37 44 45	23 79 86 57
	33		42 91		34		42 57 49	57 91 88 91
	34		71		89		45 75 53	86 89
A.	42				91		81 82 91	
	45						86 96	
	66							
	68							
	69							
	88							
Z.					13			13

A

Western Red Cedar Heartwood

Phylum	None	ACQ	DCOI			DDAC		
	0	4 kg/m^3	0.1 kg/m^3	0.3 kg/m^3	0.6 kg/m^3	1 kg/m^3	2 kg/m^3	4 kg/m^3
	Exposure time (months)							
	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24
B.	28	78	92 1	28	92	92	92 28	28
	53 33 11	7 34 7	53 53 33 33	53 33 11 33	25 53 33	11 53 11 33	37 11 7 7	32 4 7
	64 42 33	11 45 42	47 34	37 45 35	53 49	34 64 23 53	42 53 42 20	53 23 23
	89 34	12 47 49	53 53	53 47 53	53	53 72 33 70	91 45 29	33 33
	91 47	23 53 53	89 86	53 89	91	34 89	53 47	42 34
A.	53	45 94 89	91 89	91		47	91 33	53 45
	89	47 94	96			81	45	58 47
	91	58				89	53	86
						91	96	
Z.								

B

Table 5.7. Morphotaxa isolated from untreated, or ACQ-, DDAC- or DCOI-treated **(A)** sapwood or **(B)** heartwood redwood samples, after 24 months exposure in Hawaii. Each number represents a morphotaxon coded in Table 5.3. A: Ascomycete; B: Basidiomycete; Z: Zygomycete.

Redwood Sapwood

Phylum	None	ACQ	DCOI			DDAC		
	0	4 kg/m^3	0.1 kg/m^3	0.3 kg/m^3	0.6 kg/m^3	1 kg/m^3	2 kg/m^3	4 kg/m^3
	Exposure time (months)							
	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24
B.	22			21 35 21	21 21	28 28	22	78 28
						21		
A.	6 98	11 11 23	17 7 33 33	29 33 33	7 11 29	23 42 96 45	6 42 33 47	16 23 88 57
	23	17 47 53	20 16 56	33 91 57	91 23 33	86 33 96	17 45 53 57	86 57 91 58
	37	23 58	37 23 86	53 91	33 53	91 91 34	23 85 91	73 91
		73 88	42 29 91	91 96	89 91	33	53 75 98	91
			53 33 96		91	91	41 29	
			45		95		91	
			91					
Z.					13		13	13

A

Redwood Heartwood

Phylum	None	ACQ	DCOI			DDAC		
	0	4 kg/m^3	0.1 kg/m^3	0.3 kg/m^3	0.6 kg/m^3	1 kg/m^3	2 kg/m^3	4 kg/m^3
	Exposure time (months)							
	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24	6 12 18 24
B.			60	28				60 68
A.	23 11 42	37 91 7	12 47 42 33	4 44 11 7	37 23 45 23	8 7 33 34	37 23 58 45	31 11 33 33
	37 13 45	45 45	62 45 42	23 45 42 11	49 91 91 33	17 11 86 45	81 33 86 47	23 42 45
	42 42 91	91 91	42 91 45	37 91 45 33	91 45	49 23 91 86	42 91 57	29 44 86
	45 45		91 81	42 47 42	89	41 88	58	47 45 88
	91 91			89 45	71	42 91	91	86 89 96
				56	47	91	96	91 91 91
				71				96
				88				
				89				
Z.			13 13	13	13	13	13	13

B

The effects of biocide treatment were then analyzed both as a quantitative variable and as a qualitative grouping variable.

Biocide treatment as a qualitative grouping variable: MRPPs for each treatment between each wood type and their corresponding pair-wise comparisons showed that fungal composition was overlapping. Most of the A agreement statistics were below 0.09, and the p-values were mostly not significant. These results suggested that the groups were very similar and that differences observed could be due to chance (Table 5.8). Note that in MRPP p-values ≤ 0.05 do not indicate differences in fungal assemblages between two communities. For ecological purposes, differences are assumed if the A statistic ≥ 0.1 (McCune and Grace, 2002), and *if so*, differences due to

chance are identified by insignificant p-values. The p-values convey little information when the two groups are indistinguishable.

Table 5.8. Pair-wise MRPP comparisons of different biocide treatments in various wood- treatment combinations.

Wood		Teak				Redwood				WRC				Pine	
Biocide Treatment (kg/m^3)		Sapwood		Heartwood		Sapwood		Heartwood		Sapwood		Heartwood		Sapwood	
		A	p-val.	A	p-val.	A	p-val.	A	p-val.	A	p-val.	A	p-val.	A	p-val.
None	vs. DCOI 0.1	0.05	0.03	0.00	0.45	0.00	0.45	0.04	0.02	0.00	0.39	0.03	0.56	0.00	0.42
None	vs. DCOI 0.3	0.01	0.50	0.02	0.91	0.13	0.02	0.05	0.07	0.02	0.61	0.04	0.80	0.03	0.09
None	vs. DCOI 0.6	0.02	0.13	0.04	0.08	0.03	0.90	0.04	0.09	0.03	0.18	0.01	0.30	0.05	0.02
None	vs. DDAC 1	0.00	0.45	0.52	0.04	0.01	0.30	0.03	0.17	0.02	0.77	0.17	0.68	0.03	0.87
None	vs. DDAC 2	0.01	0.22	0.06	0.03	0.00	0.48	0.07	0.03	0.04	0.92	0.01	0.67	0.02	0.73
None	vs. DDAC 4	0.00	0.39	0.05	0.05	0.00	0.48	0.03	0.20	0.01	0.61	0.02	0.25	0.01	0.35
None	vs. ACQ	0.01	0.20	0.08	0.03	0.00	1.00	0.04	0.07	0.02	0.89	0.02	0.67	0.00	0.54
ACQ 4	vs. DCOI 0.1	0.07	0.02	0.06	0.03	0.02	0.10	0.00	0.47	0.09	0.01	0.05	0.09	0.04	0.00
ACQ 4	vs. DCOI 0.3	0.01	0.60	0.09	0.02	0.11	0.90	0.02	0.28	0.14	0.02	0.03	0.18	0.08	0.04
ACQ 4	vs. DCOI 0.6	0.03	0.52	0.05	0.07	0.05	0.03	0.00	0.44	0.12	0.20	0.07	0.02	0.03	0.17
ACQ 4	vs. DDAC 1	0.03	0.85	0.02	0.09	0.05	0.08	0.02	0.32	0.01	0.19	0.01	0.28	0.00	0.90
ACQ 4	vs. DDAC 2	0.01	0.20	0.05	0.01	0.03	0.80	0.02	0.12	0.01	0.31	0.06	0.92	0.00	0.40
ACQ 4	vs. DDAC 4	0.02	0.21	0.01	0.57	0.02	0.21	0.01	0.57	0.04	0.02	0.02	0.76	0.02	0.27

*Where A = agreement statistic; p-val. = p-value.

Biocide treatment as a quantitative variable: Eight NMS ordinations were run, two for each biocide at each of the time periods. Treatments were standardized as follows: untreated=0, lowest retention=1, intermediate retention=2, highest retention=3, ACQ =4. Mantel tests were used to test for associations between the quantitative value of biocide concentration and ordination scores. Biocide concentration did not have any impact on the fungal composition of the field test (Table 5.9). This suggests that chemical loading affected the overall rate of colonization, but it did not affect community composition over time.

Table 5.9. Mantel r statistic and p-values from Mantel tests used to compare the similarity between fungal communities growing in wood samples treated with different biocides treatments.

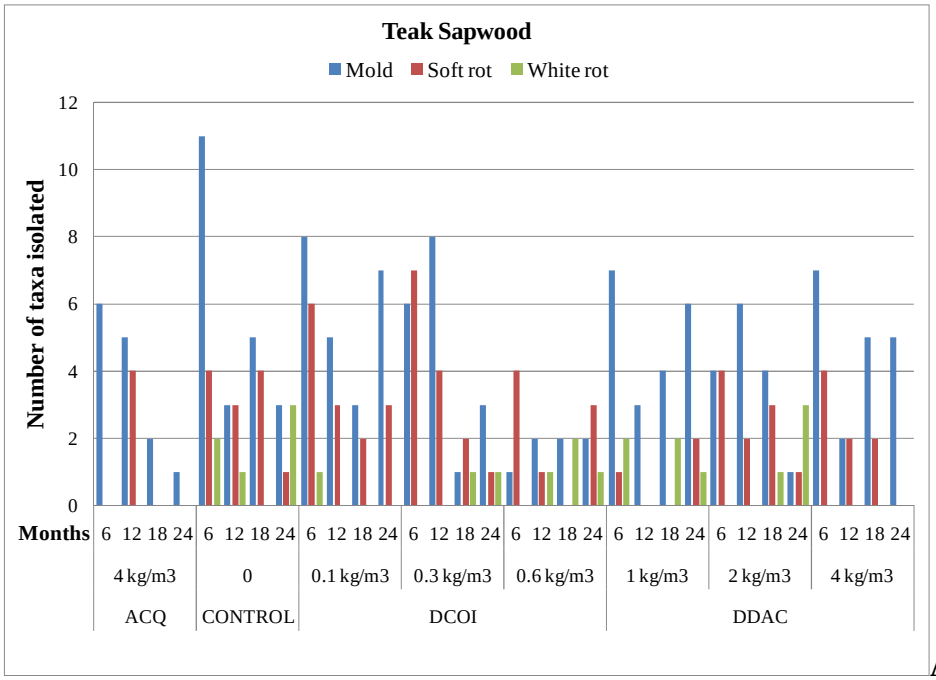
Time point	Biocide	Biocide Concentration	
		Mantel r	p-value
6 months	DCOI	-0.06	0.31
	DDAC	-0.032	0.39
12 months	DCOI	-0.00	0.93
	DDAC	-0.03	0.36
18 months	DCOI	-0.07	0.20
	DDAC	-0.03	0.10
24 months	DCOI	-0.00	0.20
	DDAC	-0.03	0.10

Wood Species: MRPP tests showed that fungal communities did not differ for most of the wood samples (6 months: A=0.01; 12 months: A=0.06; 18 months A=0.06; 24 months: A=0.04). In general, more fungal taxa appeared to be isolated from sapwood samples than from heartwood samples, but differences were not significant (Table 5.10).

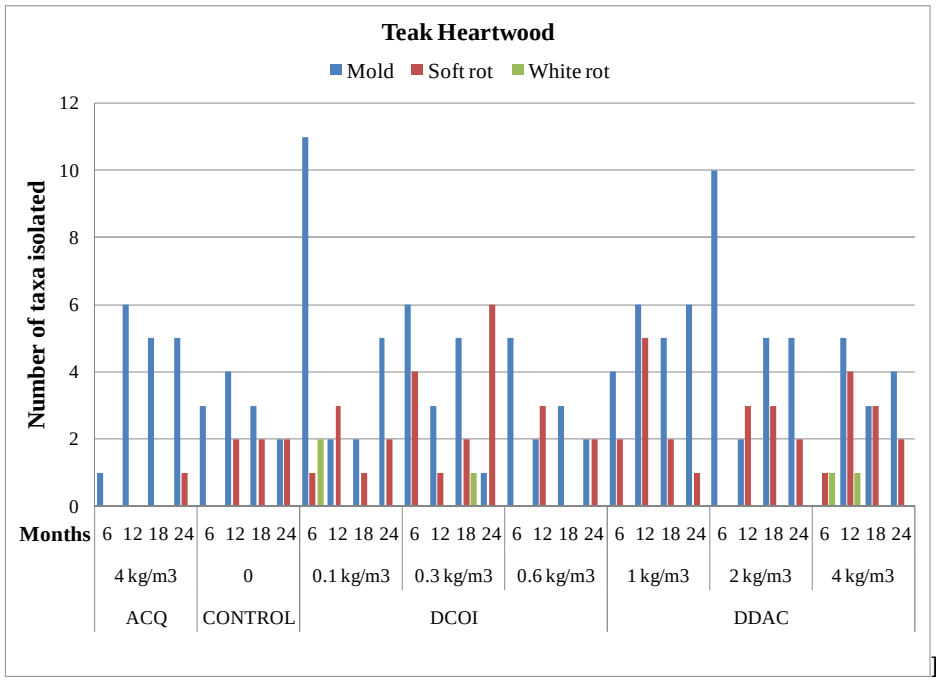
Table 5.10. Pair-wise MRPP comparisons of different biocide treatments in various wood treatments combinations. A= agreement statistics; p-val= p-value.

Month			6 months		12 months		18 months		24 months	
Pair wise comparisons			A	p-val.	A	p-val.	A	p-val.	A	p-val.
Teak heart.	vs.	Teak sap.	0.01	0.25	0.01	0.09	0.05	0.00	0.02	0.09
RW heart.	vs.	RW sap.	0.01	0.62	0.01	0.14	0.00	0.55	0.01	0.18
WRC heart.	vs.	WRC sap.	0.01	0.03	0.01	0.24	0.02	0.03	0.02	0.05

Figures 5.4 to 5.7 show the number of mold, soft rot, and white rot taxa isolated from each wood-treatment combination. In general, colonization by fungi with different decay capabilities did not follow any specific trend.

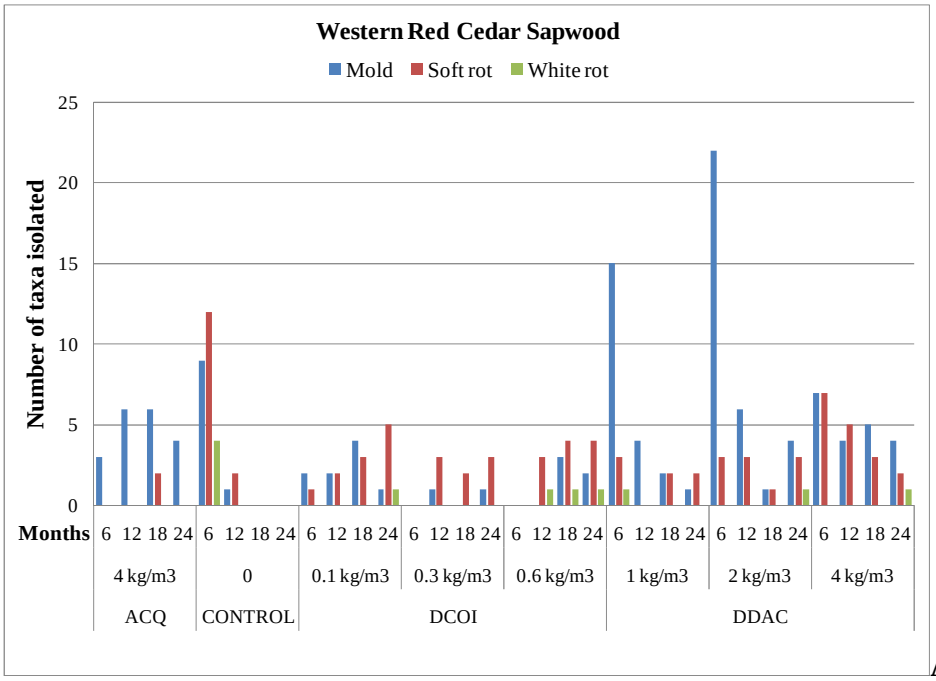


A

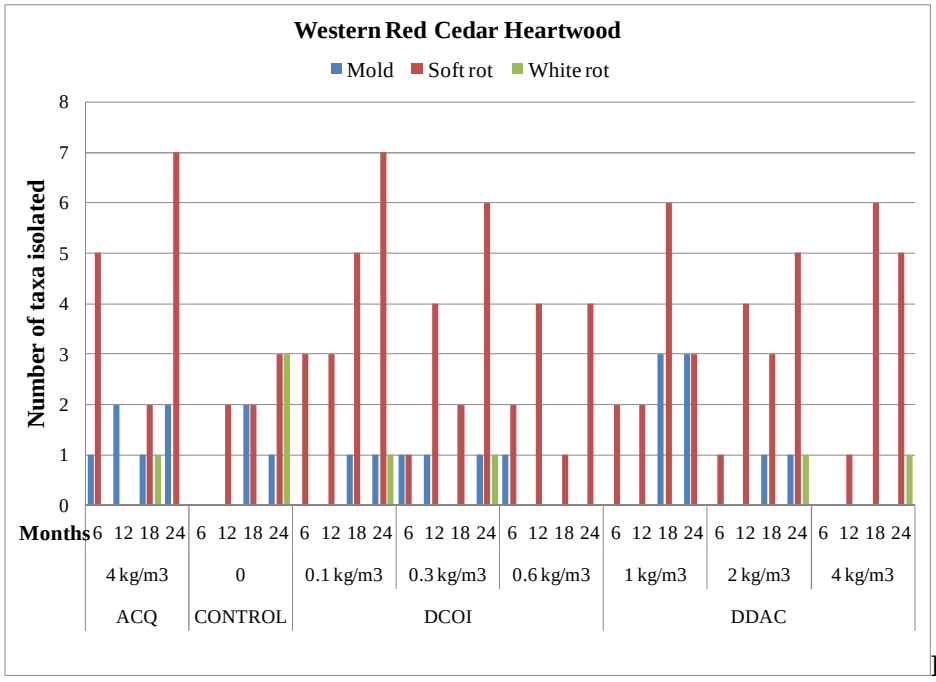


B

Figure 5.4. Number of taxa isolated from treated and untreated sapwood (**A)** or heartwood (**B**) teak samples exposed in a field test in Hawaii for 6 to 24 months, classified according to decay damage capabilities.

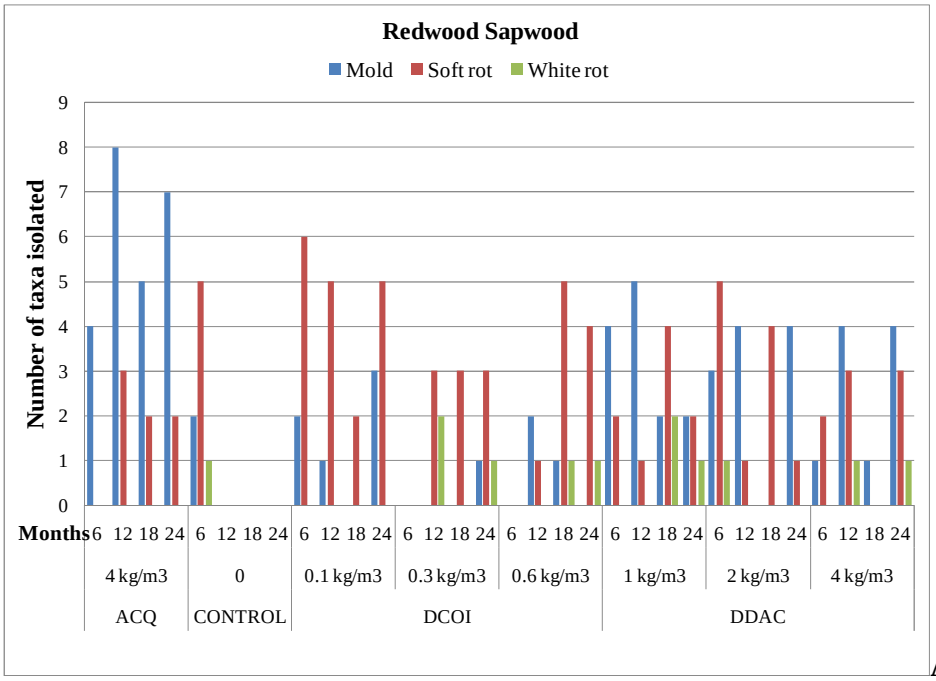


A

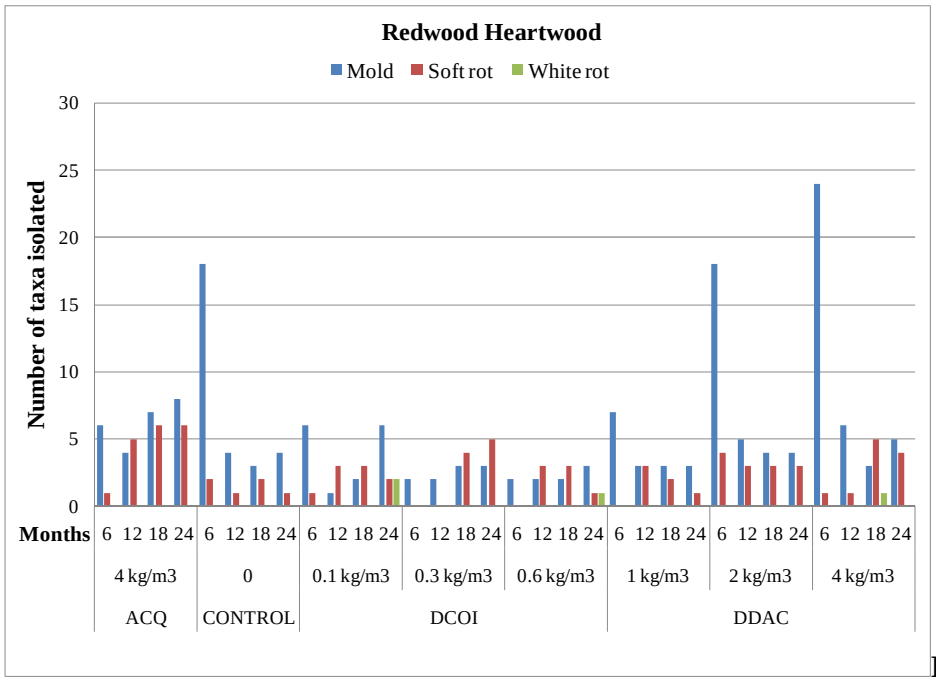


B

Figure 5.5. Number of taxa isolated from treated and untreated sapwood (**A)** or heartwood (**B**) western redcedar samples exposed in a field test in Hawaii for 6 to 24 months, classified according to decay damage capabilities.



A



B

Figure 5.6. Number of taxa isolated from treated and untreated sapwood (**A)** or heartwood (**B**) redwood samples exposed in a field test in Hawaii for 6 to 24 months, classified according to decay damage capabilities.

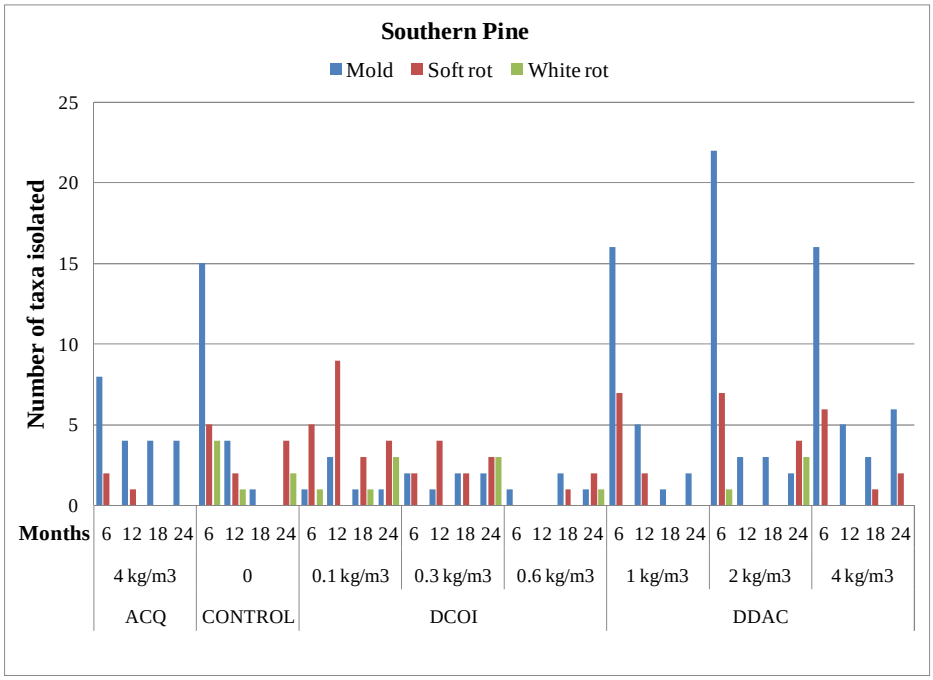


Figure 5.7. Number of taxa isolated from treated and untreated southern pine samples exposed in a field test in Hawaii for 6 to 24 months, classified according to decay damage capabilities.

5.4. Discussion

Wood samples examined in this study supported a diverse mycoflora (Table 5.2). NMS ordination indicated that the mycoflora was apparently structured, i.e., there were specific assemblages of morphotaxa (r^2=73%) and there were subtle community changes over time. However, this apparent structure could not be related to wood species, sapwood/heartwood, or biocide treatment. Fungal structure may have been driven by other factors such wood moisture content, nitrogen concentration on the wood or available sugars.

Overall, species richness (number of taxa) and fungal composition did not differ with wood type, i.e., the fungal assemblages found in each wood type were similar (Table 5.4-5.7). Communities appeared to change over time (Table 5.2; Fig 5.1); however, they

did not change towards specific fungal assemblages (Fig 5.3). Differences in fungal community compositions between 6 and 24 months of exposure were greater than those found between 6-12 or 12-18 months of exposure. Fewer morphotaxa were isolated from teak, western redcedar, and redwood heartwood samples after 6 months of exposure than after 24 months (Table 5.4-5.7). Heartwood extractives and/or anatomy probably delayed the onset of colonization. In contrast, more taxa were isolated after six months than after 24 months in sapwood of the three wood species and in the southern pine samples. This decline suggests the development of more stable communities over time. Observed changes in fungal communities with time were partly due to the influence of species that were isolated once or twice (Fig 5.3). Many species were only isolated after six months of exposure. There were more consistent patterns of association and isolation after 12, 18 and 24 months. At 24 months, most fungal assemblages contained the species putatively identified as *Resinicium friabile.* This species made the communities at month 24 appear to be farther apart than the communities at month 6 (Fig 5.3).

Another possible explanation for the apparent community change over time is that our DNA fungal isolation methodologies and protocols improved with time. For example, DNeasy Plant Mini Kit (Qiagen Sciences, Valencia, CA, USA), or a DNA extraction kit (Epoch Biolabs, Inc., Sugar Land, TX, USA), were used more frequently at months 18 and 24 than at month 6. These kits were able to filter more melanin compounds present in some of the dematiaceous fungi isolated. This allowed more successful identifications of these fungi than the successful identifications achieved when the protocol described by Pomraning *et al.*, 2009, was used.

The abilities of wood structure and chemical composition to influence decay are widely recognized, e.g., wood extractives can limit the growth of fungi. Differences in wood decay rates could be caused by differences in fungal communities (Chen *et al.*, 2002). Wood from the same tree species may, under similar environmental conditions, decompose at different rates depending upon the fungal species present (Ritschkoff,

1996; Vandegrift, 2002). However, results in this study did not indicate a change in fungal communities with wood species. This could be due to the following reasons:

- **Species richness is variable**: Fungal ecology within an ecosystem is often characterized by a few dominant fungal species, and many rare species (Callan and Carris, 2004). A consequence of rarity is a high spatial variation (Stohlgren, 2006). In this study, 52 taxa (66%) were isolated between 1 and 10 times, 12% of the taxa were isolated between 11-20 times, and 9% between 21-40 times (Chapter 4). This indicates a high spatial variation in wood blocks, even the blocks were in close proximity to one another. A taxon isolated once, for example, should have had at least the potential of being present in all of the three wood blocks sampled for the particular wood-treatment combination in which the taxa was isolated.
- **Species richness predictions are inconsistent**: We assumed that species found in a wood block have sufficient resources and tolerable conditions to live and grow there, and, if it is absent it is because of the opposite. However, there are many cases in which this is questionable. For example:
  - Residual populations: a resting spore could be isolated from a particular wood block and grow in culture media. This spore could be left over from a time when the substrate could support the survival and reproduction of the parental fungus. Any change in fungal communities because of changes in the substrate could be difficult to detect if resting spores survive.
  - Sink-source habitats: because of the proximity of the test to the soil, fungi that sporulate heavily (e.g., *Fusarium* species) will be isolated from many wood blocks even if the wood is not the ideal habitat for this species.
  - Dispersal limitation: not all fungi have an equal ability to sporulate and disperse.
- **Experimental design**: As discussed in the previous chapter, the occurrence of fungi in most of the wood blocks might reflect the relative uniformity of the samples (regardless of biocide treatment). However, new ITS morphotaxa accumulated with each additional sample in species accumulation curves (Chapter 4). Based on the

lack of asymptote in these curves, it appears that many fungal species are still to be recovered in the field test.

Spatial variability of fungi colonizing wood makes descriptions of their community structure highly complex. Improving experimental designs through higher replication and more intense cultures of a given sample could reduce the problem to a limited extent. In the present study, the spectrum of isolates recovered was biased because not all fungi growing in the wood samples could grow in the culture medium and some fungi may outcompete others in the culture medium. In addition, fungi were grouped to morphotaxa using microscopic and ITS-BLAST techniques. Additional trials to define the species boundaries might have led to a further increase in species richness. Therefore, the number of fungal species inhabiting the wood samples is very likely to be higher than revealed in the present work. Future monitoring programs, should implement different techniques for identification of species including methods that omit the cultivation step, e.g., terminal restriction fragment length polymorphism (Raberg *et al.*, 2005).

Wood decay rates differed among biocide treatments (Chapter 3) and the ordination of the compiled matrix segregated fungal communities with higher decay scores from those with lower decay scores (R^2=0.1). However, the segregation was not related to biocide treatment (Tables 5.4, 5.5). The result was probably due to an underestimation of fungi capable of producing white and brown rot decay (Chapter 4). In the current study, basidiomycetes, the main wood decomposers, were scattered among the different biocide treatments, and not associated with any particular wood species or treatment (Table 5.4-5.7).

Relationship of this study with testing of wood preservatives: Extended time periods are required to evaluate the performance of new preservative systems. The long exposure times typically required in field tests can be minimized by establishing the tests in tropical environments (Chapter 2). However, there is controversy about the

extrapolation of results from field tests in the tropics to temperate environments. Results from this study show that sixteen basidiomycetes were isolated from the samples (Tables 5.4-5.7, Chapter 4), most of which were white rot fungi. The sixteen basidiomycetes included many fungi that are also found in temperate environments (Table 5.3), suggesting that wood exposed in the tropical environment would produce comparable, but accelerated, performance data when compared with non-tropical environments. Fungal communities in all wood types contained fungal genera with wood decay capabilities across various functional types, i.e., molds, soft rot, and white rot fungi (Fig 5.4-5.7). This indicated that differences in decay rates observed in samples of the same wood type were due to biocide treatments and not to changes in fungal communities and that all samples had the same risk of decay.

5.5. Conclusions

The analysis of fungal communities of decomposing wood of teak, western redcedar, redwood, and southern pine indicated that (i) communities inhabiting different wood types in a ground proximity test in Hawaii were not different; (ii) preservative treatment did not significantly alter wood fungal colonization; (iii) there was a succession of communities from early to late decay stages, but this succession was not directed towards specific fungal assemblages; and (iv) biocide concentrations affected decay rates differentially and most of the wood blocks sampled in this study contained fungi capable of producing soft rot and white rot decay, but not all wood blocks showed signs of this type of attack.

5.6. References

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CHAPTER 6- CONCLUSIONS

6.1. Wood Treatment and Durability

The hypothesis that supplemental treatment with various levels of DCOI or DDAC increases the decay resistance of both sapwood and heartwood of teak, western redcedar and redwood was proven with some, but not all treatments combinations.

Durability of the *sapwood of the three species* was increased by treatment with DCOI at 0.6 kg/m^3 or DDAC at 4 kg/m^3. There were indications that lower concentrations of the two chemicals also improve sapwood durability. However, the observed improvements at lower treatment levels were not statistically significant, probably due to the low number of samples and the high variability among replicates. DDAC appeared to be more resistant to leaching in sapwood samples when wood was treated at 4 kg/m^3 than when it was treated at lower loadings. DCOI impregnated sapwood experienced leaching losses but there was sufficient biocide to prevent decay after 24 months.

Despite the limited uptake of preservative and some leaching over time, some treatments provided improved protection to heartwood samples. *WRC heartwood* durability was improved with 4 kg/m^3 of DDAC. There was a slight improvement in decay resistance for *redwood heartwood* samples treated with DDAC or DCOI, however, longer exposure times will be required to test the effects of biocide treatment on this wood type. *Teak heartwood* durability appeared to be reduced by biocide treatment because little decay occurred on untreated wood of this species while some decay was observed in treated samples. Increased sapwood durability would make it worth treating WRC and redwood lumber containing proportions of sapwood.

While extractive content declined in some heartwood samples after 6 months exposure in the field test, the loss was not related to biocide treatment.

6.2. Fungal Diversity and Community Analysis

Fungal diversity and abundance at the Hawaiian field site were high, but even that observed is likely underestimated. While many fungi were identified, few of these were white or brown rot fungi, even though white rot and brown rot damage was observed in many samples. The inability of basidiomycetes to compete with the mold fungi in the growth medium may have prevented their isolation in the culture media. Exposing samples further from the ground might decrease colonization by mold fungi.

High fungal diversity and abundance implied that the ground proximity test allowed rapid colonization of wood blocks regardless of biocide treatment, which is a positive characteristic of the site for testing the performance of biocide treatments. However, underestimation of species richness limits the understanding of the functional consequences of fungal succession during wood decay.

An analysis of fungal communities of decomposing wood of teak, western redcedar, redwood, or southern pine indicated that the mycoflora was apparently structured, i.e., there were specific assemblages of morphotaxa and there were subtle community changes over time. However, this apparent structure could not be related to wood species, wood type, or biocide treatment.

6.3. Overview, Recommendations, Implications, and Future Research

Wood remains one of the most important structural materials. Wood products are increasingly being replaced by 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, renewable forestry-derived wood products provide considerable potential to fulfill vital needs for structural materials in a sustainable manner. A major goal of the wood industry must be to maximize the competitiveness of wood-based materials. Slowing wood biodegradation rates is an important step in assuring the competitiveness of wood as a construction material.

Historically, naturally durable wood species have been an important resource for construction material. Species harvested from old-growth forests possessed a relatively predictable natural resistance to biodegradability, especially in above ground construction applications. However, the decay resistance of heartwood from trees grown in plantation forests is more variable and generally lower than from old-growth material. More importantly, second 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 or the potential negative impacts of the sapwood are ignored.

This project showed that supplemental treatments enhanced sapwood durability and, to a lower extent, heartwood durability of second-growth timbers of naturally durable species. The increase in sapwood durability imparted by such treatments could upgrade plantation grown material to provide it with a durability profile similar to that historically available from old-growth forests.

The results indicated that it is possible to increase the durability of second growth timbers and therefore their reliability without altering the aesthetic value of the wood and with minimal environmental effects, since low doses of the biocides tested are required and these organic biocides will slowly degrade with time. Supplemental treatment of teak, western redcedar and redwood would maximize their competitiveness.

Wood is usually protected from decay by the application of broad spectrum biocides. Little is known about the organisms that decay wood and their specific roles in the decay process. This makes it difficult to develop biocides with a reduced toxicity spectrum. Studies on fungal microbial ecology are, consequently, necessary to increase our understanding of wood decay processes. Such knowledge is crucial for environmental agencies and biocide developers to understand, dose, and optimize the

use of wood preservatives, as well as to develop new biocides with greater specificity toward wood decay microorganisms.

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APPENDICES

Appendix 1. Decay Rates per Wood Type

Table 1. Decay rates of western redcedar sapwood samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of decay score (X) given to a different numbers of replicates (N); SD: standard deviation.

Biocide		**None**	**ACQ**	**DCOI**			**DDAC**		
Dose (kg/m^3)		**0.0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
6 months	X	9.9	9.9	9.9	9.8	9.9	9.8	9.8	9.9
	sd	(0.12)	(0.09)	(0.12)	(0.26)	(0.00)	(0.21)	(0.16)	(0.09)
	N	20	20	22	22	22	22	22	22
12 months	X	8.7	9.6	10.0	9.9	9.9	9.8	9.7	9.6
	sd	(1.62)	(0.22)	(0.00)	(0.23)	(0.23)	(0.38)	(0.45)	(0.50)
	N	17	17	18	19	19	19	19	19
18 months	X	7.3	10.0	9.2	9.1	9.1	7.9	8.4	9.3
	sd	(3.15)	(0.00)	(0.98)	(1.15)	(1.29)	(1.63)	(1.36)	(1.00)
	N	15	14	16	16	16	18	16	16
24 months	X	5.8	10.0	7.3	7.4	7.9	7.2	7.2	8.4
	sd	(2.79)	(0.00)	(1.44)	(1.40)	(1.04)	(1.90)	(2.41)	(1.39)
	N	13	11	13	15	13	15	13	13
30 months	X	4.7	10.0	7.8	8.8	8.7	6.6	8.6	8.6
	sd	(4.07)	(0.00)	(1.23)	(0.89)	(1.23)	(2.21)	(0.93)	(1.12)
	N	9	8	10	12	10	12	9	10
36 months	X	6.3	10.0	7.3	8.3	9.1	5.5	8.0	9.4
	sd	(3.83)	(0.00)	(1.70)	(2.01)	(1.45)	(1.57)	(2.12)	(1.26)
	N	9	8	10	12	10	12	9	10

Table 2. Decay rates of western redcedar heartwood samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of decay score (X) given to a different numbers of replicates (N); SD: standard deviation.

Biocide		**None**	**ACQ**	**DCOI**			**DDAC**		
Dose (kg/m^3)		**0.0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
6 months	X	9.8	9.9	9.9	9.8	9.9	9.8	9.9	9.9
	sd	(0.22)	(0.09)	(0.09)	(0.22)	(0.09)	(0.14)	(0.12)	(0.09)
	N	20	20	22	22	22	22	22	22
12 months	X	9.6	9.9	10.0	10.0	9.9	9.9	9.9	9.4
	sd	(1.00)	(0.26)	(0.00)	(0.11)	(0.16)	(0.19)	(0.19)	(1.26)
	N	17	17	19	19	19	19	19	19
18 months	X	8.6	10.0	9.3	9.3	9.4	8.8	9.4	9.1
	sd	(1.24)	(0.00)	(1.00)	(1.00)	(1.03)	(1.69)	(0.81)	(1.31)
	N	15	14	16	16	16	16	16	16
24 months	X	8.3	10.0	8.5	8.3	9.0	8.1	8.9	8.5
	sd	(1.44)	(0.00)	(1.45)	(1.80)	(1.22)	(1.26)	(1.19)	(1.33)
	N	12	12	13	13	13	13	13	13
30 months	X	8.2	9.9	7.9	7.9	9.3	8.8	9.3	9.2
	sd	(1.18)	(0.17)	(1.85)	(1.80)	(0.89)	(0.98)	(0.92)	(0.85)
	N	9	9	10	10	10	10	10	10
36 months	X	7.3	10.0	7.3	6.7	7.6	6.4	8.5	9.1
	sd	(1.80)	(0.00)	(1.70)	(2.21)	(1.90)	(1.90)	(1.58)	(2.02)
	N	9	9	10	10	10	10	10	10

Table 3. Decay rates of redwood sapwood samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of decay score (X) given to a different numbers of replicates (N); SD: standard deviation.

Biocide		**None**	**ACQ**	**DCOI**			**DDAC**		
Dose (kg/m^3)		**0.0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
6 months	X	9.6	9.5	9.6	9.7	9.7	9.7	9.3	9.3
	sd	(0.35)	(0.27)	(0.41)	(0.52)	(0.44)	(0.32)	(0.30)	(0.33)
	N	20	20	22	22	22	22	22	22
12 months	X	8.2	9.5	9.1	9.6	9.3	8.9	9.4	9.3
	sd	(1.57)	(0.00)	(0.62)	(0.38)	(0.24)	(0.70)	(0.40)	(0.62)
	N	17	17	19	19	19	18	19	18
18 months	X	6.7	10.0	8.3	8.7	8.6	7.3	6.6	6.7
	sd	(2.58)	(0.00)	(1.18)	(1.08)	(1.15)	(1.40)	(2.71)	(1.79)
	N	14	14	16	16	16	16	17	17
24 months	X	7.8	10.0	7.1	7.1	7.8	7.0	7.6	7.5
	sd	(1.72)	(0.00)	(2.33)	(1.71)	(1.01)	(1.58)	(1.75)	(0.88)
	N	9	11	13	13	13	13	11	13
30 months	X	7.2	10.0	7.9	8.8	8.3	7.2	8.4	9.0
	sd	(1.83)	(0.00)	(1.05)	(0.98)	(1.64)	(2.89)	(2.03)	(0.72)
	N	6	8	9	10	10	9	8	10
36 months	X	7.6	10.0	7.7	8.2	7.9	6.6	7.8	8.2
	sd	(1.34)	(0.00)	(2.50)	(2.10)	(2.02)	(1.06)	(2.66)	(1.55)
	N	6	8	9	10	10	9	8	10

Table 4. Decay rates of redwood heartwood samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of decay score (X) given to a different numbers of replicates (N); SD: standard deviation.

Biocide		**None**	**ACQ**	**DCOI**			**DDAC**		
Dose (kg/m^3)		**0.0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
6 months	X	9.9	9.9	9.8	9.8	9.9	9.9	9.9	9.9
	sd	(0.09)	(0.00)	(0.16)	(0.16)	(0.00)	(0.09)	(0.00)	(0.09)
	N	20	19	22	22	22	22	22	21
12 months	X	9.9	10.0	9.7	9.9	9.9	9.9	9.8	9.9
	sd	(0.33)	(0.00)	(0.35)	(0.16)	(0.32)	(0.32)	(0.37)	(0.32)
	N	17	16	19	19	19	19	19	18
18 months	X	9.4	10.0	8.9	9.3	9.5	9.6	9.8	9.7
	sd	(1.28)	(0.00)	(1.20)	(1.18)	(1.03)	(0.51)	(0.40)	(0.46)
	N	14	13	16	16	16	16	16	15
24 months	X	9.4	10.0	9.1	9.1	9.5	9.2	9.5	9.7
	sd	(0.92)	(0.00)	(1.04)	(1.26)	(1.13)	(1.09)	(0.88)	(0.49)
	N	11	10	13	13	13	13	13	12
30 months	X	9.7	9.9	9.7	9.7	9.6	9.7	10.0	9.9
	sd	(0.37)	(0.19)	(0.41)	(0.42)	(0.90)	(0.34)	(0.16)	(0.22)
	N	8	7	10	10	11	10	10	9
36 months	X	8.9	10.0	10.0	9.7	9.5	9.7	10.0	10.0
	sd	(1.55)	(0.00)	(0.00)	(0.95)	(1.21)	(0.95)	(0.00)	(0.00)
	N	8	7	10	10	11	10	10	9

Table 5. Decay rates of teak sapwood samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of decay score (X) given to a different numbers of replicates (N); SD: standard deviation.

Biocide		**None**	**ACQ**	**DCOI**			**DDAC**		
Dose (kg/m^3)		**0.0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
6 months	X	9.7	9.8	9.6	9.6	9.8	9.7	9.8	9.6
	sd	(0.40)	(0.22)	(0.54)	(0.45)	(0.32)	(0.36)	(0.28)	(0.33)
	N	20	20	22	22	22	22	22	22
12 months	X	8.4	9.1	8.6	8.9	9.4	9.2	9.3	9.3
	sd	(0.94)	(0.22)	(1.12)	(1.34)	(0.21)	(0.78)	(0.59)	(0.65)
	N	17	17	19	19	19	19	19	19
18 months	X	7.4	9.8	6.5	8.1	9.2	8.0	7.5	8.8
	sd	(2.02)	(0.43)	(3.44)	(2.80)	(1.26)	(1.97)	(2.83)	(1.72)
	N	14	14	16	16	18	17	16	16
24 months	X	4.9	9.5	6.5	8.0	8.5	6.1	7.0	7.8
	sd	(3.53)	(0.90)	(3.71)	(2.22)	(2.20)	(3.68)	(1.65)	(2.01)
	N	11	12	13	12	15	14	12	13
30 months	X	3.7	9.6	5.0	7.1	8.8	7.7	8.4	8.5
	sd	(3.14)	(0.39)	(3.96)	(3.65)	(2.25)	(2.22)	(1.10)	(1.76)
	N	6	10	8	9	12	9	9	10
36 months	X	4.8	9.7	6.0	8.1	7.9	5.9	7.0	6.9
	sd	(1.50)	(0.95)	(2.45)	(2.75)	(3.18)	(2.85)	(1.50)	(3.38)
	N	6	10	8	9	12	9	9	10

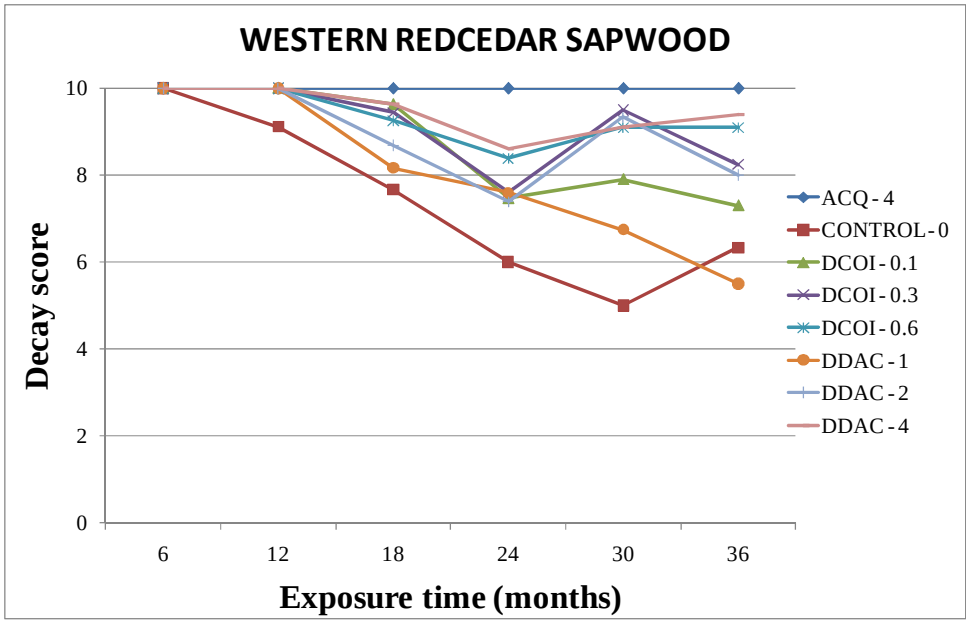
Table 6. Decay rates of teak heartwood samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of decay score (X) given to a different numbers of replicates (N); SD: standard deviation.

Biocide		**None**	**ACQ**	**DCOI**			**DDAC**		
Dose (kg/m^3)		**0.0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
6 months	X	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9
	sd	(0.09)	(0.00)	(0.12)	(0.00)	(0.09)	(0.09)	(0.00)	(0.00)
	N	20	20	22	22	22	22	22	22
12 months	X	9.7	9.9	9.6	9.5	9.6	9.6	9.4	9.5
	sd	(0.40)	(0.23)	(0.93)	(1.12)	(0.93)	(0.94)	(1.09)	(0.90)
	N	17	17	19	19	19	19	19	19
18 months	X	9.3	9.7	9.1	8.9	8.8	9.6	9.3	9.1
	sd	(0.83)	(0.47)	(1.25)	(1.36)	(1.33)	(0.81)	(0.99)	(1.29)
	N	14	14	17	16	16	16	17	16
24 months	X	9.0	9.3	9.1	8.6	8.3	9.5	9.0	8.8
	sd	(1.10)	(1.19)	(0.99)	(1.50)	(1.38)	(0.88)	(1.18)	(1.30)
	N	11	11	15	14	14	13	14	13
30 months	X	9.6	9.7	8.9	8.9	9.5	9.7	9.3	9.5
	sd	(1.06)	(0.46)	(1.19)	(1.19)	(0.42)	(0.35)	(1.17)	(0.44)
	N	8	8	12	12	11	10	11	10
36 months	X	10.0	9.6	8.8	8.5	8.6	10.0	9.2	9.4
	sd	(0.00)	(1.06)	(1.54)	(1.57)	(2.06)	(0.00)	(1.40)	(1.26)
	N	8	8	12	12	11	10	11	10

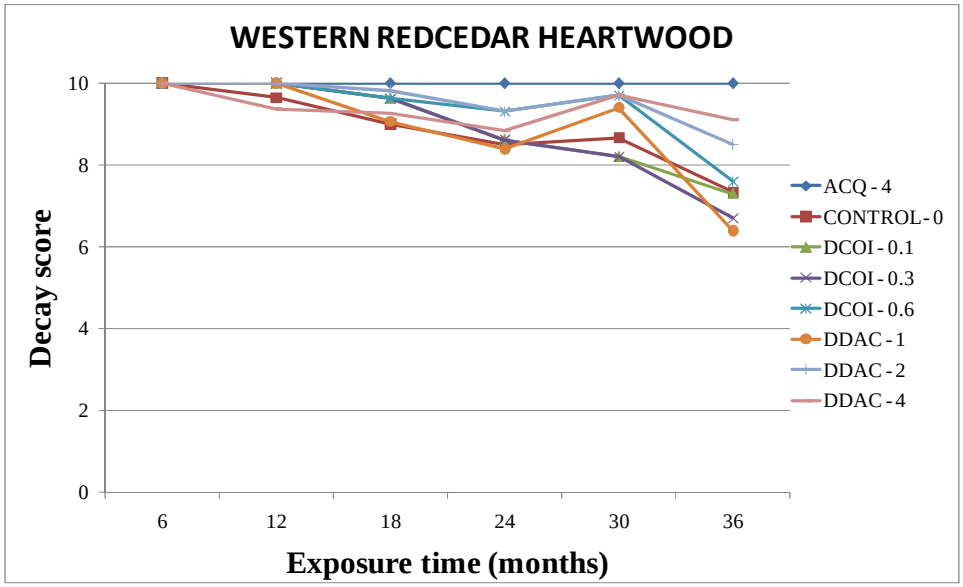
Table 7. Decay rates of yellow pine sapwood samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of decay score (X) given to a different numbers of replicates (N); SD: standard deviation.

Biocide		**None**	**ACQ**	**DCOI**			**DDAC**		
Dose (kg/m^3)		**0.0**	**4.0**	**0.1**	**0.3**	**0.6**	**1.0**	**2.0**	**4.0**
6 months	X	9.7	9.9	9.8	9.7	9.8	9.8	9.8	9.8
	sd	(0.26)	(0.12)	(0.32)	(0.29)	(0.17)	(0.28)	(0.19)	(0.29)
	N	20	20	21	21	21	21	21	21
12 months	X	9.4	10.0	9.0	9.3	9.4	8.5	9.0	9.0
	sd	(0.63)	(0.12)	(1.13)	(1.10)	(0.92)	(1.39)	(1.28)	(1.28)
	N	17	17	18	18	18	18	18	18
18 months	X	7.5	10.0	8.7	9.1	8.7	8.3	8.3	8.2
	sd	(2.38)	(0.00)	(0.99)	(1.19)	(1.40)	(1.35)	(1.44)	(1.21)
	N	14	14	14	15	16	15	15	15
24 months	X	5.3	10.0	7.5	8.3	7.8	7.9	7.5	7.4
	sd	(2.95)	(0.00)	(0.93)	(1.44)	(1.01)	(1.16)	(1.57)	(1.93)
	N	10	11	11	12	13	12	12	12
30 months	X	3.0	10.0	8.6	8.6	8.4	7.2	6.6	7.8
	sd	(3.00)	(0.00)	(0.98)	(0.88)	(0.97)	(1.54)	(2.98)	(1.77)
	N	5	8	8	9	10	9	9	9
36 months	X	1.4	10.0	4.9	6.2	6.4	5.1	5.8	7.0
	sd	(3.13)	(0.00)	(3.18)	(3.15)	(1.90)	(3.41)	(2.55)	(1.50)
	N	5	8	8	9	10	9	9	9

Appendix 2. Graphs of Decay Rates per Wood Type

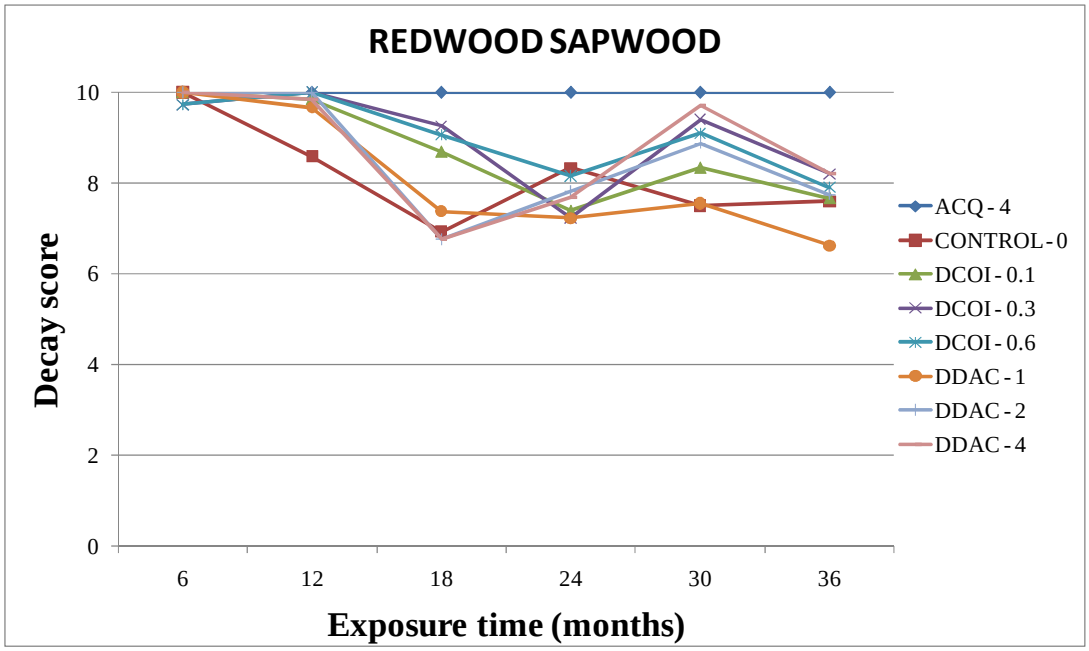


A

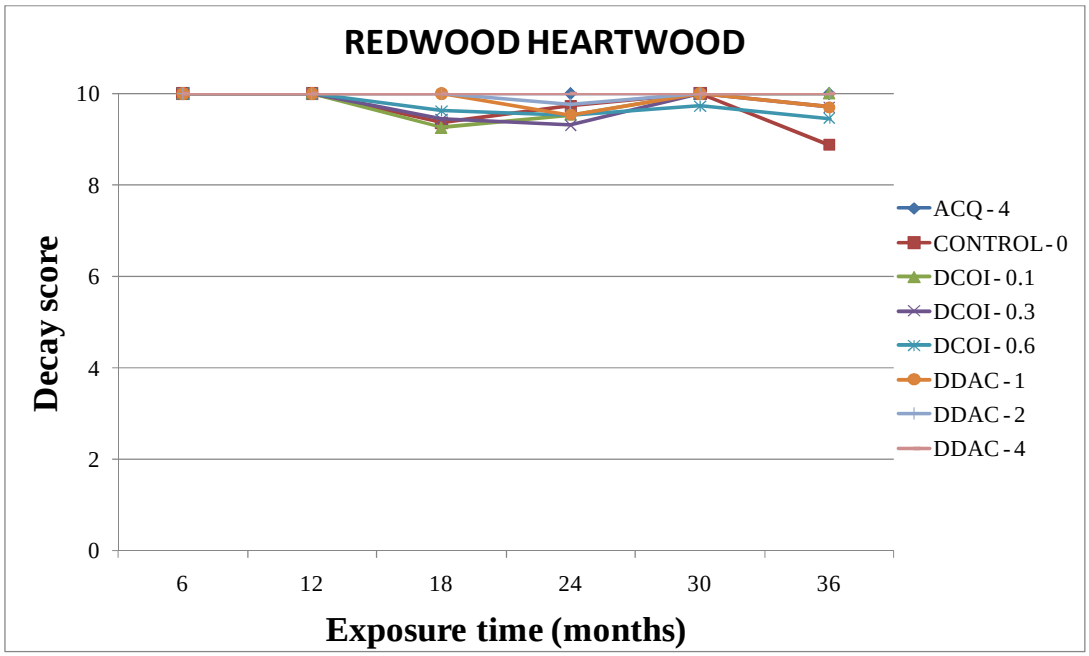


B

Figure 1. Decay rates of western redcedar sapwood (A) and heartwood (B) samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of the decay score given to a different numbers of replicates (see Apendix 1) at six months intervals.

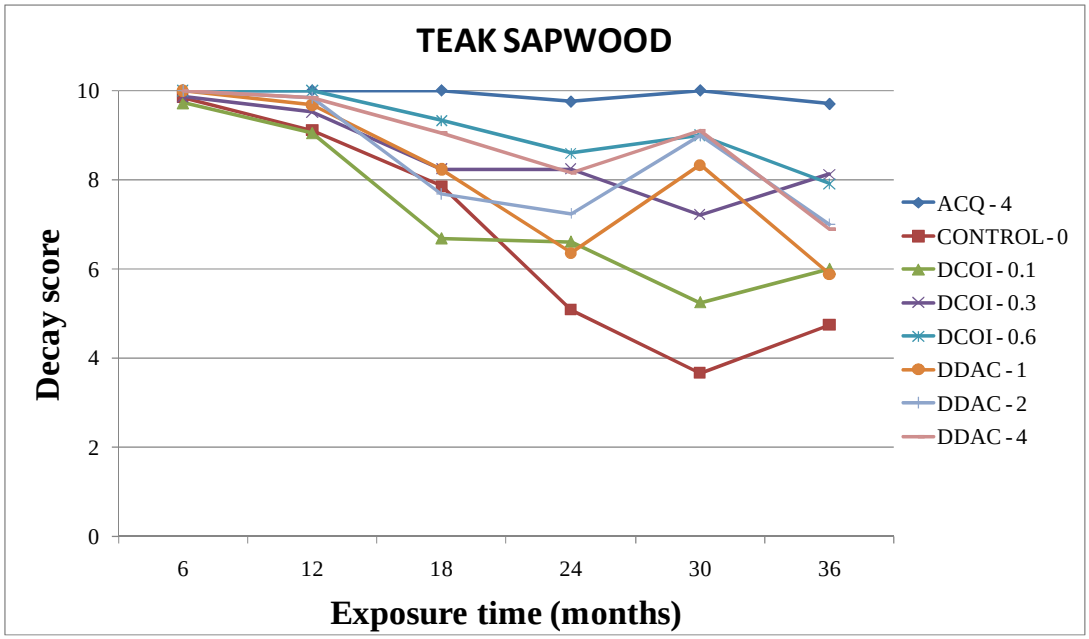


A

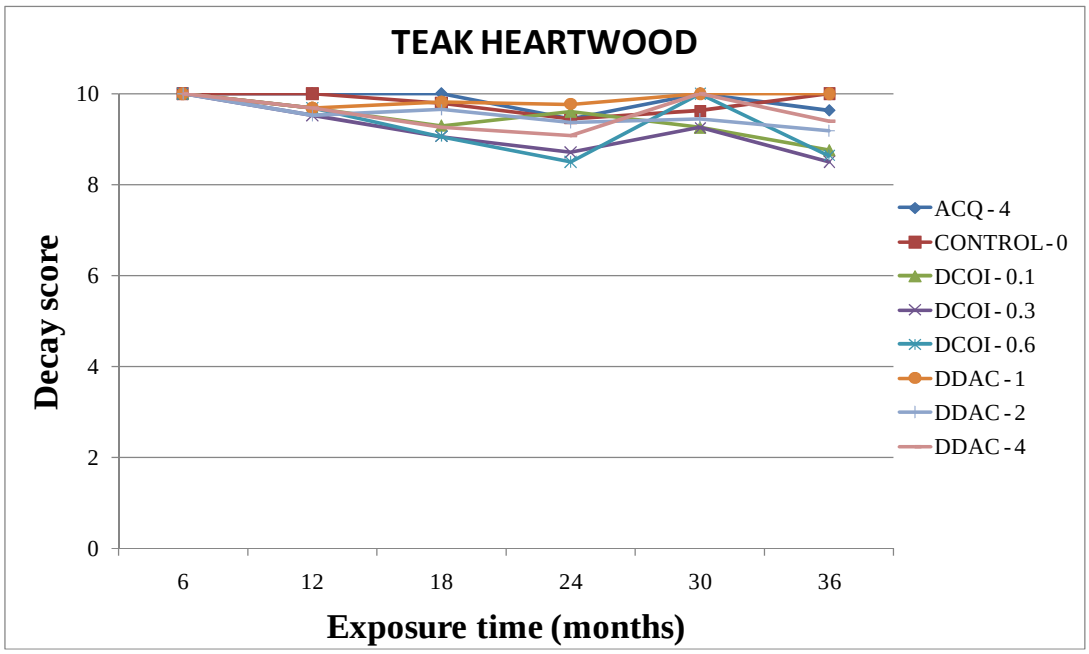


B

Figure 2. Decay rates of redwood sapwood (A) and heartwood (B) samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of the decay score given to a different numbers of replicates (see Apendix 1) at six months intervals.



A



B

Figure 3. Decay rates of teak sapwood (A) and heartwood (B) samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of the decay score given to a different numbers of replicates (see Apendix 1) at six months intervals.

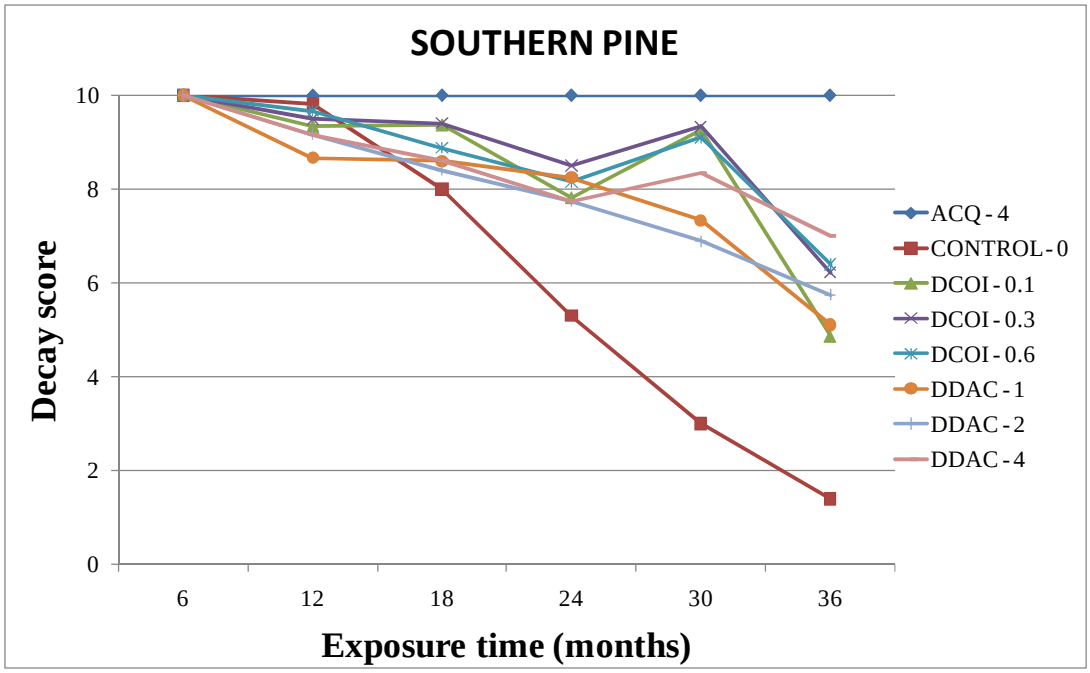


Figure 4. Decay rates of southern pine samples treated with different biocides to selected target retentions (kg/m^3) and exposed in an above ground exposure in Hilo, Hawaii for 36 months. Values represent the average of the decay score given to a different numbers of replicates (see Apendix 1) at six months intervals.

Appendix 3. Number of Fungal Isolates per Wood Type

Number of fungal isolates per wood type, classified according to its wood decay capability and time of collection (months).

Capability / Month	Number of isolates									
	6	**12**	**18**	**24**	**Total**	**6**	**12**	**18**	**24**	**Total**
Redwood	**Heartwood**					**Sapwood**				
Mold	83	27	27	36	**173**	16	24	9	21	**70**
Mold/ Soft rot	11		2	1	**14**	23	14	15	11	**63**
Soft rot	9	19	28	23	**79**	20	17	20	20	**77**
Stain	1	1	3	2	**7**	2	2		1	**5**
Unknown	4	31	21	24	**80**	1	16	13	19	**49**
White rot			1	3	**4**	2	3	3	4	**12**
Total	**108**	**78**	**82**	**89**	**357**	**65**	**76**	**60**	**76**	**277**
Teak	**Heartwood**					**Sapwood**				
Mold	40	30	31	30	**131**	50	34	26	28	**138**
Mold/ Soft rot	4	2	7	8	**21**	6	5	17	23	**51**
Soft rot	8	21	13	18	**60**	30	19	13	11	**73**
Stain	7	6	8	12	**33**	6	12	11	9	**38**
Unknown	1	20	9	17	**47**	4	17	8	10	**39**
White rot	3	1	1		**5**	5	2	6	9	**22**
Total	**63**	**80**	**69**	**85**	**297**	**101**	**89**	**81**	**90**	**361**
Western Red Cedar	**Heartwood**					**Sapwood**				
Mold	3	3	8	9	**23**	58	24	21	17	**120**
Mold/ Soft rot		1	6	10	**17**	28	3	12	15	**58**
Soft rot	14	20	27	40	**101**	26	18	17	19	**80**
Stain	2		3	2	**7**	6			3	**9**
Unknown	2	6	15	15	**38**	5	12	6	14	**37**
White rot			1	7	**8**	5	1	1	4	**11**
Total	**21**	**30**	**60**	**84**	**195**	**128**	**58**	**57**	**72**	**315**
Southern pine	**Sapwood**									
Mold	81	25	17	18	**141**					
Mold/ Soft rot	27	8	16	14	**65**					
Soft rot	34	18	7	19	**78**					
Unknown	6	13	9	15	**43**					
White rot	6	1	1	12	**20**					
Total	**163**	**67**	**50**	**78**	**358**					