Evaluating the Effect of Biodiesel on the Efficacy of the Wood Preservative Copper Naphthenate

by

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Abstract

The efficacy of biodiesel as a co-solvent for copper naphthenate wood preservative treating solutions was evaluated using two fungal decay methodologies (AWPA E10-09, British Standard Method EN113). Four fungal species (*Gloeophyllum trabeum*, *Trametes versicolor, Poria xantha, Postia placenta*) and three wood species (Douglas fir, Southern yellow pine, Western red cedar) with six replicates were utilized in both studies. Two levels of biodiesel: diesel (30:70 and 50:50) were compared to diesel-only solvent systems for copper naphthenate treating systems and treated to AWPA recommended retentions. No differences in decay efficacy between the biodiesel blends and diesel-only treatment in either the AWPA or the EN113 decay studies were detected for either standard method. Copper distribution was evaluated using SEM-EDX and no differences were noted with either solvent system. It was determined that the presence of biodiesel did not have a negative impact upon the efficacy of copper naphthenate as a wood preservative.

List of Abbreviations Used

ATCC	American Type Culture Collection
AWPA	American Wood Protection Association
AX	*Antrodia xantha*
BD	Biodiesel
30/70 BDNS	Copper naphthenate with a blended carrier of 30% biodiesel, 70% diesel without stabilizer
50/50 BDNS	Copper naphthenate with a blended carrier of 50% biodiesel, 50% diesel without stabilizer
100 BDNS	100% biodiesel without stabilizer
30/70 BDWS	Copper naphthenate with a blended carrier of 30% biodiesel, 70% diesel with stabilizer
50/50 BDWS	Copper naphthenate with a blended carrier of 50% biodiesel, 50% diesel with stabilizer
100BDWS	100% biodiesel with stabilizer
100 D	100% diesel only
CCA	Chromated copper arsenate
CML	Compound middle lamella
CuNap	Copper Naphthenate
Dcunap	Copper naphthenate with a carrier of 100% diesel
DF	Douglas fir
GT	*Gloeophyllum trabeum*
NAWPC	North American Wood Pole Council
NS	No stabilizer
PDY	Potato dextrose yeast
PP	*Postia placenta*
SEM	Scanning Electron Microscope
SYP	Southern yellow pine
TV	*Trametes versicolor*
USWAG	Utility Solid Waste Activities Group
UTS	Utility Training Service, Inc.
WRC	Western red cedar
WS	With stabilizer

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Chapter 1.0 Introduction

The treated wooden pole is “the Electric Utilities’ Material of Choice” and forms the backbone of the North American utility industry (USWAG, 2005). In North America there are currently more than 130 million poles in service with millions replaced each year (NAWPC, 2011).

The estimates for the service life of utility poles vary greatly and are dependent upon a number of factors including, but not limited to, the immediate environmental conditions around the pole, maintenance schedule, types of preservatives used, and the micro-fauna in the local environment (NAWPC, 2011). Replacement of an *in-service* pole costs an average of $2500 CAD. This would result in a cost of more than $300 billion CAD to replace all the poles that are currently in service (UTS, 2010). This cost of replacement has provided incentive to maximize the service life of poles and the use of an effective preservative can help to achieve this (NAWPC, 2011).

Poles can be treated with a variety of water-borne and oil-borne preservatives to increase their service life. These preservatives are typically delivered in a carrier system, or solvent. Historically -in North America- the non-aqueous delivery system for oil preservatives was a light petroleum oil blend. This oil was manufactured for a variety of industrial applications. One such application was as a preservative carrier oil because it met the AWPA P-9 technical specifications,

which earned it the industrial reference of ‘P-9’ oil (AWPA, 2010). However, this oil is no longer produced due to a reduced demand. Therefore, wood treaters have had to find alternative oil solvents (Murray, 2011). Diesel fuel has been used as a P-9 substitute but it has a number of issues associated with it. One such problem is a lower flash point, which can have implications for safe treating and handling (Encinar *et al*., 2005; Ramadhas *et al.,* 2005). In addition, when used as a solvent with certain oil preservative systems, the resulting products exhibit very strong odors. The addition of biodiesel (oil derived from fat sources) at a level of 30% could be a possible solution to these issues. The use of biodiesel at this level not only significantly reduces odor (Murray, 2011) but also increases the flash-point resulting in safer handling and storage of the preservative mixture (Encinar *et al*., 2005; Ramadhas *et al.,* 2005).

One such preservative mixture that would benefit from the addition of biodiesel to the carrier solution is copper naphthenate. Copper naphthenate has been marketed as a more friendly wood preservative due to its low mammalian toxicity (Freeman, 1992; United States Environmental Protection Agency, 2007) and has been noted to provide adequate protection from fungal degradation due to the presence of organometallic compounds (Freeman and McIntyre, 2008). The presence of these compounds also gives copper naphthenate preservative mixtures strong odors. The addition of biodiesel to the carrier solution may result in a more attractive and marketable product.

The use of biodiesel in the wood preserving industry has been controversial due the fact that the limited research performed to date has had conflicting results (Morrell *et al.,* 2010; Langroodi, *et al.,* 2012a; Langroodi *et al.,* 2012b). Previous research on the environmental fate of biodiesel has shown that biodiesel may be more readily decayed by microorganisms than the petroleum based diesels (Zhang *et al.,* 1998). However, it has also been indicated by Taylor and Jones (2001) that the addition of biodiesel to naphthalene-containing soils, while enhancing the remediation capabilities of the microorganisms, causes less deterioration of naphthalene than when compared to the addition of petroleum diesel to the soil. In soil block decay trials, it has been indicated by Langroodi *et al.,* (2012b) that the addition of biodiesel as a co-solvent had no impact on the preservative's efficacy. However, Morrell *et al.* (2010) indicated that in copper naphthenate preservative systems, the addition of biodiesel as a co-solvent can increase the susceptibility of the treated wood to decay.

While increased susceptibility to decay may be advantageous when remediating treated wood that has been removed from service, it is postulated that this characteristic may cause the service life of the treated products -in particular those that contain copper naphthenate- to be reduced. Therefore, due to the uncertainty of the effect of biodiesel on preservative function when used as a co-solvent with petroleum diesel, further research was warranted.

1.1 Objectives

The overall goal of this study was to evaluate the effect of biodiesel, when used as a co-solvent with diesel, on the efficacy of a copper naphthenate wood preservation treatment. This goal was achieved by employing wood decay testing using several different base media as well as utilizing the Scanning Electron Microscope (SEM) to determine any effect on the preservative penetration due to the presence of biodiesel. The specific objectives of the project were:

(i) To investigate the use of biodiesel as a co-solvent with diesel upon the efficacy of copper naphthenate against wood decay caused by four specific wood decay fungi (including copper tolerant fungi).

(ii) To investigate the effect of propyl gallate on the efficacy of copper naphthenate against wood decay caused by four specific wood decay fungi (including copper tolerant fungi) when biodiesel was used as a co-solvent with diesel.

(iii) To investigate the use of biodiesel as a co-solvent with diesel on the distribution of copper naphthenate within the cell structure of wood.

Chapter 2.0 Literature Review

Wood has been an important resource to mankind throughout history. Since *Homo erectus* learned to control fire more than 300,000 years ago, wood has been used as a fuel source to cook food and provide heat (Roebroeks and Villa, 2011). As the species evolved to the Neolithic era, the role of wood in society evolved as well. In addition to fuel, it is also used to create tools, weapons, furniture, boats, and other wood structures (Muller, 2012; Tegel *et al.,* 2012; Fibiger *et al*., 2013). The versatility of wood helped to shape our society and remains an important resource today.

Many modern products are made of wood, however, wood is also the carbon source of choice for many organisms (Gilbertson, 1980). These organisms can inflict costly damage to wood structures, making them unsafe and requiring maintenance or replacement. To combat this damage and increase product longevity, a large variety of wood preservatives have been developed (AWPA, 2010). In order to gain a better understanding of how these organisms damage wood products and how wood preservatives stop or reduce the attack, a basic understanding of wood structure is essential.

2.1 Wood

Wood typically refers to the tissue that is harvested from the trunks and branches of trees and shrubs, and is comprised of many different components that serve a

variety of physiological purposes within the living tree (Wiedenhoeft and Miller, 2005). The outer bark provides protection from physical damage to the interior structures of the tree (Delvaux *et al*., 2010), while a portion of the inner bark - referred to as the secondary phloem- serves to transport the products of photosynthesis (sugars) from the needles or leaves to the rest of the tree (Magel *et al.,* 2000). Next to the inner bark is the vascular cambium (Philipson *et al*., 1971). The vascular cambium is unique as it consists of a single layer of embryonic cells. These cells grow and differentiate into either the bark of the tree or become the xylem, or wood, of the tree (Philipson *et al*., 1971).

The xylem of the tree can be further classified as either sapwood or heartwood (Pinto *et al.,* 2004). The sapwood is located next to the vascular cambium (Figure 2.1). In the living tree, sapwood cells that are closest to the vascular cambium serve to transport liquids (such as water and sap), but sapwood cells also function to store starches (Myburg and Sederoff, 2001; Pinto *et al.,* 2004). The sapwood can vary in thickness depending on the species of tree (Ryan, 1989). Moving towards the interior of the tree, the next portion of xylem is referred to as the heartwood. The development of heartwood is a natural transition of living sapwood cells to the nonliving cells of heartwood. Cellular extractives accumulate in these cells and this can cause the heartwood in some species to become much darker than the surrounding sapwood (Pinto *et al.,* 2004). While the heartwood is considered non-living, it is theorized that its presence still aids the living tree in several ways. The sapwood portion of the living tree demands

high levels of energy reserves, so it is theorized that the presence of heartwood helps to conserve energy while still contributing strength and structural support to the tree (Banber, 1976). These extractives, such as phenolic compounds, that are present in the heartwood also lend a natural durability to this tissue helping to reduce attack by wood decaying organisms (Taylor *et al.,* 2002). Moving from the heartwood to the interior of the tree is a group of parenchyma cells referred to as the pith. These cells develop during the first year of growth of the tree (Fritts, 1976).

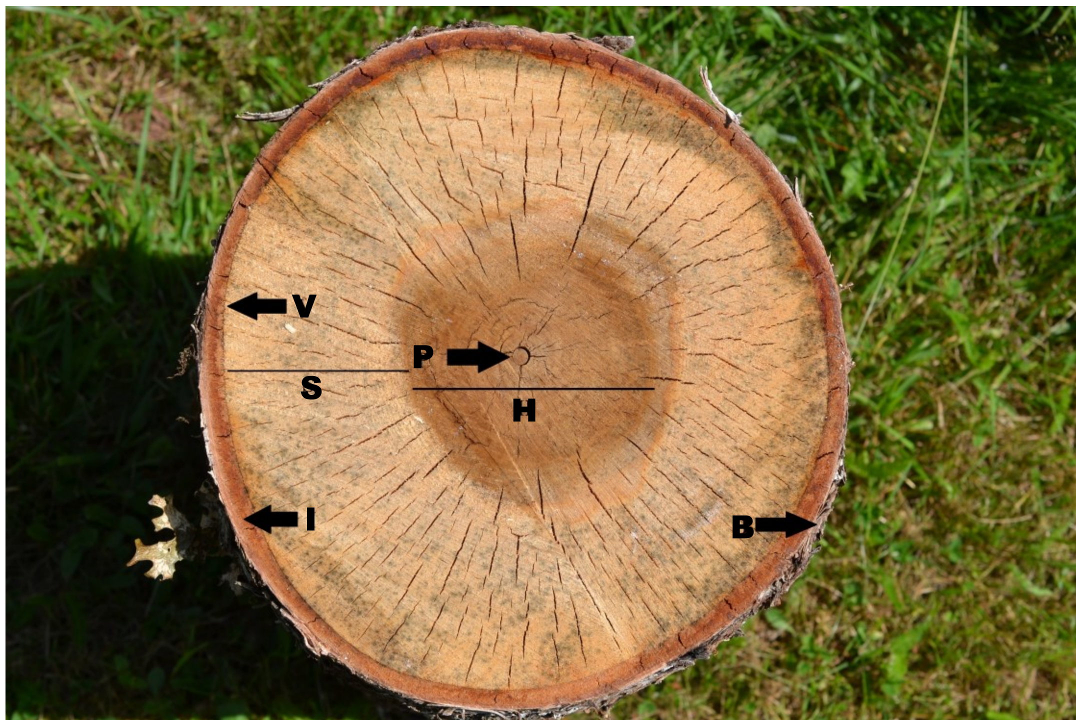

Figure 2.1: Cross-section of a tree illustrating the (B) outer bark, (I) inner bark, (V) vascular cambium, (S) sapwood, (H) heartwood, and (P) pith. Scale- tree diameter is 30 cm.

When looking at the cross section of the trunk of a tree -in addition to viewing the heartwood and sapwood- the presence of growth rings will also be observed. In some species these rings may not be as clearly defined. Each ring represents a year of growth (Stokes and Smiley, 1996). The interior part of the growth ring section is referred to as early wood (Figure 2.2). The cells in this region are formed during the beginning of the growing season and possess large cell lumens and thin cell walls (Plomion *et al.,* 2001). The cells that are formed at the end of the growing season are referred to as late wood. These cells possess much thicker cell walls and smaller lumens. The transition from early to late sapwood can be abrupt or it can be gradual. In softwood (such as Southern yellow pine and Douglas fir) and ring porous hardwoods (ash, oak, and elm) have very clear contrast between early and late wood (Harada and Cote, 1985).

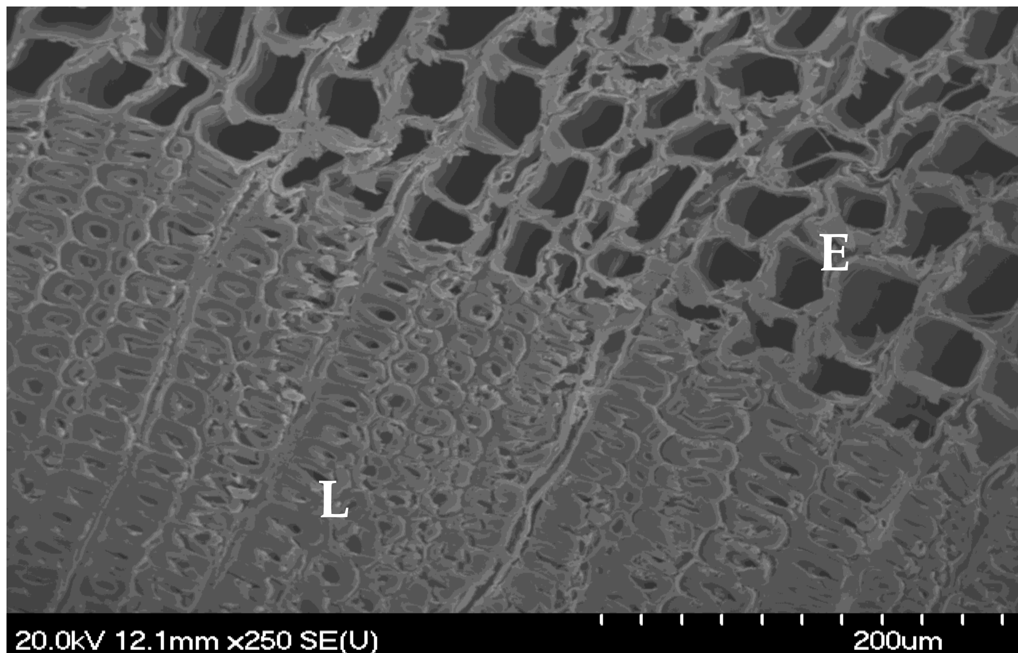

Figure 2.2: Scanning electron microscope image of a Douglas fir tracheid cross-section showing the early wood (E) and late wood (L) tracheid cells

Xylem cell structure can be divided into the two main categories of softwood and hardwood. Gymnosperms, (e.g. Southern yellow pine, Douglas fir, and Western red cedar) are classified as softwoods, while angiosperms, (e.g Red maple and Yellow birch) are classified as hardwoods (Wiendenhoef and Miller, 2005). The cellular structures of softwood are less complex than their hardwood counterparts in that they possess only two basic cell types: axial tracheids and parenchymal ray cells (Figure 2.3) (Harada and Cote, 1985). This design results in a more homogenous structure. The elongated structure of tracheid cells function in supporting the wood of the gymnosperm as well as transporting water and dissolved minerals from the roots upwards to the needles of the tree (Smith, 1993; Sperry *et al.,* 2006). Parenchymal ray cells are brick-like in shape,

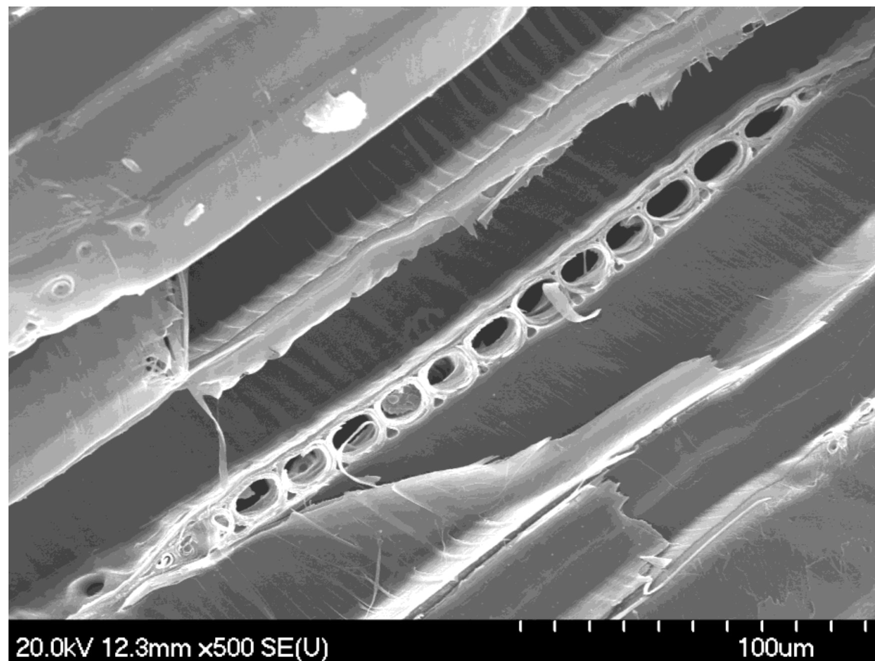

Figure 2.3: Scanning electron microscope image of a parenchymal ray cell, taken at 500x magnification

form the rays found in softwoods, and are responsible for the production of a variety of biochemicals (Figure 2. 3). The parenchymal cells also serve as storage cells and provide lateral transport of biochemical products (as well as small amounts of water) within the xylem (Wiedenhoef and Miller, 2005).

Angiosperms have more complicated and varied cell types (Sperry *et al.,* 2006). They possess four basic types of wood cells: fibres, vessels, axial parenchyma, and ray cells. Fibres serve to provide mechanical support for the hardwood tree, much like a softwood tracheid; however, they are much shorter in length than a tracheid cell (Wiedenhoef and Miller, 2005; Rodriguez *et al.,* 2011). Fibres also differ in shape, the fibre cross-section is round, whereas the tracheid cell cross-section tends to be square in appearance (Sperry *et al.,* 2006).

Angiosperms have also developed specialized conduction cells, referred to as vessels (Sperry *et al.,* 2006). These cells are much larger than any of the other types of cells and can be arranged in different patterns within the wood. Arrangement of vessels spread evenly through the sapwood is referred to as diffuse-porous, while in some angiosperms the vessels can be arranged in a ring porous fashion (Myburg *et al.,* 2001). In ring porous angiosperms the vessels that are located in the early sapwood are much larger and more concentrated while only small vessels are located in the late wood of the tree (Hitz *et al*., 2008).

The axial parenchyma is a cell type that is rarely found in softwoods. In hardwoods, however, they are found in abundance and in specific patterns (Wiendenhoeft and Miller, 2005).These thin-walled, living cells are typically situated around gum canals and ray cells, serve to transport sugars to the network of parenchymal cells within the xylem, and are responsible for the storage of starch (Carlquist, 2007).

2.1.1 Pits

The cells of both the angiosperm and the gymnosperm require the ability to communicate with each other by transporting products between cells, and coordinating functions within the living tree. This is achieved via modifications of the cell wall referred to as a pit (Jansen *et al*., 2009). There are a variety of pit types, including simple pits, bordered pits (Figure 2.4), and half bordered pits, which all contain common elements such as a pit membrane, pit aperture, and pit chamber (Kolavali, 2013). The semi-porous remains of the primary wall comprise the pit membrane. The composition of the pit membrane is intriguing as it is comprised of a carbohydrate membrane as opposed to the typical phospholipid membrane common to many cells (Jansen *et al*., 2009). The pit aperture is appropriately described as it is the opening leading into the open space of the pit: the pit chamber (Phillips, 1933; Wiendenhoeft and Miller, 2005).

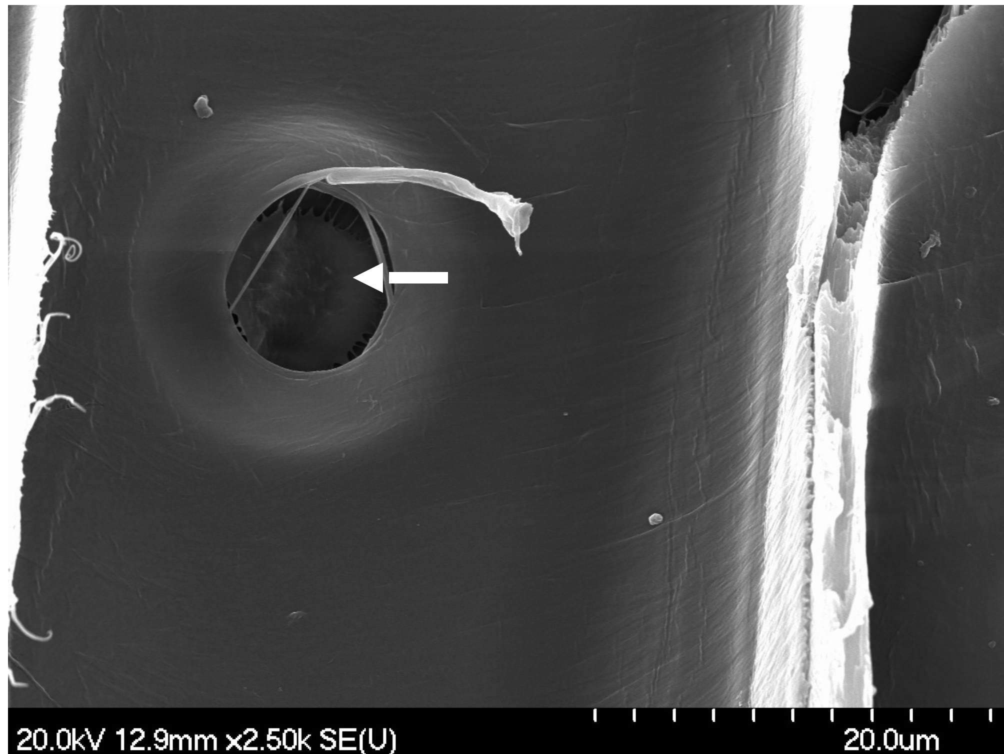

Figure 2.4: Scanning electron microscope image of a bordered pit from a Douglas fir wood cell. The web like structure within the bordered pit is referred to as the pit membrane (arrow).

Pits will typically form in what is referred to as a ‘pit pair’. The pits are formed separately in adjacent cells, and will form in a location so that the pit structure from one cell will align with the adjacent cell, forming a ‘pit pair’ (Wiendenhoeft and Miller, 2005). It is very uncommon for a ‘blind pit’ (a singular pit with no corresponding pit in an adjacent cell) to be formed in wood (Dute and Rushing, 1987). Where these pits are located in the cells and how they are formed determine what type of pit will be present.

Bordered pits are the most complex of the pit types and always form between conducting cells (Wiendenhoeft and Miller, 2005).They will, on occasion, form between other thick-walled cells. The border of the pit is created by the cell wall arching partially over the opening of the pit, giving it a raised round shape, similar to an inner tube (Jansen *et al*., 2009) (Figure 2.4).

Half bordered pits are essential to the living tree. These types of pits are located between a parenchymal cell and a conducting cell, which allows the living tree to communicate between the living cells of the parenchyma and the conducting cells of the tree (Côté, 1963).

Simple pits are often found between parenchymal cells (Baas and Rijksherbarium, 1986). They lack a border around the pit aperture and are simply straight-walled. Their pit pairs are congruent in formation (Baas and Rijksherbarium, 1986).

2.1.2 Cell Wall

Despite the differences in cell types, the walls of the cells share a similar construction and are comprised of three main regions: the middle lamella, the primary wall, and the secondary wall (Figures 2.5) (Booker and Sell, 1998). The middle lamella is the outer most layer of the wood cell and permits the cells to adhere to one another in an organized fashion, resulting in a unified structure and function to create the living tree (Kretschmann, 2003). The middle lamella in

non-woody cells contains pectin, however, it is highly lignified in woody cells (Rioux *et al*., 1998).

The region adjacent to the middle lamella is the primary wall, which is the first layer to be secreted. It is thin and flexible during cell growth and is comprised mainly of cellulose fibers randomly arranged in a hemicellulose and pectin matrix (Plomion *et al*., 2001). As the cell completes the growth phase, the primary wall becomes lignified and difficult to distinguish from the middle lamella. As such, the combined structures are often referred to as the compound middle lamella (Meshitsuka and Nakano, 1985).

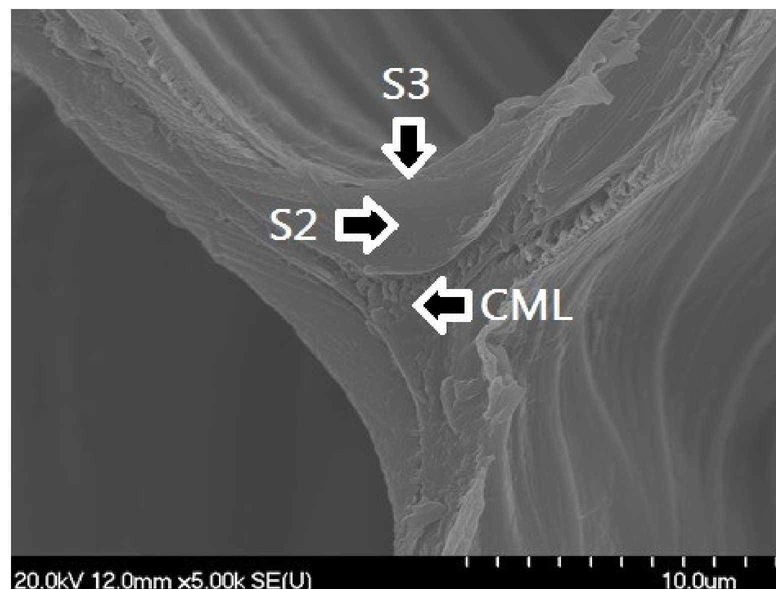

Figure 2.5: The corner of a tracheid cell wall showing the S_2 layer, S_3 layer and the compound middle lamella (CML). See text for explanation.

The third region is the secondary wall region and it is comprised of three separate layers, each with its own distinct construction (Yano *et al*., 1997). The first secondary wall layer is the S_1 layer. This is a relatively thin layer, comprising only 10-15% of the secondary wall region thickness and possesses a high percentage of lignin (Donaldson, 1994). The next layer in the secondary wall is the S_2 layer. This area represents approximately 85% of the secondary wall thickness and imparts the majority of the characteristics to the cell wall, and in turn to the properties of the resulting timber (Gierlinger and Schwanninger, 2006). The S_2 layer also possesses a high percentage of the cell's cellulose (Barnett and Bonham, 2004). The third layer in the secondary wall is the S_3. This region is very thin in comparison to the S_1 and S_2 layers, making up only 5% of the secondary wall thickness (Brandstrom, 2001). The S_3 layer contains very little lignin and is comprised mainly of hemicellulose and cellulose (Zhong and Ye, 2009).

While wood cells may be variable in size, shape, and function, on a macromolecular level they contain three macromolecules that are considered to be the building blocks of the wood cell: cellulose, hemicellulose, and lignin (Pérez *et al*., 2002). Cellulose, the most common organic compound on Earth, is also the most plentiful polysaccharide in wood, making up approximately 50% of the wood's composition by weight (O'Sullivan, 1997). Cellulose is produced by the polymerization of glucose units linked together in a β(1-4) formation. In wood, the number of glucose units present in a cellulose molecule can vary from 7,000 to 15,000 units in length (Di Lazzaro *et al.,* 2010) and approximately 70% of the

cellulose in wood is found in the crystalline form (Bertran and Dale, 1986). Cellulose is responsible for giving wood its strength and is very resistant to tensile force (Hinterstoisser *et al.*, 2003). This strength is due to the covalent bonding within the pyranose ring (Hinterstoisser *et al*., 2003).

Hemicellulose, by comparison, has far less strength than cellulose. It has a tendency to form a gel-like layer around cellulose fibers in the wood cell (Dahlman *et al*., 2003) due to its low level of polymerization and poor ability to form a crystalline structure (Barrañon, 2006). This is due to the fact that hemicellulose is a far more heterogeneous polysaccharide than cellulose. Unlike cellulose, hemicellulose contains not only glucose, but also mannose, galactose, xylose, arabinose, 4-O- methylglutaconic acid, and galacturonic acid (Barrañon, 2006). The polymers that are formed are also shorter than that of cellulose; the chains formed are typically between 500-3,000 saccharide units in length (Di Lazzaro *et al.,* 2010). The heterogeneity of hemicellulose also tends to cause the structures to be branched and the bonds that are formed between the heteropolymers can be easily hydrolyzed (Jacobsen and Wyman, 2000). While hemicellulose may have less strength than cellulose it appears that it may be the connecting material between cellulose and lignin (Andersen *et al*., 2008).

Lignin is a large, three dimensional phenylpropanol polymer that has the ability to covalently bond with hemicellulose (Wang *et al*., 2009). It is also a hydrophobic structure. By bonding with hemicellulose lignin is able to act as an encrusting

agent thereby limiting the exposure of the carbohydrate fraction of the wood cell to water. This protects the hydrogen bonds from water thereby inhibiting the interference of the bonds and leaving the tensile strength of the polymers intact (Reid, 1995). The composition of lignin will vary depending upon the wood species that synthesizes it. In softwoods, lignin is derived from the polymerization of coniferyl alcohol and is known as guaiacyl lignin (Minami and Saka, 2003). In hardwoods, syringyl units are formed (from sinapyl alcohol) as a copolymer with guaiacyl units (Minami and Saka, 2003).

2.2 Degradation

In the environment dead or dying trees are recycled by incorporating their components back into the carbon cycle (Gilbertson, 1980). While this may be advantageous in a natural setting, it is not as advantageous with regard to the commercial applications of wood such as buildings, structures, tools, and furniture. In order to be able to combat deterioration, it is important to understand these processes and to be able to intervene with effective methods which will increase product longevity.

Degradation of wood can be broken down into two main categories: abiotic and biotic. Abiotic refers to the non-biochemical and physical factors that result in wood decay. Weathering, while primarily cosmetic, causes unprotected wood to undergo slow chemical and physical degradation (Zabel and Morrell, 1992). It is primarily caused by exposure to ultraviolet light and oxidation reactions on the

surface of the wood, however, environmental factors such as seasonal freezing and thawing can also contribute to weathering of wood. Wood that has undergone several years of weathering will have the characteristic gray color as well as a rough texture. While the effects of weathering can be easily visualized, it typically causes very little effect on the strength of the wood (Zabel and Morrell, 1992). Mechanical wear can contribute to decay as well as causing breakage and erosion of the surface of wood. Thermal decomposition via distillation or burning can also affect the structure of wood. Chemical decomposition, while not as common, can occur either through exposure to strong acids, strong bases, or strong oxidizing agents (Zabel and Morrell, 1992). Mechanical destruction can also occur, such as boring and surface rasping by marine borers or tunneling and excavation by insects such as termites, boring beetles, and carpenter ants.

Biotic degradation can be caused by bacteria etching and tunneling wood cell walls, by surface molds, and sapwood staining fungi (Zabel and Morrell, 1992). While there are many types of biotic deterioration, such as the breakdown of cellulose by the microbes within the gut of termites, fungi are the main category of micro-organisms that actually cause decay of wood through enzymatic breakdown of the wood cell components (Keilich *et al,* 1970).

2.2.1 Decay Fungi

Wood decay fungi are one of the primary concerns when dealing with wooden structures since they can cause structural failure within a short period of time. A

large variety of wood decay fungi exist, however, most require moisture, oxygen, and specific temperature and pH ranges, in order for optimum decay to occur. If these requirements are not met, then decay will not proceed. While all wood decay fungi have similar aspects, they can be categorized into three main functional groups characterized by the type of decay that they produce: soft-rot, brown-rot, and white-rot.

2.2.1.1 Soft-Rot Fungi

Soft-rot in wood is typically caused by fungi that belong to the phylum *Ascomycota* (Bouws *et al*., 2008). The decay that is produced by soft-rot fungi is quite unique in comparison to other wood rotting fungi. Wood that has been exposed to soft-rot fungi will exhibit a brown, soft exterior that is prone to cracking and checking once it is dry (Jacobsen and Wyman, 2000). On a cellular level, degradation can be categorized as either type I or type II decay (Boer *et al*., 2005). With type I decay, the fungi produce biconical or cylindrical cavities via hyphal tunneling through the lignified portions of the wood cell wall. This type of decay can degrade cellulose, hemicellulose, and lignin (Boer *et al*., 2005). Type II decay is a result of the fungi eroding the secondary wall of the wood cell. It originates within the interior of the cell (within the lumen) and then progresses through to the middle lamella (Boer *et al*., 2005). Even in the advanced stages of decay, the middle lamella may be the only portion of the wood cell that remains intact while the secondary wall may be totally degraded (Blanchette, 2000). This

is most likely due to the type II soft-rot fungi's limited ability to degrade lignin (Tuomela *et al.,* 2000).

Soft-rots have also adapted the unique ability to grow in environments with limited oxygen (such as aqueous environments). While this ability makes soft-rot fungi a major concern for wooden structures such as boats, pilings, and wharves, brown- and white-rot fungi are of concern for wooden products that are located within non-aqueous environments (Kim and Singh, 2000).

2.2.1.2 Brown-Rot Fungi

Brown-rot fungi refer to a portion of the fungi belonging to the phylum *Basidiomycota (*Blanchette, 1995). Brown-rots are typically the most destructive of the three types of rots because they cause a drastic reduction in the bending and impact strength of wood (Green and Highley, 1997). This is accomplished via the preferential degradation of cellulose and hemicellulose while leaving behind the slightly modified lignin (Filley *et al*., 2002). The modified lignin left behind is very brittle, will crack into cubes, and eventually crumble into a powder. This lignin also imparts the characteristic brown color and consistency associated with wood decayed by brown-rot fungi (Goodell, 2003).

Brown-rot fungi begin their attack on the wood cells by first entering the ray cells. They then progress into the longitudinal tissues using microhyphae to pass through the pits of the wood cells allowing them to gain entry into the cell lumen

(Green and Highley, 1997). Once here, the hyphae continue to grow in close contact with the outer portion of the secondary layer: the S_3 layer (Illman, 1991).

In the early stages of brown-rot decay, however, the exact mechanism of entry into the wood cell has not been clearly determined. It is thought that the brown-rot fungi utilize hydroxyl radicals [·OH] from the Fenton reaction [$H_2O_2 + Fe^{2+} + H+ \rightarrow H_2O + Fe^{3+} + \cdot OH$] to penetrate the wood cell wall, which begins the enzymatic process of cellulose and hemicellulose degradation (Green and Highley, 1997).

The hemicellulose components of the wood cell are degraded first resulting in a significant loss in strength of the wood cell (Curling *et al.,* 2002). As previously mentioned, hemicellulose has a tendency to form a gel-like envelope around cellulose fibers. The loss of this protective and supportive gel contributes to an increase in wood cell porosity (Green *et al.,* 1991). This allows the cellulose component of the wood cell to become accessible to the larger cellulolytic enzymes allowing the cellulose to be hydrolyzed (Green *et al.,* 1991). Oxalic acid, which is produced by certain brown-rot fungi, plays a role in wood cell degradation by lowering the pH of the surrounding tissue, as well as in aiding with the de-polymerization of hemicellulose and cellulose (Green *et al.,* 1991). The production of oxalic acid by certain species of brown-rot has given these fungi the unique ability to tolerate certain metals that would otherwise be toxic (Gadd,1993; De Groot and Woodward, 1999).

If an organism is able to endure exposure to a toxin or an unfavorable environmental factor, it is then defined as tolerant. Some species of brown-rot fungi, such as *Postia placenta* and *Antrodia xanthan,* can be extremely tolerant to metals (ie. copper) that would normally be toxic to fungi (Green and Clausen, 2003; AWPA, 2010). It is thought that the drop in pH caused by the production of oxalic acid results in the precipitation of copper into insoluble copper oxalate crystals resulting in an innocuous copper metabolite (Green and Clausen, 2003).

2.2.1.3 White-Rot Fungi

White-rot fungi are a functional grouping of wood degrading basidiomycetes that have the ability to degrade lignin completely to carbon dioxide and water, referred to as mineralization (Ten Have and Teunissen, 2001). With the exception of a select few other microorganisms that are capable of partial lignin degradation, the white-rot fungi are the only organisms that are able to completely depolymerize and mineralize lignin (Pointing, 2001). White-rot fungi preferentially degrade lignin in the wood cell, causing the cellulose and hemicellulose to become bleached in appearance and the wood to flake apart (Blanchette, 1984). All white-rot basidiomycetes, however, do not degrade lignin, cellulose, and hemicellulose at the same rate. They can be classified into two separate groups according to the decay patterns of simultaneous rot and selective delignification (Blanchette, 1984).

Simultaneous rot is the process by which white-rot fungi will simultaneously decompose the lignin, cellulose, and hemicellulose contained within the wood cell (Pandey and Pitman, 2003). With this type of decay, the wood cell is penetrated by the microhyphae, resulting in holes in the secondary wall of the wood cell. As the decay process continues, the hole formed by the hyphae becomes enlarged. It is also possible for the hyphae to grow within the cell lumen (much like a brown-rot fungi) and to maintain contact with the S_3 layer of the wood cell (Wilcox, 1973). As decay continues, the hyphae excrete degradation enzymes and a zone of cellular degradation -or lysis zone- develops. This occurs because the enzymes that are secreted have activity only within the proximity of the hyphae (Cowling and Brown, 1969).

White-rot fungi can exhibit a second type of decay pattern whereby the lignin in the wood cell is preferentially degraded before the cellulose and hemicellulose fractions (Blanchette, 1991). This is known as selective delignification but has also been referred to as corrosive rot (Blanchette, 1991). In this type of decay the hyphae invade the lumen of the wood cells and low weight molecular substances are secreted by the hyphae. These substances diffuse through the S_2 layer of the wood cell and have the potential to dissolve lignin from adjacent wood cell walls (Kirk and Cullen, 1998). In other instances of selective delignification by white-rot, fungal hyphae have caused a separation of the wood cells by penetrating the cell walls and delignifying the middle lamella (Schwarze and Baum, 2000). In most cases the cellulose and hemicellulose components of the wood cell are

decomposed after delignification, but extreme cases have been observed in which delignification has occurred throughout the entire wood structure (Srebotnik and Messner, 1994).

2.3 Wood Preservation

As long as mankind has been constructing items from wood there have been attempts made to extend the life of the finished product. In ancient times, the Greeks and Romans used a variety of preservatives, such as oils, tar, resins, and extracts from resistant timbers to preserve various structures such as bridges (Larsen and Marstein, 2000). When dealing with timber that would come into contact with the ground, they would also employ a technique of gently burning the wood to produce a charred layer. This method would provide excellent protection of the wood from biological decay (Larsen and Marstein, 2000).

These methods of preservation remained unchanged for centuries, however, the industrial revolution led the way for change for many things including wood preservation. The invention of tanks that could withstand high pressure was made possible with the technology that arose from the development of the steam engine. Coal production was also increasing and with it factories that produced products from coal such as creosote. These events led to John Bethell developing a patent process of injecting a coal-tar creosote preservative into wood under pressure in a cylinder (Larsen and Marstein, 2000). This process is

still in use today to deliver many different types of preservative solutions (Larsen and Marstein, 2000).

2.3.1 Full Cell Treating

Bethell's treatment process was granted a patent in 1838 (Bethell, 1838). The primary concept behind this type of preservative loading process is to actually fill the lumen wood cells with preservative (Figure 2.6). For this reason, it is commonly referred to as the 'full cell' process. To achieve 'full cell' loading, first a vacuum is applied to the treatment cylinder to draw the air and free water out of the wood cell. Many different levels of vacuum may be applied for varying time periods, however, the AWPA (2010) does specify a minimum vacuum of -77kPa for not less than 30 minutes. Next, the preservative is drawn into the cylinder,

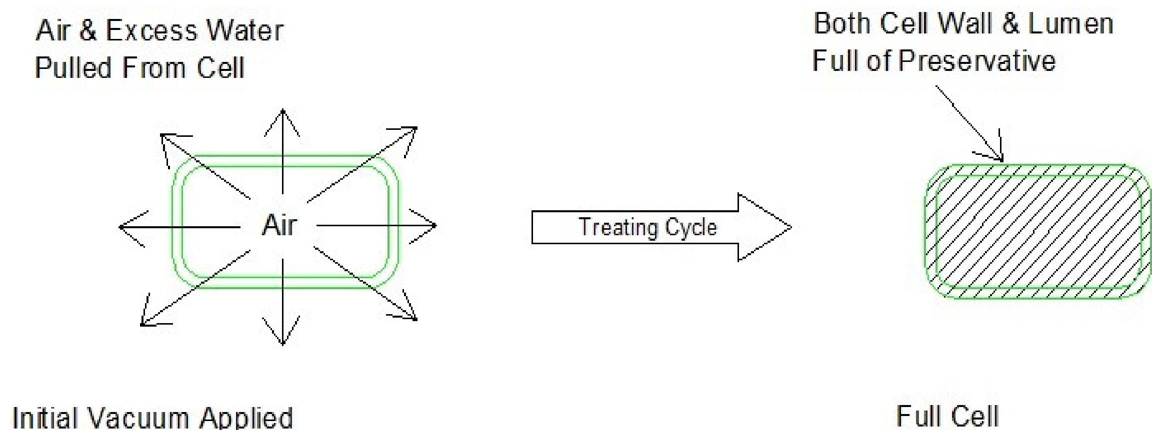

Figure 2.6: A diagram of the full cell process demonstrating the deposition of the preservative solution (modified from Hiziroglu, 2013).

without breaking the vacuum. Once the cylinder is filled with treating solution pressure is applied. Again, this pressure period can be for varying lengths of times at various pressures depending on the wood products to be treated. This causes the preservative to be driven into the empty cell lumens. After the pressure period has been completed, the preservative will be drained from the cylinder. A final vacuum stage is normally applied to help draw off any excess preservative; however, a final vacuum does not need to be applied to complete the process.

While the Bethell process was originally employed to deliver oil-borne preservatives, such as creosote, today it is typically used to treat wood with water-borne preservatives, such as CCA (Hiziroglu, 2013). Bethell's method was the first effective treatment method, however, it had several economic and environmental drawbacks. This method uses a large amount of preservative, which costs the manufacturer more to treat the product, and it is prone to product bleeding and leaching, allowing more preservative to enter the local environment around the installed treated product (Richardson, 1993). Rueping and Lowery later developed methods that addressed these concerns.

2.3.2 Empty Cell Treating

The empty cell process was pioneered by Rueping and Lowry, who were granted their patents for their impregnation processes in 1902 (Rueping, 1902) and 1906 (Lowry, 1906). In contrast to the full cell method, these methods utilize trapped

air within the lumen to only coat the interior of the cells instead of filling them with preservative (Figure 2.7). The cycle begins with initial pressure being applied. Typically, 35-40 psi of pressure is applied (depending on which wood species is being treated) to the wood cell causing the cell lumens and free water within the cells to become compressed. The duration of this phase will also vary, from minutes to an hour, depending on the products being treated. The preservative is then introduced under pressure to the treatment cylinder. Once the cylinder has been filled, the pressure is increased, depending on the product being treated, to a range of 140 to 150 psi. This pressure is maintained until the preservative retention level required has been met. This can take several hours to achieve. Once the pressure portion of the cycle has been completed the pressure is released and allowed to return to 0 psi. This allows the air within the lumens to expand, pushing the preservative out of the cells, leaving a coating of preservative within the cell. A vacuum is then applied to remove any excess preservative that may remain (Hiziroglu, 2013).

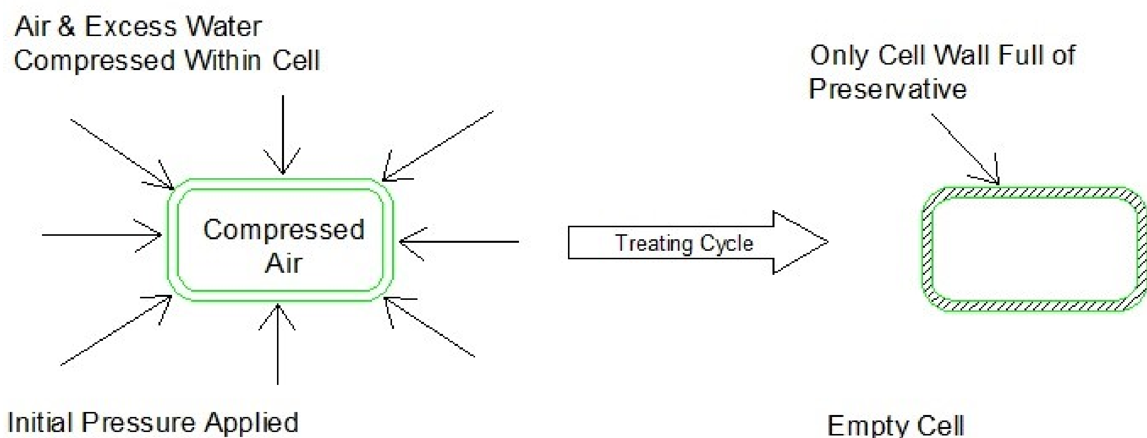

Figure 2.7: A diagram of the empty cell process demonstrating the deposition of the preservative solution (modified from Hiziroglu, 2013).

The empty cell process has both economic and environmental benefits (Hiziroglu, 2013). From an economic standpoint, more preservative can be recovered after the treating process (anywhere from 20-60% of the initial preservative applied), so smaller amounts of preservative can be used to achieve adequate protection against decay (Hiziroglu, 2013). From an environmental viewpoint (because less preservative is utilized and left behind within the cell) this reduces the amount of preservative that may be leached into the local environment (Richardson, 1993). It also reduces the amount of preservative that will have to be recycled at the end of the treated product's working lifespan. The empty cell process is typically utilized to deliver oil-borne preservatives, such as creosote, pentachlorophenol, and organometallic compounds like copper naphthenate (Hiziroglu, 2013).

2.3.3 Copper Naphthenate

Von Wolniewicz had originally proposed the use of metal naphthenates in 1898, but it wasn't until 1911 that a copper naphthenate solution, called Cuprinol, was marketed in Denmark for industrial use (Richardson, 1993). From there it was introduced to the Swedish markets in 1920, and then brought into England that same year. It wasn't until the 1940s that the product was introduced into North American markets (Richardson, 1993).

Copper naphthenate (Figure 2.8) is an organometallic compound with a distinct green color. When it is used as a preservative it will impart a vivid green color to

the wood; after a period of weathering however, the wood will turn to varying shades of brown, depending on the amounts of pressure and temperature that the wood was originally treated with (Cheremisinoff and Rosenfeld, 2010).

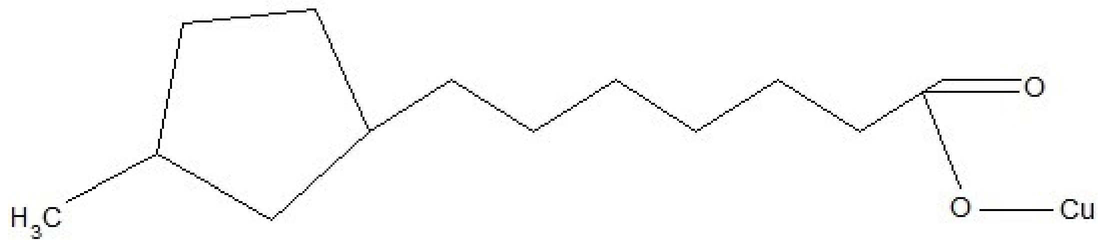

Figure 2.8: A diagram of one of the many postulated chemical formations of copper naphthenate (after EPA, 2007).

Copper naphthenate can be manufactured in several different ways. It can be produced by heating naphthenic acid and dissolving copper oxide or copper carbonate into it (fusion method) or it can be produced by mixing sodium naphthenate with copper sulphate in an aqueous solution (Richardson, 1993).

Solvents that are used for copper naphthenate must comply with the AWPA Standard P-9 (AWPA, 2010). In the past, the primary solvent was ‘P-9 oil’ but with the cessation of its production by its major distributor, the solvent that has been primarily used to replace it has been diesel (Murray, 2011). However, there are many issues associated with using diesel as a solvent, such as a higher flash point, lower solvency effect, and odor, resulting in the need for an alternate

carrier solvent. The use of biodiesel as a co-solvent has been proposed as a more suitable replacement.

2.3.4 Biodiesel

Biodiesel is a fuel derived from the trans-esterification of vegetable oils or animal fat (Knothe *et al.,* 2005). In order to achieve this, vegetable oils or animal fat are reacted in the presence of a base with an alcohol to yield the long chain alkyl esters that comprise biodiesel (Figure 2.9). Biodiesel is created from a variety of carbon sources, such as palm, soybean, peanut, rapeseed/canola, sunflower, safflower, and coconut oils as well as animal fats (typically tallow) and even waste fats such as frying oil (Knothe *et al.,* 2005).

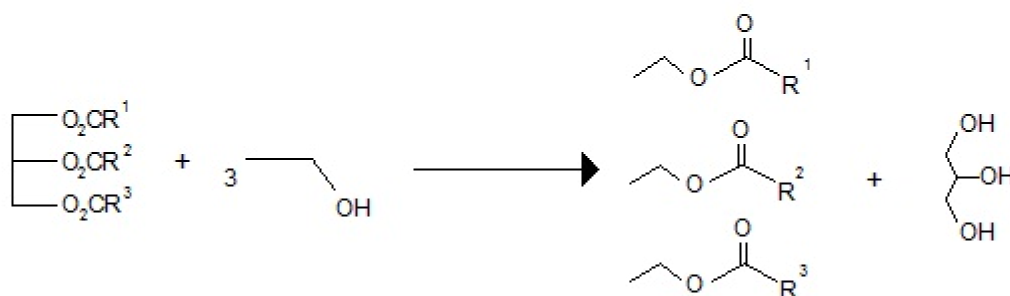

Figure 2.9: A simplified diagram of the trans-esterification chemical process (modified from Rifleman, 2010). R^1, R^2, and R^3 represent alkyl groups.

Biodiesel is used in the wood preserving industry as a co-solvent in P-9 Type A solvent systems. Typically, biodiesel makes up 35% or less of the P-9 Type A solvent, however at this time there is no limit on the amount of co-solvents that

can be used as long as the mixture meets the physical testing criteria outlined by the AWPA (Morgan, 2010).

Using biodiesel as a co-solvent has many benefits when compared to petroleum diesel. Murray (2011) has noted that the addition of biodiesel as a co-solvent at the level of 30% significantly reduces the odor of various treatment solutions. Biodiesel blends also have a higher flash point than petroleum diesel leading to safer storage and handling conditions. Biodiesel is also derived from a renewable domestic resource, which reduces the dependence on petroleum products (Knothe *et al.,* 2005).

Biodiesel also has several disadvantages, including increased cost and a lower oxidative stability than petroleum diesel (Knothe *et al.,* 2005; Xin *et al.,* 2009). This reduced oxidative stability is attributed to its high content of unsaturated methyl esters, which can be easily oxidized. Susceptibility to oxidative degradation can alter the physical properties of biodiesel as well as pose issues for storage (Knothe *et al.,* 2005; Xin *et al.,* 2009). A solution to this issue is the addition of antioxidants such as propyl gallate, because these have been shown to enhance the stability of biodiesel (Xin *et al.,* 2009).

Propyl gallate (Figure 2.10) (an ester of gallic acid) is a commonly used antioxidant in the food industry (Becker, 2007) and due to its ability to inhibit the actions of oxygen it is also referred to as a ‘stabilizer’. It is generally accepted as

a safe antioxidant (Becker, 2007). In addition to its excellent antioxidant capacity in foods and vegetable oils it has been shown by Hsu *et al.* (2009) that propyl gallate demonstrates anti-fungal properties as well as other protective mechanisms during soil block testing. While the exact mechanism is not known, it is hypothesized by Schultz and Nicholas (2002) that propyl gallate has a trinary (fungicidal/antioxidant/metal chelator) protective action.

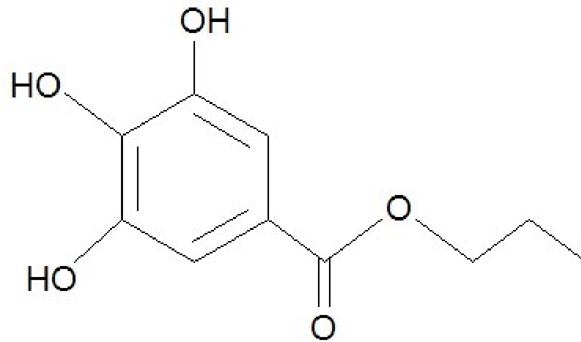

Figure 2.10: Chemical structure of propyl gallate

2.4 Utility Pole Industry

Since the advent of the trans-continental telegraph (which was constructed in 1861) wood poles have been employed to suspend utility wires. By the turn of the 20th century, wood poles were utilized to help deliver electricity to residential customers. Today the majority of transmission poles and utility poles are treated wood. More than 130 million poles are estimated to be in service in North America (NAWPC, 2011).

There are several wood species that are utilized for utility poles. According to a survey conducted by Mankowski *et al.,* (2002) it is estimated that approximately 69% of the utility poles in service in the United States are Southern yellow pine (*Pinus* spp), 15% are Douglas fir (*Pseudotsuga menziesii*) and 13% are Western red cedar (*Thuja plicata*). Of these species, Southern yellow pine is most often used for distribution poles (Figure 2.11), due to their availability for use in smaller pole classes, while Douglas fir and Western red cedar are more commonly utilized for larger transmission poles (Figure 2.12) (Wolfe and Moody, 1997).

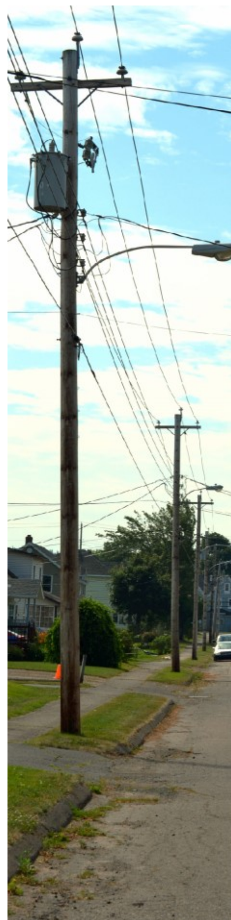

Figure 2.11: An example of a pine distribution pole in service

Figure 2.12: An example of Douglas fir transmission poles in service

While some species, such as Western red cedar, are considered to be more durable than others (Scheffer and Cowling, 1966) the majority of utility poles are treated with preservative to extend their service life. Carrier solutions, and their effect on the efficacy of the preservatives they are utilized with, have been researched in the past (Duncan and Audrey, 1950; Muin *et al.,* 2003). However, the use of biodiesel as a co-solvent in the preservative industry is a relatively new procedure. As such, research regarding the use of biodiesel as a co-solvent is limited, and further research is required.

Chapter 3.0 Experimental Methods and Materials

A series of biological decay trials were utilized to evaluate the efficacy of biodiesel as a co-solvent. Scanning electron microscopy (SEM) was also used to determine if there was a difference in preservative distribution within the wood cell when different solvent systems are used.

3.1 Wood Species Evaluated

Three wood species of economic importance to the utility pole industry were selected for evaluation: Southern yellow pine (SYP), Western red cedar (WRC), and Douglas fir (DF). The AWPA standard testing methods typically suggest the use of a non-durable coniferous species such as Southern yellow pine (AWPA, 2010), however, Western red cedar and Douglas fir were also of interest due to their commercial use in the utility pole industry.

3.2 Sapwood Test Block Preparation

Soil block testing is a screening evaluation that was developed as a standard method by the AWPA to determine minimum preservative retentions that would be adequate for further field tests. This screening test is currently the most common test that is utilized in the North American wood preserving industry. Sections 3.3, 3.4, 3.5, 3.6, and 3.7 outline the procedure that was performed.

A series of blocks were milled from sapwood samples of each species (SYP, WRC, and DF). These test blocks were treated with preservative and then exposed to select decay fungi for a given period of time. The test blocks were milled as accurately as possible into 14 mm cubes to yield an approximate volume of 2.7 cm^3 as per AWPA Standard Method E10-09. The dimension of each of the cubes were taken and recorded. The test blocks were then pressure treated with one of 10 preservative treatments:

- 30% soy biodiesel with stabilizer, 70% diesel, and copper naphthenate (30/70BDWS)
- 30% soy biodiesel without stabilizer, 70% diesel, and copper naphthenate (30/70BDNS)
- 50% soy biodiesel with stabilizer, 50% diesel, and copper naphthenate (50/50BDWS)
- 50% soy biodiesel without stabilizer, 50% diesel, and copper naphthenate (50/50BDNS)
- all soy biodiesel (0% diesel) with stabilizer and no copper naphthenate (100BDWS)
- all soy biodiesel (0% diesel) without stabilizer and no copper naphthenate (100BDNS)
- diesel only (100%), and copper naphthenate (Dcunap)
- diesel only (100%), and no copper naphthenate (100D)
- chromated copper arsenate (CCA) only
- untreated control (Control)

The stabilizer used was propyl gallate at a level of 0.01% (w/w) (H and A (Canada) Industrial Inc., ON, Lot number 201012023).The soy-based biodiesel was obtained from Alpha Chemical Ltd (Dartmouth, NS). The diesel was sourced from Suncor Energy Inc. (Toronto, ON). The ratios of diesel to biodiesel were added as volume percentages. The copper naphthenate concentrate was obtained from MERICHEM (Houston, TX) and CCA was obtained from Arch Lonza (Allendale, NJ). Replicates of 10 were used. Prior to preservative treatment and placement into the decay microcosms, the blocks were subjected to a period of conditioning (AWPA E10-09).

The blocks were placed in a conditioning room at a temperature of 24°C ± 1°C and a relative humidity of 60% ± 5% and a subset was weighed daily. The blocks were maintained in this room until they achieved moisture equilibrium and constant masses were obtained (approximately 21 days).

Due to the variation in treatment permeability and pre-determined levels of preservative thresholds, the AWPA (2010) has outlined minimum preservative retentions which can be found in the section U1-10 (2010) for both copper naphthenate and CCA. As these retentions are the industry standard they were utilized for this research. The following target retentions were used for each wood species:

- Douglas fir
    - Copper naphthenate: 1.52 kg/m^3
    - CCA: 9.6 kg/m^3
- Southern yellow pine
    - Copper naphthenate: 1.28 kg/m^3
    - CCA: 9.6 kg/m^3
- Western red cedar
    - Copper naphthenate: 1.92 kg/m^3
    - CCA: 9.6 kg/m^3

After treatment the treated blocks were placed in sealed bags for a 24 hour period. They were then removed from the bags and the excess preservative was blotted from the surface of the blocks using paper towel. The cubes were immediately weighed and this weight was used to determine the preservative retention of the blocks. The blocks were then re-wrapped with tinfoil and returned to the conditioning room to slowly dry the cubes for one week. The blocks were then unwrapped and the cubes allowed to dry in the conditioning room for 21 days after which the weights of the blocks were obtained.

After being weighed, the blocks were packaged using 4 mil thick polyethylene (Figure 3.1). The air was removed from the packaging, the packages were sealed, and then the packages were sterilized by exposure to 2.5 mrad of

ionizing radiation from a cobalt 60 source (Nordion, QC). The blocks were stored until they were to be aseptically transferred to the soil culture bottles.

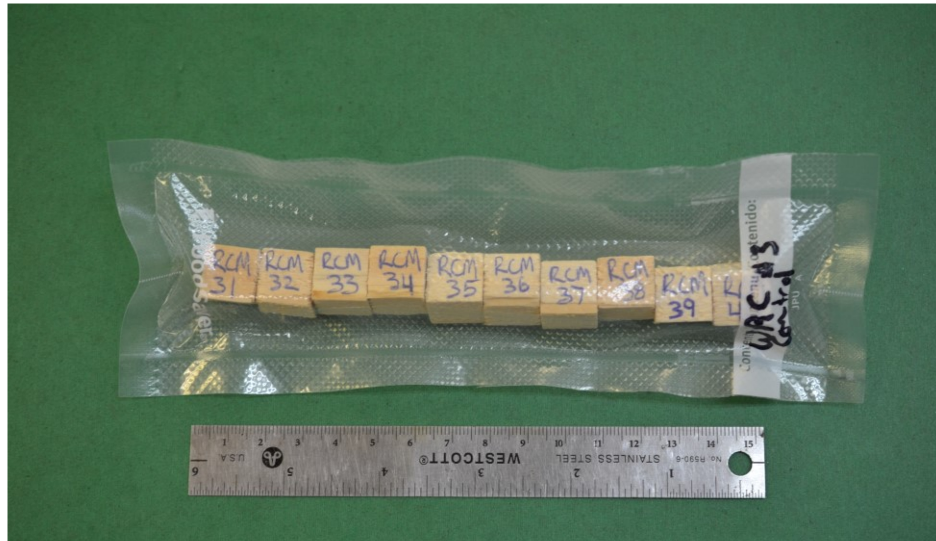

Figure 3.1: Packing of the treatment blocks

3.3 Selection of Test Fungi

Four species of fungi were utilized for this test: three brown-rot fungal species (*Gloeophyllum trabeum, Postia placenta*, and *Antrodia xantha*) and one white-rot fungus (*Trametes versicolor)*. *Gloeophyllum trabeum* was chosen for its tolerance to phenolic and arsenic compounds (AWPA, 2010). In addition, it is a species that the AWPA (2010) suggests to include in softwood soil block culture tests. *Postia placenta* was chosen because it is particularly tolerant to copper and zinc compounds. The AWPA (2010) also recommends the use of this fungus when testing copper containing preservatives. *Antrodia xantha* was used in this

test as well due to its copper tolerance (Duncan, 1958). The white-rot fungus *Trametes versicolor* was included due to its rapid growth rate (Schmidt, 2006).

3.4 Fungal Culture Preparation and Maintenance

The four fungal cultures previously described were obtained from the American Type Culture Collection. The strains used were *Gloeophyllum trabeum* (ATCC 11539), *Postia placenta* (ATCC 11538), *Poria xantha* (ATCC 11086) and *Trametes versicolor* (ATCC 42462). The stock cultures of *Gloeophyllum trabeum* (GT) and *Trametes versicolor* (TV) cultures were maintained on Difco ™ Potato Dextrose Agar (39 g/L) with the addition of Bacto ™ yeast extract (0.5g/L). *Postia placenta* (PP) and *Poria xantha* (PX) stock cultures were maintained on Difco™ Yeast Mold (YM) agar.

3.5 Determination of the Preservative Solution Concentrations

In order to achieve the desired preservative retentions of CCA and copper naphthenate, a series of preservative solutions were prepared. Sapwood blocks of each wood species were prepared in the same manner as previously described in Section 3.2. These test cubes were treated using three different preservative strengths and replicated 30 times for a total of 90 treated sapwood cubes for each species and preservative type. The retentions from these treated cubes were calculated by weight. The resulting retentions underwent regression analysis to determine an equation that could be used to predict the treatment

solution strength required to obtain the desired retention levels of CCA and copper naphthenate for each wood species (Pennie, 2010).

3.6 Preservative Treatment Cycle

The preservative treatments were delivered to the blocks utilizing a miniature treating cylinder (Figure 3.2) to simulate the process of wood treatment in a commercial scale treating cylinder.

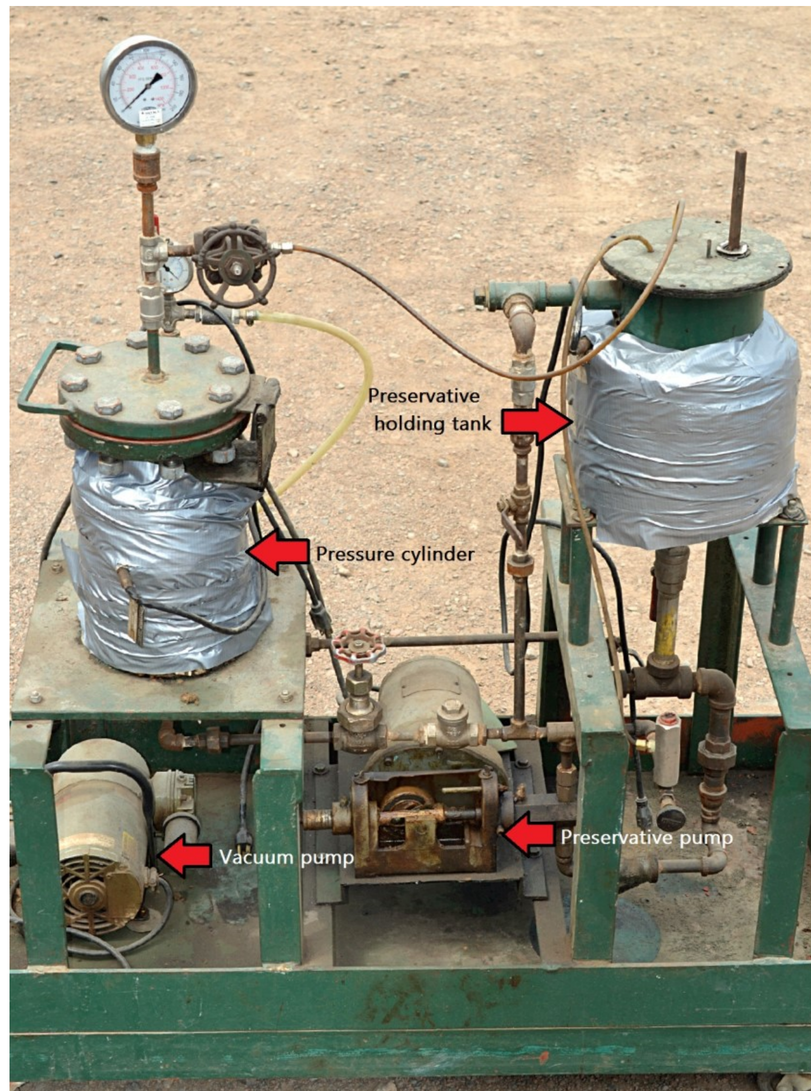

Figure 3.2: The miniature treating cylinder used to treat the test blocks

The preservative cycle used was a version modified from the AWPA standard E10-09 as developed by Pennie (2010). The treatment cycle consisted of:

- A 20-minute vacuum at -7.73 kPa
- A pressure period of 1033 kPa for 45 minutes
- A 10-minute period at atmospheric pressure to allow for kick back

The cubes were then placed in airtight bags and allowed to equilibrate for 24 hours, after which the surface of the cubes were blotted dry to remove any excess preservative and immediately weighed to determine the preservative retention.

3.7 Determination of Preservative Retention

The calculation to determine the preservative retention of the cubes was taken from AWPA standard E10-09 and is as follows:

$$\text{Preservative retention (kg/m}^3\text{)} = \frac{GC}{V} \times 10^*$$

In this equation:

- G represents the amount of preservative absorbed by the sapwood block (final weight of the preservative treated cube minus the initial weight of the cube in grams)
- C represents the amount of preservative (in grams) in 100 g of treating solution
- V represents the volume of the cube in cubic centimeters (cm^3)

* This is a correction factor used in the conversion to the final unit kg/m^3

3.8 Soil Block Study

The method used to determine the efficacy of the copper naphthenate and CCA preservative solutions was a modification of E10 (revised in 2009) Standard Method of Testing Wood Preservatives by Laboratory Soil Block Cultures (AWPA, 2010). This method was used to determine the efficacy of the CCA and copper naphthenate preservative treatments in resisting accelerated fungal decay under laboratory conditions.

3.8.1 Preparation of Microcosms

A soil substrate of pH 4.5 ± 0.5 was used to prepare individual fungal microcosms. The soil was excavated from a field that had not been utilized for agricultural activities for more than 50 years. This location was chosen instead of a forested area in order to avoid the introduction of indigenous wood decay fungal species into the microcosms. The soil pH was not amended as per AWPA (2010) recommendation because amending the soil could have potentially inhibited the growth of the fungi in the microcosms (Little *et al*., 2010). The pH was determined as per the method described by Tran and van Lierop (1993). The average water holding capacity of the soil to be used was 38% as determined by the method outlined in the AWPA (2010) standard procedure E10-09.

3.8.1.1 pH Determination of the Soil

The pH was determined using the SMP single buffer method described by Tran

and van Lierop (1993). Ten grams of air dried soil was placed into a beaker, and 10 mL of water was added to the soil. The slurry was then mixed continually for 30 minutes. The sample was then measured using a standard glass pH electrode.

3.8.1.2 Soil Preparation and Water Holding Capacity Determination

The soil substrate was mixed to ensure homogeneity and to break up all clumps and sifted through 2mm mesh. The soil was then added to 500 mL Mason jars (at the weight of 250 g). Sawdust was added to the jars in place of wood feeder strips (as suggested by AWPA E10-09). The sawdust from the same species of wood block to be tested was used. For example, if the wood block to be tested was made from Douglas fir sapwood then Douglas fir sawdust was added to the microcosm. The weight of sawdust that was added was 2.5 g which represented the approximate weight of a feeder strip. Distilled water was then added to the microcosm to bring the moisture content of the soil to 130% of the water holding capacity. A modified lid of three layers of 7.0 cm slow flow rate Whatman number 2 fine porosity filters were used for the center of the Mason jar lids (Pennie, 2010). The finished microcosms were autoclaved for 40 minutes at 103.4 kPa and a temperature of 121°C.

3.8.2 Inoculation and Incubation of Microcosms

Each of the microcosms was inoculated after autoclaving, with one of the four test species of fungi. A 12 mm square section of agar covered in fungal mycelia

was aseptically cut using a scalpel and was then aseptically transferred to the microcosm. The microcosms were then placed in an incubation room (24°C ± 1°C) with a relative humidity of 65% (± 5%) (Figure 3.3). The microcosms were then incubated until the sawdust was covered with mycelium. Once this was achieved, treated wood cubes were aseptically transferred to the microcosms (Figure 3.4). The microcosms inoculated with brown-rot fungal species were incubated for 8 weeks and the microcosms inoculated with white-rot fungus were incubated for 16 weeks. The microcosms were evaluated periodically for the duration of the study, to ensure that there was an adequate amount of moisture in the jars. Due to considerable moisture loss, a 0.23% malt extract broth was aseptically added to all microcosms, as required, to bring the soil back to 130% of the water holding capacity (as per AWPA, 2010). This was determined by weighing the microcosms weekly and recording their mass losses.

Figure 3.3: Soil microcosms within the incubation room.

Figure 3.4: Example of a soil microcosm (Pennie, 2010 used with permission).

3.8.3 Measuring Decay

At the end of the incubation period, the blocks were taken from the microcosms and all visible mycelia were carefully removed by peeling the fungal mat back with forceps and carefully removing any remaining mycelia with a small synthetic brush. The blocks were then returned to the conditioning room. Once the conditioning period was complete (21 days) each block was weighed to the nearest 0.01 g and the percent weight loss was calculated as a measure of decay. Figure 3.5 shows blocks that have been cleaned and conditioned prior to massing.

Figure 3.5: Wood blocks that have been cleaned after incubation. The treatments from left to right are as follows: Control, 100D, Dcunap, 30/70BDWS, 50/50BDWS, 100BDWS, and CCA.

3.9 Plate Studies

The plate studies were conducted in the same manner as the previously described soil block study (Section 3.8) with the exception of the preparation of soil microcosms. Agar plates with the addition of malt extract were used in place of the soil microcosms (Kamdem *et al.,* 2002).

3.9.1 Malt Media Evaluation

A test was devised to evaluate the performance of the four wood decay fungi (*Postia placenta*, *Antrodia xantha*,*Trametes versicolor,* and *Gloeophyllum trabeum*) using varying malt media compositions. The goal of this study was to verify that the level of malt referenced in British Standard EN 113 (1997) would be appropriate to sustain the test fungi while achieving the required degradation for the control cubes. Five levels of malt concentrations were prepared containing 0%, 1%, 2%, 3%, and 4% malt w/v. In addition to the malt, each media preparation contained 20 g of granulated agar per litre of prepared media (British Standard 113, 1997). Each test concentration was then inoculated with one of four test fungi. The plates were incubated until the growth of the mycelia covered approximately half of the surface of the malt media (approximately 7 days). At that time sterile sapwood blocks of WRC, SYP, or DF were added to the test plate.

3.9.2 Sapwood Test Block Preparation

Sapwood cubes were milled (14 mm x 14 mm x 7 mm) from the sapwood of SYP, WRC, and DF. These sapwood blocks were then conditioned in a forced

draft oven (35 °C) until the blocks achieved a constant weight. Final dry weights were recorded after cooling. The blocks were steam sterilized (103.4 kPa, 121°C, 20 min) (AWPA, 2010). They were then aseptically transferred to the petri dishes containing the inoculated malt media. The experimental design was a factorial with three factors (malt level, fungi type, and wood species) in 6 replicates, resulting in a total of 360 malt-based microcosms.

3.9.3 Measuring Decay

At the end of the trial the mycelia were carefully removed by peeling the fungal mat back with forceps and carefully removing any remaining mycelia with a small synthetic brush. The blocks were dried again in a force draft oven (35 °C) until a constant weight was achieved. The final weights were then recorded and the percent weight loss was calculated as a measure of decay.

3.9.4 Malt Decay Study

The malt decay study was conducted in the same manner as the soil block study that has been previously described in Section 3.8, with the exception of preparation of agar plates instead of soil microcosms. The fungal species *Postia placenta*, *Antrodia xantha*,*Trametes versicolor,* and *Gloeophyllum trabeum* were cultured on the optimum percentage of malt that was previously determined (Section 3.9.1).

3.9.5 Weathering Procedure

Sixty 14 mm cubes of Douglas fir sapwood blocks were milled and were allocated to one of 6 treatments:

- 30% biodiesel with stabilizer and 70% diesel (30/70)
- All diesel (100%) with copper naphthenate (Dcunap)
- Biodiesel (100%) with stabilizer only (no copper naphthenate) (100BDWS)
- Diesel only (100D)
- CCA only
- Untreated control (CW)
- Control that did not undergo the weathering process (Control)

Three days after treatment, the blocks were then weathered as per the AWPA (2010) E10-09 weathering procedure for oil-based preservatives. Blocks of the same treatment were spaced equally on stainless steel mesh supports in 600 mL beakers. The blocks were weighted down utilizing stainless steel mesh. Distilled water was added to fill the beaker and then held at room temperature (24°C ± 1°C) for 2 hours. Once the time had elapsed the water was emptied from the beakers and the blocks were incubated (weathered) for 334 hours at 49°C. Once the weathering was completed the blocks were sterilized by exposure to 2.5 mrad of ionizing radiation from a colbalt 60 source (Nordion, QC). The blocks were then aseptically transferred to malt agar plates prepared in the same manner as previously described, with the exception that only *Postia placenta* mycelium was used to inoculate the microcosms. The malt agar plates were then

incubated as previously described (Section 3.8.2) and the percent decay was calculated in the same manner as previously described (Section 3.8.3).

3.10 Scanning Electron Microscopy (SEM) Analysis

Douglas fir sapwood blocks were evaluated using the SEM to determine if the addition of biodiesel to the carrier solution for copper naphthenate had an effect on the distribution of copper within the wood cells.

The sapwood blocks were prepared in the same manner as in Section 3.2 and were assigned one of three treatments (6 reps):

- 30% soy biodiesel with stabilizer, 70% diesel, and copper naphthenate (30/70BDWS)
- diesel only (100%) and copper naphthenate (Dcunap)
- untreated control (Control)

The cubes were conditioned for two weeks (24°C ± 1°C; 60% ± 5% RH, Section 3.2). The cubes were then further conditioned in a force draft oven (30 °C for 30 days) to drive off any volatile fractions of the preservative and reduce preservative smearing during the sectioning process.

The dimensions of the cubes were taken using digital calipers; the center of the cube was determined (± 0.01 cm) and scored with a sharp dissection probe. Sections were then taken from the radial, cross-section, and tangential planes in

a manner similar to that outlined by Dawson-Andoh and Kamden (1998). To avoid transfer of the preservative a new surgical blade was used for each cut.

The cut sections were then affixed to aluminum stubs using carbon cement. The paste was allowed to cure for a 24-hour period in a dust-free environment (24°C ± 1°C). Samples were cleared of any contaminants using compressed air and were then coated with gold and palladium using a Polaron Range SC7620 Sputter coater (Quorum Technologies, Kent, UK) (18µA for 120 seconds) to yield a standard thickness of 27 µm.

3.10.1 Measurement of Copper

The copper was quantified using a Hitachi S-4700 cold field emission SEM with an integrated Oxford x-ray system controlled by INCA software. The SEM was optimized for copper and the accelerating beam was set for 20 keV/10µAS and a dead time of 20-30%. The detection limit was 7% (weight).

All quantifications were run at 2500x. For the radial section, two bordered pits closest to the ray cell structures were selected. Three measurements were taken on the rim of the border pit, then 10 µm and 20 µm away from the border pit. For the cross-section, two tracheid cells were randomly selected. The S_2 layer, S_3 layer, and compound middle lamella of the corner of the tracheid cells were measured.

3.11 Statistical Design and Analysis of Experiments

The soil block trial (Section 3.2) was designed as a full factorial (3 wood species x 4 fungal species x 10 wood preservative treatments x 10 reps = 1200 cubes). Each wood species was analyzed separately using a two-way ANOVA. This was performed using the general Linear Model procedure in Minitab 16 (Minitab, 2010). The SYP data were normally distributed; the WRC and DF, however, were not. In order to satisfy the assumptions of normality, a square root transformation was performed on the DF data and a log transformation was performed on the WRC data.

In order to determine the optimal preservative treatment solution that would result in the desired preservative target retentions, regression analyses were performed on the data obtained in Section 3.5. These analyses were performed using Minitab 16 (Minitab, 2010). Each regression was comprised of 3 preservative concentration levels with 30 reps per concentration. This was performed for each of the 4 copper containing treatments and for each of the three wood species (3 concentrations x 30 reps x 4 treatments x 3 wood species = 1080 cubes). From these regression analyses equations were obtained in order to predict the preservative concentrations required to produce the desired target retentions.

The malt media evaluation (Section 3.9.1) was analyzed with an ANOVA, as the intent was to determine the level of malt that would produce adequate levels of

untreated sapwood decay for all fungal species tested. The General Linear Model procedure in Minitab 16 (Minitab, 2010) was utilized.

The Malt Decay Study (Section 3.9.4) was also designed as a full factorial (3 wood species x 4 fungal species x 7 wood preservative treatments x 10 reps = 840 cubes). The data from this trial were analyzed in the same manner as the data from the Soil Block Study. Each wood species was analyzed separately using a two-way ANOVA. This was performed using the General Linear Model procedure in the Minitab 16 (Minitab, 2010). The data obtained from DF and WRC met the assumptions of normality, however the SYP data was not normal. In order to meet these assumptions a square root transformation was performed on this data prior to analysis.

The Weathering Procedure (Section 3.9.5) was also designed as a full factorial (8 preservative treatments x 1 wood species x 1 fungal species x 10 reps = 80 cubes), and analyzed using the General Linear Model procedure in Minitab 16 (Minitab, 2010). In order to meet the assumptions of normality a $y^{0.75}$ transformation was performed prior to statistical analysis.

A two-way ANOVA procedure was also utilized to analyze the data from the SEM analysis (Section 3.10.1). As the data was found to be non-normal, a cube root transformation was performed to meet the assumptions of normality. This design

was also a full factorial (3 treatments x 9 locations x 4 reps = 108 measurements of copper).

In order to determine which means were statistically different, all ANOVA procedures were performed using Tukey's Studentized Range Test. An α of 0.05 was used to determine statistical significance.

Chapter 4.0 Results and Discussion

The results presented in this section correspond to the Materials and Methods presented in Chapter 3.0.

4.1 Determination of Preservative Solutions

In order to determine the appropriate level of copper naphthenate and CCA to be added to their respective carrier solutions so that the target retentions for each wood species and preservative combination were achieved, a regression analysis was performed. The equations obtained from these regression analyses and their corresponding R^2 values are reported in Table 4.1. Each wood species had 30 cubes treated at three preservative concentrations with each of the four preservative combinations. This resulted in 1080 cubes in total being treated.

As shown in Table 4.1, the R^2 values are very high, indicating a high level of correlation between the data set and the prediction equations. All p-values were less than 0.05 indicating that all regressions were significant. Due to the high level of predictability potential, these equations were utilized to formulate the preservative solutions in order to achieve the target retentions outlined in Section 3.2.

Table 4.1: A summary of the formulas resulting from the regression analysis used to determine the percentage of stock solution to be added to the carrier solutions to achieve the targeted preservative retentions

Treatment	Species	Formula[1]	Treatment Level[2]	R^2
Dcunap	WRC	$1.92\ kg/m^3 = -0.0068 + 5.88x$	0.328%	99.8
Dcunap	SYP	$1.28\ kg/m^3 = 0.0894 + 4.48x$	0.266%	99.6
Dcunap	DF	$1.52\ kg/m^3 = -0.0495 + 4.62x$	0.340%	98.2
30/70BDWS	WRC	$1.92\ kg/m^3 = -0.0769 + 6.33x$	0.315%	99.7
30/70BDWS	SYP	$1.28\ kg/m^3 = 0.0140 + 4.92x$	0.257%	97.7
30/70BDWS	DF	$1.52\ kg/m^3 = -0.0400 + 4.06x$	0.380%	97.1
50/50BDWS	WRC	$1.92\ kg/m^3 = 0.0539 + 5.77x$	0.320%	99.7
50/50BDWS	SYP	$1.28\ kg/m^3 = 0.0220 + 4.34x$	0.290%	98.8
50/50BDWS	DF	$1.52\ kg/m^3 = -0.0527 + 4.26x$	0.369%	98.0
CCA	WRC	$9.6\ kg/m^3 = -2.95 + 8.93x$	1.41%	98.1
CCA	SYP	$9.6\ kg/m^3 = -3.96 + 7.58x$	1.79%	93.8
CCA	DF	$9.6\ kg/m^3 = -6.06 + 9.06x$	1.73%	99.6

[1] x = Treatment Level
[2] The treatment level indicates what percentage of stock solution needs to be added to the carrier solution
For treatment abbreviations see List of Abbreviations on page ix

4.2 Soil Block Study

A modified version of the AWPA Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures E10-09 (AWPA, 2010) was utilized to evaluate the effect of using biodiesel as a co-solvent on the fungal activity of four common North American fungal species: *Postia placenta*, *Poria xantha*, *Gloephylum trabeum*, and *Tramedes versicolor*. Each of the three wood species tested (SYP, DF, and WRC) were analyzed separately as differences

between the wood species were anticipated and each wood species has different industrial applications.

4.2.1 The Degradation of Douglas Fir Sapwood Blocks

The majority of transmission poles in service are comprised of Douglas fir, and as such it is considered to be of major importance to the wood utility pole industry (Manowski *et al.,* 2002). Therefore, it was of interest to examine the decay resistance of DF sapwood when treated with copper napthenate preservatives that incorporated biodiesel as a co-solvent.

Due to DF sapwood block availability, the 30/70BDNS treatment was not available for this portion of the study and is therefore missing from Table 4.2. As previously mentioned, the DF data set was not normal, so to meet the assumptions of normality, the data set was transformed using a square root transformation. Once these assumptions were satisfied the data was analyzed using a two-way ANOVA. Tukey’s HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized.

All main effects had a p value of 0.00, indicating that they were statistically significant. There was also an interaction between the fungal species and preservative treatment (p value 0.00), therefore the results from the highest order interaction are presented in the following Table 4.2.

Table 4.2 Mass losses of DF sapwood blocks treated with Cu preservatives

Treatment	Fungus*	Mean (% mass loss)	Standard error	Tukey's grouping**
Control	PP	37.00	4.45	A
100D	PP	8.14	1.67	EFGHI
Dcunap	PP	12.55	0.68	CDEFGH
30/70BDWS	PP	10.32	0.53	CDEFGH
50/50BDWS	PP	7.41	0.36	GHIJKL
50/50BDNS	PP	7.03	0.27	HGHIJK
100BDWS	PP	3.94	0.95	IJKLM
100BDNS	PP	5.91	0.86	HIJKL
CCA	PP	0.17	0.07	N
Control	PX	26.95	4.98	AB
100D	PX	7.74	1.39	DEFGHIJ
Dcunap	PX	14.71	0.72	CDEF
30/70BDWS	PX	9.26	0.47	CDEFGH
50/50BDWS	PX	6.77	0.35	GHIJKL
50/50BDNS	PX	6.37	0.58	HIJKL
100BDWS	PX	1.46	0.61	MN
100BDNS	PX	2.50	0.70	KLM
CCA	PX	0.04	0.10	N
Control	GT	35.14	3.67	A
100D	GT	8.30	1.43	FGHI
Dcunap	GT	14.86	0.42	C
30/70BDWS	GT	9.66	0.59	CDEFGH
50/50BDWS	GT	6.42	0.63	HIJKL
50/50BDNS	GT	6.04	0.33	HIJKL
100BDWS	GT	1.16	0.56	MN
100BDNS	GT	2.73	0.82	LM
CCA	GT	0.08	0.07	N
Control	TV	35.39	1.96	A
100D	TV	11.02	0.73	CDEFGH
Dcunap	TV	14.28	0.62	CDE
30/70BDWS	TV	9.40	0.47	CDEFGH
50/50BDWS	TV	10.77	0.60	CDEFGH
50/50BDNS	TV	9.44	0.54	CDEFGH
100BDWS	TV	10.26	1.60	CDEFGH
100BDNS	TV	15.83	2.15	BCDE
CCA	TV	3.06	0.07	JKLM

* Brown-rot fungi were the following codes: PP(*Postia placenta*), PX (*Poria xantha*), GT (*Gloephylum trabeum*). The white-rot fungus was denoted as TV (*Trametes versicolor*)
**Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page ix

It was observed that when exposed to each of the four fungal species, the 30/70BDWS treatment had statistically the same resistance to fungal metabolic activity. It was also noted that the addition of 50% biodiesel as a co-solvent, regardless of the addition of the stabilizer propyl gallate, had statistically the same resistance to fungal metabolic activity as that of the 30/70BDWS treatment when exposed to each of the four test fungi. Interestingly, when comparing the performance of the biodiesel containing co-solvent preservative blends to that of copper naphthenate using diesel only as a solvent (Dcunap), the biodiesel blended co-solvent preservative solutions performed statistically as well as the Dcunap solutions with one notable exception. When examining the inhibition capability of Dcunap and the copper naphthenate 50% biodiesel blended preservative solutions (both with and without stabilizer) after exposure to the copper tolerant fungal species *P. xantha*, the 50% biodiesel blended solutions both had a statistically lower rate of mass loss due to fungal metabolic activity than that of Dcunap.

At this time, there are no journal publications elucidating the effect of biodiesel as a co-solvent on the performance of copper naphthenate with which to compare the results of this study. There are, however, industry funded studies that have been published in non-peer reviewed sources on this topic. Morrell *et al.,* (2010) examined the effect of biodiesel on the efficacy of copper naphthenate using the AWPA Standard Method E10-09. The experiment utilized SYP as the wood test species and the sapwood test blocks were exposed to *P. placenta* and *G.*

trabeum to evaluate preservative performance through measurement of mass loss. The conclusion drawn from this study was that biodiesel had a negative effect upon the performance of copper naphthenate. When attempting to compare the results obtained by Morrell *et al.,* (2010) to the results obtained in this study it was noted that the data reported did not undergo statistical analysis. The means and standard deviations were reported and trends were noted by the authors. Upon examination of the standard deviations, many overlapped. Without statistical analyses it is not possible to determine if the means obtained are statistically different or similar to one another, or that the trends noted were statistically significant. As such, the results obtained by *Morrell et al.,* (2010) could not be compared to the results of this study.

The 2012(b) study of Langroodi *et al.,* however, had findings similar to this study. While the Soil Block Study utilized PCP (pentachlorophenol) as the fungal inhibiting preservative, the study also utilized a biodiesel blended carrier solution. They also found that the addition of biodiesel to the carrier solution resulted in the same performance as that of the PCP preservative solution, which utilized diesel as a carrier solution. These findings also suggest that the addition of biodiesel to the carrier solution does not have a negative impact on the ability of the preservative solution to deter fungal metabolic activity.

Sapwood cubes that were treated with diesel-only and biodiesel-only were included to observe if the carrier solution imparted any fungal inhibitory

characteristics without the addition of preservatives to the solutions. It was noted that when exposed to each of the four test fungi that the biodiesel-only treated cubes (both with and without stabilizer) had a significantly lower mass loss when compared to the diesel only treated sapwood blocks. These results would suggest that biodiesel is providing some resistance to fungal decay when compared to diesel. It is postulated that this resistance is attributed to the biodiesel providing an alternate, more readily accessible form of carbon for the fungal species to metabolize as it was observed that a mat of fungal mycelia was encapsulating the exterior of these sapwood cubes with little visible damage occurring to the sapwood beneath (Figure 4.1). Further study would be required to elucidate this issue.

Other interesting observations included the comparison of all the copper naphthenate containing solutions to that of CCA. None of the copper naphthenate containing solutions performed as well as CCA, which provided the highest level of inhibition of fungal metabolic activity when compared to all other treatments. CCA is one of the most widely used preservative solutions in the wood preserving industry and has been noted to have a broad range of fungal inhibition (Micklewright, 1994). This is most likely due to the arsenic component of CCA, as Lebow *et al.,* (2000) noted that the addition of arsenic provided additional protection against fungal metabolic activity.

Figure 4.1 : A Douglas fir sapwood block treated with 100BDWS showing the encapsulating layer of fungal mycelia of *P. placenta*.

It was also observed that when the sapwood cubes were treated with CCA and exposed to the four test fungi, exposure to *T. versicolor* produced significantly higher mass losses than the CCA treated sapwood cubes exposed to the three brown-rot fungi. *T. versicolor* has been noted as a fungal species that is tolerant to CCA (Guillen *et al.,* 2009) and this finding appears to coincide with what was found in this study.

4.2.2 The Degradation of Southern Yellow Pine Sapwood Blocks

Southern yellow pine (SYP) was another wood species of interest as it is of significant economic importance. Manowski *et al.,* (2002) found that 69% of the wood utility poles in service were SYP poles, and that the majority of these were distribution poles. Due to the high number of these poles in service, it was of

interest to examine the decay resistance of SYP sapwood when treated with copper naphthenate preservatives that utilized biodiesel as a co-solvent.

As previously mentioned in Section 3.11, this data set had a normal distribution and so the data was analyzed using a two-way ANOVA. Tukey's HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized. All main effects had a p value of 0.00 indicating that they were statistically significant. There was also an interaction between the fungal species and preservative treatment (p value 0.00), therefore the results from the highest order interaction are presented in Table 4.3.

As shown in Table 4.3 all of the SYP sapwood blocks treated with copper naphthenate containing solutions experienced similar rates of mass loss regardless of the percentage of biodiesel included in the carrier solution when exposed to the four fungal test species, with one exception. When these sapwood blocks were exposed to *G. trabeum* the mass loss incurred by Dcunap was significantly larger than that of 50/50BDWS. This may be in part due to the presence of propyl gallate. Hsu *et al.,* (2009) noted an antifungal effect of propyl gallate during soil block testing and while the 30/70BDWS would have propyl gallate present, the amount would be smaller than that of 50/50BDWS due to the lower content of biodiesel in the blended carrier solution.

Table 4.3 Mass losses of SYP sapwood blocks treated with Cu preservatives

Treatment	Fungus*	Mean (% mass loss)	Standard error	Tukey's grouping**
Control	PP	33.39	2.62	AB
100D	PP	13.45	1.15	DEFG
Dcunap	PP	15.55	0.46	DE
30/70BDNS	PP	12.94	0.79	DEFG
30/70BDWS	PP	13.06	0.62	DEFG
50/50BDWS	PP	11.11	0.46	EFGH
50/50BDNS	PP	12.22	0.39	DEFG
100BDWS	PP	4.85	0.88	IJK
100BDNS	PP	6.57	1.19	HIJ
CCA	PP	0.41	0.13	K
Control	PX	39.09	2.28	A
100D	PX	14.77	1.15	DEF
Dcunap	PX	14.96	0.87	DE
30/70BDNS	PX	13.03	0.57	DEFG
30/70BDWS	PX	13.73	0.59	DEFG
50/50BDWS	PX	12.50	0.46	DEFG
50/50BDNS	PX	11.88	0.58	DEFGH
100BDWS	PX	4.15	0.59	IJK
100BDNS	PX	5.49	0.79	IJK
CCA	PX	0.43	0.10	K
Control	GT	22.49	2.10	C
100D	GT	13.49	0.85	DEFG
Dcunap	GT	16.89	0.41	D
30/70BDNS	GT	12.68	0.76	DEFG
30/70BDWS	GT	12.54	1.29	DEFG
50/50BDWS	GT	11.21	0.75	EFGH
50/50BDNS	GT	14.02	1.36	DEFG
100BDWS	GT	4.17	1.33	IJK
100BDNS	GT	5.18	0.75	IJK
CCA	GT	0.61	0.39	K
Control	TV	29.69	1.58	B
100D	TV	12.53	1.43	DEFG
Dcunap	TV	15.01	0.53	DE
30/70BDNS	TV	13.08	0.70	DEFG
30/70BDWS	TV	13.71	0.73	DEFG
50/50BDWS	TV	12.58	0.92	DEFG
50/50BDNS	TV	12.07	0.61	DEFG
100BDWS	TV	9.02	1.38	GHI
100BDNS	TV	8.94	0.75	FGHIJ
CCA	TV	3.19	0.07	JK

* Brown-rot fungi were the following codes: PP(*Postia placenta*), PX (*Poria xanthan*), GT (*Gloephylum trabeum*). The white-rot fungus was denoted as TV (*Trametes versicolor*)
**Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page ix

It was also noted that when compared to each other, the 30/70 and 50/50 biodiesel-blended preservative treatments had the same mass loss regardless of the addition of stabilizer. This was also true no matter which of the test fungi that these treated sapwood blocks were exposed to.

When comparing the metabolic activity of the individual solvent components 100D, 100BDWS, and 100BDNS it was observed that the three brown-rot species *G. trabeum*, *P. xantha*, and *P. placenta* each had more metabolic activity associated with sapwood cubes treated with 100D than that of 100BDWS or 100BDNS. Sapwood cubes exposed to the white-rot, *T. versicolor*, however had the same mass loss regardless of being treated with 100D, 100BDWS, or 100BDNS. This may be due to the capability of *T. versicolor* to mineralize a wide variety of substrates. This is achieved through a non-specific enzyme system and this capability has led to *T. versicolor* being utilized extensively in bioremediation (Novotny *et al.,* 1997; Pointing, 2001).

It was also noted that none of the copper naphthenate containing solutions performed as well at inhibiting fungal metabolic activity as the CCA treated sapwood blocks. This was a result similar to that reported for DF and is thought to be attributed to the arsenic component of the preservative.

4.2.3 The Degradation of Western Red Cedar Sapwood Blocks

Western red cedar is also a wood species that is currently utilized for distribution poles. While only accounting for 13% of the poles in service, it is the third most common species utilized for utility poles (Manowski *et al.,* 2002).

This data set was non-normal and in order to meet the assumptions of normality, a log transformation of the data was performed prior to analysis. Tukey's HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized. All main effects had a p value of 0.00 indicating that they were statistically significant. There was also an interaction between the fungal species and preservative treatment (p value 0.00), therefore the results from the highest order interaction are presented in Table 4.4.

In this study it was observed that the sapwood blocks treated with copper naphthenate containing solutions experienced similar rates of mass loss regardless of the percentage of biodiesel included in the carrier solution and regardless of which of the four fungal test species the sapwood cubes were exposed to. This is similar to the results found for DF and SYP as well as to the findings of Langroodi *et al.,* (2012b) that were previously described in section 4.2.1.

Table 4.4 Mass losses of WRC sapwood blocks treated with Cu preservatives

Treatment	Fungus*	Mean (% mass loss)	Standard error	Tukey's grouping**
Control	PP	34.42	4.47	AB
100D	PP	17.28	0.78	CDEFG
Dcunap	PP	19.05	0.33	BCDEF
30/70BDNS	PP	13.15	0.27	EFGH
30/70BDWS	PP	14.50	0.90	EFGH
50/50BDWS	PP	14.82	0.73	DEFGH
50/50BDNS	PP	11.68	0.69	EFGHIJ
100BDWS	PP	14.46	2.17	EFGH
100BDNS	PP	5.00	0.84	L
CCA	PP	1.18	0.20	M
Control	PX	28.43	4.27	ABCD
100D	PX	15.79	0.46	DEFG
Dcunap	PX	19.25	0.97	BCDEF
30/70BDNS	PX	14.42	0.51	EFGH
30/70BDWS	PX	15.62	0.59	DEFG
50/50BDWS	PX	14.50	0.40	EFGH
50/50BDNS	PX	12.88	0.56	EFGHI
100BDWS	PX	4.20	0.44	L
100BDNS	PX	13.59	2.01	EFGHI
CCA	PX	7.18	1.44	JKL
Control	GT	44.91	5.77	A
100D	GT	17.35	0.53	CDEFG
Dcunap	GT	16.82	1.21	DEFG
30/70BDNS	GT	13.80	0.66	EFGH
30/70BDWS	GT	15.37	0.69	DEFG
50/50BDWS	GT	11.50	1.05	GHIJ
50/50BDNS	GT	11.60	0.65	FGHIJ
100BDWS	GT	8.44	1.24	IKJL
100BDNS	GT	6.70	1.15	KL
CCA	GT	4.84	1.02	L
Control	TV	31.42	4.69	ABC
100D	TV	14.42	1.02	EFGH
Dcunap	TV	15.08	0.48	DEFGH
30/70BDNS	TV	13.63	1.16	EFGHI
30/70BDWS	TV	16.79	1.29	CDEFG
50/50BDWS	TV	12.72	0.80	EFGHI
50/50BDNS	TV	13.56	0.66	EFGH
100BDWS	TV	16.11	0.44	DEFGH
100BDNS	TV	22.03	3.25	BCDE
CCA	TV	8.87	0.79	HIJK

* Brown-rot fungi were the following codes: PP(*Postia placenta*), PX (*Poria xanthan*), GT (*Gloephylum trabeum*). The white-rot fungus was denoted as TV (*Trametes versicolor*)
**Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page ix

Interestingly, when the sapwood blocks treated with copper naphthenate solutions were exposed to *T. versicolor* they achieved a similar mass loss to that of the CCA treated blocks. This may not be due to a better efficacy of the copper naphthenate treated sapwood blocks, but may be related to a reduced efficacy of CCA when exposed to *T. versicolor*. This may be due to the ability of *T. versicolor* to utilize a non-specific enzyme system that is capable of mineralizing a wide variety of substrates (Novotny *et al.,* 1997; Pointing, 2001).

When comparing the metabolic activity of the individual solvent components 100D, 100BDWS, and 100BDNS it was observed that the 100% biodiesel treated sapwood blocks performed as well as, or better than, the 100% diesel treated sapwood blocks.

4.2.4 Summary of the Soil Block Study

When examining the results of the mean mass losses of each of the three wood species, there are three similarities across all three wood species that were analyzed:

- Both of the copper naphthenate biodiesel-blended carrier solutions (30/70 and 50/50) had the same mean mass losses regardless of the addition of stabilizer.
- All of the copper naphthenate biodiesel-blended solutions performed as well as, or better than, the copper naphthenate preservative solution that contained 100% diesel as a carrier.

- Of the individual carrier components, 100% biodiesel (regardless of the addition of stabilizer) had the same or lower mass loss due to fungal metabolism than that of the 100% diesel component.

These results imply that the addition of biodiesel as a co-solvent does not have a negative impact on the efficacy of copper naphthenate. As previously mentioned in Section 4.2.1, these results are similar to those obtained by Langroodi *et al.,* (2012b).

4.3 Malt Study

As the preparation of the soil microcosms was both labor and time intensive, an alternate test system was sought. A 4% malt agar is utilized in the British Standard EN113, a test method for determining the test preservative effectiveness against wood destroying basidiomycetes; the applicability of this level was determined in a screening trial. To verify that this level of malt was the level most suited to produce adequate levels of decay amongst the fungal cultures in use in this trial, a series of malt levels were evaluated. All four species of wood decay fungi previously described were tested as well as all three wood species.

In order to utilize the same concentration for all fungal species and wood types, the data were pooled and only the main effect of malt was evaluated. This data set had a normal distribution; therefore a one-way ANOVA was performed on the

data set. Tukey's HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized. The main effect of malt had a p value of 0.00 indicating that they were statistically significant. The results from this trial are presented in Table 4.5 below.

Table 4.5: Summary of mean mass losses after 8 weeks incubation in the fungal microcosm

Malt % in Agar	Mean mass loss (%)	Standard Deviation	Tukey's grouping*
4	27.72	13.3	A
3	20.12	13.27	B
2	13.48	12.78	C
1	9.27	12.20	C D
0	7.13	13.34	D

*Treatments sharing the same letter are not statistically different.

As shown in Table 4.6 the 4% malt agar facilitated the largest amount of mass loss when compared to all other malt levels. The second highest mass loss was obtained with the 3% malt level; this was followed by 1% and 2% which were not statistically different from each other. The 1% level was also not statistically different from the 0% malt level. While the results were statistically significant, the variability for this trial was very high, as shown in the standard deviations and the R^2 was also low at 51.24%. For the purpose of the following study, the 4% level of malt was utilized as it produced the largest mass losses and exceeded the minimum 20% mass loss required by British Standard EN 113. However, finding another medium that produces high rates of mass loss with lower rates of variability in all strains of wood decay fungi utilized is a recommended area for further study.

4.4 Plate Study

The plate study was conducted as a means to address some of the issues that arose with the implementation of the Soil Block Study (Section 4.2). An alternate media was sought that was less labor intensive to prepare, that was able to be easily monitored for contamination, that would produce less variability of the results, and that would have comparable results to those obtained by the Soil Block Study. The British Standard EN 113 Test Method for Determining the Protective Effectiveness Against Wood Destroying Basidiomycetes (1997) was chosen to evaluate the effect of using biodiesel as a co-solvent on the fungal activity of four common North American fungal species: *Postia placenta*, *Poria xantha*, *Gloephylum trabeum*, and *Tramedes versicolor*. Each of the three wood species tested (SYP, DF, and WRC) were analyzed separately as differences between the wood species were anticipated. The data obtained from the decay of *P. xantha* was not included in these results as the untreated sapwood control blocks did not reach the minimum level of mass loss set out by BS EN113 (1997) (20% mass loss) for the results to be considered valid. The same treatments utilized in the Soil Block Study (Section 4.2) were also used in this study with one notable exception; biodiesel treatments that did not include stabilizer were not included in the experimental design.

4.4.1 The Degradation of Douglas Fir Sapwood Blocks on Malt Agar Media

It was estimated by Manowski *et al.,* (2002) that 15% of the utility poles in service in North America were comprised of Douglas fir (the majority of which are transmission poles), making this wood species of particular interest to the wood preserving industry. As such, it was of interest to examine the decay resistance of DF sapwood when treated with copper naphthenate preservatives that incorporated biodiesel as a co-solvent.

This data satisfied the assumptions of normality. The data was analyzed using a two-way ANOVA and Tukey's HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized. The results from *P. xantha* were not included in this analysis as the minimum level of decay (20%) was not achieved by the control blocks and therefore could not be considered valid as set out in the BS EN 113 (1997) standard method.

All main effects had a p value of 0.00 indicating that they were statistically significant. There was also an interaction between the fungal species and preservative treatment (p value 0.00), therefore the results from the highest order interaction are presented in the following Table 4.6.

Table 4.6 Decay resistance of DF sapwood blocks treated with copper-containing preservatives incubated in a plate microcosm

Treatment	Fungus*	Mean (% mass loss)	Standard error	Tukey's grouping**
Control	PP	54.82	2.15	A
100D	PP	15.16	0.98	D
Dcunap	PP	10.87	0.31	FG
30/70BDWS	PP	11.07	0.47	FG
50/50BDWS	PP	10.56	0.27	FGH
100BDWS	PP	2.92	0.58	J
CCA	PP	8.98	0.80	GH
Control	GT	32.50	0.90	B
100D	GT	14.85	0.52	DE
Dcunap	GT	11.52	0.41	EFG
30/70BDWS	GT	7.44	0.41	HI
50/50BDWS	GT	9.68	0.27	GH
100BDWS	GT	4.51	0.73	IJ
CCA	GT	9.87	0.52	FGH
Control	TV	27.61	2.39	C
100D	TV	14.85	0.52	DE
Dcunap	TV	10.16	0.19	FGH
30/70BDWS	TV	7.07	0.24	HI
50/50BDWS	TV	9.00	0.27	GH
100BDWS	TV	2.743	0.52	J
CCA	TV	8.57	0.46	GH

* Brown-rot fungi were the following codes: PP(*Postia placenta*), PX (*Poria xanthan*), GT (*Gloephylum trabeum*). The white-rot fungus was denoted as TV (*Trametes versicolor*)
**Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page ix

It was observed in this study that there were no differences between the 30/70BDWS and 50/50BDWS treated sapwood blocks when exposed to each of the test fungi. It was also noted that these treatments performed as well as Dcunap, with one exception. This occurred when the treated sapwood blocks were exposed to *G. trabeum.* In this instance the 30/70BDWS treated sapwood blocks achieved a lower mean mass loss than the Dcunap treated blocks. These

results were similar to those found by Langroodi *et al.,* (2012b) whose study also observed that the addition of biodiesel as a co-solvent did not affect the efficacy of the oil-borne preservative pentachlorophenol.

It was also noted that when comparing the mean mass losses of the copper naphthenate containing preservative treated blocks (regardless of the presence of biodiesel co-solvent) to that of the CCA preservative treated blocks it was found that these mean mass losses were similar.

Additionally, when comparing the metabolic activity of the individual solvent components 100D and 100BDWS it was observed that the 100% biodiesel-treated sapwood blocks performed better than the 100% diesel-treated sapwood blocks.

These results suggest that the addition of biodiesel as a co-solvent does not have a negative impact on the efficacy of copper naphthenate when treating DF sapwood. This result is similar to those obtained in the Soil Block Study.

4.4.2 The Degradation of Southern Yellow Pine Sapwood Blocks on Malt Agar Media

According to Manowski *et al.,*(2002) the majority of wood utility poles in service (69 %) were reported to be SYP, making it a wood species of significant economic importance to the wood preserving industry. Thus it was of interest to

examine the decay resistance of SYP sapwood when treated with copper naphthenate preservatives that utilized biodiesel as a co-solvent.

As previously mentioned in section 3.11, this data had a non-normal distribution. In order to satisfy the assumptions of normality, a square root transformation was performed prior to analysis with a two-way ANOVA. Tukey's HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized. The results from *P. xantha* were not included in this analysis as the minimum level of decay (20%) was not achieved by the control blocks and therefore could not be considered valid as set out in the BS EN 113 (1997) standard method.

All main effects had a p value of 0.00 indicating that they were statistically significant. There was also an interaction between the fungal species and preservative treatment (p value 0.00), therefore the results from the highest order interaction are presented in the following Table 4.7.

Table 4.7 Decay resistance of SYP sapwood blocks treated with copper-containing preservatives incubated in a plate microcosm

Treatment	Fungus*	Mean (% mass loss)	Standard error	Tukey's grouping**
Control	PP	57.56	0.23	A
100D	PP	16.38	0.13	C
Dcunap	PP	12.26	0.14	CD
30/70BDWS	PP	10.49	0.45	CD
50/50BDWS	PP	13.36	0.14	CD
100BDWS	PP	4.59	0.19	FG
CCA	PP	5.28	0.18	EFG
Control	GT	35.80	0.27	B
100D	GT	12.67	0.23	CD
Dcunap	GT	11.50	0.14	CD
30/70BDWS	GT	9.40	0.14	DE
50/50BDWS	GT	11.71	0.49	CD
100BDWS	GT	5.49	0.24	EFG
CCA	GT	4.01	0.17	G
Control	TV	33.41	0.30	B
100D	TV	13.86	0.18	CD
Dcunap	TV	12.92	0.11	CD
30/70BDWS	TV	8.76	0.33	DEF
50/50BDWS	TV	11.21	0.40	CD
100BDWS	TV	2.86	0.15	G
CCA	TV	4.26	0.09	G

* Brown-rot fungi were the following codes: PP(*Postia placenta*), PX (*Poria xanthan*), GT (*Gloephylum trabeum*). The white-rot fungus was denoted as TV (*Trametes versicolor*)
**Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page ix

When observing the results it was noted that both the 30/70BDWS and 50/50BDWS treated sapwood blocks had the same mean mass losses. It was also observed that the biodiesel blended co-solvent copper naphthenate treated sapwood blocks performed as well as the sapwood blocks treated with Dcunap.

It was also observed that the solvent component 100BDWS treated sapwood blocks performed better than the 100% diesel treated sapwood blocks.

It was also noted that none of the copper naphthenate containing preservative treatment (regardless of the proportion of biodiesel contained in the co-solvent) performed as well as the CCA treated sapwood blocks.

These results indicate that the addition of biodiesel as a co-solvent does not have a negative impact on the efficacy of the preservative copper naphthenate when treating SYP sapwood. These findings are similar to those obtained by Langroodi *et al.,* (2012b) and by the SYP soil block study.

4.4.3 The Degradation of Western Red Cedar Sapwood Blocks on Malt Agar Media

Western red cedar is currently the third most common species utilized for utility poles (Manowski *et al.,* 2002) and as such was a wood species of interest for this study.

This data set had a normal distribution and was analyzed using a two-way ANOVA. Tukey's HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized. Only the main effect of treatment was found to be significant ($p = 0.00$), while the main effect of fungal species was not ($p = 0.784$). There was also no interaction effect found ($p = 0.091$), as such only the main effect of treatment is presented in Table 4.8. Therefore, the data presented are pooled data for all fungi.

Table 4.8 Decay resistance of WRC sapwood blocks treated with copper-containing preservatives incubated in a plate microcosm

Treatment	Mean (% mass loss)	Standard error	Tukey's grouping*
Control	31.06	0.93	A
100D	17.49	0.45	B
Dcunap	12.82	0.29	C
30/70BDWS	10.66	0.29	D
50/50BDWS	10.38	0.32	D
100BDWS	1.61	0.38	F
CCA	6.14	0.59	E

*Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page ix

It was noted in this study that both of the biodiesel co-solvent blends (30/70BDWS and 50/50BDWS) had the same rates of mean mass losses. It was also observed that these biodiesel-blended preservative treatments had a lower rate of mean mass loss than that of Dcunap.

As well, it was observed that the solvent component 100BDWS treated sapwood blocks performed better than the 100% diesel treated sapwood blocks.

Furthermore, it was noted that none of the copper naphthenate containing preservative treatment (regardless of the proportion of biodiesel contained in the co-solvent) performed as well as the CCA treated sapwood blocks.

These results indicate that the addition of biodiesel as a co-solvent to a copper naphthenate preservative mixture may be more advantageous at reducing mass loss due to fungal metabolism when treating WRC sapwood. Further research is

required to fully elucidate the effect of biodiesel as an oil-borne preservative co-solvent.

4.4.4 Summary of the Plate Study

When examining the results of the mean mass losses of each of the three wood species there are three similarities across all three wood species that were analyzed:

- Both of the copper naphthenate biodiesel blended carrier solutions (30/70 and 50/50) had the same mean mass losses regardless of the concentration of biodiesel present.
- All of the copper naphthenate biodiesel blended solutions performed as well as, or better than, the copper naphthenate preservative solution that contained 100% diesel as a carrier.
- Of the individual carrier components 100% biodiesel had a lower mass loss due to fungal metabolism than that of the 100% diesel component.

These results imply that the addition of biodiesel as a co-solvent does not have a negative impact on the efficacy of copper naphthenate, and may in fact have a positive effect in certain instances. As previously mentioned in section 4.2.1 these results are similar to those obtained by Langroodi *et al.,* (2012b).

These results are very similar to those obtained from the Soil Block Study previously described in Section 4.2 despite the differences in media. Although

these two studies were not directly compared statistically, a few general observations can be made about the two testing methods. It would appear overall that there were higher rates of degradation of the preservative-treated sapwood blocks occurring within the Soil Block Study. The soil microcosm environment appears to be more challenging to the preservative treatments and as such, would theoretically make any differences in the preservative efficacies more apparent. This observation was also described by Schilling and Jacobson (2011), however, Archer *et al.,* (1993) determined that despite the differences in the two test methods, both gave results that approximated the preservative efficacy needed to protect in-service wood.

Both methods have their advantages. The plate method is easily prepared, the media is easier to control, and is more easily monitored. However, the soil block method appears to be more challenging to the preservative solutions tested as well and takes less time to achieve the desired mass loss in the control blocks than the agar block method. The choice of which method to employ will depend on the specific goals of the study.

4.5 Weathering of Douglas Fir Sapwood

An accelerated weathering procedure (outlined in Section 3.9) was utilized to evaluate the performance of the preservatives after undergoing a weathering process using DF sapwood cubes. After the weathering procedure, the sapwood

cubes were incubated within microcosms inoculated with *P. placenta* for a period of 12 weeks.

This data set was non-normal, so in order to meet the assumptions of normality a y^ 0.75 transformation was performed. Following the transformation, the data set was analyzed using a one-way ANOVA. Tukey's HSD was utilized to differentiate between the means as this test minimizes Type I error. An α of 0.05 was utilized. The main effect of treatment was found to be significant ($p = 0.00$).

Table 4.9 illustrates the mass loss of the sapwood cubes caused by exposure to *P. placenta*.

Table 4.9: Mass loss of DF sapwood cubes after 12 weeks incubation within the fungal microcosm

Treatment	Mean (% mass loss)	Standard deviation	Tukey's grouping*
CW	35.93	1.85	A
Control	32.11	1.14	A
100D	2.91	0.31	B
Dcunap	3.59	0.23	B
30/70BDWS	4.10	1.59	B
100BDWS	4.47	1.12	B
CCA	2.78	1.15	B

*Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page v

The weathered (CW) and un-weathered (Control) sapwood control blocks achieved statistically the same mean mass losses. This suggests that the weathering procedure is neither enhancing nor detracting from the ability of *P. placenta* to degrade the untreated sapwood cubes. The weathering procedure did, however, affect the mean mass losses of the preservative treated cubes. All

treated cubes, while statistically lower than both control groups, were not statistically different from each other. It can be inferred from these results that weathering of the sapwood cubes puts each preservative treatment at the same disadvantage. As this was a small study, further research is needed to explore the effects of weathering on the preservative mixtures.

4.6 Scanning Electron Microscopy Analysis of Preservative Distribution

Scanning electron microscopy was performed on three treatment groups of DF sapwood blocks (untreated, treated with 30/70 biodiesel/diesel, and 100% diesel copper naphthenate preservative solutions). The samples were prepared for analysis as outlined in Section 3.12. All data collected was measured as a ratio of copper to oxygen. A sample spectrum from sample 9b (Dcunap cross-section) is shown below in Figure 4.2. A summary of the results is shown below (Table 4.10).

Table 4.10: Summary of the means obtained from SEM analysis, where copper was measured as a ratio of copper to oxygen

Treatment	Mean (% Cu)	Standard Deviation	Tukey's grouping*
Control	0.003	0.0004	B
Dcunap	0.34	0.010	A
30/70BDWS	0.33	0.082	A

*Treatments sharing the same letter are not statistically different.
For treatment abbreviations see List of Abbreviations on page ix

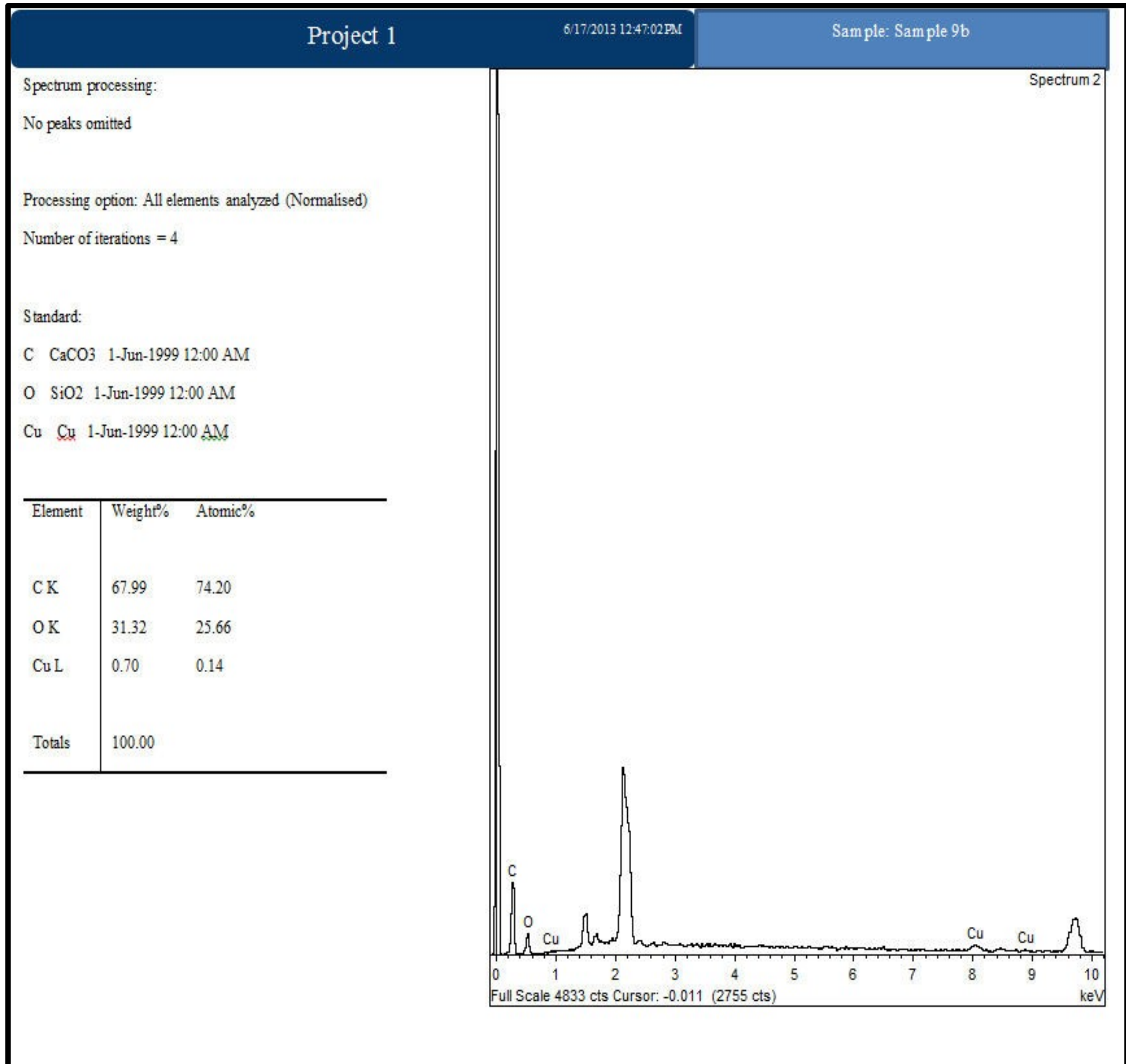

Figure 4.2: A sample spectrum taken from a Dcunap treated sapwood cross-section

Measurements were taken at several locations cut from the centre of the sapwood cubes from the tangential, radial, and longitudinal planes and were taken at set distances away from specific structures within the wood cell (Section 3.10). The location at which the copper was sampled from was not significant, however, there was a treatment effect observed. As shown in Table 4.13, the control group had the least amount of copper measured. This was as expected

and, from the low levels of copper measured, it can be inferred that the untreated Douglas fir sapwood contains very minimal amounts of copper. The Dcunap and 30/70 treatments had significantly greater amounts of measurable copper and were not statistically different from each other. It is also important to note that there was no significant effect from the location at which the measurements were taken from. These results from this trial indicate that both Dcunap and 30/70 equally distribute copper within the center of the sapwood cubes. Further study is recommended to examine the copper distribution of both preservatives within differing locations and species of sapwood.

5.0 Summary of Experiments

Despite the use of differing media and design of microcosms, the results had key similarities, while differing in a few aspects, such as mass loss attributed to specific fungal species and differences in the durability of each sapwood species used. In both the soil block and plate studies, when examining the efficacy of the copper naphthenate preservative solutions (that used either a blend of 30% biodiesel and 70% diesel or 50% biodiesel and 50% diesel) in relation to the decay caused by the four fungal species, each blend had statistically similar performances. In both trials, all copper naphthenate solutions that utilized a diesel/biodiesel-blended carrier solution performed as well as, or better than, the copper naphthenate solution that utilized 100% diesel only as a carrier.

When examining the Soil Block Study, the addition of stabilizer in all cases resulted in equal or better performance than that of its treatment counterpart which did not include stabilizer.

When examining the data of the weathering trial, similar results were observed in that both the diesel and diesel/biodiesel-blended copper naphthenate solutions had statistically the same performance. In this instance, however, both treatments performed as well as the CCA treatment.

In the SEM analysis, the location at which the measurements were taken was not statistically significant. This analysis did reveal that overall, when treated to the

same target retention, neither the use of a 100% diesel or 30% biodiesel and 70% diesel blend as a carrier solution affected the distribution of copper within the center of the treated sapwood block.

6.0 Conclusions and Recommendations

The goal of this research was to examine whether the addition of biodiesel as a co-solvent in copper naphthenate had an effect on the efficacy of copper naphthenate as a wood preservative. Based on the results from these trials several conclusions can be made.

It can be concluded that the addition of biodiesel to the carrier solution did not negatively affect the protective ability of copper naphthenate. In addition, the use of the stabilizer propyl gallate did not negatively impact the antifungal capability of copper naphthenate. The use of biodiesel as a co-solvent under weathering conditions did not negatively impact the performance of copper naphthenate. It was also concluded that the addition of biodiesel to the carrier solution at the 30% level did not negatively impact the distribution of copper within the wood cells.

From these results it can be concluded that the addition of biodiesel at a rate of 30% and 50% to the carrier solution does not negatively affect the performance of copper naphthenate as a wood preservative when exposed to samples of brown-rot and white-rot wood decaying fungi. The copper tolerance of the test fungi did not have an impact on the efficacy of the biodiesel blends either.

This research could benefit from further exploration in certain areas, such as the refinement of AWPA Standard Method E10-09 so that it could more accurately

represent the decay that occurs with in-service wood products. This would include the development of an accelerated weathering procedure that could more accurately represent the weathering process that occurs in in-service treated wood products.

The British Standard EN113 could also benefit from further research. The development of a more suitable malt medium to produce more virulent and consistent results warrants further exploration.

Further exploration of the use of Scanning Electron Microscopy could be utilized to track the progression of preservatives through different penetration levels of sapwood. As well, further exploration into sample cutting and preparation is warranted.

This research has provided validation of the use of biodiesel as a co-solvent in copper naphthenate solutions and has confirmed that the presence of biodiesel within the carrier solution does not result in a reduction of the copper naphthenate's preservative efficacy.

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