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Jeffrey J. Morrell

Copper is an excellent biocide which has a long history of usage for protecting wood from deterioration, but some organisms have developed tolerance for this chemical. Copper tolerance among wood decay fungi is a poorly understood phenomenon that is gaining importance as cofactors such as arsenic and chromium are removed from industrial wood preservatives. Currently, the occurrence and methods for detecting copper tolerance are varied and relatively subjective.

This study used a quantitative, non-destructive and automated method for testing the ability of a potentially copper tolerant soil to degrade copper treated wood *in vivo*. Flexural stiffness properties of small western hemlock samples treated to two retentions with six common wood preservatives were measured to assess microbial damage over one year of exposure to Oregon soil in an active hop yard.

While mean control stiffness of untreated controls declined markedly over one year, all preservatives conferred a high degree of protection to the samples over

the test period. Microscopic investigation revealed that a moderate amount of soft rot and general decay existed in some of the preservative treated stakes, while the controls showed widespread damage from both soft rot and general decay.

The results indicate that while the exposure period allowed fungi to colonize treated wood, these organisms had not developed to the extent that they affected properties of wood treated with copper-based biocides.

Performance of Copper Based Biocides in Potentially Copper Tolerant Soils

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Andrew B. Chang

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Approved:

Redacted for Privacy

Major Professor representing Wood Science

Redacted for Privacy

Chair of Department of Wood Science and Engineering

Redacted for Privacy

Dean of Graduate School

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Performance of Copper Based Biocides in Potentially Copper Tolerant Soils

1 INTRODUCTION

1.1 GENERAL ASPECTS OF WOOD DECAY

"Almost every chemical procedure or compound of any plausibility has been suggested in the course of the last five years, and submitted either to the admiralty or navy boards; but the multiplicity and contradiction of opinions formed nearly an inextricable labyrinth. "
--William Chapman, "Treatise on the Preservation of Timber," 1817

These cautionary words presciently describe the dilemma of wood preservation. Wood is prized for its strength, appearance, economy, overall versatility and, more recently, its renewability. Durable wood structures can be fabricated using relatively unsophisticated methods, and timber is readily available in most temperate regions. Sustainably harvested wood is an also energetically efficient and environmentally low impact building material compared to steel and concrete (Koch, 1992).

Despite its many useful attributes, wood has limitations as a structural material. It is susceptible to serious degradation by a wide variety of insects, bacteria and fungi in soil contact or at elevated moisture. As the supply of timber shifts from heartwood-rich old growth to second growth trees with higher proportions of sapwood, we may also see an increase in decay risk. Sapwood is generally easier to degrade than heartwood and increasing use of sapwood-rich plantation trees accentuates the need for effective wood preservative methods (Wilkinson, 1979; Preston, 2000).

1.2 WOOD PROPERTIES

An understanding of wood's chemical and structural composition is essential to decay investigations. Wood is predominantly composed of cellulose microfibrils surrounded by hemicellulose and lignin polymers. These fibers give wood its very broad suitability for a variety of human items, whether for pulp, composite panels or solid wood. Microstructure and chemical composition of wood also plays a critical role in determining susceptibility to biodeterioration. Briefly, cellulose and hemicellulose are more readily attacked polymers, while lignin is generally considered much more resistant to biodegradation (Rayner and Boddy, 1988).

1.3 WOOD DECAY

Wood is susceptible to chemical attack by microorganisms seeking to capture and use the energy contained by the chemical bonds in wood. While complete pyrolysis of a kilogram of wood would release an estimated 4400 kCal of heat, fungal decomposition releases 3000 kCal from that same kilogram (Bech-Andersen, 1993). Fungi are regarded as the most aggressive of wood decay organisms and can cause rapid and catastrophic damage under certain conditions (Zabel and Morrell, 1992).

Lignicolous fungi can further be differentiated as brown rots, white rots or soft rots according to the type of wood damage resulting from fungal attack. This method is useful because it is faster than identification through microscopic examination of fungal features, and there are no developed genetic techniques of identification for wood decay. Classification of fungal type according to damage has

proven useful, and a 1997 comparison of lignicolous fungi concluded that of 78 species of wood decay fungi surveyed, all could be fit into one of the three major types of fungi. White rots are generally considered to be capable of lignin attack, while brown rots and soft rot fungi are capable of only modest lignin decomposition (Worrall et al., 1997). Soft rot decay may also be caused by bacteria, but the decay is usually slow and overshadowed by fungal agents (Rayner and Boddy, 1988).

1.3.1 Soft rots

Soft rots were originally so named because they produce a softening and graying of the surface wood fibers. The term soft rot has been expanded to denote wood decay that begins as distinct elongated cavities in the S2 layer of the woody cell wall irrespective of surface softening (Levy, 1965). It is generally caused by micro-fungi belonging to the Ascomycetes or Deuteromycetes, but under specific regimes, bacteria can also cause erosion troughs classified as soft rot in the S2 layer (Singh and Butcher, 1985; Daniel and Nilsson, 1989; Baecker and King, 1985). The diversity of soft rot causal organisms is such that soft rot damage is somewhat heterogeneous and the anatomy of the damage may be a reflection of wood structure (Rayner and Boddy, 1988).

1.3.1.1 Occurrence of soft rot in wood

Soft rot fungi occur in a diverse array of environments where Basidiomycetes have difficulty establishing, such as those with low oxygen levels or frequent wet and dry cycles. Exogenous nutrient loadings also seem to help soft rot development

(Morrell, 1981). Unlike white and brown rots, the soft rots *Chaetomium globosum* and *Scytalidium lignicola* attacked birch most aggressively in the presence of relatively high concentrations of nutrients such as nitrogen, phosphorous and magnesium (Worrall et al., 1991). Soft rot promoting conditions are typical of wood in soil contact, and investigations indicate that soft rot is indeed the most frequently encountered decay type found on treated wood in soil contact (Wakeling and Singh, 1993).

1.3.1.2 Etiology of soft rot in wood

The decay morphology of soft rot differs markedly from brown rots. Differences in polymer composition between the six major sub-units of the woody cell wall exert a major influence on the progression of soft rot. Soft rot fungi preferentially attack those regions of the wood cell that are low in lignin, ie the S2 and S1 walls. The middle lamella (ML) and S3 layers initially suffer only minor damage and in fact were initially thought to be immune to soft rot (Liese, 1970). Although the ML and S3 are not readily attacked, microfungi eventually will attack these cell layers as well, resulting in wholesale degradation of the cell.

Soft rot fungi produce characteristic decay patterns known as Type 1 and Type 2 rots. Type 2 attack is generally similar to localized white-rot attack, resulting in v-shaped grooves eroded by hyphae. Type 1 attack consists of chains of acutely pointed cavities which can be visualized as a series of appressed hexagons connected end to end parallel to the microfibrils at an angle of about 45° to the tracheid axis. The cavities are readily apparent when viewed under polarized light either from the

transverse or radial wood section at magnifications of 200X or greater (Figure 4.3). Continued exposure to soft rot fungi will see the cavities multiply and eventually merge with one another, but the ultimate degree of decay is variable and likely hinges on environmental factors like wood chemical composition, ambient gases, nutrient loadings and pH regimes (Duncan, 1960; Levy, 1965). Soft rot fungi are generally thought to be less susceptible to copper containing biocides (Morrell, 1991).

1.3.1.3 Bacteria-caused soft rot

Under specific conditions, bacteria can attack all chemical components of wood. Bacterial soft rot is generally distinct from fungal soft rot, with bacteria forming erosion troughs starting in the S3 layer. It should be noted, however, that Actinomycete bacteria can produce wood decay that is morphologically very similar to that caused by true fungal soft rots, although the initial cavities are markedly smaller (Baecker and King, 1985). Furthermore, the heterogeneous conditions in which soft rot occurs may allow the simultaneous colonization of treated wood by both bacteria and fungi. At present, bacteria are considered to be slower colonizers of wood, and are therefore overshadowed by the greater impact of fungi (Rayner and Boddy, 1988).

1.3.2 White Rots

The white rots are members of the Order Aphyllophorales of the Phylum Basidiomycota that possess the ability to attack and mineralize all components of

woody tissue, including the refractory lignin. Many of these fungi employ large enzymes that are closely held to the fungal hyphae as they extend through the woody substrate. This results in a characteristic pattern of localized erosion decay (Rayner and Boddy, 1988).

While the ability of white rots to degrade phenolic type compounds suggests that these organisms may degrade organic preservatives such as creosote and pentachlorophenol, their ability to tolerate copper has not been noted (Morrell, 1991).

1.3.3 Brown Rots

Brown rot is caused by fungi belonging to the order Aphyllophorales and is unique amongst decay types because it produces extensive depolymerization of the polysaccharide wall components while leaving the lignin relatively intact. This diffuse attack does not leave localized decay features like the soft and white rots (Worrall et al. 1997).

Because of the diffuse and rapid attack, brown rots pose a greater threat to wood than other decay fungi (Highley et al, 1992). Furthermore, fungal species belonging to the broad grouping *Poria* (*sensu lato*) have long been noted for their resistance to copper toxicants (Zabel, 1954). The brown rots *Postia placenta*, *Antrodia vaillantii* and *Wolfiporia cocos* are the most commonly studied copper tolerant wood decay fungi. All of these were once classified as members of the genus *Poria*.

Brown rot fungi are aggressive wood decay fungi, producing weight losses that can reach 70% in samples (Bech-Andersen, 1993). Attack produces a characteristic brown discoloration, culminating in friable wood that cracks in cubical patterns visible to the naked eye. Lignin is only partially degraded, but nearly all of the hemicellulose and cellulose is removed. On a microscopic level, brown rot produces an indistinct, diffuse decay. The diffuse decay pattern has led to speculation that the chemical agents responsible for brown rot attack are relatively low molecular weight, and cannot be large enzymes like those possessed by white rot (Worrall et al., 1997).

In contrast to the largely enzymatic and hyphal-limited attack by white rot, brown rot is probably caused by readily diffusible degradation agents such as oxidizing species and oxalic acid. These smaller and more mobile agents cause wood decay in advance of the growing fungal hyphae. White rot decay is localized because the composite nature of the cell walls with their differential lignin, hemicellulose and cellulose makes it difficult for the enzymes to move freely. Woody structure appears to afford no such barrier to the brown rots (Jensen et al., 2001; Schmidt et al., 1981; Alexopoulos, 1996).

1.4 SUMMARY

Brown rots and soft rots pose the greatest threat to copper-treated wood. While the topic of preservative tolerance in white rot might be important in pentachlorophenol-treated and creosote treated wood, white rots are not thought to present a hazard to metallic-treated wood.

Of the two classes of decay fungi thought to possess copper-tolerant members, brown rots are the more aggressive and can cause very rapid loss of prized wood properties. Soft rots, while slower to act, are able to degrade wood under environmental conditions that inhibit the more evolved Basidiomycetes.

2. LITERATURE REVIEW

2.1 WOOD PRESERVATION

Intensive and sustained efforts to thwart wood decay organisms have led to the development of a wide variety of chemical treatments. From primitive attempts with charring to more sophisticated efforts that inactivate the enzymes specific to wood decomposers, the search for effective wood preservation techniques has continued since humans first perceived the degradation of wood under certain conditions.

Chemicals ranging from carbon-based compounds to heavy metals have been evaluated as wood preservatives over the past 200 years for their efficacy, safety, longevity and cost effectiveness. More recently imposed criteria are concerns about the environmental impact and disposal of treated wood. Shaped by these challenging and sometimes confounding performance needs, dominance in wood preservatives has progressed from creosote through pentachlorophenol to the current preeminence of metallic biocides in the U.S. wood preservation industry.

2.1.1 Copper based wood preservatives

Copper-containing compounds are among the best wood preservatives because of their outstanding combination of low cost, availability and effectiveness. Copper has long been recognized as a fungicide since the serendipitous discovery that Bordeaux mixture, consisting of copper sulphate (blue vitriol) and lime, achieved not only the intended effect of reducing human pilferage, but also

protection against the downy mildew that plagued French viticulturalists (Ainsworth, 1981; Butler, 1914). Boulton (1930) reported that copper sulphate was recommended for wood preservation as early as 1769.

Formulations of copper chromium arsenate (CCA) are currently the most widely used wood preservatives in the United States because of effectiveness and low cost. CCA resists leaching and is effective against most insects and decay fungi and when CCA was first developed by Kamesam in India, its application cost was one-sixth that of creosote (Wilkinson, 1979). This combination of characteristics led to a six-fold increase in CCA usage between 1971 and 1998 (Preston, 2000). By 1991, CCA impregnation was used for nearly three-quarters of the 17 million cubic meters of pressure-treated wood products produced annually in the U.S. (Mickelwright, 1993).

In addition to CCA, a number of other waterborne copper systems have been developed for specific applications, including ammoniacal copper zinc arsenate (ACZA), ammoniacal copper quaternary (ACQ) and copper citrate. Oilborne copper naphthenate and copper-8-quinolinolate have also become more widely applied as users seek alternatives to the traditional, but presently environmentally eschewed oilborne creosote or pentachlorophenol (PCP). This predominance of copper-based preservatives has led to concerns that copper tolerant wood decay organisms will emerge to become an important performance issue.

2.1.2 Potential shortcomings of copper based preservatives

Despite the outstanding features and widespread acceptance of metallic wood preservatives, there are two concerns about these systems. The first is the growing concern about the environmental impact of wood that has been treated with heavy metals. The toxicity of arsenic, copper, and chromium are well documented and heavy metals cannot be mineralized like organic toxicants. Excessive arsenic exposure has been linked to human cancer and copper compounds are particularly toxic to aquatic organisms. While the toxicity of metallic preservatives is important, a full treatment of this aspect is beyond the scope of this review and can be found in other summaries (Sorenson, 1991; Braunbeck et al., 1998; Weas, 1990).

The other potential problem with metallic biocides stems from the wide diversity of wood decay organisms, some of which have the ability to colonize and degrade wood treated with copper-based biocides. These organisms and the damage that they cause to copper-treated wood samples were the focus of the following study.

2.1.3 Copper tolerance

While brown rot fungi and soft rot fungi are the primary organisms implicated in damage to copper treated wood, comparatively little is known about possible mechanisms of tolerance apart from oxalic acid secretion in brown rots. From the cellular perspective, copper tolerance in non-wood decay fungi has been better studied.

Fungal mechanisms of resisting high copper levels can be lumped into either extracellular or intracellular coping methods. Acid secretion, H_2S secretion and binding of copper to the hyphal sheath or mucilage excretions are examples of physical exclusion that render copper less toxic. In tandem with the exclusion approach, fungal cells use a variety of intracellular pathways to detoxify and transport the copper from the cell.

2.1.3.1 Copper and fungal physiology

For the living cell, copper is both a necessary micronutrient at low concentrations and a toxicant at higher concentrations. Copper generally occurs in the environment as Cu^{2+} and is transported into cells as the Cu^{+} form. As a transition metal with possible valance states of 0, +1 and +2, copper is a used in a diverse number of biological processes including respiration, destruction of free radicals and ironically, as an enzymatic cofactor in the degradation of wood (Sorenson, 1991). Copper is therefore an ubiquitous micronutrient found in fungi as well as in all other living organisms.

Yeasts are known to contain 8 μg of copper per gram of biomass and copper deprivation leads to abnormal growth in yeasts (Linder and Hazegh-Azam, 1996; Shatzman and Kosman, 1978). Experiments on yeasts and Zygomycetes have indicated that modest amounts of copper are needed for energy transfer in respiration. Cytochrome C, oxidase CuA and CuB transfer electrons from oxygen to cytochrome C during respiration in both mitochondria and the membranes of bacteria.

Copper-zinc superoxide dismutases (SODs) are also an important part of an organism's defense system against free radicals that produce superoxides and other destructive oxygen species (free radicals). If unchecked, these oxygen species can attack plasma membranes and genetic material. Copper containing SODs can convert two O_2^- superoxides and water to O_2 and H_2O_2, preventing the formation of damaging free radicals (Pena et al., 1998).

2.1.3.2 The role of copper in wood biodegradation

In addition to its fundamental role in fungal metabolism, small amounts of copper are also needed by Basidiomycetes to decay wood. A non-enzymatic lignin degradation system using copper II/4-aminopyridine complex to depolymerize lignin has been proposed as a mechanism for wood degradation by white rot fungi (Fackler et al., 2001). Under some controlled tests, elevated copper levels appear to stimulate the production of wood degrading laccase, a white rot enzyme that attacks the more decay-resistant lignin. In light of copper's widespread use as an anti-fungal toxicant, it is ironic that *Trametes pubescens* increases laccase production in the presence of 1.5mM of $CuSO_4$ (Galhaup and Haltrich, 2001).

Fenton reactions have also been implicated as agents used by brown rots to produce readily diffusible oxidative species that attack wood. While copper has yet to be explicitly implicated in formation of Fenton-type reactions, studies have found that *Gloeophyllum trabeum* uses extracellular Fe^{2+} to produce H_2O_2 that attacks the wood substrate and copper could serve in a similar capacity (Jensen et al., 2001).

2.2 OCCURRENCE OF COPPER TOLERANCE

Copper tolerance by fungi has been examined by mycologists, toxicologists, environmental engineers and wood technologists. Ashida (1965), Morrell (1991), Gadd (1993) and Cervantes and Gutierrez-Corona (1994) have reviewed the literature regarding copper tolerance in fungi.

Copper tolerant behavior is a complex phenomenon which may be influenced by soil conditions, preservative type, and fungal strain. Test sites often contain pockets of copper tolerance, but the phenomenon is not always reproducible (Morrell, 1991).

Numerous species of fungi have been studied for copper tolerance and molds, yeasts, brown rots, soft rots and dematiacious fungi have been found to exhibit copper tolerant behavior. It therefore seems that copper tolerance is shared across the fungal kingdom (Aishida, 1965; Morrell, 1991; Gadd, 1993; Cervantes and Gutierrez-Corona, 1994). Furthermore, fungi of known copper tolerance such as *Wolfiporia cocos* have been observed in numerous regions within the United States (Davidson, and Campbell, 1954; Gilbertson, 1981). Further indicating the widespread distribution of copper tolerance is the large number of locations where failure of copper treated wood has been noted, including Europe, North America, New Zealand and Africa (Morrell, 1991; Wakeling and Singh, 1993; Morris, 1995).

The widespread occurrence and diversity of copper tolerant species would seem to make studies of this phenomenon easier, but while many species possess a level of copper tolerance, damage caused to copper-treated wood in service is not consistently observed, even on the same site (Schultz et al., 2000).

The sporadic failure of copper-treated wood products probably stems from the fact that intraspecies variation in copper tolerance has frequently been observed (Osborne and DaCosta, 1970; Woodward and DeGroot, 1999) coupled with soil conditions whose physical, chemical and biological properties can vary widely within a small area (Shultz et al,. 2000).

2.2.1 Copper toxicity to fungi

While copper is an essential micronutrient, high concentrations of copper act to disrupt normal metabolic processes. The toxicity of copper to wood decay fungi is generally attributed to the disruption of enzymes. Molecular biologists concerned with deficiencies in human copper homeostasis and also bioremediation of heavy metal contamination have provided a growing understanding of the threat that elevated levels of copper present to the living cell (Silver, 1990; Linder and Hazegh-Azam, 1996). Specifically, copper causes outright damage through the formation of uncontrolled reactive oxygen species (free radicals) via Fenton-class reactions that interfere with normal cell function through protein oxidation, DNA and RNA cleavage, and membrane damage (Halliwell and Gutteridge, 1999).

2.2.2 Mechanisms of copper tolerance

2.2.2.1 Extracellular mechanisms of copper tolerance

Fungi can cope with excess copper before it enters the cells using one of two allied approaches. The fungus can sequester the copper, binding it to melanin pigments, mucilage or other hyphal components outside the cell. Alternatively, fungi may produce extracellular chemicals that reduce heavy metal toxicity.

Adsorption and sequestration of copper occurs on the hyphal sheath via binding to melanin or other sites, thereby limiting uptake and exposure of cytoplasmic organelles to excess copper. One advantage of this adsorption strategy is that it is always functioning and does not need induced metabolic changes to offer protection. Adsorption of Cu^{2+} is attributed to gluconuric acid carboxyl groups on the fungal cell walls and may be aided by the production of H_2S convert metals into a sulfide species that is then immobilized on the cell wall (Gardea-Torresdey et al.,1997). Hyphal adsorption of heavy metal species may also provide the fungus with a toxic exclusion barrier against other microorganisms (Rizzo and Blanchette, 1992; Caesar-Tonthat et al., 1995; Venkateswerlu and Stotzky, 1989a; Gadd, 1984).

Acid secretion is also associated with an increase in survival of fungi in copper-rich conditions (Young, 1961; Gadd and White, 1985). As early as 1931, workers had associated the production of oxalic acid by *Poria* species (*sensu lato*) with copper tolerance, but even today, the exact role of oxalic acid produced by copper tolerant brown rots is not known (Clausen et al., 2000).

The general consensus is that oxalic acid reacts with copper compounds to

form insoluble and less toxic copper oxalate. This copper oxalate may then be bypassed or removed from the decay region (Williams and Fox, 1994, Sutter et al., 1983).

2.2.2.2 Cellular mechanisms of copper tolerance

Some species such as *S. cerevisiae* also possess cell metabolic methods, rather than cell-wall sequestration to control copper uptake (Gadd, 1984). Under normal conditions, the fungal organism effectively regulates the level of copper through small cysteine-rich protein carriers called metallothioneins. Copper exclusion mechanisms function to environmentally defined conditions, beyond which the fungal cell must depend on internal regulatory mechanisms.

Much of the investigative work on the effect of copper within the fungal cell has been performed on the ubiquitous yeast genera *Saccharomyces* and *Schizosaccharomyces* although there have also been experiments with other Ascomycetes, Basidiomycetes and Zygomycetes.

Intracellular copper binders including cysteine-rich CUP1 and metallothionein (MT) are protein-based. Some fungi including *S. cerevisiae* can respond to environmental copper with a ten-fold or greater increase in expression of CUP1 (Pena et al., 1998). Other workers have found that copper-resistant strains *of S. cerevisiae* contain two to seven times as many copies of the CUP1 resistance locus as copper-sensitive strains (Cervantes and Gutierrez-Corona 1994).

Proteins imbedded in the cell membrane play a role in copper distribution within the fungal cell. Transmembrane proteins Ctr1 through 5 function as copper

transport complexes and are found in both *S. cerevisiae* and *Schizosaccharomyces pombe*. At present, however, knowledge about these proteins and the control of their production is incomplete (Pena, et al. 2000; Zhou and Thiele, 2001).

2.2.3 Summary

In spite of numerous studies of copper tolerance over the last 70 years, our understanding of the causes of this resistance is incomplete in almost all aspects. Most studies on the biology of copper tolerance have used *Schizosaccharomyces* and *Saccharomyces*, but the same microbiological techniques have yet to be applied to copper tolerant wood decay fungi. In addition, the sporadic occurrence of copper tolerance in the field makes it difficult for wood technologists to properly assess the threat of this phenomenon to treated wood.

An updated and cross-disciplinary review of copper tolerance along the lines of Ashida (1969) and Morrell (1991) would be very valuable in pointing the out the direction that wood preservationists should take with this subject. Previous studies have proven that wood decay is a very complex system and copper tolerance is just as complex. Good progress on elucidating both intra and extra-cellular copper resistance mechanisms has been made, but the work has primarily been *in vitro* and may not accurately predict *in situ* behavior.

There is relatively little data on the occurrence of copper tolerant microbial attack under true field conditions. More heterogeneous tests using soil beds have been performed, but these tests still present an elevated decay hazard to treated samples and may not represent the biodiversity found *in situ*.

Also, international standards of testing for copper tolerance have not been developed. Established protocols for assaying for copper tolerance need to be developed for efficient evaluation of threats posed by copper tolerant fungi.

In previous tests, we have identified an agricultural soil that appears to exhibits considerable tolerance to some copper compounds, but the nature of the tolerance is unknown (Smith and Morrell, 1995, Chang and Morrell, 1997). The widespread nature of this tolerance, however, provides an opportunity to study further this phenomenon in more detail and was the subject of this study.

2.3 SMALL SCALE BENDING TESTS AS A DECAY ASSESSMENT METHOD

Previous studies on copper tolerant wood decay fungi have followed three overall methodologies. The first is to observe copper treated wood failing in service and find evidence of microbial attack through the microscopic examination of the wood samples (Singh and Butcher, 1985, Singh and Wakeling, 1993). In the second approach, workers inoculate cultures of known copper tolerant species onto copper treated wood samples or culture media under sterile conditions and observe the resulting damage (Baecker and King, 1985; Hale and Eaton, 1989; Daniel and Nilsson, 1989). More recently, work in Europe has focused on comparing stiffness losses in preservative-treated specimens exposed to soil beds. These tests could prove invaluable in assessing the copper tolerance threat to wood under more heterogeneous conditions (Machek et al., 1998; Militz et al. 1996; Williams and Caswell, 1993).

Despite the large volume of copper-treated wood in the United States, this topic has received less attention in North America. Additionally, none of these approaches expose wood treated with accepted formulations to actual field decay conditions. The current literature therefore cites copper tolerance by one or perhaps a few organisms rather than the multitude of decay agents that exists *in vivo*.

The soil block test and visual assessment of buried samples are the most widely accepted methods for assessing the efficacy of wood preservatives (Zabel and Morrell, 1992). The validity of these methods for assessing the efficacy of wood preservatives has been established for more than half a century and their value is proven by the fact that all of the currently used preservation formulations have been developed using these tests.

2.3.1 Soil block tests

The soil block test was adopted first in Europe and later on in the United States (Leutritz, 1946). The weight of a block of wood is measured on an oven dry basis prior to and after exposure to decay organisms. These threats may be single isolates of fungi selected as aggressive representatives of their type of decay such as *Postia placenta* (brown rots) or *Trametes versicolor* (white rots). Alternatively, the samples may be exposed in soil beds under strictly controlled moisture and nutrient conditions. The decay resistance of the sample is defined according to the weight loss of the oven dried block following fungal exposure.

This method has the drawback that it does not represent the natural heterogeneous decay environment, which is a more complex system of microbial

communities and varied conditions (Rayner and Boddy, 1988). It also tends to overlook more subtle changes in material properties that occur early in the decay process (Crawford, 1995).

The soil block method also does not address one of the most important aspects of preservative efficacy, namely the ability of the preservative to extend the structural properties of wood under conditions conducive to fungal attack. Many fungi can cause substantial loss in wood properties while producing only slight weight losses and little visual evidence of damage (Wilcox, 1978; Crawford, 1995).

2.3.2 Field stake tests

Field stake tests are also widely used to assess wood preservative efficacy (Blew, 1948). Preservative-treated stakes are buried in soil and visually inspected at set time intervals for wood decay. The condition of the preservative treated stake is then compared to untreated controls as well as stakes treated with reference preservatives.

A disadvantage of stake tests is the time required for wood decay fungi to produce meaningful results. For example, field stake tests can last from three to 20 years depending on the wood species, treatment chemicals and exposure conditions (Morrell and Zabel, 1992). Additionally, the visual assessment of wood decay is subjective and restricted to the surface. Subsurface damage may therefore be overlooked, and the reproducibility between trials at different sites and observers is limited.

2.3.3 Summary of copper tolerant assessment methods

A key deficiency of conventional methods is their inability to assess the strength loss caused by wood decay. The rationale behind treating wood to protect against decay stems from a desire to extend useful structural life, yet the widely practiced methods of assessing preservative efficacy do not directly measure wood strength properties. While both soil block and stake field tests could be adapted to allow for strength loss determinations, the adaptation is not generally made.

In light of the drawbacks to the two conventional preservative efficacy test methods, an alternative method for decay assessment would be desirable. While no method can compensate for the varied decay threat posed by infinite combinations of decay factors and conditions, methodologies have been developed to quantify the mechanical effects of wood decay.

2.4 OVERVIEW OF STRENGTH ASSESSMENT TECHNIQUES

Mechanical tests to assay the mechanical impacts of decay have been explored at least since 1926 when Longyear investigated the correlation between weight loss and strength decrease, concluding that significant strength loss preceded noticeable weight loss.

2.4.1 Destructive mechanical strength tests

Subsequently, two general reviews by Mateus (1957) and Wilcox (1978) have described a number of mechanical testing alternatives to weight loss or visual

assessment. Toughness, or the ability of wood to withstand impacts is generally the first mechanical property to be affected by wood decay. Generally, a 10% weight loss in specimens will result in a greater than 60% loss in impact resistance (Richards, 1954). In addition to toughness, hardness, modulus of rupture (MOR) and tension testing can all be used to assess wood decay (Mateus, 1957).

Toughness, hardness and MOR and tension testing have the disadvantage of being destructive tests: in the process of testing the samples material properties are irrevocably changed as the stresses placed on the sample exceed the proportional limit (Panshin and de Zeuuw, 1980).

2.4.2 Static bending (MOE) tests

Modulus of Elasticity (MOE) testing, however, is non-destructive and allows several operational advantages. The decayed sample can theoretically be replaced into the decay test after evaluation, although it is unclear what impact temporary removal might have on microbial decay. Non-destructive tests also permit detection of systematic errors and the retesting of samples.

A four point mechanical testing methodology measuring stiffness below the proportional limit was used to assess wood preservative as early as 1957. While the concept attracted favorable comments when first presented, the method did not supplant the soil block method (Mateus, 1957). More recently, research in the Netherlands has shown that vibrational energy initiated through stakes can also be used to dynamically determine the MOE loss due to decay (Machek, et al., 2001). If acceptably consistent, this technique would provide an marked by the ability of

researchers to repeatedly test samples while minimizing the impacts of sampling on decay etiology.

Nicholas et al. (1991) and Crawford (1995) developed a three point small scale bend testing apparatus to measure stiffness reduction as a possible means for rapid quantitative assessment of decay. While Winandy and Morrell (1993) found that a four-point bending test produced a more uniform bending force which minimized the effects of localized decay, Nicholas and Crawford's system provided the advantage of precisely controlled and repeatable deflections, as well as a constant point of bending stress contact on the sample.

Crawford found that this method allowed efficient testing of small samples of wood both prior to and after exposure to decay fungi. Stiffness retention can be expressed as a percent of the original stiffness, producing a non-destructive assessment of a quantifiable wood property. The developmental investigations suggested that MOE testing procedures allow fungal attack to be detected earlier than would be possible using either visual or weight loss methods (Crawford, 1995). This technique might be useful for assessing microbial effects on various treated stakes exposed in a copper tolerant environment.

2.5 OBJECTIVE OF STUDY

This study used small sample stiffness tests and microscopic examination to assess the performance of western hemlock samples treated with copper-based biocides and exposed to agricultural soils in the Willamette Valley region of Oregon.

3 MATERIALS AND METHODS

3.1 MATERIALS SELECTION AND CONDITIONING

Western hemlock (*Tsuga heterophylla*) was selected as the test material because the heartwood and sapwood of this species tends to be indistinguishable and uniformly treatable (Kumar and Morrell, 1989, Panshin and de Zeeuw, 1980). In addition, untreated western hemlock wood has been classified as slightly or non-resistant to decay (USDA, 1987). This lack of natural decay resistance meant that increases in strength retention could be attributed to the test preservative rather than to inherent variation in natural decay resistance within the species. Finally, low decay resistance was expected to translate into more rapid stiffness loss in the untreated control samples, reducing the time needed to obtain meaningful results.

Flat grain boards were selected from kiln-dried western hemlock purchased from a Corvallis lumber retailer. Thirty two hundred stakes (150mm X 17mm X 3mm in the axial, radial and tangential directions, respectively) were cut from the boards.

The stakes were conditioned to a constant weight at 65% relative humidity and 19° C, and weighed (nearest 0.01g). A weight distribution curve was constructed for the stakes, and 1820 stakes falling between a range of 3.357 grams and 4.227 grams were selected as the stiffness test stakes. This range corresponded to the mean weight of the original 3200 stakes ± 1 standard deviation (Figure3.2). The intent of this procedure was to reduce weight variation among samples and reduce the potential influence of density variations on decay rate and stiffness.

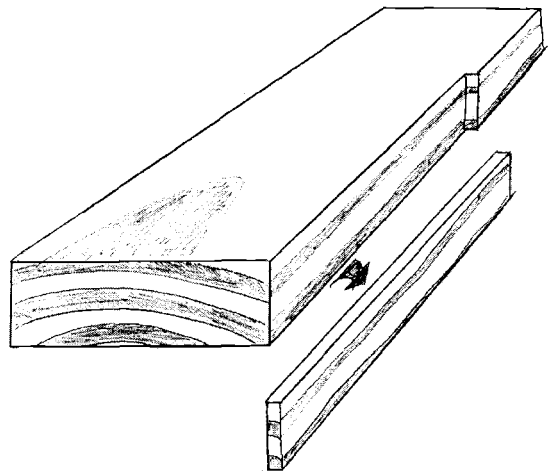

Figure 3.1. Pattern for cutting stakes from kiln-dried western hemlock 1"x6" stock

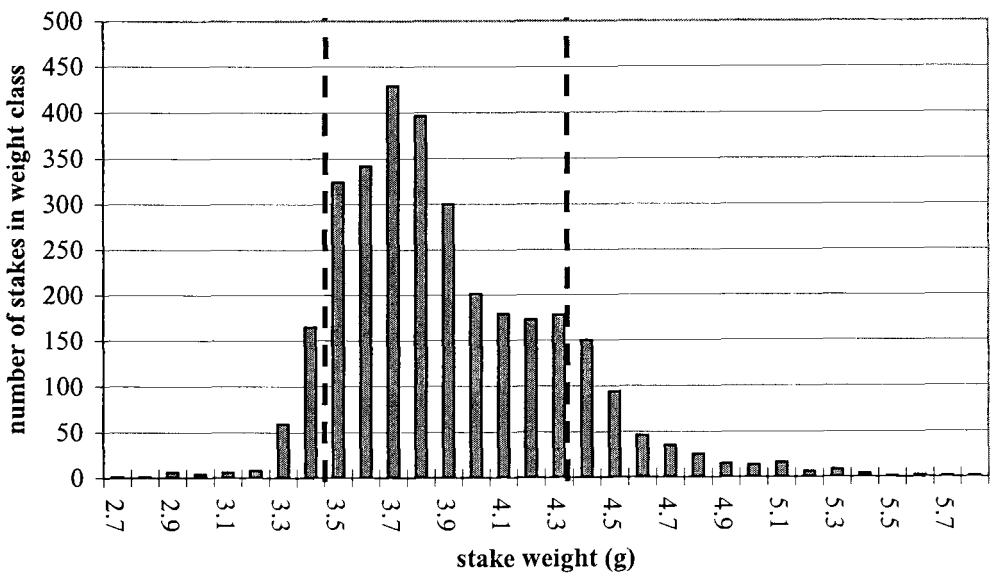

Figure 3.2. Weight distribution for 3193 untreated western hemlock stakes. Dashed lines indicate those stakes falling between 3.4 g and 4.3 g used for stiffness testing.

These 1820 stakes were randomly assigned as modulus of elasticity samples to 14 treatment groups using a random number generator, to produce 14 groups of 130 stakes each.

3.2 PRESERVATIVE TREATMENTS

Six wood preservatives were evaluated at two commonly used concentrations for a total of 12 preservative treatments (Table 3.3). Waterborne chemicals were evaluated at the two AWPA C-2 standard retention levels of 6.4 kilograms/ cubic meter (0.40 pounds per cubic foot (pcf)) and 4.0 kg/m^3 (0.25 pcf). Oilborne chemicals were evaluated at retentions according to the AWPA C-2 standards (1999). These were 0.96 kg/m^3 (0.040 pcf) and 0.48 kg/m^3 (0.020 pcf) for copper naphthenate and 0.96 kg/m^3 (0.040 pcf) and 0.48 kg/m^3 0.020 pcf for copper-8-quinolinolate. During treatment, the AWPA standard pound/cubic foot (pcf) retention units were converted to grams of preservative per liter of wood for convenience. While all biocides contained a copper component, many also had co-biocides intended to protect the wood from copper tolerant fungi. It was thought that the different formulations might provide insights concerning the copper tolerant fungi that might be present in the soil. In addition, the use of chemicals and retentions according to current AWPA treating standards provided an assessment of the real world preservative effect afforded to the wood samples.

Diesel oil and water controls were pressure-treated to establish the solvent sorption of the test stakes. For each treatment, 130 stakes for each treatment were placed in a tank and entirely submerged in either diesel or water using coated lead

weights. The treating tank was sealed in the treatment vessel. The stakes were then subjected to a modified full-cell process beginning with a 4.76 Kpa vacuum for 25 minutes, after which, pressure was raised to 862 Kpa and held for 50 minutes (AWPA, 1999). The pressure was released and the vessel was opened. Stakes were wiped dry and weighed to determine net solution absorption.

A one liter volume of hemlock stakes absorbed 708.9g of water or 515.1g of diesel oil. From these trials, it was established that the 130 stakes, representing 0.9740L of volume would be totally immersed during all phases of the pressure treatment with 2200g of diesel oil or 2500g of water (Table 3.4). These weights of solvents were used for all treatments, and the weights of preservative active ingredients were adjusted to the target the concentrations (Table 3.4). One hundred thirty randomly selected stakes were treated with each formulation using the full-cell process. Following treatment, any stakes floating on the surface of the treatment solution were considered insufficiently treated and discarded. The remaining stakes were weighed to determine net retention (Table 3.4) and stored for 24 hours at 21-23° C to allow fixation reactions to proceed. The stakes were then air-dried to allow solvent evaporation.

A Kron® label maker was used to create weather and UV-resistant plastic laminate identification labels that were stapled to the wide face of each stake using corrosion-resistant Monel® staples.

Table 3.1. Stock solutions of chemical preservatives used to treat western hemlock stakes.

Chemical	Abbrev.	Stock Conc. (%)	AWPA Standard	Carrier	Trade Name	Source
Ammoniacal Copper Zinc Arsenate	ACZA	11.47	C2	Water	Chemonite II	J. H. Baxter, San Mateo, California
Copper Chromium Arsenate, Type C	CCA	50.0	C2	Water	K-33	Osmose, Buffalo, New York
Ammoniacal Copper Citrate	CuCit	9.0	C2	Water	Copper Citrate	Osmose Buffalo, New York
Ammoniacal Copper Quaternary	ACQ	10.0 50.0	C2	Water	ACQ	CSI Charlotte, North Carolina
Copper Naphthenate	CuN	8 as CuO	C2	Diesel	MGARD S-520	OMG Cleveland, Ohio
Copper-8-Quinolinolate	Cu8	0.6 as CuO	C2	Diesel	PQ-15	ISK-Biotech Sciences, Memphis, Tennessee

3.3 PRE-EXPOSURE STATIC BENDING STRENGTH TESTING

Prior to field exposure, all samples were subjected to a stiffness test as described by Crawford (1995). The stiffness test was a third point load test performed on a small-scale bend testing apparatus developed by Dr. Darrel Nicholas at the Mississippi State University Forest Products Research and Utilization Lab. This apparatus allowed for a repeatable loading rate and measured the strain response of the stake at a fixed deflection and is shown in Figure 3.4.

Table 3.2. Average retentions of selected wood preservatives in western hemlock stakes treated using a full cell process.

Treatment	Target Retention kg/m^3	Treatment Solution, Conc. (%)	Net Solution Retention, g/stake	Net Preservative retention, kg/m^3
ACZA	6.4	0.896	5.91 (0.35)	6.19 (0.37)
	4.0	0.562	5.87 (0.29)	3.86 (0.19)
CCA	6.4	0.896	5.86 (0.44)	6.14 (0.46)
	4.0	0.562	5.89 (0.33)	3.87 (0.22)
CuCit	6.4	0.896	5.87 (0.41)	6.15 (0.43)
	4.0	0.562	5.88 (0.33)	3.86 (0.22)
ACQ	6.4	0.896	5.91 (0.32)	6.19 (0.34)
	4.0	0.562	5.80 (0.31)	4.35 (0.62)
CuN	0.57	0.00125	3.82 (0.69)	0.56 (0.09)
	0.28	0.00063	3.94 (0.60)	0.290 (0.10)
Cu8	0.64	0.001333	3.85 (0.62)	0.60 (0.10)
	0.32	0.000667	3.90 (0.68)	0.30 (0.05)
#2 Diesel (control)	N/A	N/A	4.04 (0.60)	N/A
Water (control)	N/A	N/A	5.58 (0.49)	N/A

a Values represent means of 130 replicates. Figures in parentheses denote one standard deviation

Water saturated stakes were used because bending properties become more stable above the fiber saturation point (USDA, 1999). Samples were submerged in water and subjected to a vacuum of 94.79 kpa for 30 minutes then pressure was raised to 1034 kpa and held for 50 minutes.

The saturated stakes were then individually placed on the bending apparatus (Figure 3.3a), which allowed precise control of both loading rate and maximum

sample deflection (Crawford, 1995). The bend testing was essentially automated, with the electronic controls providing repeatable loading rate and deflection. Each stake was manually positioned so that the span distance between the two rollers was 140 mm. A plunger was then advanced using an electronically driven ball screw drive at a loading rate of 104.5 mm/min until the center of the stake deflected to a maximum of 2.50 mm past its relaxed position (Figure 3.3b). Crawford established these settings in southern yellow pine samples as the best compromise that neither exceeded the proportional limit nor suffered from excessive electrical "noise" which would decrease the sensitivity of the measurements. A Transducer Technologies™ load cell in the plunger measured the strain exerted by the flexed stake on the plunger. This information was relayed to the software that provided a profile of each flex test and recorded the maximum strain at 2.50 mm. The machine was calibrated using a spring steel calibration bar after every thirty stakes. This standard produced a strain of 1140 to 1150 grams at 2.50mm of deflection. Each sample was measured twice to reduce the risk of operator-caused errors.

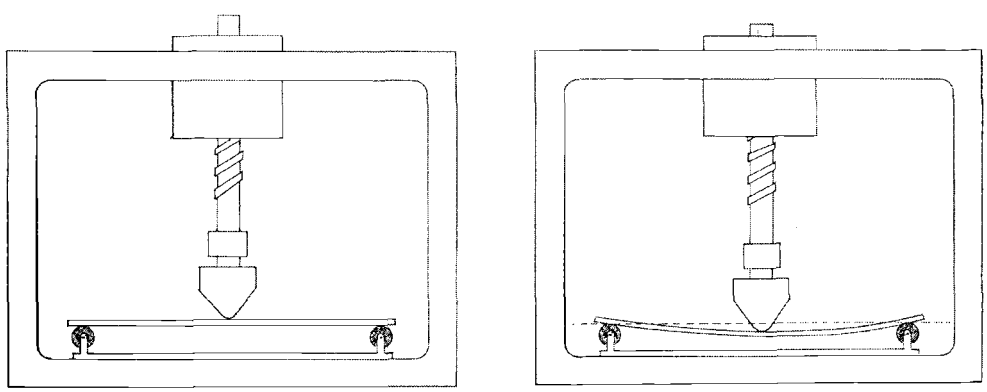

Figure 3.3. Bend testing apparatus with sample a) relaxed or b) deflected to 2.5mm.

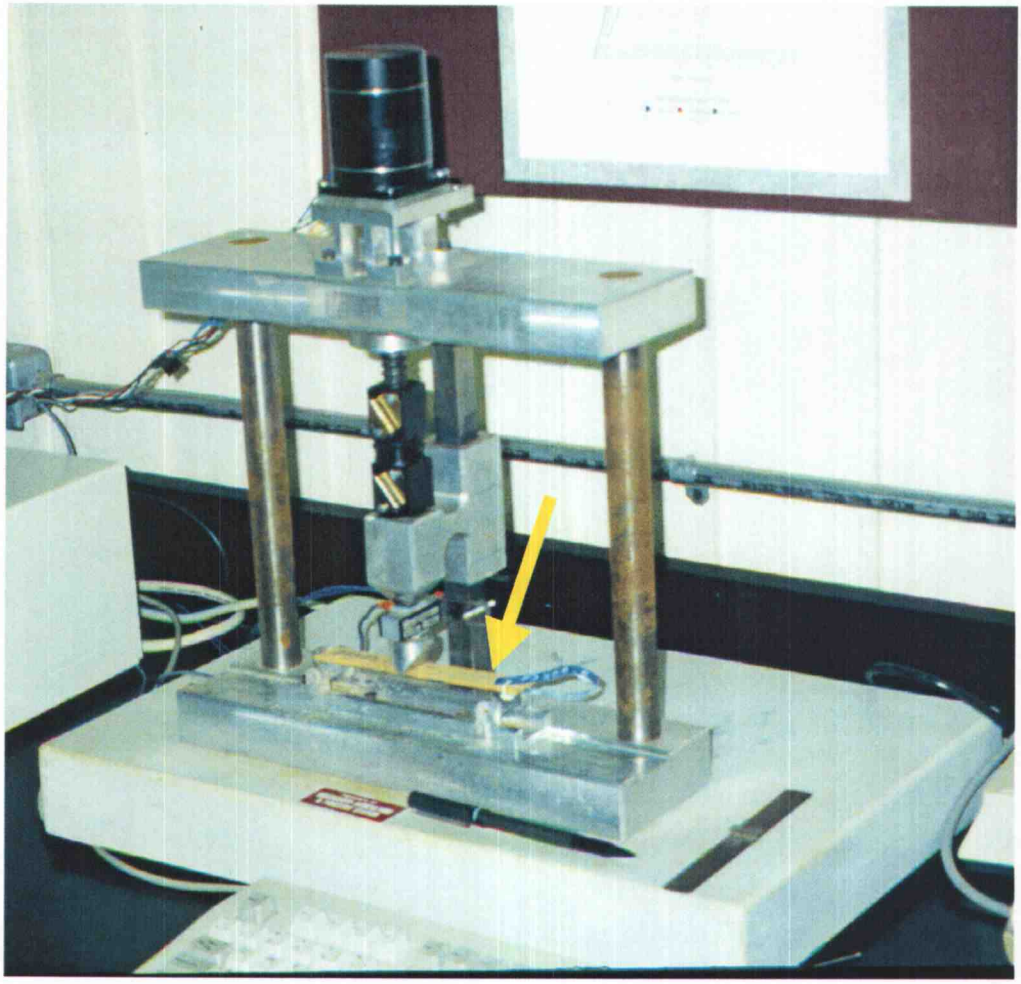

Figure 3.4. Photo of Bend Test Apparatus: MSU 1997. The stake to be tested is indicated by the arrow. (Ballpoint pen at base of apparatus for scale.)

3.4 FIELD PLACEMENT

The stakes were exposed to soil in two fields on a hop farm located near Brooks, Oregon. The sites were chosen because previous studies had suggested that the soil micro-organisms in this area were especially copper tolerant (Smith and Morrell, 1995, Chang and Morrell, 1997). Hop production involves applications of foliar copper sprays to control the growth of powdery mildew. In addition, hop vines

are supported by copper naphthenate or copper-8-quinolinolate treated kraft paper twine. High copper inputs and previous tests showing rapid degradation of copper treated twine led to speculation that long-term copper inputs had conditioned local soil fungi to relative copper tolerance.

The lower field was in the Willamette River floodplain (N45°02'37.9," W123°01'04.8"), while the upper site was approximately 20 meters above the lower site in elevation (N45°02'49.7", W122°59'25.1"). The stakes were planted amongst the perennial hop plants so that damage from agricultural equipment would be minimized. Additionally, it was anticipated that the hops yards would be irrigated over the dry summer months, thereby preventing dry conditions that might retard fungal decay rate.

Both field sites were level and received similar quantities of solar radiation, but the two fields were at slightly different elevations. The lower field was essentially in the Willamette Valley floodplain and the upper field was approximately 20 meters higher in elevation. In addition, the soil structure of the two fields differed. The lower field frequently showed extensive areas of standing water during the rainy season while the upper field was better drained.

Soil Conservation Service soil maps for Marion county showed that the lower field site consisted of either Chehalis silty clay loam (Ch) or Cloquato silt loam (Cm) soil series. The Ch soils are slightly acidic (pH 6.6) and moderately permeable, while the Cm soils are moderately acidic (pH 6.0) and moderately permeable. The soil type of the upper field was an Amity silt loam of pH 6.0 and moderately low

drainage. The soil map descriptions of the two fields did not differ markedly from one another, although the lower field was rated as more fertile (Williams, 1972).

Sixty-five stakes from each treatment were located in each of two fields. The fourteen treatments were randomly mixed in a grid of 910 randomly ordered stakes in each field. Stakes were inserted in the soil 150mm apart from each other to a depth of 130mm. Assignment of a given stake to its grid location was randomized and independent of treatment. The location of each stake was mapped so that harvests could be scheduled through random selection.

3.5 SAMPLE EXTRACTIONS

Stakes were removed at 7 intervals between one and 12 months after burial. The removal schedule was based upon the bending test results from previous removals. At each time point, five unbroken stakes for each treatment were carefully extracted from the soil .

Residual soil was washed from the stakes which were subsequently dried at 30°C to arrest any further decay. Post exposure bending test procedures were identical to pre-exposure procedures. The samples were shipped to Mississippi State University for testing in the same bending test apparatus using the method described (Figure 3.4) by Crawford (1995).

Samples tested prior to exposure had stiffness values ranging from 950g to 2700g at 2.50mm of deflection. To compensate for this wide range in inherent bending strength, stiffness loss was expressed as a percentage of original stiffness.

This allowed the values for the different stakes to be averaged and the means of treatment groups to be compared. The time interval between extraction, testing and return, would have made it impossible to replace the stakes in the field without seriously influencing decay progression.

3.6 DATA ANALYSIS

The experimental design used each field as a completely randomized block. Two ANOVA tests were performed to test for significant differences between treatment means. Means were compared using the Tukey-Kramer procedure. P-values of less than 0.05 were considered to be significant, while p-values between 0.050 and 0.10 were interpreted as suggestive, but inconclusive. Because of the randomized block design, and the likelihood of unequal sample sizes, the GLM procedure was used in SAS. Initially, the procedure was run with field location to test for significant differences between fields. Once field site was determined to be statistically similar, the data from these two sites were combined; then the effects of treatment and time were modeled.

3.7 MICROSCOPIC INSPECTION

Polarized light microscopy was used to examine stakes for evidence of fungal attack. Damage was assessed on stakes removed after 12 months of exposure. Two specimens from each lower retention and the water/diesel controls were examined using light microscopy for soft-rot or other forms of decay. Transverse sections (50μm thick) were cut from each stake at 45mm below the top of each sampled stake

using a sledge microtome. In addition, tangential/radial sections (25μm thickness) were prepared to provide an axial profile view of the cell walls. All sections were mounted in a 50:50 mixture of ethanol and glycerin and placed under coverslips. A Nikon Labophot-2 compound light microscope with a swing-out polarizing filter was used for microscopic examination of the sections. The fields were examined at 40X, 200X and 400X magnification. The polarizing filter increased the contrast between soft rot cavities and surrounding sound wood for tangential sections.

Decay assessment of the radial and transverse sections was necessarily subjective. Two stakes from the lower retention of each treatment were used from each field. Each stake was given a visual assessment score for general decay and soft rot. Each of the slides was scanned over its entire surface to provide an overall assessment of cell wall damage. Scores for each stake within a treatment were averaged for a mean of n=4. For example, the water treatment scored a mean of 3 and 5 for general and soft rot decays respectively. These scores were added for a total mean decay rating of 8, the greatest decay level of any of the tested stakes.

3.7.1 Soft rot rating

1. **No Decay**—cell wall material entirely intact, no discoloration or staining visible. Decay extent assigned a value of 0.
2. **Minimal Decay**—Cell walls largely intact; Type 1 soft rot cavities take up less than 10% of the estimated total surface area of cell walls in transverse sections. Decay extent assigned a value of 1.0.
3. **Moderate Decay**—Type 1 soft rot cavities present on greater than 10% but

less than 30% of the surface area of cell walls in the tranverse section. Cavities are also still confined with the cell wall S2 region. Decay extent assigned a value of 2.0.

4. **Extensive Decay**—Type 1 soft rot cavities occupy 30 to 70% of available surface are of cell walls in the transverse section. Decay extent assigned a value of 3.0.
5. **Complete Decay**—Type 1 soft rot cavities pervasive on greater than 70% of available cell wall surface area in the transverse section. Cavities have proceeded through the S2 layer and into the S1, S3 and middle lamella regions, resulting in near collapse. Decay extent assigned a value of 4.0.

3.7.2 General decay

1. **No Decay**—cell wall material entirely intact, no discoloration or staining visible. Decay extent assigned a value of 0.
2. **Minimal Decay**—Cell walls largely intact; Faint cracking or occasional splitting allowed with small amount of discoloration. Decay extent assigned a value of 1.
3. **Moderate Decay**—Pitting and/or cracking evident in cell wall; rays darkening. Decay extent assigned a value of 2.
4. **Extensive Decay**—Type 1 soft rot cavities occupy 30 to 70% of available surface are of cell walls in the transverse section. Decay extent assigned a value of 3.

5. **Complete Decay**: Extensive decay past the S2 layer and into surrounding areas. Large portions of the cell wall attacked and decomposed. Decay extent assigned a value of 4.

An mean aggregate score for decay in a given treatment was determined by calculating the means for both soft rot and general decay index for each of the four stakes sampled within a treatment Minimum and maximum aggregate soft rot decomposition ratings were therefore 0 and 8 respectively.

Evidence of soft rot type cavities in both the transverse and radial sections was recorded using a Nikon FDX-35 camera and a Nikon H-III adapter between the camera and compound microscope.

4 RESULTS AND DISCUSSION

Stake stiffness values initially increased from their original pre-exposure states. This behavior was consistent with earlier observations (Nicholas, pers. comm.). The controls initially gained strength and then rapidly lost stiffness as the trial progressed. Preservative treated stakes, however, showed relatively little decline in stiffness over 12 months. Degradation apparent to the naked eye was confined to those stakes that had been treated with the water control. The untreated water controls stakes became increasingly difficult to remove intact from the ground as exposure time increased. The water control stakes were noticeably more friable than the diesel controls and all of the treatments after twelve months of exposure, but a cursory comparison of percent weight loss between the treatments revealed no meaningful quantitative difference between the treatments. All treated stakes were generally in good condition, although the heavier treatments of copper naphthenate appeared to be somewhat softened.

4.1 SITE EXPOSURE FACTORS

4.1.1 Stake attrition

The test site was an active hop yard which experienced regular tilling and other manipulation. An excess of stakes were installed to compensate for any stakes that might be destroyed by field machinery. Despite this precaution, serial trips to the hop yards revealed that agricultural machinery destroyed nearly half of the stakes

from one treatment (Figure 4.2). The high attrition rate caused intact stakes to become increasingly difficult to locate within the stake beds, and more than seven extractions would have been possible only if some of the treatments were omitted.

Stakes were placed in an active hop yard to ensure that soil moisture was not limiting. During the fourth, sixth and eighth month extractions, however, it was clear that the soil dried out quite thoroughly, showing desiccation down to a depth of 150 mm (Figure 4.2). During the rainy months, the lower field was frequently water-saturated with standing water on the surface of the field (Figure 4.1). The upper field was generally better drained, and no standing water was observed.

4.1.2 Weather conditions

Willamette Valley soil moisture conditions typically are wet for about 4 to 5 months and then dry for the remainder of the year. Figures 4.1 and 4.2 illustrate the extremes of western Oregon soil moisture conditions. Considering the assumptions that brown and white rots are most active under sustained moisture contents between 40 and 80% MC, it is possible that the extremes in moisture conditions experienced by the stakes in 1998 favored soft rot attack.

Much of the original interest in soft rots stemmed from the attack of wood in cooling towers, and based on this, soft rot fungi are thought to become a significant component of wood decay during frequent wet/dry cycles (Morrell, 1981; Rayner and Boddy, 1988).

To compare 1998 weather conditions to typical Willamette Valley conditions, precipitation and temperature records from the Salem, Oregon airport were located

(Oregon Climate Services website). These precipitation and temperature records indicated that 1998 weather was slightly but not substantially different than the thirty year average for the years 1961 through 1990 (Tables 4.1 and 4.2). Overall, there was slightly more precipitation in 1998; the rainy season saw more precipitation while the dry season say slightly less precipitation. In addition, the average temperatures for 1998 were slightly higher than the surrounding years.

In summary, a comparison of typical Salem weather records to the weather encountered by the stakes in 1998 indicates that the stakes experienced close to normal precipitation and rain.

Figure 4.1. Lower field test site on Willamette flood plain, January 1998 showing standing water in tire tracks.

Figure 4.2. Photograph of dry summer soil conditions and test stakes destroyed by agricultural machinery. Photograph taken during stake extractions at month 8 in August, 1998.

Table 4.1. Monthly mean temperature (°C) at the Salem WSO, Airport from the Oregon Climate Services

	Temperature (°C)												
Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Ann. Tot.
1996	4.8	5.3	8.7	11.9	12.9	17.6	22.2	21.3	16.6	12.2	7.5	5.4	12.2
1997	5.0	6.1	8.2	10.6	16.8	17.0	20.5	22.0	18.8	12.1	9.9	5.0	12.7
1998	**6.1**	**7.8**	**9.3**	**11.5**	**13.5**	**17.4**	**21.6**	**21.7**	**19.4**	**12.4**	**9.4**	**4.7**	**12.9**
1999	6.0	6.5	7.7	10.4	12.7	16.2	19.5	20.6	18.1	12.8	10.1	6.6	12.3
Avg.*	4.1	5.9	7.7	9.4	12.6	16.2	19.0	19.2	16.3	11.4	7.2	4.4	11.1

*Avg. represents historical mean of years 1961-1990.

Table 4.2. Monthly mean rainfall (mm) at the Salem WSO, Airport from the Oregon Climate Services.

	Precipitation (mm)												
Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Ann. Tot.
1996	210	330	78	145	82	19	23	5	49	121	257	381	1701
1997	230	153	186	87	61	47	9	29	97	164	112	80	1162
1998	**230**	**157**	**126**	**41**	**141**	**25**	**3**	**1**	**17**	**64**	**297**	**297**	**1323**
1999	244	290	129	34	48	36	4	17	2	62	184	137	1187
Avg.*	150	114	106	61	48	34	14	19	39	76	160	173	995

*Avg. represents historical mean of years 1961-1990.

4.2 EFFECT OF TEST SITE ON FIELD CONDITIONS

The field trial was designed as a randomized block because it was thought that the soil microbes specific to the two fields might cause significantly different strength losses between identical treatments in the upper and lower field sites. An inspection of the mean strength loss curves suggests that this speculation might be supported by empirical evidence, with the lower fields showing a more rapid reduction in stiffness (Figure 4.3, Table 4.3 and Table 4.4). Within a given treatment, mean lower field retained stiffness was generally lower than its counterpart from the upper field, although the standard deviations were large compared to the means.

To test for a field location effect, an ANOVA comparing identical treatments for each of the two soil fields was conducted with Statistical Analysis Systems (SAS)

v.8 software using the Tukey Kramer comparison (Table 4.5) at the α=0.05 level. These comparisons showed that there were few significant differences with the exception of those stakes extracted after two months of exposure, where 5 of 14 comparisons indicated a strongly suggestive or significant difference between the upper and lower test.

Table 4.3. Stiffness retention percent means of treated and untreated western hemlock stakes exposed for a maximum of 12 months in an Oregon soil. (mean ± standard deviation; n = 5). See Materials and Methods section for exact chemical concentrations and retentions.

			% of Original Strength						
			Exposure Period (Months)						
Trt.	Targ. Reten. kg/m^3	Field Site	**1**	**2**	**3**	**4**	**6**	**8**	**12**
CuN	0.57	Upper	103(1)	98(1)	93(4)	104(5)	103(1)	98(3)	104(2)
	0.57	Lower	101(1)	103(1)	93(2)	98(5)	101(3)	107(3)	100(6)
	0.28	Upper	102(4)	99(2)	96(3)	89(14)	101(1)	99(4)	100(4)
	0.28	Lower	99(1)	103(1)	92(1)	94(13)	102(2)	89(5)	103(2)
Cu8	0.60	Upper	97(10)	97(1)	88(4)	97(3)	101(2)	93(10)	95(4)
	0.60	Lower	100(1)	101(2)	91(2)	96(6)	98(2)	85(8)	97(4)
	0.28	Upper	103(1)	98(1)	89(20)	89(8)	103(9)	94(9)	92(19)
	0.28	Lower	100(1)	101(1)	92(5)	96(4)	99(4)	94(13)	94(5)
Diesel	N/A	Upper	103(2)	96(3)	93(1)	91(10)	99(3)	82(12)	81(13)
	N/A	Lower	102(6)	102(1)	91(1)	86(16)	94(3)	81(12)	77(10)
Water	N/A	Upper	106(3)	99(2)	92(3)	84(8)	82(7)	64(4)	44(15)
	N/A	Lower	101(1)	100(4)	86(1)	80(6)	66(12)	46(8)	26(25)

Table 4.4. Stiffness retention percent means exposed for a maximum of 12 months in an Oregon soil. (mean ± standard deviation; n = 5). See Materials and Methods section for exact chemical concentrations and retentions.

			% of Original Strength						
			Exposure Period (Months)						
Trt.	Targ. Reten. kg/m^3	Field Site	1	2	3	4	6	8	12
ACZA	6.4	Upper	108(2)	103(4)	97(3)	106(1)	107(2)	106(4)	108(2)
	6.4	Lower	108(4)	108(2)	97(1)	107(4)	106(4)	92(6)	103(2)
	4.0	Upper	101(1)	103(2)	92(7)	102(3)	100(2)	91(4)	86(4)
	4.0	Lower	103(1)	101(1)	92(3)	94(1)	105(3)	95(16)	98(5)
CCA	6.4	Upper	108(1)	103(1)	97(1)	106(2)	107(1)	106(2)	108(3)
	6.4	Lower	108(2)	108(3)	97(1)	107(2)	106(2)	92(9)	104(8)
	4.0	Upper	103(1)	101(1)	92(2)	97(14)	105(1)	95(8)	98(10)
	4.0	Lower	101(1)	103(1)	92(3)	102(2)	100(3)	91(12)	86(7)
CuCit	6.4	Upper	99(1)	99(1)	95(6)	97(5)	99(2)	98(2)	98(4)
	6.4	Lower	99(1)	100(1)	92(1)	99(3)	98(2)	96(3)	97(2)
	4.0	Upper	102(1)	99(2)	83(17)	100(1)	102(1)	97(6)	97(5)
	4.0	Lower	101(1)	102(2)	92(2)	98(4)	101(1)	94(9)	102(3)
ACQ	6.4	Upper	105(4)	101(1)	95(1)	102(4)	101(4)	104(2)	106(3)
	6.4	Lower	104(1)	106(2)	95(1)	102(2)	104(3)	104(5)	104(4)
	4.0	Upper	99(1)	98(1)	92(1)	95(7)	98(2)	94(1)	98(4)
	4.0	Lower	98(1)	99(1)	88(3)	97(2)	98(1)	76(8)	99(4)
Diesel	N/A	Upper	103(2)	96(3)	93(1)	91(10)	99(3)	82(12)	81(13)
	N/A	Lower	101(6)	102(1)	91(1)	86(16)	94(3)	81(12)	76(10)
Water	N/A	Upper	106(3)	99(2)	92(3)	84(8)	82(7)	64(4)	44(15)
	N/A	Lower	101(1)	100(4)	87(1)	80(6)	66(12)	46(8)	26(25)

The lack of significant difference in decay between fields at other times can be attributed to the large standard deviations between the means as well as the similarity of the two test sites. This variability was not unexpected and has been reported in other biodeterioration trials (Schultz, Nicholas, 2001). While the mean trends suggest that field location affected strength loss, the large variation in strength retention within means generally results in no significant difference. The effect of stake location was therefore neglected and further comparisons were made only between treatments and dates.

Table 4.5. P-values for comparisons of strength retention means between identical treatments exposed in the upper and lower fields.

Treatment	Retention Level kg/m^3	P-values after exposure						
		1 mo.	**2 mo.**	**3 mo.**	**4 mo.**	**6 mo.**	**8 mo.**	**12 mo.**
ACZA	6.4	1.000	0.0001	1.000	1.000	0.9978	0.5427	1.000
	4.0	1.000	0.9970	0.6460	1.000	1.000	0.8934	1.000
CCA	6.4	1.000	0.0016	1.000	1.000	1.000	0.7271	1.000
	4.0	1.000	0.9729	1.000	0.9920	1.000	1.000	0.9213
CuCit	6.4	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	4.0	1.000	0.2748	0.8670	1.000	1.000	1.000	1.000
ACQ	6.4	1.000	0.0537	1.000	1.000	1.000	1.000	1.000
	4.0	1.000	0.9999	1.000	1.000	0.9983	0.0701	1.000
CuN	0.57	1.000	0.0010	1.000	0.9998	1.000	0.9947	1.000
	0.28	1.000	0.1737	1.000	1.000	1.000	0.9669	1.000
Cu8	0.64	1.000	0.1693	1.000	1.000	0.9666	0.9948	1.000
	0.32	1.000	0.5189	1.000	1.000	0.0001	1.000	1.000
Diesel	N/A	1.000	.0016	1.000	0.9999	1.000	1.000	1.000
Water	N/A	1.000	1.000	0.9985	1.000	1.000	0.2734	0.1608

p-values from Tukey Kramer test

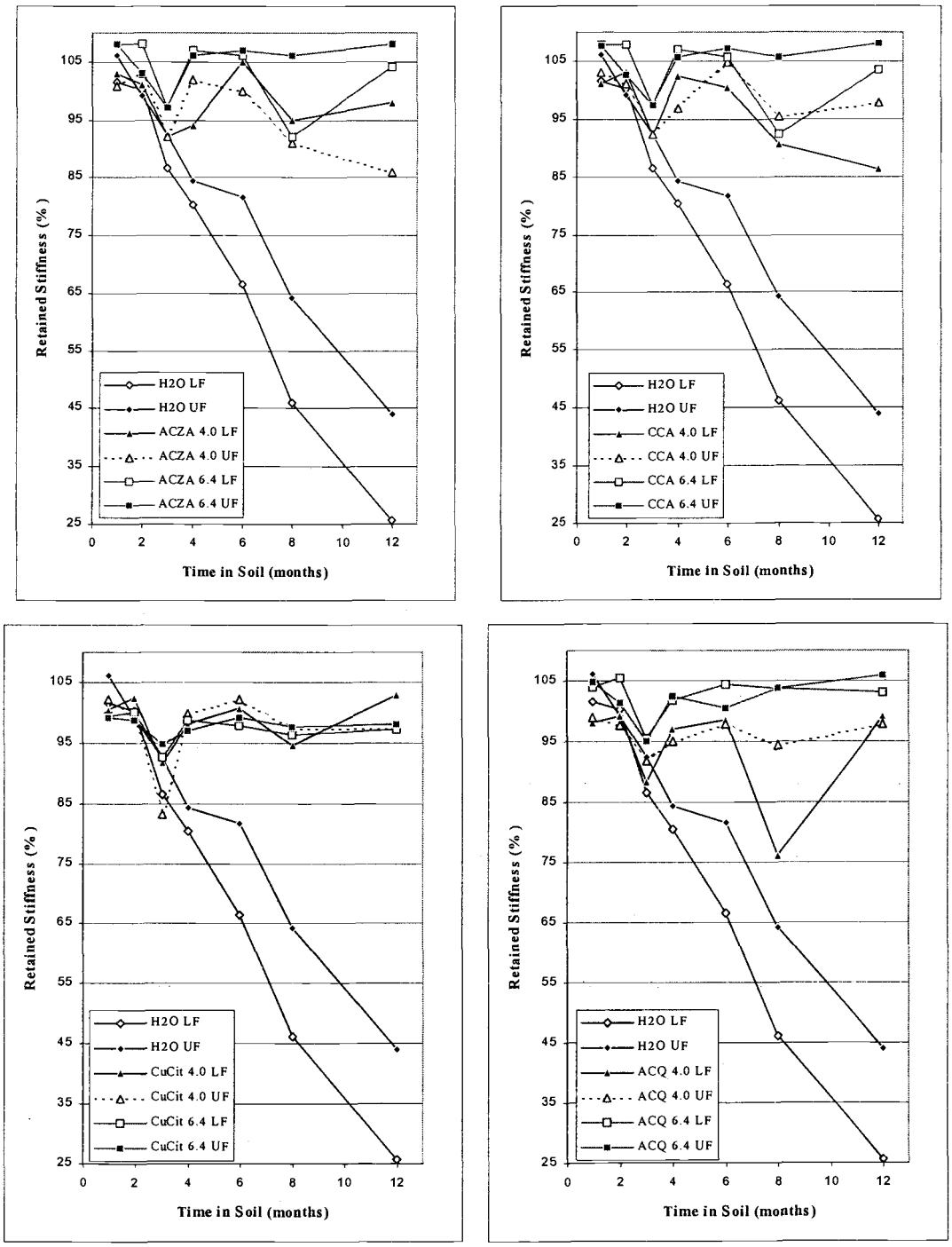

Figure 4.3. Relative stiffness of preservative treated western hemlock stakes over 12 months exposure in an Oregon soil.

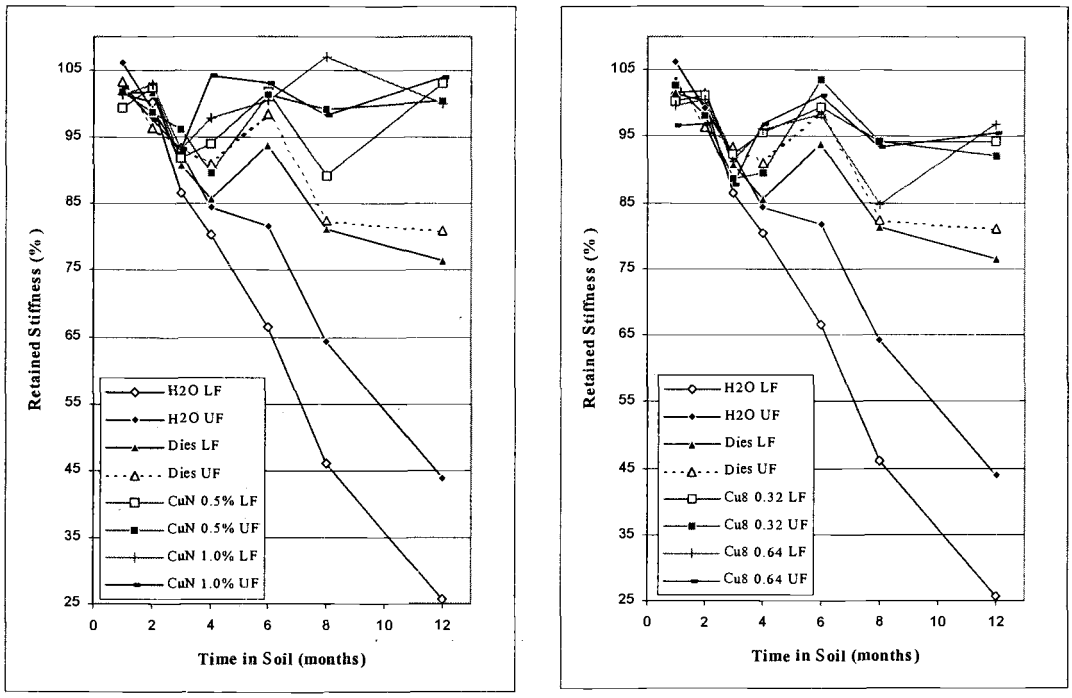

Figure 4.3. (Continued)

4.3 COMPARISONS BETWEEN TREATMENTS AND EXPOSURE PERIOD

Strength retention means for the two fields combined are summarized in Table 4.6. Trends for the combined field mean retentions are presented in Figure 4.4. Mean stake stiffness steadily declined for the diesel and water controls over the 12 month field exposure. Water treated stakes, however, clearly suffered the greatest strength loss, retaining only 34.8% of their original strength compared to 78.7% retention of stiffness for diesel treated samples after 12 months.

Preservative treated stakes did not experience obvious strength declines except for stakes treated with CCA to a retention of 4.0 kg/m^3. The remaining waterborne treatments retained virtually all of their original stiffness at the end of

twelve months. Oilborne copper naphthenate treated stakes retained over 100% of their original stiffness, while copper quinolinolate retained nearly 95% at both retentions evaluated.

Stiffness among means were compared using the Tukey-Kramer procedure using SAS v.8 software. Mean retention strengths for the upper and lower fields combined (n =10) were compared between treatments and within treatments by dates. Means were reported as significantly different if the p-values were less than α=0.05.

Tukey-Kramer comparisons indicated that only the CCA treatment at 4.0 kg/m^3 showed a significant decline in stiffness over the 12 month exposure period (Table 4.6). Inter-treatment comparisons showed no significant differences between any of the wood preservatives.

The retention of stiffness at levels near 100% indicates that Oregon soil fungi in the Brooks area were unable to effect substantial strength reduction in wood treated with various copper-based preservatives over a one year exposure. From the results, it seems likely that the exposure time was insufficient for allowing differences in wood preservative efficacy to manifest themselves. The large standard deviations may also have obscured any statistically significant differences that might have been present at 12 months.

Table 4.6. Percent retained stiffness of treated and untreated western hemlock stakes after 12 months in soil contact. See Materials and Methods for exact chemical concentrations and retentions.

		% of Original Strength						
		Exposure Period (Months)						
Chemical	Target Reten. kg/m^3	1	2	3	4	6	8	12
ACZA	6.4	105(3)^{a}	102(5)^{a}	94(3)^{b}	102(3)^{a}	104(3)^{a}	92(9)^{a*}	102(2)^{abST}
ACZA	4.0	101(1)^{a}	101(2)^{a}	89(7)^{b}	99(4)^{a}	101(3)^{a}	95(12)^{ab}	99(6)^{aST}
CCA	6.4	108(2)^{a}	105(4)^{a}	97(1)^{b}	106(2)^{a}	106(2)^{a}	99(10)^{b}	106(6)^{aST}
CCA	4.0	102(1)^{ac}	102(2)^{ac}	92(3)^{b}	98(10)^{abc}	103(3)^{ac}	93(10)^{b}	92(10)^{bS}
CuCit	6.4	99(1)^{a}	99(1)^{a}	94(4)^{b}	98(4)^{a}	98(2)^{a}	97(2)^{ab}	98(3)^{aST}
CuCit	4.0	101(1)^{a}	102(3)^{a}	87(12)	99(3)^{a}	101(1)^{a}	96(7)^{a}	100(5)^{aST}
ACQ	6.4	105(3)^{a}	103(3)^{a}	95(1)	102(3)^{a}	102(4)^{a}	104(3)^{aSWZ}	105(4)^{aST}
ACQ	4.0	99(1)^{ac}	98(1)^{ac}	90(3)^{bcd}	96(5)^{abc}	98(1)^{ac}	85(11)$^{bdTU XYZ}$	98(4)^{acST}
Water	N/A	104(3)^{ab}	100(3)^{ab}	89(4)^{abc}	82(7)^{bc}	74(12)^{bc}	53(11)	35(21)

Table 4.6. (Continued)

Chemical	Target Reten. kg/m^3	% of Original Strength Exposure Period (Months) 1	2	3	4	6	8	12
CuN	0.57	102(1)^{a}	100(3)^{a}	93(3)	101(5)^{a}	102(2)^{a}	103(5)$^{aSTD EH}$	102(5)^{aST}
CuN	0.28	101(3)^{a}	101(2)^{a}	94(3)^{ac}	92(13)^{c}	102(2)^{a}	94(7)^{aSTUV}	102(3)^{aST}
Cu8	0.64	98(7)^{a}	98(3)^{a}	89(3)^{bc}	96(5)^{ac}	100(2)^{a}	89(10)^{bc}	96(4)^{aST}
Cu8	0.32	101(1)^{a}	100(2)^{a}	90(14)^{a}	93(6)^{a}	101(7)^{a}	94(11)^{a}	93(13)^{aST}
Diesel	N/A	102(4)^{abc}	99(4)^{abcd}	92(2)^{abcde}	88(13)^{bcdef}	96(4)^{abcd}	82(12)^{cdef}	79(11)^{def}
Water (repeat)	N/A	104(3)^{ab}	100(3)^{ab}	89(4)^{abc}	82(7)^{bc}	74(12)^{bc}	55(11)	35(21)

Superscript lower case letters shared by means of different dates within the same treatment denote no significant difference at the 0.05 level (differences between any two retentions based on Tukey test.)

Superscript capital letters shared by means of different treatments within the same date denote no significant difference at the 0.05 level (differences between any two retentions based on Tukey test.)

* denotes no significant difference between any preservative treatments at the 0.05 level.

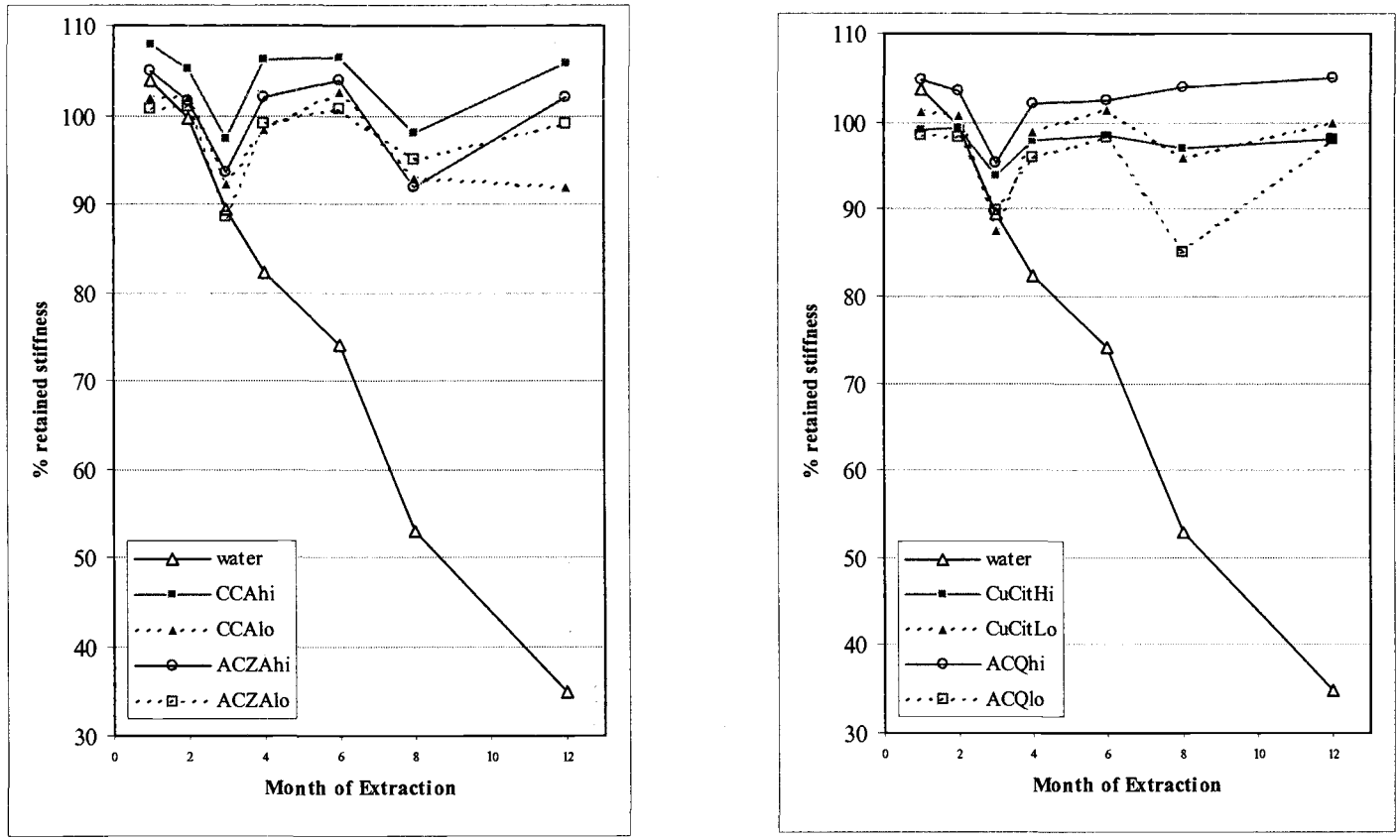

Figure 4.4. Strength retention of treated and untreated western hemlock stakes exposed over 1 year of soil contact.
A) CCA and ACZA treatments contrasted with the water controls.
B) Copper citrate and ACQ treatments contrasted with the water controls.

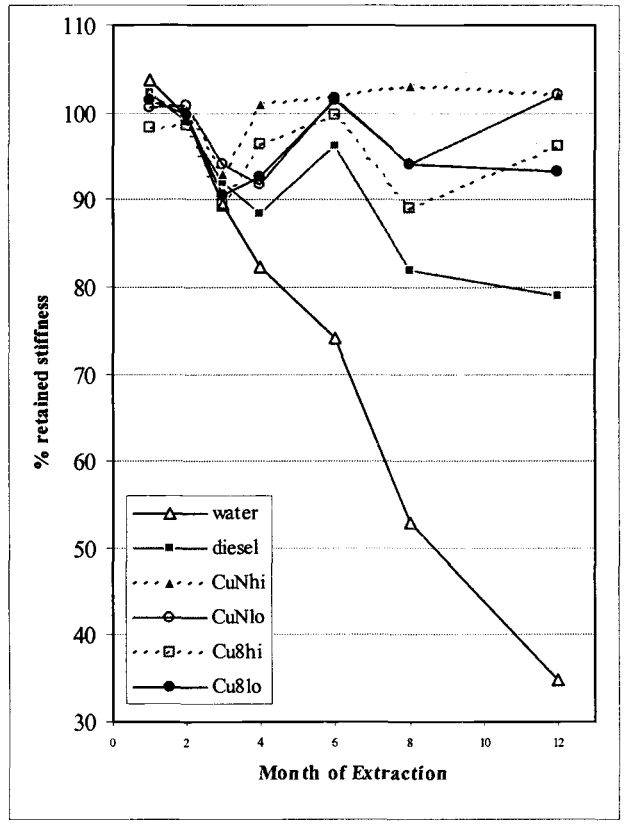

Figure 4.4. Continued.
(A) Copper naphthenate and copper quinolinolate percent stiffness rententions over 12 months contrasted with diesel and water controls.

4.4 MICROSCOPIC EVALUATION OF WOOD DECAY

To determine if stiffness loss was an adequate indicator of wood decay, a microscopic assessment of visual damage was made on the low retention stakes exposed in both fields. Damage to stakes was divided into two categories. Diffuse (non-soft rot) damage such as discoloration, general erosion or cell wall fracturing was considered to be "general decay."

Signs of soft rot were also noted. Type 1 bore holes in the S2 layer were considered soft rot. These cavities appeared as elongated tubes with appressed ends running at an angle to the axis of the tracheids in the tangential and radial sections (Figure 4.5).

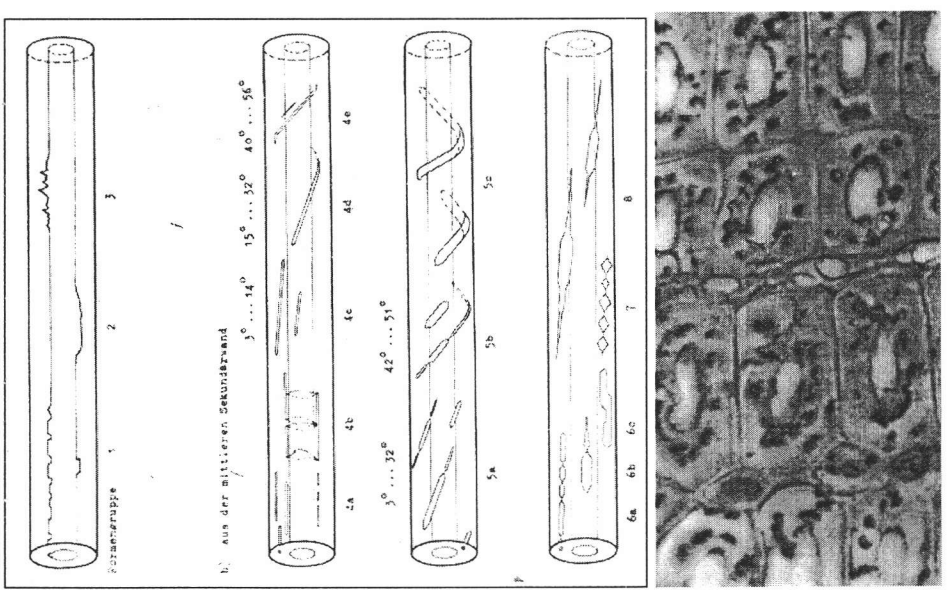

Figure 4.5. Soft rot as depicted in previous studies. Left image (Curtois, 1963) shows soft rot erosion on the far left vessel while the three vessels to the right show Type 1 cavities. Right image (Levy, 1965) shows a transverse section *Pinus sylvestris* at 1520X. Black dots are Type 1 soft rot cavities extending out of the plane of the image.

A magnification of 200X was adequate to confirm soft rot presence, especially on the tangential/radial sections where the activity of soft rot was easily discerned from the surrounding sound wood. Higher magnification was useful for detail, but the oil immersion 1000X objective provided marginal resolving power. A polarized light filter was most useful for highlighting soft rot damage when used to view radial sections.

Tangential sections of diesel (Figure 4.11) and water treated controls (Figures 4.12 and 4.13) both contained extensive soft rot cavities, although the damage was noticeably more severe in the water controls. No Type 1 soft rot cavities were seen in either tangential or transverse sections of ACZA, CCA, ACQ, copper citrate and copper naphthenate treated samples extracted after 12 months of exposure (Figures 4.6 and 4.10). A limited, but unmistakable number of Type 1 cavities were, however, seen in one of the copper-8-quinolinolate treatments (Figure 4.14). This slide revealed the characteristic cavities in the upper right corner, with numerous fungal hyphae visible throughout the slide.

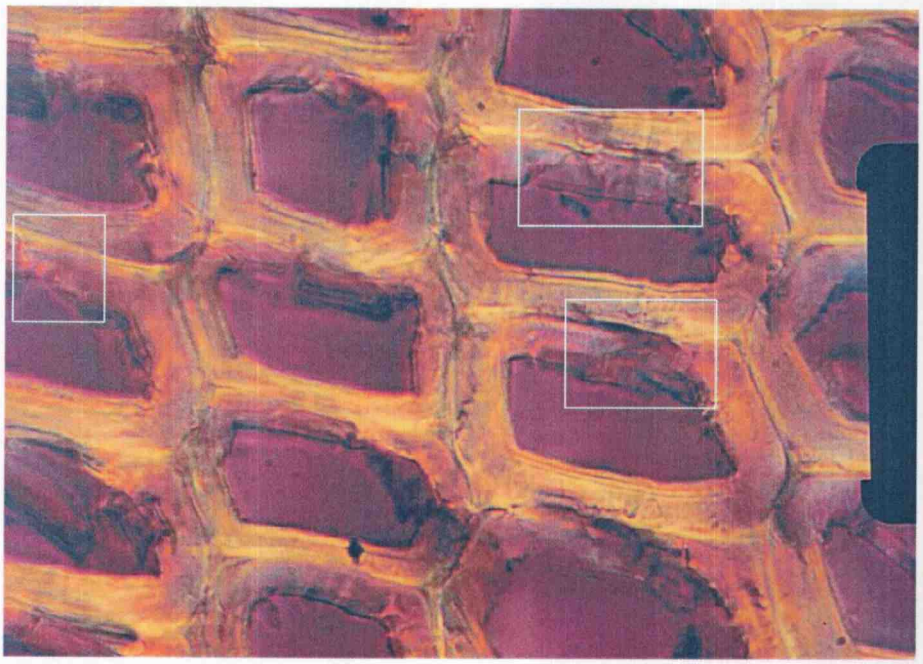

Figure 4.6. Transverse section of ACZA 4.0 kg/m3 treated western hemlock after 12 months of exposure to soil. Boxes indicate erosion damage to cell walls. (1000X, polarized illumination)

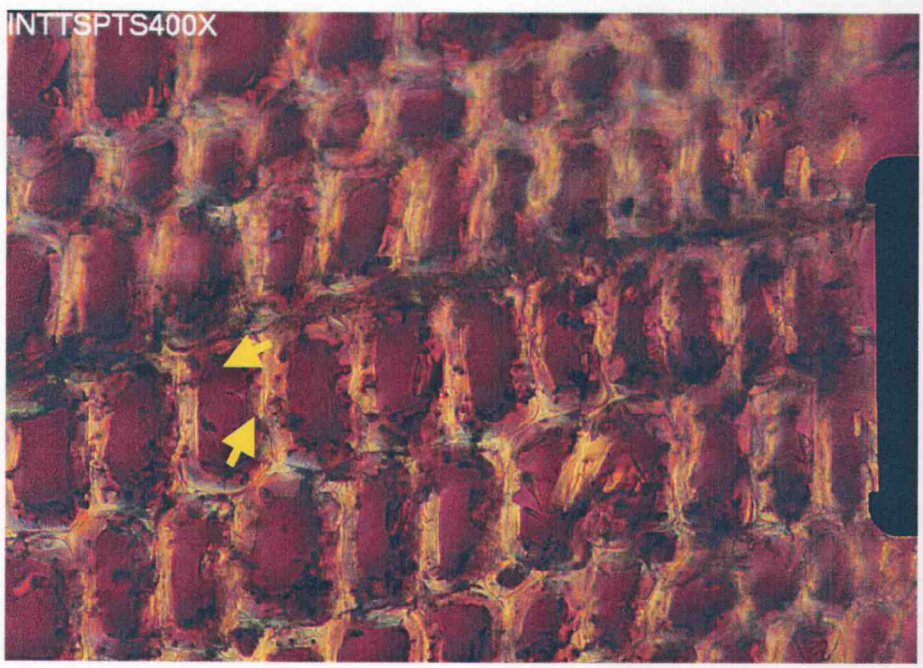

Figure 4.7. Transverse section of water treated control after 12 months of soil exposure showing extensive Type 1 soft rot cavities indicated by arrows. (400X, polarized illumination)

Figure 4.8. Exterior transverse section of diesel treated western hemlock showing soft rot cavities after 12 months of exposure to soil. (400X, polarized illumination)

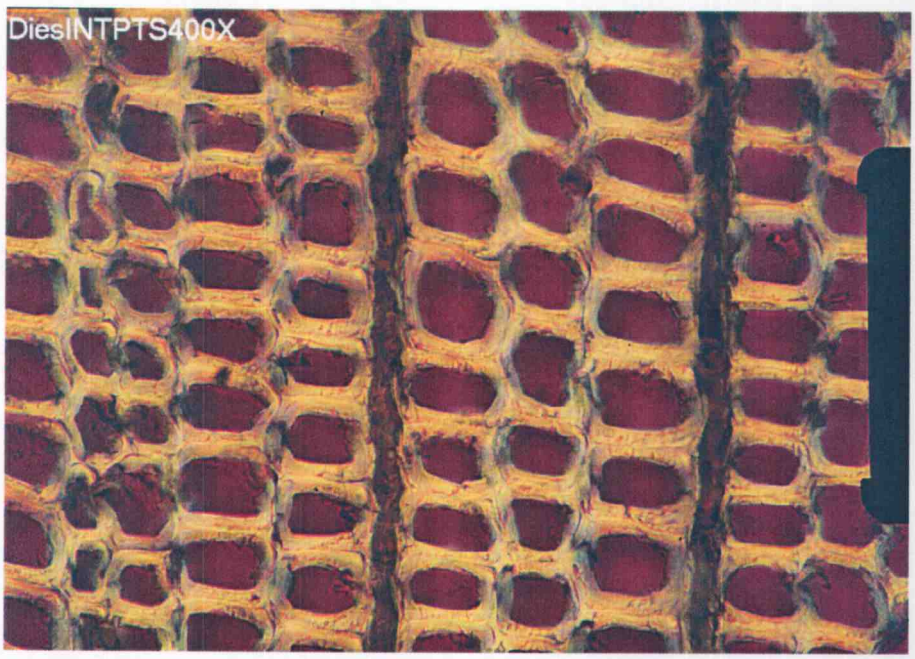

Figure 4.9. Interior view of same diesel treated stake as Fig 4.3.3; after 12 months of exposure to soil. Cell wall erosion and Type 1 cavities were present but in less abundance than the surface. (400X)

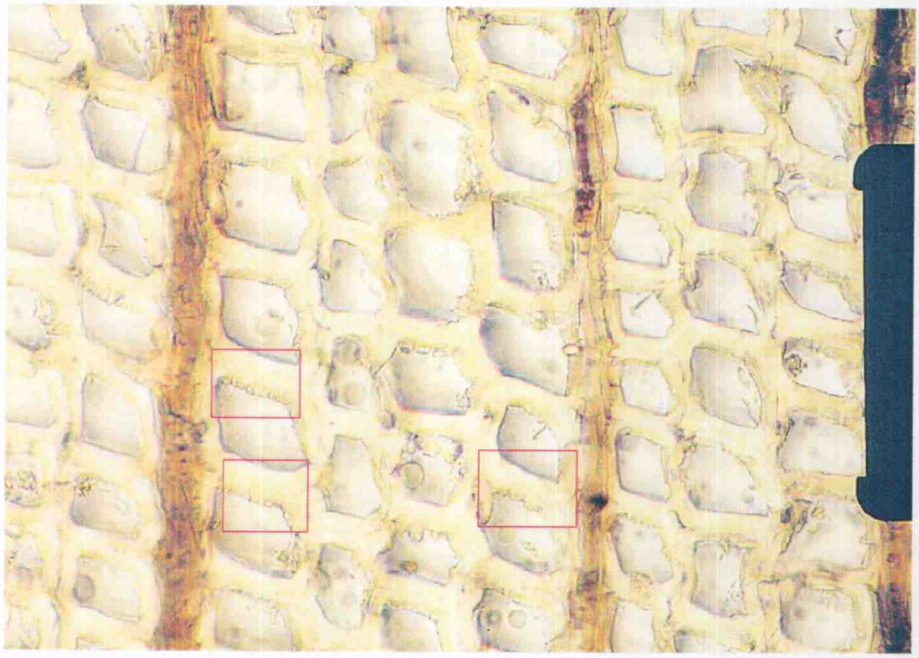

Figure 4.10. Transverse section from an ACZA-treated stake after 12 months of exposure to soil. Boxed areas denote slight erosion damage; also visible is discoloration of rays. (400X)

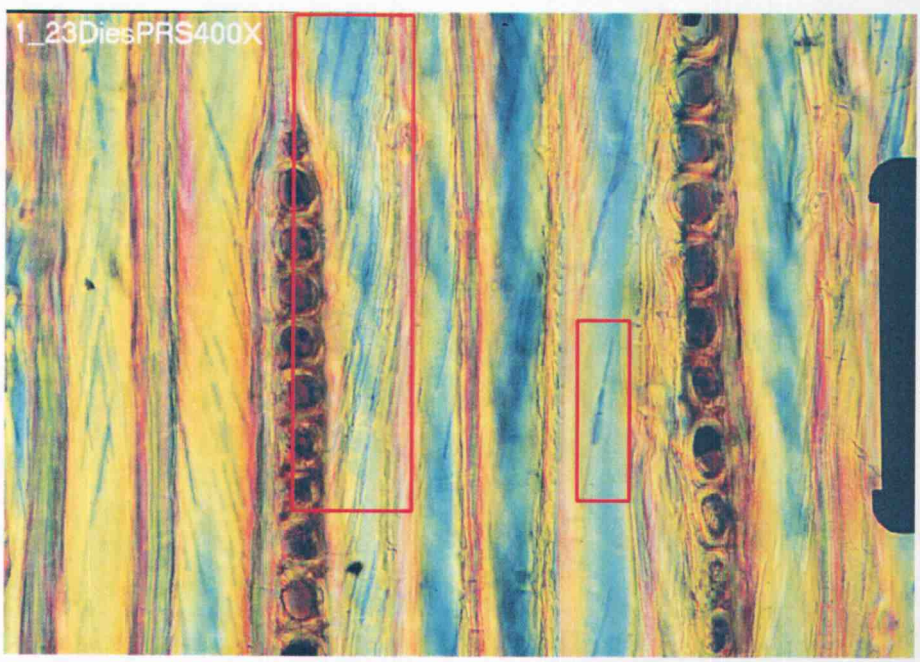

Figure 4.11. Tangential view of diesel treated stake after 12 months of soil exposure showing extensive Type 1 soft rot cavities in boxed areas. (400X, polarized illumination)

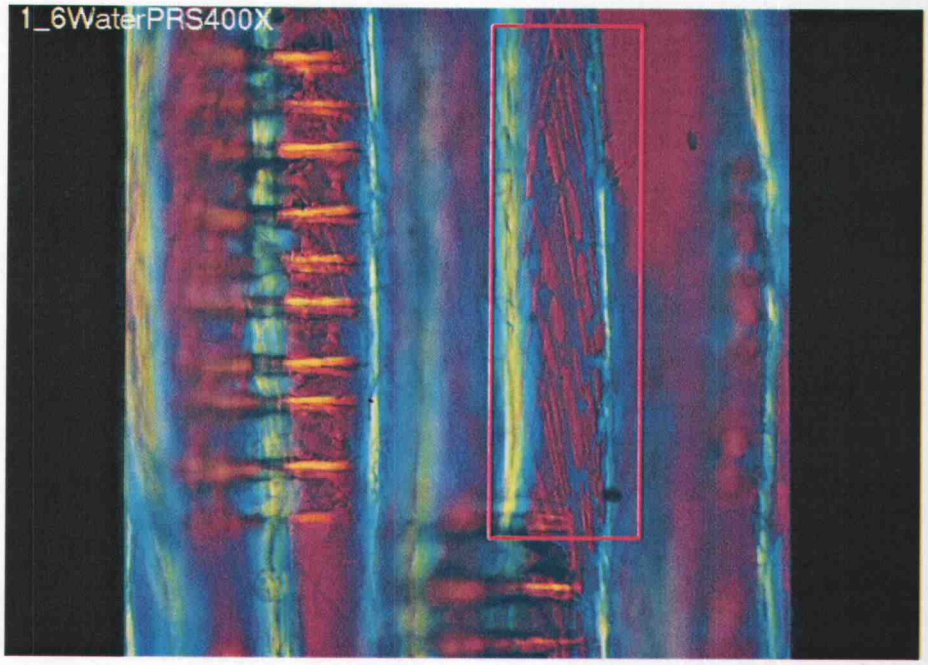

Figure 4.12. Tangential view of water treated stake after 12 months of soil exposure showing extensive Type 1 soft rot damage. (400X, polarized illumination)

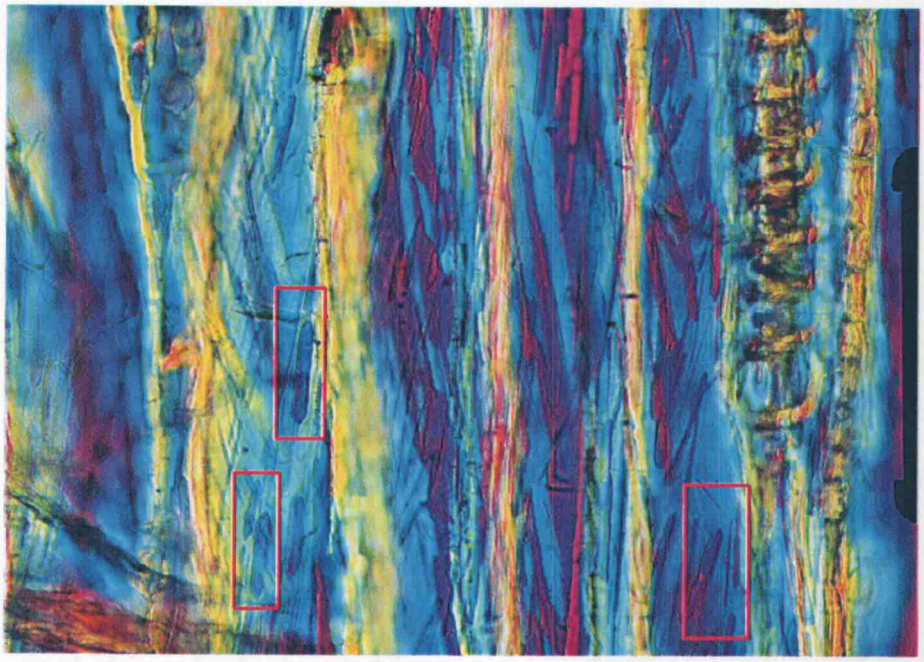

Figure 4.13. Tangential view of water treated stake after 12 months exposure to soil showing extensive Type I soft rot channels. (400X, polarized illumination)

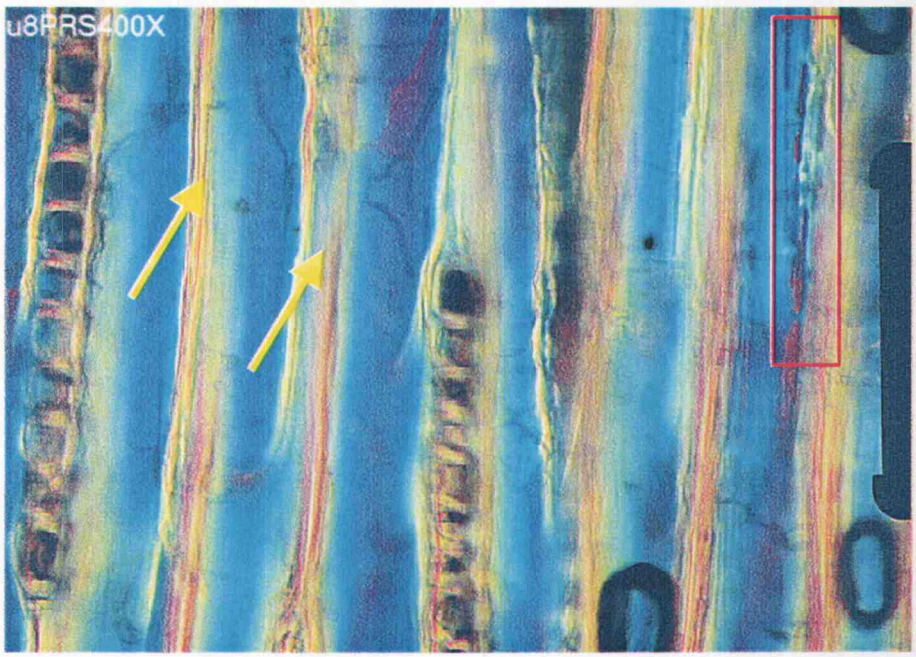

Figure 4.14. Tangential view of Copper quinolinolate treated stake after 12 months of soil exposure showing scattered soft rot cavities (boxed) and hyphae in the lumens (arrows). (400X, polarized illumination)

4.4.1 Transverse sections

The four controls treated with only water showed clear evidence of Type I soft rot decay (Figures 4.7, 4.12 and 4.13). Many stakes showed signs of diffuse cell wall degradation and several preservative-treated samples contained hyphae in the lumens (Figure 4.14). Diesel controls also showed signs of extensive soft rot attack, but suffered less visible damage than the water treatments (Figure 4.8, 4.9 and 4.11).

Figures 4.6 and 4.7 highlight the differences between a typical water control stake and the 4.0 kg/m^3 treatment of ACZA. While the ACZA treated stake had some erosion on lumen surfaces, there was little evidence of pitting or tunneling in the S2 layer. In contrast, the water control contained extensive bore holes from Type

I soft rot activity.

Control stakes treated only with the diesel solvent show widespread decay but the damage was less severe than the water treated controls. One of the notable aspects of the diesel treated control stakes observed was the apparent gradient in soft rot intensity between the surface and interior of the stake (Figures 4.8 and 4.9).

Soft rot type 1 decay was evidenced by cavities in the cell walls, primarily in the earlywood/latewood transition region (Curtois (1963) and Levy (1965). The decay patterns exhibited differed from those reported by Singh and Butcher (1985) for erosion troughs caused by bacteria in CCA treated radiata pine posts, suggesting that fungi were the most probable source of the decay.

4.4.2 Scoring soft rot for a given treatment

Table 4.7 summarizes the decay scores that characterize the visible decay damage to the sampled stakes.

The diesel treatment, while less decayed than the water treatment, still received a considerably higher combined visual decay rating than any of the preservative treatments. All of the preservative-treated stakes had lower decay ratings, although ACQ and Cu8 were more decayed than the other treatments.

The summary table indicates that with the exception of one Cu8 treatment, no soft rot damage was observed on treated samples. In the case of the Cu8 treatment showing soft rot, the damage was relatively sparse (Figure 4.14). Soft rot fungi tend to be important initial colonizers of preservative treated wood in soil contact (Jin and Archer, 1991). Their general absence in the treated exposed stakes suggests that the

wood has not yet begun to experience substantial degradation. These observations support the strength data and indicate that all the compounds appear to provide protection, although some treatments have clearly begun to experience decay.

Table 4.7. Characteristics of microbial damage on treated and untreated western hemlock stakes exposed in soil contact for 12 months as determined by light microscopy.

Treatment	% Mean Residual Strength[a]	Degree of Damage[ab]		Cumulative Mean Decay[ac]
		Decay	Soft Rot	
Water	29	3.0 (0.0)	4.0 (0.0)	7.0 (0.0)
CCA	82	1.0 (0.0)	0.0 (0.0)	1.0 (0.0)
ACZA	90	1.0 (0.0)	0.0 (0.0)	1.0 (0.0)
CuCit	93	1.2 (0.5)	0.0 (0.0)	1.8 (0.5)
ACQ	87	1.2 (0.5)	0.0 (0.0)	2.2 (0.5)
Diesel	63	2.0 (0.0)	2.8 (0.5)	4.8 (0.5)
CuN	90	1.0 (0.0)	0.0 (0.0)	1.0 (0.0)
Cu8	81	1.2 (0.5)	0.5 (1.0)	2.8 (0.7)

[a]Means with standard deviations in parentheses
[b]Values represent means of 4 replications when 0 equals no damage And 5 equals total destruction.
[c]Values represent means of 4 replications when 0 equals no damage And 10 equals total destruction.

4.4.3 Summary for microscopy

Microscopic evaluation of a selection of the stakes treated to the lower retention with the test preservatives correlated with stiffness losses in the stakes. All

of the diesel and water control stakes showed extensive soft rot damage, while the preservative treatments had little evidence of damage. Preservative-treated stakes suffered minor cell wall erosion, but only one of the sampled stakes contained Type 1 soft rot damage.

5. CONCLUSIONS

Results generally indicated that one year of exposure did not produce significant differences in tensile strength reduction between the different copper-containing treatments. Even the poorest performing of the preservatives (CCA 4.0 kg/m^3) lost only eight percent of its stiffness. There were many signs that soil microbes had successfully colonized the stakes, however, and soft rot was observed in one copper quinolinolate treated sample.

In many respects, the Oregon agricultural field conditions represent a challenge to wood decay fungi. Wood in typical service may provide an environment more conducive to decay.

Previous studies (Smith and Morrell 1995; Chang and Morrell, 1997) indicated that microbes present in the test soils were capable of attacking cellulosic string treated with copper naphthenate and copper quinolinolate. This suggests that the chemical structure of wood fibers, with its complex architecture of lignin, cellulose and hemicellulose presented an additional challenge to wood decay organisms. The clear presence of Type I soft rot cavities in one of the copper-8-quinolinolate treated samples, however, suggests that fungi can colonize at least some copper treatments of wood.

The decline in stiffness for the water and diesel-saturated stakes over the twelve months indicated that microbes capable of aggressive wood decay were present in the soil. Microscopic inspection of the control stakes confirmed

widespread attack of the cell walls by soft rot fungi, although the diesel treatment appeared to afford limited protection for the 12-month trial.

The stake tests gave repeatable results, and were a useful method of assessing wood decay. There was a close correlation between microscopic degradation and stiffness reduction in the controls, indicating that bending strength provided a useful indicator of decay damage, even when the decay environment was relatively variable.

One anticipated advantage of the stiffness reduction method, however, did not manifest itself in samples that had been treated with AWPA-standardized preservatives. While the mean stiffness of solvent controls (diesel and water) declined rapidly, there was no significant reduction in preservative treated stakes strength over the year of exposure. This outcome indicates that stiffness reduction may not be suitable for assessing preservative efficacy *in situ* for exposure periods of less than a year.

FUTURE RECOMMENDATIONS

The results indicates that future investigations into copper tolerant fungi endemic to temperate regions will require longer decay intervals than the 12 month interval used for this experiment, even with assessment techniques optimized for early decay detection. Methodology for assessing copper tolerance should be used in tandem with isolations of fungi colonizing stakes and laboratory tests of these isolates for copper tolerance. Stiffness tests should also be carried out for a longer exposure periods, or under conditions that are more conducive to intense fungal activity. Wood samples could also be exposed using decay beds with soil taken from fields similar to those in this study, but under controlled moisture and temperature regimes.

While the use of commercially accepted wood preservatives such as CCA and ACZA was interesting from a simulation standpoint and increases real world relevance, stakes treated with varying levels of $CuSO_4$ and amine copper should be used so that copper alone is assessed for preservative efficacy.

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