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Assessment of the mechanical properties of lodgepole pine in the incipient stage of decay by a white -rot fungus

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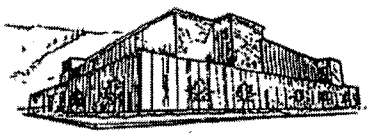

Assessment of the Mechanical Properties of Lodgepole Pine in the Incipient Stage of Decay by a White-Rot Fungus

by

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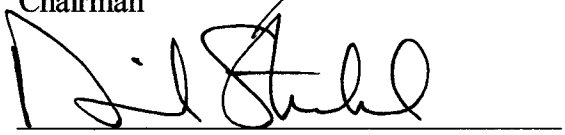

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Assessment of the Mechanical Properties of Lodgepole Pine in the Incipient Stage of Decay by a White-Rot Fungus (117 pp.)

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ABSTRACT

This study quantified the effects of volume and stage of a wood decay fungus (*Phellinus pini* (Thore Lloyd) on the mechanical strength of lodgepole pine and its impact on the current standard of visual stress grading. To improve detection and identification of decay fungi at the species level during the incipient decay stage, a fungal ribosomal DNA assay was designed. An effective extraction method for isolating fungal DNA from wood also was developed by integrating the glass bead system, organic solvents and an extraction buffer.

A Basidiomycete, *Phellinus pini* and an Ascomycete, *Byssochlamys nivea* were successfully identified from fungal cultures by directly sequencing with ITS-1F (specific for higher fungi) and ITS-4 (universal primer) primers. This study also demonstrated that direct sequencing can correctly identify these decay fungi, avoiding incorrect identification due to morphological variation within and between these species.

The average percentage reduction in modulus of elasticity (MOE) and modulus of rupture (MOR) were significantly affected by decay volume and discoloration, but the interaction of decay volume and discoloration had little effect on these properties. The average percentage reduction of specific gravity was affected by only discoloration, while the average reduction percentage of compression parallel to the grain increased significantly with increased decay volume. The reduction of MOR increased more rapidly than did the reduction in MOE. MOR showed an average maximum reduction of 34% at the combination of high discoloration and 67-100% decay volume while MOE exhibited a 20% average maximum reduction. Compression parallel to the grain displayed a 15% average maximum reduction at 67-100% decay volume.

The results indicate that the maximum 34% reduction of MOR corresponds to the maximum allowable reduction of MOR of No 1. structural grade for beam-stringers in the Western Wood Products Association (WWPA) grading rules. The strength reduction of MOR is large enough to be of serious concern. While, the reductions in MOE and compression parallel to grain do not impact the current visual stress grading rules, the reduction in MOR calls for further investigation into potential impact on grading rules.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDIX LISTS

INTRODUCTION

Wood is economically vital throughout the world as construction materials, furniture, manufacture, home furnishings, energy production, pulps and paper making as well as other biochemical products. In the United States, wood accounts for nearly 25% of industrial materials use (National Research Council, 1990). The consumption of wood as timber products in the United States has increased more than 5 billion cubic feet from 1950 to 1980 (Ulrich, 1983) and is still increasing annually. Unlike petroleum products, wood can be naturally and consistently produced from forests around the world. This renewable ability means that with proper forest management, world economies, their industries and consumers can be assured of a continuous supply of raw materials and products.

Wood provides high mechanical strength with relatively low weight and can be easily reshaped, glued, and mechanically fastened for many purposes. Its flexibility and strength makes wood the major structural material of houses for more than 70% of American families. Although wood has many advantages, it also has some disadvantages that can limit the usefulness for some purposes. These disadvantages are its biodegradability, dimensional instability, combustibility, and its inherent variability in its physical and mechanical properties. Among these disadvantages, biodegradation, caused by fungi, bacteria, insects and other biological agents, is the most important factor that adversely affects the strength of wood.

Compared with other biological causes of wood damage, discoloration and decay caused by fungi account for the largest volume loss of both standing timber and wood in use. Fifteen to twenty five percentage of the nation's marketable wood volume in

standing timber and 10 to 15% of wood products in production and storage are degraded each year. It is estimated that over 10 % of the timber products consumed in the United States are replaced because they have been rendered unserviceable by fungal decay (Zabel and Morrell, 1992). The cost of insect damage and decay in wood approaches $400 million per year in California alone (Brier *et al.*, 1988), and much more can be expected in the future. Decay damage not only causes large monetary losses but it is also results in indirect losses that have a heavy impact on the national economy. The cost associated with the replacement process is quite often higher than the cost of the replacement material and several times higher than the initial material and labor costs of the original placement. Until wood is more purposefully protected against decay damage, direct and indirect losses will continue to be economically burdensome.

Decay occurs in living trees, dead standing trees, merchandized logs, lumber or wood in service when the proper growth conditions required by the decaying fungi are reached. Most fungal decay occurs only within a narrow range of temperature and wood moisture content. While the range of temperature favorable to decay includes that found both inside and outside habitable structures for at least a portion of the year, the relatively high moisture content required for decay growth must, in most instances, be introduced from the environment. For wood in service, this can be in the form of ground-contact moisture, atmospheric precipitation, condensation, plumbing leaks, landscaping irrigation or other external sources.

Fungal spores enter living and dead trees in a forest stand via wounds or insect attacks. Some pathogenic fungi infect living trees through root contact from tree to tree.

Wood's mechanical strength can be reduced by as much as 75 percent with less than a 5 percent weight loss during the incipient stage of decay without a significant change in outward appearance of the wood. A quantified relationship between the amount of red heart and the reduction in structural and mechanical properties of lodgepole pine has yet to be adequately defined. In addition, grade rules offer no methods for rating the amount or degree of infection through visual assessment of the affected area(s). Currently, the two extreme solutions, one of not allowing material with visual signs of incipient decay and the other of not limiting its use, can either considerable waste of a valuable resource or use of material with unknown mechanical properties. Therefore, grading agencies, wood microbiologists, wood engineers and end users really like to know the effects of fungal incipient decay on wood strength properties.

In order to prevent the incipient decay from causing more damage on the wood in service, wood engineers, microbiologists and tree pathologists need to effectively detect and identify incipient decay. It is important, therefore, to develop a method to detect the incipient decay stage and identify the fungal species responsible for the decay.

Several methods for detecting fungi exist, including the direct culturing of fungi, chemical staining, and the use of electrical resistance, magnetic resonance, Immunobloting and Enzyme-linked Immunosorbent Assay (ELISA). These methods have all been used to detect incipient decay, but their accuracy can be reduced by wood extractives, fungal species and environmental conditions. For the identification of fungi during the incipient stage, the traditional method is to isolate and grown fungi in different media, and to compare these cultures to a culture of known species, using

general appearance, reaction to different growth media, growth rate and morphological structures of the fungi. These procedures require at least 8-12 weeks to be completed and an experienced mycologist to accomplish the identification. In addition, the complexity of fungal life cycles also makes the identification even more difficult. The polymerase chain reaction (PCR) techniques and the availability of fungal DNA sequence database have made identification more feasible and accurate. This makes it possible to integrate culture and PCR as an effective procedure to identify unknown fungi. Unlike the Restriction Fragment Length Polymorphism (RFLP) method that uses enzymes to cleave the DNA sequence amplified by the PCR into smaller fragments and compares this unidentified DNA fragment profile to an identified fragment profile (White e*t al.*, 1990; Jasalavich *et al.*, 2000; Schmidt and Moreth, 2000; Germain *et al.*, 2002), using direct sequencing for fungal identification does not require DNA samples from known fungi.

The purpose of this research was, first, to quantify the effects of fungal decay stage, as determined by discoloration, and volume of decay, on the mechanical properties of lodgepole pine. The second goal was to compare the results of bending and compression strength tests to other strength reduction factors as related to visual stress grading standards. Third, this study also evaluated the impact of these strength reductions on the current visual grading rules of western conifer species. Furthermore, in order to improve the detection and identification of decay fungi during the incipient decay stage, protocols need to be developed for fungal DNA isolation, purification, Polymerase Chain Reaction (PCR) with specific primers, and direct sequencing for the ribosomal DNA of internal transcribed spacer (ITS) regions.

The results of this research will provide grading agencies and wood microbiologists with usable guides for the identification and evaluation of firm red heart and the engineering community with design values for a range of decay severities. In addition, new methods of test-material collection were developed, and improvements to current grading rules benefiting lumber producers and users alike are suggested. To paraphrase an official of a western United States lumber-grading agency, "We really do not know what its (firm red heart) effects on strength are". The improved fungal identification procedure also will offer the tree pathologist, mycologist and wood engineer another useful tool for the investigation of the biology of wood-decaying fungi.

LITERATURE CITED

Brier, A. N., W. A. Dost, and W. W. Wilcox. 1988. Characteristics of decay and insect attack in California homes. California Agriculture. 42 (5):21.22.

Germain, H. G. Laflamme, L. Bernier, B. Boulet, and R. C. Hamelin. 2002. DNA polymorphism and molecular diagnosis in *Inonotus* spp. Can. J. Plant Pathol. 24:194-199.

Jasalavich, C. A., A. Ostrofsky, and J. Jellison. 2000. Detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding rRNA. Appl. Environ. Microbiol. 66(11):4725-4734.

National Research Council. 1990. Forestry research: A mandate of change. National Academy Press, Washington, D.C.

Schmidt, O., and U. Moreth. 2000. Species-specific PCR primers in the rDNA-ITS region as a diagnostic tool for *Serpular lacrymans*. Mycol. Res. 14:69-72.

Ulrich, A. H. 1983. U.S. timber production, trade and price statistics. USDA. For. Serv. Misc. Publ. 1424.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315-322. *In* M. A. Innis, D. H. Gelfan, J. J. Sninsky, and T. J. White (ed.) PCR protocols: a guide to methods and applications. Academic Press, San Diego, Calif.

CHAPTER ONE

Identification and Detection of Decay Fungi from Lodgepole Pine (*Pinus contorta* Dougl. ex Loud.) by DNA Assay

LITERATURE REVIEW

The reliable and effective identification and detection of incipient and early decay in wood is a primary concern for many lumber graders, wood engineers and microbiologists (Zabel and Morrell, 1992). Culturing fungi from wood samples and microscopic examination of fungal structures has been the traditional method for detection and identification of the wood decay species during the incipient and early decay stages. (Nobles, 1948; Nobles, 1965; Gilberson, 1974; Stalpers, 1978; Wang and Zabel, 1990). The morphological variation of fungal structures within a particular species, however, can cause difficulty in accurate identification (Davidson *et al.*, 1942; Nobles, 1948).

The techniques of molecular investigation have been developed and continuously improved since 1990 and can be used in many applications such as medicine, plant pathology, microbiology and forensic science. During this period, these molecular-based identification tools have been receiving significant attention as means for identification of wood decay fungi. (Jellison and Goodell, 1988; Gardes and Bruns, 1993; Benello *et al.*, 1998; Jasalavich *et al.*, 2000; Schmidt and Moreth, 2000).

Several molecular-based identification techniques, including Restriction Fragment Length Polymorphism (RFLP), Enzyme-linked Immunosorbent Assay (ELISA) and Immunoblotting have all been used to detect or identify incipient decay, but the accuracy

of these methods are reduced by wood extractives, environmental conditions or fungal species. Polymerase Chain Reaction (PCR) is the technique used to replicate a fragment of DNA to produce many copies of a particular DNA sequence for post-PCR analysis such as Restriction Fragment Length Polymorphism (RFLP) analysis or direct DNA sequencing. During the PCR, two strands of the DNA are separated by heating and short sequences of a single DNA strand (primers) are added, together with a supply of free nucleotides and DNA polymerase obtained from an extreme heat-resistant bacterium. In a serious of heating and cooling cycles, the DNA sequence is rapidly amplified and doubled with each cycle.

Restriction Fragment Length Polymorphism (RFLP) applies several DNA digesting enzymes to cleave the DNA sequence amplified by the Polymerase Chain Reaction (PCR) into several small DNA fragments, which can then be used to produce a DNA fingerprint for comparing similarities and differences between fungal species or other organisms (McPherson and Møller, 2000). However, the RFLP analysis does not determine the DNA code for each DNA fragment.

DNA sequencing is the determination of the precise sequence of nucleotides in a sample of DNA that is synthesized from four deoxynucleotide triphosphates (dATP, dCTP, dGTP, and dTTP). The DNA sequence is then defined by the four bases (Adenine (A), Guanine (G), Cytosine (C) and Thymine (T)) of deoxynucleotide triphosphates. In general, DNA sequencing provides more information than RFLP analysis (McPherson and Møller, 2000).

Although Restriction Fragment Length Polymorphism (RFLP) analysis is useful for studying the diversity of fungal communities (Johsson *et al.*, 1999) and confirming

the identity of organisms among species, this technique requires known DNA sequences or a database of RFLP profiles to be able to identify unknown fungal species. In addition, the resolution of RFLP can decrease with short DNA fragments.

Since the rapid development of commercial automated sequencing systems and the availability of internet-based fungal gene databases such as the one offered by the National Center for Biotechnology Information (NCBI) (Altschul *et al.*, 1997), sequencing PCR products is now more effective than using RFLP for fungal identification, especially when identified RFLP profiles are not available.

An efficient method for DNA extraction and purification is another requirement for a successful PCR and post PCR analysis. Several studies have described extraction and purification procedures for fungal DNA (Wilson, 1987; Chow and Käfer, 1993; Wieland, 1997). The cetyltrimethylammonium bromide (CTAB) method and the method using organic solvents such as phenol-chloroform have become two principal procedures (Wieland, 1997). However, most CTAB methods for extracting fungal DNA were modified from the original CTAB method developed for plant tissues (Taylor *et al.*, 1993). Weiland (1997) reported that the CTAB method was considered superior at removing unwanted carbohydrates compared the method with employing organic solvents such as phenol and chloroform, but the method using pheno-cholorform is often faster than the CTAB method. Additionally, most methods used to process fungal nucleic acid are relatively tedious and can handle only a small number of samples at a time. More recently, Weiland (1997) developed a rapid procedure using liquid nitrogen and grounding mortar and pestle with quartz sand followed by extraction using organic solvents and alcohol precipitation. Although this method is relatively expeditious, it still

requires grinding of mycelium in frozen or lyophilized form and use of toxic organic solvents during the extraction process. In order to increase efficiency and to process many samples at once, a method for isolation of total nucleic acid from the fungus, *Aspergillus nidulans,* was developed by Chow and Käfer (1993). Compared with the methods of Weiland (1997) and Jasalavich *et al.*(2000), this older method uses an integrated glass bead grinding system, organic solvents and an extraction buffer, and includes the use of a beating tube that reduces the hazard of exposure to toxic organic solvents. It also replaces the step involving grinding and is able to process many samples simultaneously. Very recently, Cassago *et al.* (2002) has also used Chow and Käfer's method to extract DNA from the filamentous fungus, *Trichoderma reesei.* Their results also showed that this method extracted DNA more quickly than CTAB method and was able to produce many high quality DNA samples at the same time.

The internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) have been used to analyze the phylogenetic relationships for different microorganisms (Wose and Olsen, 1986; Medlin *et al.*, 1988; Jorgensen and Cluster, 1989; White *et al.*, 1990). The ITS regions of fungal rDNA contain two non-coding regions (ITS1 and ITS2) that are within the three highly conserved rDNA repeat units (18S, 5.8S and 26S) (White *et al.*, 1990; Gardes and Bruns, 1993). Compared with the slowly evolving nuclear small-subunit rDNA sequences, the ITS regions vary highly among fungal species within a genus (Gardes *et al.*, 1991; Lee and Taylor, 1992; Gardes and Bruns, 1993). Therefore, this highly variable ITS region is the most-widely sequenced fungal DNA and is suitable as a systematic study at species level or even within species. In addition, Gardes and Bruns (1993) also found that the ITS regions provide two advantages for PCR

amplification. The advantages are: 1.) the ITS regions are about 600-800 base pairs long and are ready to be amplified by universal primers that are complementary to sequences within the rRNA genes and 2.) the nature of the multi-copy makes very dilute concentrations of the ITS regions easily amplified.

In order to effectively amplify the ITS region for sequencing, the selection of the primer pair is important because the primers determine the location and length of sequence to be amplified from DNA (McPherson and Møller, 2000). Several ITS specific primers have been used to amplify the fungal ITS region of rDNA (Grades *et al.* 1991; Lee and Taylor, 1992, Grades and Bruns, 1993; Jasalavich *et al.*, 2000; Germain *et al.*, 2002; Paulin-Mahady *et al.*, 2002). Among those primers, the universal primers (ITS1, ITS4), combined with the fungal specific primers (ITS1-F, ITS4-B), are the most frequently used primers for decay fungi. Gardes and Bruns (1993) indicated that using the Basidiomycetes specific primer (ITS4-B) with either the universal primer (ITS1) or higher fungal specific primer (ITS1-F) can amplify rDNA from all basidiomycetes but offers no amplification of Ascomycetes. Another combination that used the fungal specific primer (ITS1-F) with the universal primer (ITS4) showed this primer pair to be able to effectively amplify ITS regions from both Ascomycetes and Basidiomycetes. Lately, Jasalavich *et al.* (2000) conducted a Restriction Fragment Length Polymorphism (RFLP) analysis for fourteen decay fungi and twenty-five wood-inhabiting fungi inoculated into spruce wood. The results were similar to those of Gardes and Bruns (1993). Their study also indicated that the PCR with these ITS primers could detect incipient decay before any measurable weight loss of the wood block.

OBJECTIVES

- Develop an effectively DNA assay to identify and detect decay fungi during incipient decay stage
- Compare the reliability of identification methods (Morphology VS. DNA Assay)

MATERIALS AND METHODS

Raw Material Selection

Lodgepole pine (*Pinus contorta* Dougl. ex Loud.) is a species native to western North America. Its geographic range extends from New Mexico to north of the Arctic circle and from the eastern foothills of the Rocky Mountains to the Pacific Ocean (Koch, 1996).

Ten states process large volumes of lodgepole pine (Koch, 1996). In total, 26,697 million cubic feet (756 million m^3) of growing stock can be found in forests of these ten states, including 76,280 million board feet (300 million m^3) of saw timber. The Rocky Mountain region (six states) provides about 80 percent of the growing stock and 73 percent of the saw timber. The Pacific Northwest region has the second largest growing stock volume (3.2%), while the Pacific Southwest produces only 1.9 percent of the growing volume. In the Rocky Mountain region, Montana has the highest net volume of growing stock (9,141 million cubic feet) and net saw timber volume (20,033 million cubic feet).

Lodgepole pine (*Pinus contorta*) is known for growing in dense fire and insect-generated stands, which pose significant management problems for forest managers. In the lodgepole pine forests, stem decays by wood-rotting fungi are common in trees

wounded by fire or insects. For example, red ring rot, caused by the fungus *Phellinus (Fomes) pini,* is one of the most widespread stem decays of lodgepole pine forest in Northwest U.S. and Canada. Therefore, the common utilization of fire-wounded and fungus-attacked lodgepole pine is of considerable concern.

Lodgepole pine is one of the major timber resources for structural lumber, log homes, rustic furniture and secondary wood products throughout its range. Recently, the secondary product industry has also begun increased utilization of this tight-grained wood with characteristically small knots for interior and exposed parts in furniture, cabinets and solid lumber panels.

Because lodgepole pine is subject to catastrophic fires and is often utilized after it has died from insect attack and disease such as *Phellinus pini,* this species was chosen as the subject for this study.

Media Preparations

The wood samples were placed onto malt extract agar (MEA) to culture fungi (Davidson *et al.*, 1938; Nobles, 1948; Wang and Zabel, 1990), because malt extract agar (MEA) provides good recovery of wood decay fungi. Gallic acid agar (GAA) was used to differentiate the white-rot and brown-rot fungi (Davidson *et al.,* 1938; Nobles, 1948; Wang and Zabel, 1990). The white-rot fungi produce dark brown discoloration on gallic acid agar while the brown-rot fungi do not affect the color of gallic acid agar. The preparations of MEA and GAA are list in Appendix 1.1.

Fungal Isolation Procedure

Four wood samples (1.0 x 1.0 x 1.0 inches / 2.54 x 2.54 x 2.54 cm) were randomly obtained from clear wood and each discoloration class (low, medium and high)

of each log, with a total sample size of isolation was three hundred and twenty wood blocks (4 x 4 x 20 = 320). Samples were trimmed and labeled (Table 1.1) in a sterilized petri dish with a sterilized knife down to 0.6 x 0.6 x 0.6 inches (1.52 x 1.52 x 1.52 cm), and surface sterilized by the following procedure before being placed into MEA in petri dishes for incubation:

- 10% bleach solution (5 ml bleach / 45 ml sterilized water) for 1 min.
- Sterile water rinse for 1 min.
- Sterile water rinse for 1 min.
- Absorb extra water on surface with a small piece of sterilized filter paper.

Each 60 mm MEA dish contained only one wood block (Figure 1.1). Based on the methods of Nobles (1948), all the MEA plates were sealed with paraffin film and incubated in a growth chamber at 25 °C in darkness for 10 days. Once the cultures were actively growing, a three mm cube was transferred to a fresh 100 mm MEA plate. These MEA plates were sealed with paraffin film and incubated in the dark at 25 °C for six weeks. Plates were examined every week for growth rate by counting the number of weeks for fungal mycelium to cover the MEA plate.

Identification by Morphology

Fungal identification procedures were based on the wood decay fungi identification manual (Nobles, 1948). Culture growth rate, appearance of culture, color reaction in GAA and fungal morphology were used as the identification keys. Fungal cultures growing in MEA were examined at the end of six weeks. Safrain-O was used to stain fungi for viewing of microscopic characters. The staining solution is list in Appendix 1.2. The staining procedure is as follows (Johansen, 1940):

1. Deliver 1 droplet of stain solution on the slide.
2. Place about 0.5 mm square of fungal hyphae or spores on the slide.

3. Spread the hyphae or spores with a needle.
4. Let the sample totally immerse in the stain solution for 30 seconds
5. Deliver one droplet of 2.5% alcohol/water solution to dilute the extra stain solution.
6. Place a cover glass over the sample, gently press the cover glass, and remove excessive stain solution from the slide before examining under light microscope.
7. Use a 3500 K blue color filter on the light resource of light microscope for better contrast.

The GAA (Nobles, 1948) was used to differentiate white-rot fungi from brown-rot fungi. Fungal cultures from MEA plates were transferred to the GAA plates and incubated in the dark for two weeks prior to examine.

Table 1.1. The log label abbreviation and its general descriptions.

Log Label	Description
CAB	CAB = CABCO; a local log sorting yard dealing primarily in pulpwood for the Smurfit-Stone Corporation's Frenchtown, MT Kraft pulp mill. The logs primarily come from private landowners within 100 miles of Missoula.
RLH	Northwest Manufacturing Inc: a local log home construction company. It is the Montana manufacturing operation of Real Log Homes, Inc., headquartered in Vermont. This company produces pre-cut log home packages and obtains its raw material from locations throughout the Rocky Mountains of the U.S. and Canada.
STIM	Stimson Lumber Company: a private forest products and natural resource company based in Portland, Oregon. With its roots dating back to the 1850s, the company is one of the oldest, continuously operating forest products companies in the United States. Its has assets and operations in four Western States: Idaho, Montana, Oregon, and Washington. Its raw material for the small log sawmill comes primarily from private industrial forest lands in Idaho and Montana

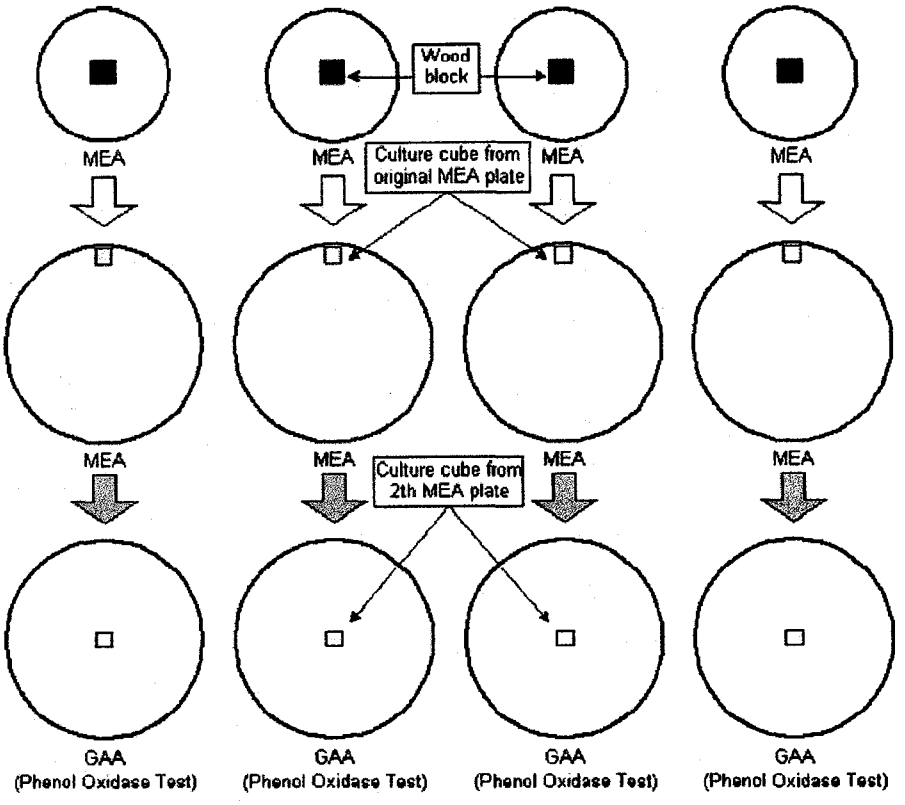

Figure 1.1. The flow chart and diagram of the placements of wood samples and culture cubes in MEA and GAA plates during the fungal isolation and phenol oxidase test.

DNA Extraction and Purification

The DNA extraction was modified from the methods of Smalla *et al.*(1993) and Chow and Käfer (1993). One-tenth of a gram of mycelium was collected and resuspended in a 2.0 ml bead beating tube with 0.3 ml of an extraction buffer (50mM $NaPO_4$ (pH=8), 5mM EDTA, 3% SDS),0.4 gm of sterilized acid washed glass beads (0.3-0.4 micron), and 0.3 ml of phenol:chloroform (1:1 volume/volume) was added into the mycelial suspension. The mixture was then vortexed at top speed for 5 minutes. The tube was beaten on a bead beater for 2 minutes at 4 °C and was cooled on ice for 1 min after beating. The process was then repeated. An additional 0.2 ml of extraction buffer solution and 0.2 ml phenol: chloroform (1:1) were added to the vortexed mixture. The

solution was mixed by vortexing for 20 seconds and then centrifuged at 14,000 rpm and 4 °C for 30 sec to separate the phases. The aqueous phase was removed and re-extracted in a new 2.0 ml Eppendorf tube with 0.3 ml of phenol: chloroform. This mixture was centrifuged at 14,000 rpm and 4 °C for another 30 sec, and the aqueous phase was removed and placed into a new 1.5 ml Eppendorf tube.

The precipitation of nucleic acid was performed by adding one volume of ice-cold 95% ethanol and letting the tube set at -20°C for 45 minutes. The nucleic acid was pelleted by centrifugation at 14,000 rpm and 4 °C for 30 minutes. The supernatant was decanted, leaving the pellet of nucleic acid at the bottom of the tube. The pellet was washed with 400 µl of ice-cold 70% ethanol, and resuspended by gently shaking the tube. The tube was centrifuged at 14,000 rpm and 4 °C for 20 minutes, and the supernatant was decanted. The nucleic acid was then pelleted in the same tube by centrifugation at 14,000 rpm and 4 °C for 5 minutes. Any excessive supernatant was removed by a pipet. The tube was dried in a vacuum-centrifuge at 3,500 rpm and 25 °C for 20 minutes. The dried nucleic acid pellet was dissolved in 300 µl of sterilized double distilled water.

In order to remove the RNA from the extraction solution, 15 µl RNase (1mg/ml solution) was added to the 300µl nucleic acid solution and placed in a 37 °C water bath for 30 minutes. After the removal of RNA, a volume of 300µl phenol:chloroform (1:1) was added to the tube, and the tube was vortexed for 45 seconds before centrifuged at 14,000 rpm and 4 °C for 60 seconds. The aqueous phase was transferred and re-extracted in a new tube with 300 µl of phenol:chloroform (1:1). This mixture was centrifuged for another 30 sec and the aqueous phase was removed and placed into a new tube. The DNA

was precipitated by adding 0.1 volumes of 3 M NaAcetate (pH=5.2) and 2.5 volumes of ice-cold 95% ethanol and storing at –20 °C for at least 45 minutes.

Following the precipitation, DNA was then pelleted by centrifugation at 14,000 RPM and 4 °C for 30 minutes. The pellet was washed with ice-cold 70% ethanol and dried in a vacuum-centrifuge at 3,500 rpm and 25 °C for 20 minutes. The dried DNA pellet was dissolved in 100 µl of sterilized double distilled water or storage buffer (6 mM Tris HCl, 0.1 mM EDTA, pH=8.0).

Polymerase Chain Reaction (PCR) Amplification

The two internal transcribed spacer (ITS) primers, ITS1F and ITS4 were used (White *et al.*, 1990; Gardes *et al.*, 1991; Gardes and Bruns, 1993). The PCR positive controls used the eukaryotic 18S universal primers (536F and 907R) for DNA from CAB9 or RLH4 (Holben *et al.*, 2002). Amplifications were performed in 50 µl reactions tubes containing 5.0 µl PCR buffer [Roche] (100 mM Tris/HCl, 15 mM $MaCl_2$, 500mM KCl, pH = 8.3), 2.0 µl dNTP [Roche] (10 mM of each deoxyribonucleotide: dATP, dCTP, dGTP and dTTP), 5.0 µl of each ITS primer (50 pmole/µl), 0.5 µl DNA Tag polymerase [Roche] (5 units/µl), 30.5 µl double distilled water and 2.0 µl diluted DNA template (10^{-1}X). A PCR reaction without DNA templates was included in all the PCR processes as a negative control for monitoring contaminations. The temperature cycles were carried out using a Thermocycler (MJ Research Model PTC-100), with the settings shown in Table 1.2.

PCR products were examined by running electrophoresis for 30 minutes, and 4 µl of each unpurified product was analyzed in 2% (weight/volume) agarose gels in 1X TBE (89 mM tris-borate, 89 mM boric acid, 2mM EDTA) with ethidium bromide (EtBr) at

100 ng/ml in both running buffer and gel. 4 μl low mass marker and 4μl negative control product were served as the references for quality control and to quantify purpose. DNA bands were visualized under ultra violet (UV) light and photographed with a digital camera.

DNA Sequencing

PCR products were purified using a commercial DNA purification kit (Qiagen Company) and were sequenced using an Applied Biosystems model 373 DNA sequencer with a Big Dye Filter wheel and the Big Dye Terminator v.30 sequencing chemistry at the Murdock molecular biology facility-The University of Montana. The sequencing results were then compared to the existing databases of fungal nucleotide acid by BLAST (Altschul *et al.*, 1997), a network program for DNA analysis and annotation from the National Center for Biotechnology Information (NCBI).

Table 1.2. The settings of temperature cycles for PCR.

Step 1	94 °C for 95 seconds
Step 2	95 °C for 45 seconds
Step 3	55 °C for 55 seconds
Step 4	72 °C for 45 seconds
Step 5	Go To Step 2 for 12 times
Step 6	95 °C for 45 seconds
Step 7	53 °C for 55 seconds
Step 8	72 °C for 90 seconds
Step 9	Go To Step 6 for 21 times
Step 10	72 °C for 10 minutes
Step 11	4 °C for infinite time

RESULTS

Culture Grouping and Preliminary Fungal Identification

Fungal cultures were successfully isolated from 18 of 20 lodgepole pine logs containing firm red heart. The samples from the discolored wood produced cultures whereas the samples from clear wood did not. The samples from log CAB2 with high discoloration yielded two different species, while the other logs only produced one type of fungal species. These cultures were grouped into six morphological groups (Table 1.3) by their cultural and hyphae characteristics as described in the identification manual of wood decay fungi (Nobles, 1948). Four groups (A, B, C, D, E) produced bsidiospores except group F. Three groups (A, B, C) that presented swelling on hyphae and showed only little variation in culture appearance were keyed to *Phellinus pini* (red ring rot or white pocket rot). Two groups (D, E) did not present swellings on hyphae, and keyed to *Fomes nigrolimitatus* Egel. However, the lack of swellings on hyphae sometime can occur with *Phellinus pini* growing in agar media (Nobles, 1948). Group F, on the other hand, its culture only produced simple septa hyphae without any types of spores. Therefore, the information from the culture was not enough to key the fungi to any species described in identification manual. A unique feature of group F was an enlargement on the apex of hyphae. No fungi were isolated from samples of clear wood and fungal hyphae were not found in microscopic examination of clear wood samples.

Table 1.3. The lists of logs and identification of fungi from each group. (*Two different cultures were isolated from the log: CAB2).

Group	Log Label	Species
A	CAB1, 2*, 3, 4, 8, 10	*Phellinus pini*
B	CAB9; RLH10, 11, 13	*Phellinus pini*
C	STIM2, 5, 7; RLH17	*Phellinus pini*
D	RLH4, 5	*Fomes nigrolimitatus*
E	STIM14, RLH15	*Fomes nigrolimitatus*
F	CAB2*	Unknown

Culture and hyphae characters

For the groups A, B, C, D, and E, growth was slow to very slow (5-8 cm in six weeks). Advancing zones for fungi in these groups were even and the mycelia were white to milky white in color. The mycelia both penetrated the agar and grew aerially. The center area of the mycelium changed color from white to yellow or dark brown with a wooly to cottony texture with the mycelium raised much higher than the advancing zone after five weeks of growth (Figure 1.2 and 1.3). MEA colorized by these fungi showed only slight brown discoloration or no color change at the end of six weeks. The color of GAA turned dark brown (positive reaction) after these fungi were grown on it for one week (Figure 1.4). Thin-walled hairline hyphae and thick-walled contextual hyphae were found and both hyphal types possessed simple septae (Figure 1.5 and 1.6). Clamp connections were not found in cultures from these five groups. However, Nobles (1948) also failed to find clamp connections. Chlamydospores, setate, conidia and oidia were not found in these groups. Abundant oval spores (6-8 µl long and 4-7 µl wide) developed after seven to eight weeks of growth in both MEA and GAA plates in these five groups

(Figure 1.7). Group A, B and C showed swelling on the hyphae (Figure 1.8), but group D and E did not present any swelling on hyphae.

For group F, growth rate was very slow, and mycelium only covered about 50-60% of the plate surface after six weeks. The advanced zone was even and mycelium remained white even after eight weeks of growth (Figure 1.9). The aerial mycelium did not rise as high as for the other five groups and showed a much finer and smoother cottony texture. The mycelium submerged into the agar. MEA showed no color change after six weeks of incubation. No color change occurred on GAA. Thin walled hyphae (2-5μ in diameter) and aerial hyphae (4-8 μ in diameter) contained simple septa structure, but no clam connections were found (Figure 1.10). No chlamydospores, conidia, or oidia were produced. An enlargement (ascus-like structure) was found on the apex of hyphae (Figure 1.11). However, the morphology was insufficient to identify this culture.

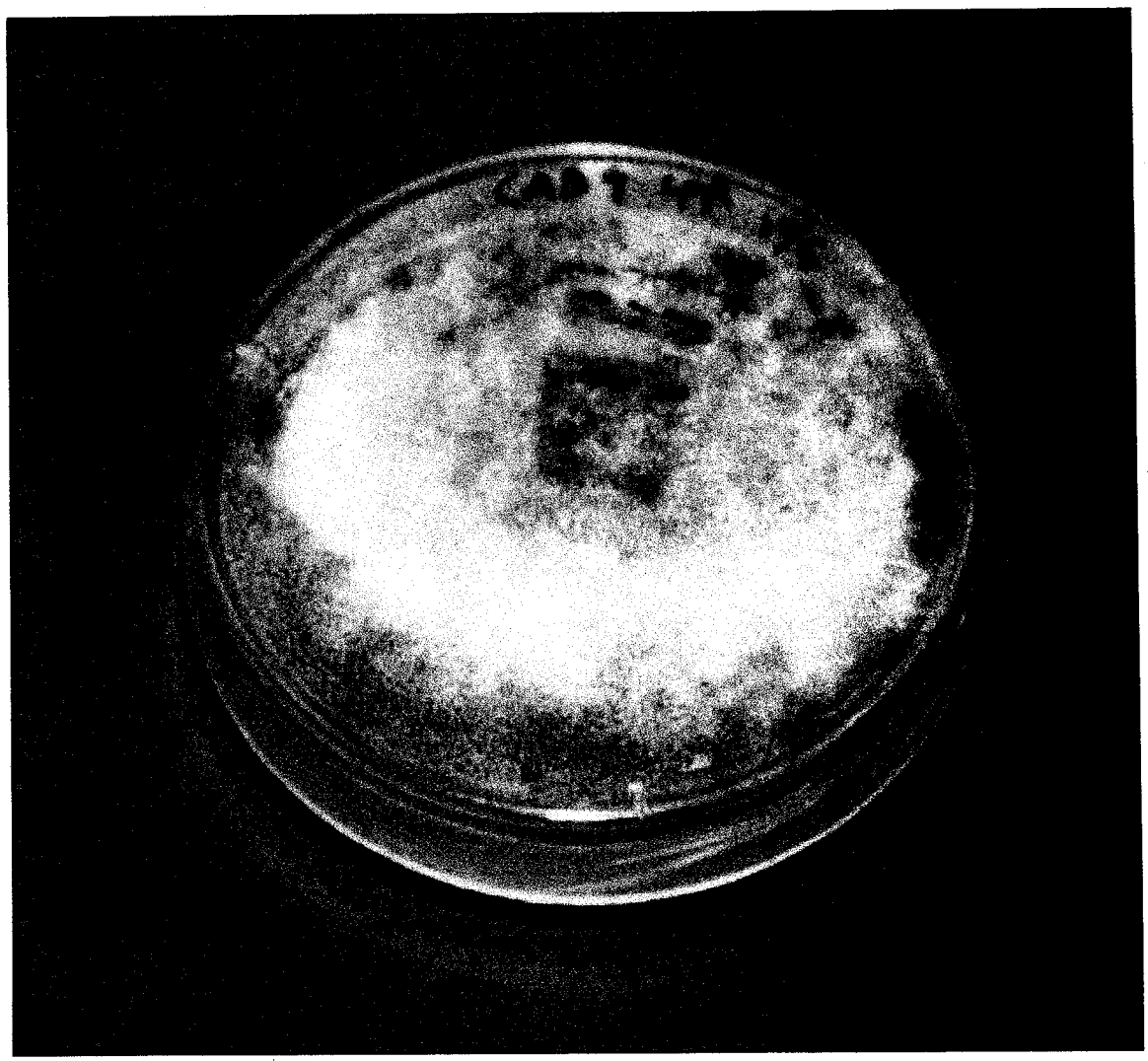

Figure 1.2. MEA plate containing wood sample from lodgepole pine log (CAB9) and mature mycelium of *Phellinus pini* after 7 weeks of growth.

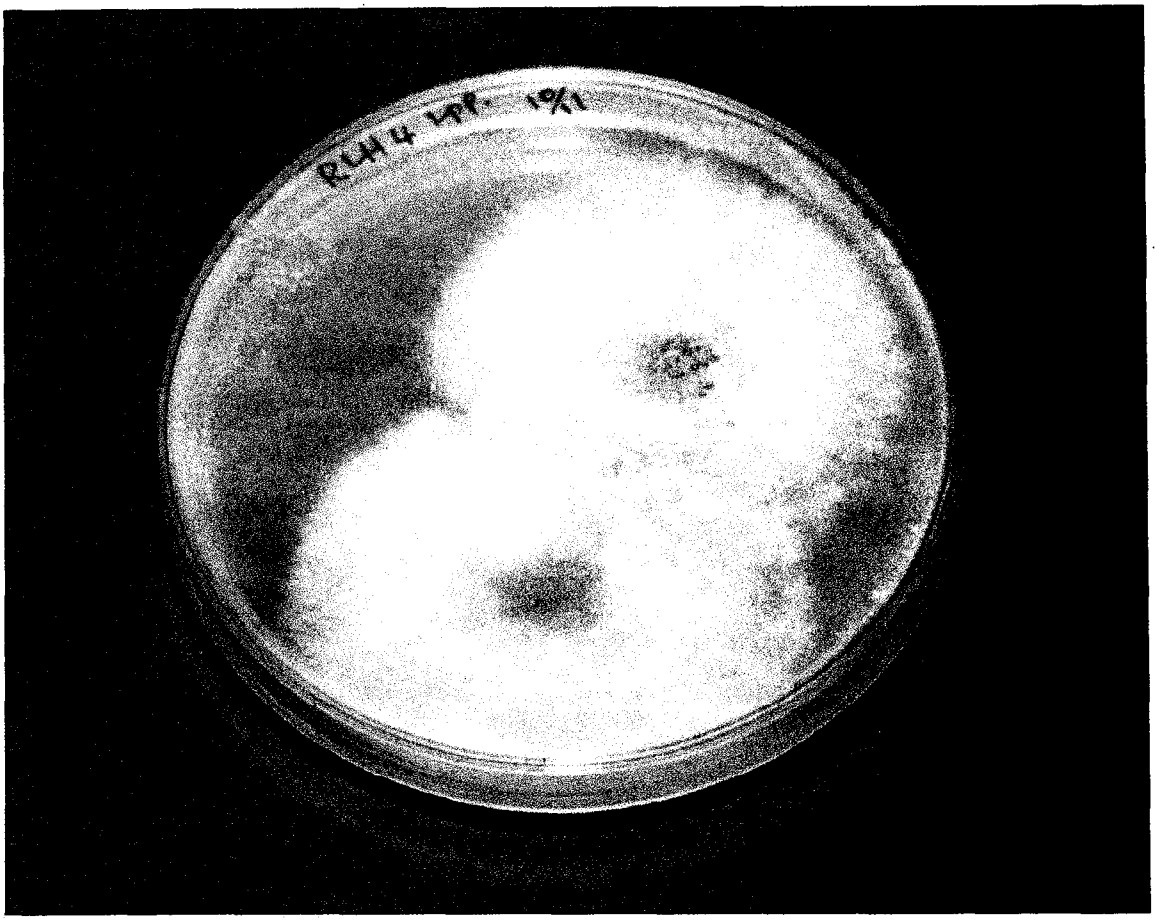

Figure 1.3. MEA plate containing two *Phellinus pini* cultures isolated from the lodgepole pine log (RLH4) with the two cultures merged after 2 weeks of growth. Center of each culture turned yellow to brown color. MEA showed almost no discoloration.

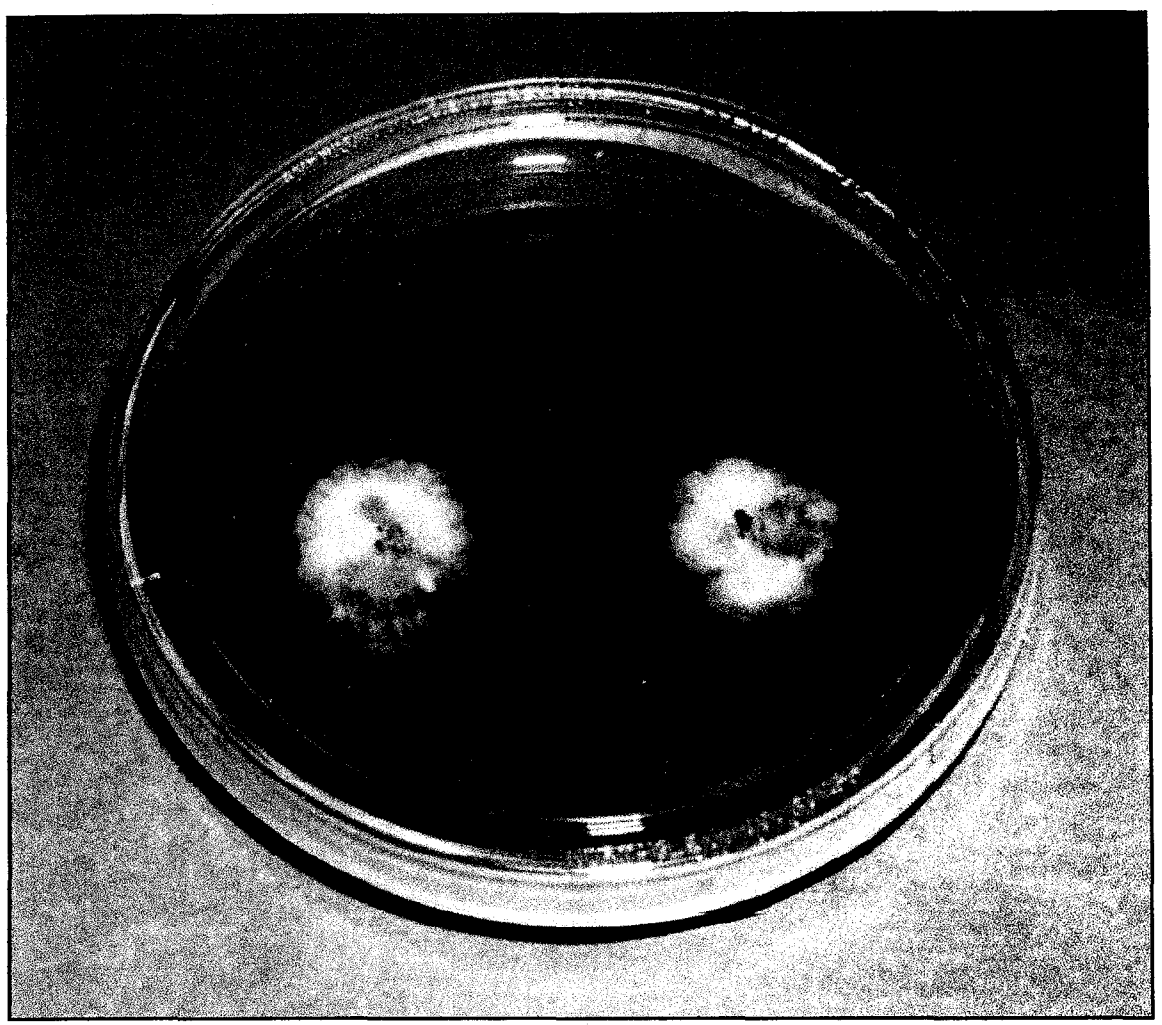

Figure 1.4. GAA plate containing fungal cultures from the lodgepole pine log (RLH4) after one week of growth with the dark brown discoloration of agar caused by the phenol oxidase reaction from *Phellinus pini.*

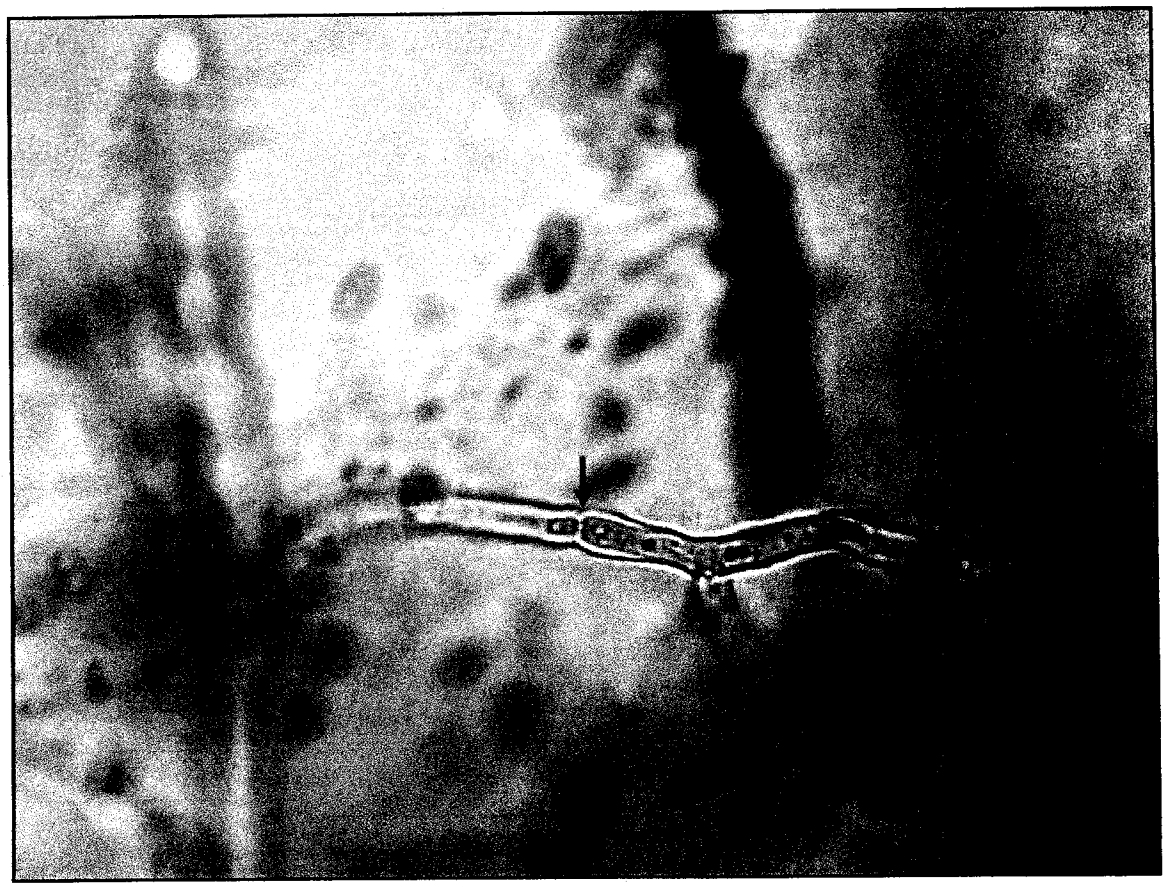

Figure 1.5. Fungal hypha with a simple septum and thin wall structure (culture of *Phellinus pini* isolated from the lodgepole pine log: CAB9).

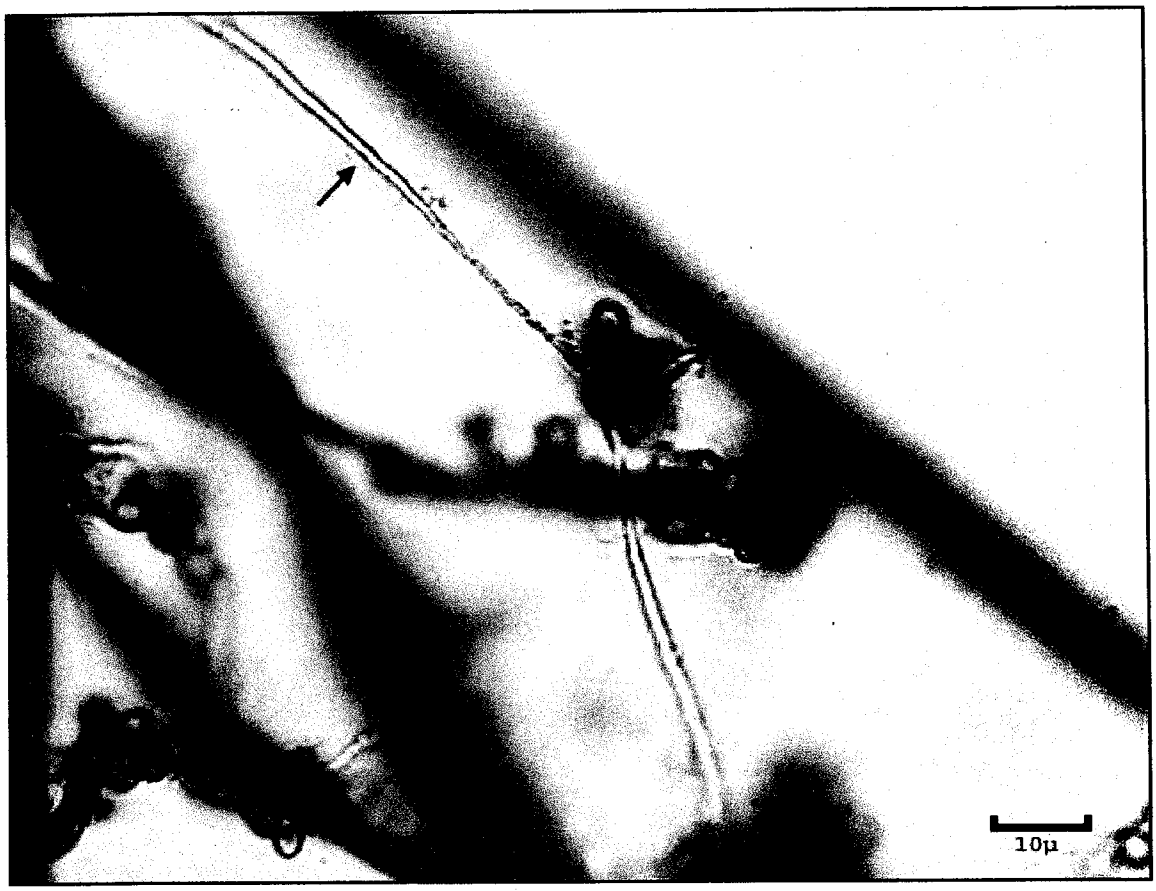

Figure 1.6. The hairline hyphae structure of *Phellinus pini* isolated from the lodgepole pine log (CAB9).

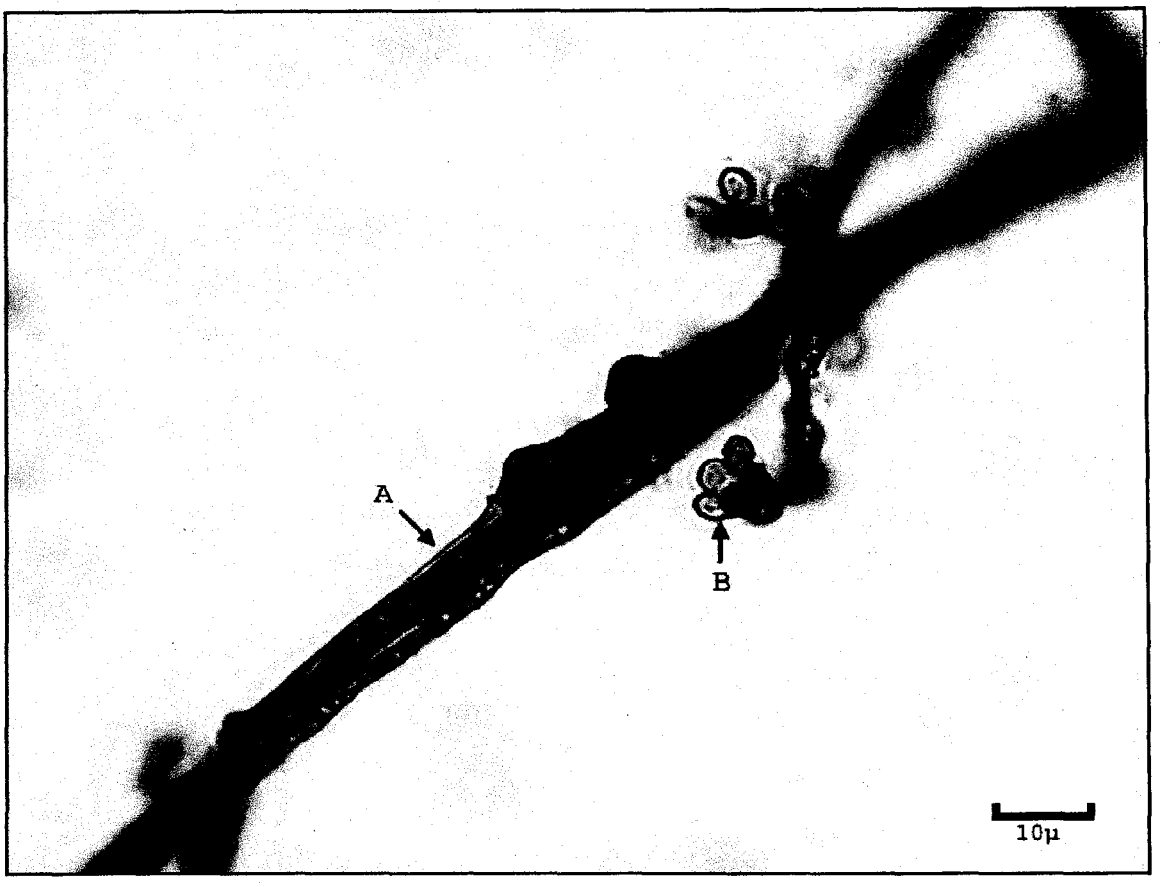

Figure 1.7. Thick walled hypha (arrow A) and basidiospore (arrow B) of *Phellinus pini* isolated from the lodgepole pine log (RLH4).

Figure 1.8. A swelling structure on the small diameter hyphae of *Phellinus pini* isolated from the lodgepole pine log (CAB9).

Figure 1.9. Mycelia of unknown species (group F) isolated from CAB2 after 8 weeks of growth on the MEA plate.

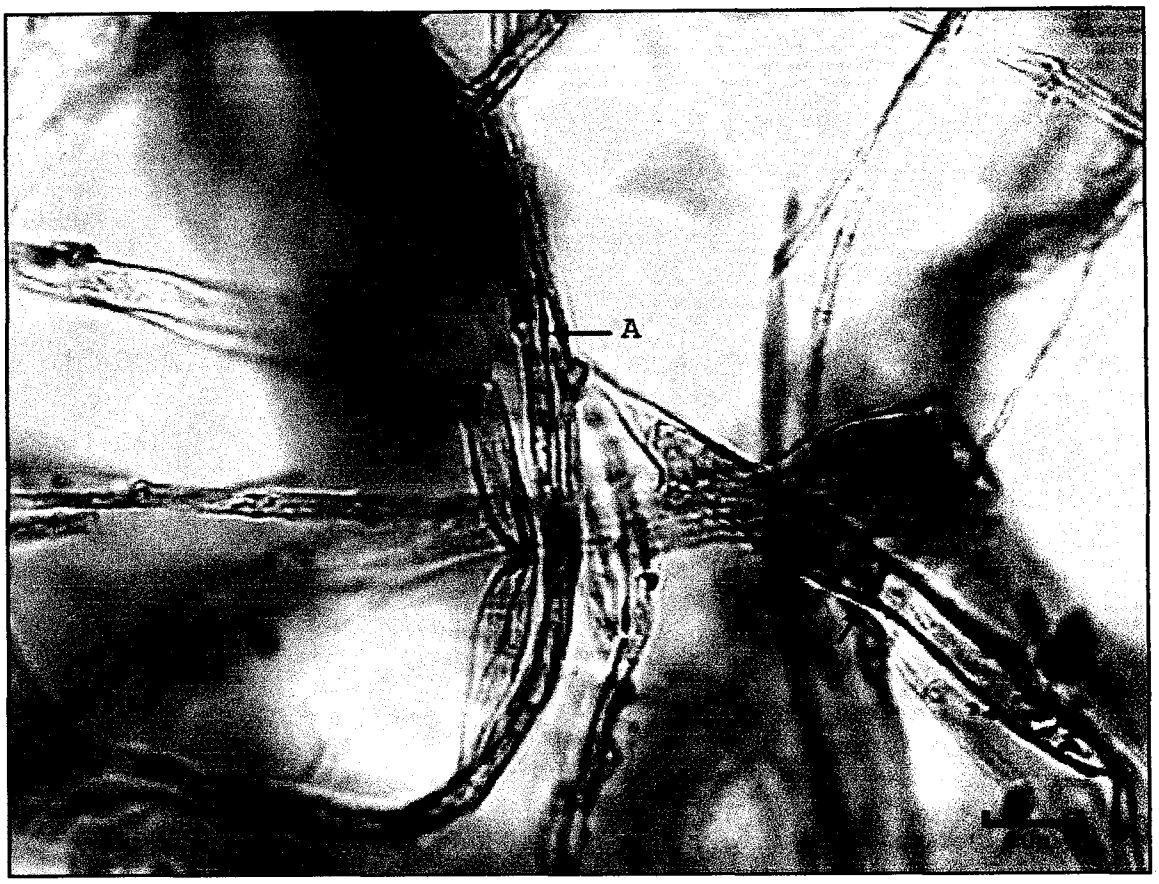

Figure 1.10. Thin wall hyphae with simple septa structure (arrow B) and smaller diameter hyphae (arrow A) of unknown species (group F) was isolated from CAB2 log.

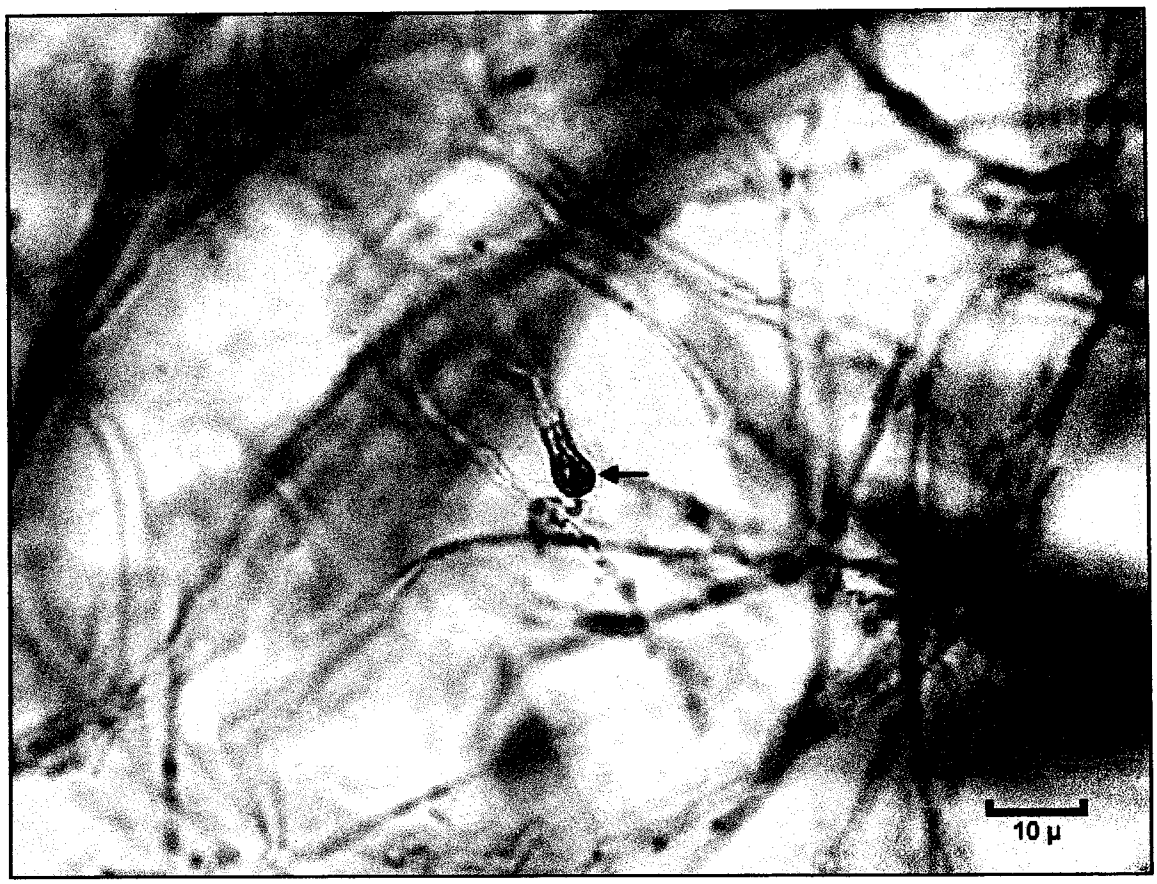

Figure 1.11. An enlargement (ascus-like structure) on the apex of smaller diameter hyphae of unknown species (group F) was isolated from CAB2 log.

DNA Extraction, Purification and PCR

DNA extraction and purification-This modified method for DNA extraction and purification was able to successfully extract DNA and RNA from each representative fungal culture of all six groups. The integration of phenol-chloroform with an extraction buffer yielded an adequate quantity of total DNA from pure fungal cultures. Due to the RNA present in all the DNA extractions, the extra purification using RNase, followed by phenol-chloroform extraction to eliminate RNA and remaining RNase, was required for the amplification by PCR. This study found that the fresh mycelium from advance zone of a fungal culture is preferred for DNA isolation rather than mycelium from the very old growth zone that did not consistently yield enough DNA.

DNA precipitated by adding 0.1 volumes of 3 M NaAcetate (pH 5.2) and 2.5 volumes of ice-cold 95% ethanol and storing at –20 °C for at least 45 minutes was necessary to recover enough DNA for PCR. Overnight ethanol precipitation at a lower temperature (-70°C) did not outperform the method using incubation at –20 °C for 45 minutes.

PCR results and optimization-The PCR results showed that the primers ITS1-F (specific primer for higher fungi) and ITS4 (universal primer) amplified only one band from each DNA template (Figure 1.12). The PCR products amplified from groups A, B, C, D and E were about 720-780 base pairs (bp) long, while the PCR product from the group F was approximately 920-990 bp. The positive control used the DNA templates from group D, and it was amplified by the 18S universal primers (536F and 907S). The negative control was performed without DNA template in the reaction solution. The positive control showed one amplification band (about 560-580 bp) on the agarose gel.

A series of annealing temperatures of different time lengths were tested. It was determined that the combination of two annealing temperatures (55 °C for 55 seconds and 53 °C for 55 seconds) generated the cleanest and most reliable results. The performance of PCR amplification diminished when the annealing temperature was above 56 °C or below 52 °C. Meanwhile, extending the time beyond 55 seconds for annealing yielded no advantage.

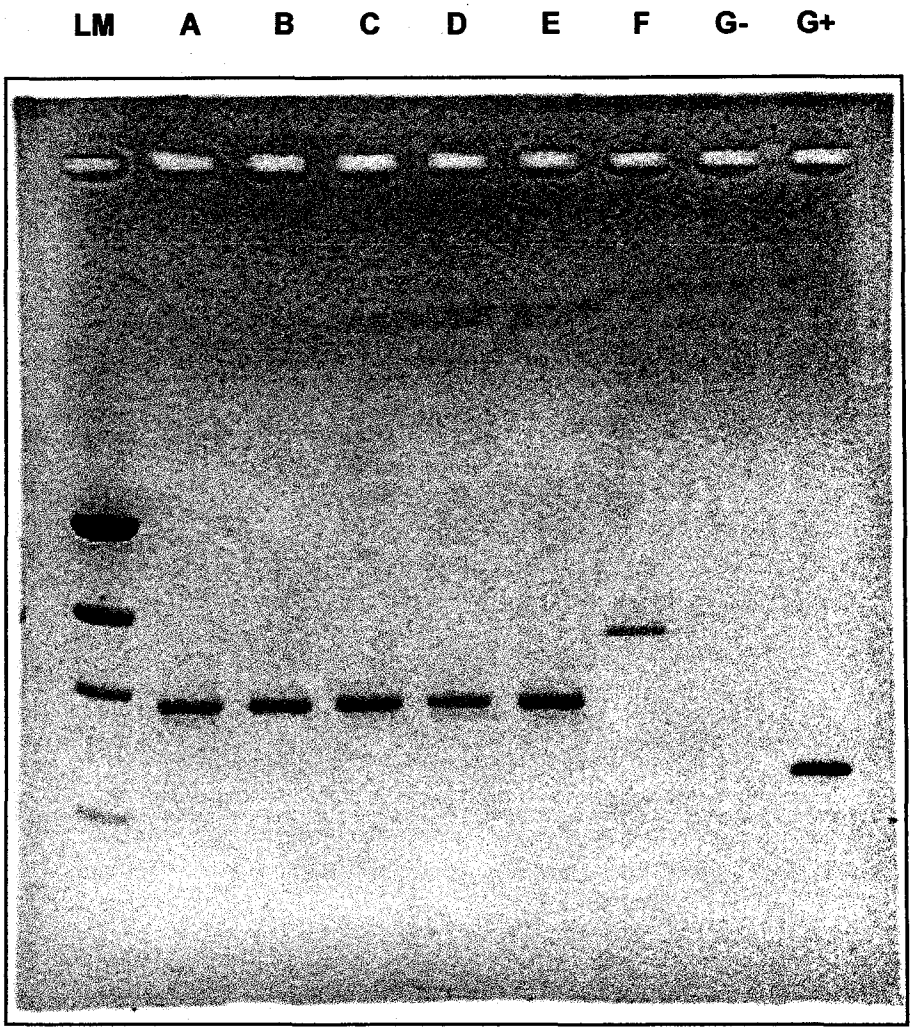

Figure 1.12. Purified PCR products having run in electrophoresis for 30 minutes. 4 µl of each product was analyzed in 2% (weight/volume) agarose gels in 1X TBE (89 mM tris-borate, 89 mM boric acid, 2mM EDTA) with ethidium bromide (EtBr) at 100 ng/ml in both running buffer and gel. From left to right of the gel: **LM**. 4 µl low mass marker; **A**. Group A: CAB4 (ITS1F-ITS4); **B**. Group B: CAB9 (ITS1F-ITS4); **C**. Group C: STIM2 (ITS1F-ITS4); **D**. Group D: RLH4 (ITS1F-ITS4); **E**. Group E: RLH15 (ITS1F-ITS4); **F**. Group F: CAB2 (ITS1F-ITS4); **G-**. Negative control: No DNA template (ITS1F-ITS4); **G+**. Positive control: RLH4 (536F-907R).

DNA Sequencing and Fungal Identification

The raw sequence codes for the fungi sequenced in this study are presented in Appendix 1.3. Pair sequences (forward and reverse) from six groups were compared to the fungal DNA databases from NCBI. The results indicated that cultures from group A, B, C, D and E had the 98-99% similarity to the Basidiomycetes fungus, *Phellinus pini* (Table 1.4). The sequencing identification results were different from the identification by fungal culture and hyphal characteristics. Group D and E were *Phellinus pini* and not *Fomes nigrolimitatus* (Table 1.4). Group F, which could not be identified using morphology had a DNA sequence with a 94.1% similarity to be the Ascomycetes fungus, *Byssochlamys nivea* (Table 1.4). The sequences were submitted to GenBank of NCBI and published with the following access numbers presented in table 1.4.

Table 1.4. The fungal identification results by DNA sequences and the sequence similarity percentages to the database from the National Center for Biotechnology Information (NCBI).

Group	Identified species	Similarity	GenBank access number
A	*Phellinus pini* (*Porodaedalea pini*)	98.7 %	AY265221
B	*Phellinus pini* (*Porodaedalea pini*)	99.2 %	AY265221
C	*Phellinus pini (Porodaedalea pini*)	99.1 %	AY265221
D	*Phellinus pini* (*Porodaedalea pini*)	98.4 %	AY265221
E	*Phellinus pini* (*Porodaedalea pini*)	98.3 %	AY265221
F	*Byssochlamys nivea*	94.1 %	AY265223

DISCUSSION AND CONCLUSION

Although the DNA assay developed in this study requiring phenol-chloroform extraction, the capability and efficiency of this DNA method for accurately processing large numbers of samples from fungal cultures make it superior to other methods. In addition to use in the identification of wood decay fungi, this method's ability to produce consistently high quality PCR product is of great value for many fungal research applications. This study demonstrates that comparing the sequences from the fungal ITS regions can correctly identify decay fungi, while fungal identification relying on the culture and hyphae features has difficulty in distinguishing different samples of the same species showing morphological variation.

The ITS1-F and ITS4 primers were used in this study because they showed a reliable PCR amplification with the temperature cycles described in this study. On the other hand, the quality of the PCR products amplified by ITS1-F and ITS4-B (basidiomycetes specific) was too low for sequencing and required reamplification before any post PCR analysis. Also, the ITS1-F and ITS4-B primers could miss wood-inhabiting Ascomycetes fungi that sometimes also cause the discoloration of wood.

A final concentration 1μM (10 pmole/μl) of primers did not work for all samples, even with different DNA template concentrations. The relative concentration of MgCl to other components in the PCR reaction solution also plays an important role for a successful PCR optimization. Several concentrations of MgCl had been tried in this study, and the concentration of 15 mM produced the best results at annealing temperatures of 55°C and 53°C. This method can be best described as a scaled down version of touchdown PCR thermal cycles (McPherson and Møller, 2000). This two-step

annealing process provided a wider annealing window for the primers and DNA templates, and it also produced more dependable outcomes than those using a single annealing temperature.

In conclusion, this modified DNA extraction method effectively yielded sufficient DNA for PCR amplification with ITS primers. Meanwhile, direct sequencing the ITS region of fungal ribosomal DNA demonstrated a more reliable and effective approach for identifying decay fungi than did the culture assay. Although this method worked in this study, the DNA assay for the identification of wood decay fungi still has many areas that needs improvement and refinement. Jasalavich *et al.* (2000) presented results of extracting fungal DNA directly from inoculated wood blocks, but their study was not focused on field samples containing an incipient stage of decay. A study of rapid PCR detection of stained fungi (Kim *et al.*, 1999c) and a study of molecular diagnosis in *Inonotus* spp. (Germain *et al.*, 2002) showed the potential of species-specific primers and DNA extraction by microwave, but their DNA samples were either isolated from fungi with fruiting bodies, which is unlikely to be found in wood during the incipient decay stage. Based on this and previous studies (Kim *et al.*, 1999a; Kim *et al.*, 1999b; Kim *et al.*, 1999c; Jasalavich *et al.*, 2000; Germain *et al.*, 2002), three major improvements for the PCR identification of fungi are needed. First, in order to eliminate the step of culturing fungi from a wood sample before DNA extraction, a method of isolating the fungal DNA directly from the field wood with incipient decay still needs to be developed and incorporated with a more effective DNA extraction method. Second, in order to detect and identify a certain species of fungus from a sample containing mixed species without sequencing or post PCR analysis such as RFLP, it is crucial to develop species-

specific primers. Third, the effects of PCR inhibitors from treated wood and wood products are still not fully understood and require further study.

The DNA extraction method in this study showed good DNA recovery for the samples from pure cultures, but the effectiveness is still not fully understood when processing directly from naturally decayed wood, treated wood and other solid and composite wood products. The inhibitors from decayed wood, or chemical compounds in preservative-treated wood could present problems during the PCR reaction by decreasing the concentration of DNA below that required for PCR reaction. While studies have been conducted on isolating fungal DNA from inoculated wood blocks in the laboratory (Jasalavich *et al.*, 2000; Kim *et al.*, 1999c), no studies using direct DNA isolation from wood with natural incipient decay have been conducted. The technique used in this research has the potential of opening new lines of research and development.

LITERATURE CITED

Adair, S., S. H. Kim, C. Breuil, and S. H. Kim. 2002. A molecular approach for early monitoring of decay basidiomycetes in wood chips. FEMS. Microbiol. Letter. 211(1):117-122.

Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25:3389-3402.

American Society for Testing and Materials (ASTM). 2002. Testing small clear specimens of timber. ASTM D143-94. ASTM, West Conshohocken, Pa.

Berlyn, G.P. and J.P. Miksche. 1976. Botanical microtechnique and cytochemistry. 326 pp.

Benello, P., T. D. Bruns, and M. Gardes. 1998. Genetic structure of natural population of the ectmycorrhizal fungus *Suillus pungens*. New Phytol. 138:533-542.

Cassago, A., R. A. Panepucci, A.M.T. Baião and F. Henrique-Silva. 2002. Cellophane based mini-prep method for DNA extraction from the filamentous fungus *Trichoderma reesei*. BMC Microbiology 2:14.

Chow, T. Y. K. and E. Käfer. 1993. A rapid method for isolation of total nucleic acids from *Aspergillus nidulans*. Fungal genetics newsletter 40: 25-27.

Davidson, R. W., W. A. Campbell, and D. B. Vaughn. 1942. Fungi causing decay of living oaks in the eastern United States and their cultural identification. U.S. Dept. Agric. Tech. Bull. No. 785. 65 p.

Davidson, R. W., W. A. Campbell, and D. J. Blasidell. 1938. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. Agric. Res. 57:683-695.

Dix, N. J. and J. Webster. 1995. Fungal Ecology. Chapman Hall Press.

Gardes, M, and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113-118.

Gardes, M., T. J. White., J. A. Fortin., T. D. Bruns, and J. W. Taylor. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Can. J. Bot. 69:180-190.

Gilberson, R. L. 1974. Fungi that Decay Ponderosa Pine. University of Arizona Press. 197 pp.

Gilberston, R. L., F. F. Lombard, and E. R. Canfield. 1975. Gum guaiac in field tests for extracellular phenol oxidases of wood-rotting fungi and other *Basidiomycetes.* USDA. Forest Service Research Paper FPL 269. Madison, Wisconsin.

Germain, H. G. Laflamme, L. Bernier, B. Boulet, and R. C. Hamelin. 2002. DNA polymorphism and molecular diagnosis in *Inonotus* spp. Can. J. Plant Pathol. 24:194-199.

Holben, W. E., P. Williams, M. Saarinen, L. K. Särkilahti, and J. H. A. Apajalahti. 2002. Phylogenetic Analysis of intestinal microflora indicates a novel mycoplasma phylotype in farmed and wild salmon. Microb. Ecol. 44:175-185.

Jasalavich, C. A., A. Ostrofsky, and J. Jellison. 2000. Detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding rRNA. Appl. Environ. Microbiol. 66(11):4725-4734.

Jellison, J., and B. Goodell. 1998. Immunological detection of decay in wood. Wood Sci. Technol. 22:293-297.

Johansen, D. L. 1940. Plant Microtechnique. McGraw-Hill. 523 pp.

Jorgensen, R. A., and P. D. Cluster. 1989. Modes and tempos in the evolution of nuclear ribosomal DNA: new characters for evolutionary studies and new markers for genetic and population studies. Ann. Mo. Bot. Gard. 75:1238-1247.

Johsson, L., A. Dahlberg, M. C. Nelson, O. Zackrisson, and O. Karen. 1999. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests and their composition following wildfire. Mol. Ecol. 8(2):205-215.

Kim, S.H., A. Han, A. Uzunovic and C. Breuil. 1999a. Specificity of the universal ribosomal DNA primers against softwood sapstain fungi. Material and Organism 32:183-193.

Kim, S.H., A. Han, J. Kronstad, and C. Breuil. 1999b. Differentiation of sapstain fungi by restriction fragment length polymorphism patterns in the nuclear small subunit ribosomal DNA. FEMS Microbiology Letters 177:151-157.

Kim, S.H., A. Uzunovic and C. Breuil. 1999c. Rapid detection of *Ophiostoma piceae* and *O. quercus* in stained wood using PCR. Appl. Environ. Microbiol. 65:287-290.

Koch, Peter. 1996. Lodgepole Pine in North America. Forests Products Society. Madison, WI. Vol. 1. 343 pp.

Krahmer, R. L., R. C. DeGroot and E. C. Lowell. 1982. Detecting incipient brown rot with florescence microscopy. Wood Science. 15(2): 78-80.

Kuo, M. J., F. McClelland, S. Luo, P. L. Chien, R. D. Walker and C. Y. She. 1988. Applications of photoacustic spectroscopy for wood samples. Wood and Fiber Science. 20(1): 132-145.

Lee, J. S., K. S. Ko, and H. S. Jung. 2000. Phylogenetic analysis of *Xylaria* based on nuclear ribosomal ITS1-5.8S-ITS2 sequences. FEMS Microbiol. Letters. 187:89-93.

Lee, S. B. and J. W. Taylor. 1992. Phylogeny of five fungus-like prototistant Phytophtora species, inferred from the internal transcribed spacers of ribosomal DNA. Molecular Biology and Evolution. 9:636-653.

Medlin, L., H. J. Elwood, S. Stickel, and M. L. Sogin. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene. 71:491-499.

McPherson, M. J. and S. G. Møller. 2000. PCR. The Basics from Background to Bench. Springer Press. 276 pp.

Nobles, M. K. 1948. Studies in forestry pathology. VI. Identification of cultures of wood-rotting fungi. Canad. J. Res., Sec. C. 26:281-431.

Nobles, M. K. 1965. Identification of cultures of wood-inhabiting Hymenomycetes. Canad. J. Bot. 43:1007-1139.

Paulin-Mahady, A. E., T. C. Harrington, and D. McNew. 2002. Phylogenetic and taxonomic evaluation of *Chalara, Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. Mycologia. 91(1):62-72.

Smalla, K., Cresswell, N., Mendoca-Hagler, L.C. Wolters, A., and J.D. van Elsas. 1993. Rapid DNA extraction protocol from soil for polymerase chain reaction-mediated amplification. Journal of Appl. Bacteriology. **74:** 78–85.

Schmidt, O., and U. Moreth. 2000. Species-specific PCR primers in the rDNA-ITS region as a diagnostic tool for *Serpular lacrymans*. Mycol. Res. 14:69-72.

Stalpers, J. A. 1978. Identification of wood-inhabiting Aphyllophorales in pure culture. Stud. Mycol. 16:1-248.

Taylor, B. H., J. R. Manhart, and R. M. Amasino. 1993. Isolization and characterization of plant DNAs, p. 37-56. In B. R. Glick and J. E. Thompson (ed.), Methods in Plant Molecular Biology and Biotechnology. CRC Press, Ann Arbor, Mich.

Wang, C. J. K., and R. A. Zabel. 1990. Identification Manual for Fungi from Utility Poles in the Eastern United States. American Type Culture Collection. Allen Press. P. 1-23.

Wieland, J.J. 1997. Rapid procedure for the extraction of DNA from fungal spores and mycelia. Fungal Genetics Newsletter 44: 60-63.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315-322. *In* M. A. Innis, D. H. Gelfan, J. J. Sninsky, and T. J. White (ed.) PCR Protocols: A guide to Methods and Applications. Academic Press, San Diego, Calif.

Wilson, K. 1987. Preparation of genomic DNA from bacteria, P. 2.4.1-2.4.5. *In* F. M. Ausubel, R. Brent, R. E. kingston, D. D. Moore, J. G. Seidmand, J. A. Smith, and K. Struhl (ed.), Current Protocols in Molecular Biology. John Wiley & Sons, Inc., New York, N.Y.

Wose, C. R. and G. J. Olsen. 1986. Archaebacterial phylogeny: perspectives on the kingdoms. Syst. Appl. Microbiol. 7:161-177.

Zabel, R. A., and J. J. Morrell. 1992. Wood Microbiology: Decay and Its Prevention. Academic Press, Inc., New York, N. Y. 476 pp.

CHAPTER TWO

The Mechanical Properties of Clear Specimens of Lodgepole Pine with Incipient Decay Caused by *Phellinus Pini* (Thore) Lloyd

LITERATURE REVIEW

Decay Fungi- Wood decay fungi deteriorate wood by producing enzymes that dissolve the cell components as their energy sources. Fungi penetrate cell wall structures such as pits or through bore holes in the cell wall (Zable and Morrell, 1992). The primary components of wood that decay fungi utilize, are lignin (20-35%), cellulose (40-50%) and hemicellulose (25-40%) (Haygreen and Bowyer, 1989). Lignin, a major structural element of wood, is a natural plastic with high molecular weight and serves as a binding agent in cell wall structures and is brown in color. Cellulose and hemicellulose are low weight polysaccharides (Haygreen and Bowyer, 1989), are light colored and provide a crystalline framework and inherent tension strength for the cell wall. Decay fungi can be categorized into three groups based on the cell wall component utilized and the characteristics of the decayed wood (Zable and Morrell, 1992). These categories include the white-rot fungi, the brown-rot fungi and the soft-rot fungi.

White-rot fungi- The common white-rot fungi consist of include *Phellinus (Fomes) pini, Phellinus pseudo-pini, Traemetes versicolor, Stereume sanguinolentum, Inonotus (Polyporous) tomentosus, Dichomitus squalens (Polyporous anceps)* and *Armillaria* spp. (Koch 1996), and primarily attack decay-free heartwood. White-rot fungi break down lignin and some hemicellulose, but leave most cellulose and hemicellulose, giving the light-colored decayed wood its name. This simultaneous decomposition

causes wood to become progressively more fragile over time. Although white-rot is caused by a variety of fungi, most white-rot fungi are members of the Basidiomycetes. The Basidiomycotina is a large class of fungi that bear spores resulting from sexual reproduction exterior to a basidum and produce visible reproductive structures such as mushrooms and shelf conks (Manion, 1991).

Brown-rot fungi- The common brown-rot fungi include *Coniophora puteana, Phaeolus (Polyporous) scheinitzii and Phellinus weirii* (Koch, 1996). Like the white-rot fungi, the brown-rot fungi are capable of degrading cellulose and hemicellulose, but are unable to digest the lignin component of wood. In the case of brown-rot, in the advanced stage of decay, the lignin remains intact and appears as a brown, crumbly matrix. Unlike the soft-rots and white-rots, the brown-rot fungi are relatively few in number, comprising less than 6% of all wood-decay fungi (Zable and Morrell, 1992). The brown-rots are most prevalent in coniferous woods of the northern hemisphere (Manion, 1991).

Soft-rotting fungi- This group contains species that are capable of degrading cellulose and hemicellulose and may partially digest lignin (Worrall *et al*., 1997; Anagnost, 1998). The soft-rots are particularly prevalent during the early stages of wood decay and in conditions of high moisture and increased nitrogen content. They, therefore, play an important role in the decomposition of fence posts, building timbers, window frames, and other structural components of homes (Wang and Zable, 1990). Wood affected by soft rot may appear wet, spongy, or pitted. There are over 300 known species of soft-rot fungi. These include many filamentous micro fungi (the molds) from the Deuteromycetes, and some belong to the Ascomycetes (Wang and Zable, 1990). Unlike the brown-rot or white-rot fungi, the soft-rot fungi attack only the surface of the wood

and break down the cellulose or hemicellulose in the cell walls. They do much less damage to the heartwood of decay resistant species (Koch, 1996).

Decay Processes

Decay processes consist of both enzymatic and physical attack during the fungal hyphal penetration of the cell wall. White-rot and brown-rot fungi use different enzymatic mechanisms to decompose cell wall. Most previous studies dealing with enzymatic processes of decay fungi only focused on either the cellulose decomposition or lignin decomposition. To date no study has completely described the interrelated enzymatic processes of cellulose, hemicellulose and lignin degradation. Most studies used (Green and Highley, 1997) only cotton cellulose, pure hemicellulose or lignin medium to examine the activities of enzymes, and most of them were not able to duplicate the enzymatic processes taking place in wood tissues. Further, the mechanisms of hemicellulose degradation are not fully understood (Highley and Dashek, 1998).

Cellulose decomposition by white-rot fungi- Studies conducted by Eriksson *et al.* (1990) suggested that at least three enzymes are involved in the primary hydrolytic process that reduce cellulose to glucose. However, this research raised some questions related to cellulose decomposition in wood, because most studies were based only on cotton cellulose. The white-rot fungus, *Phanerochaete chrysosporium*, has been intensively studied for the decomposition of cellulose in the laboratory. The enzyme reacting sites on cellulose are mainly β-1,4 glucosidic linkages between two adjacent glucose units (Figure 2.1). Eriksson (1978 and 1990) reported that the mechanisms of cellulose decomposition include three primary and two secondary enzymes. The primary enzymes are endo β-1, 4 glucanase, exo β-1, 4 glucanase and β-1, 4 glycosidases, while

the secondary enzymes (oxidative enzymes) are oxidoredutase (cellobiose dehydrogenase) and cellobiose oxidase (cellobiose to cellobiose acid). The attacking sites on the cellulose polymer unit were described by Alder (1997) as seen in Figure 2.1 and the pathways of enzyme mechanisms were shown in Figure 2.2.

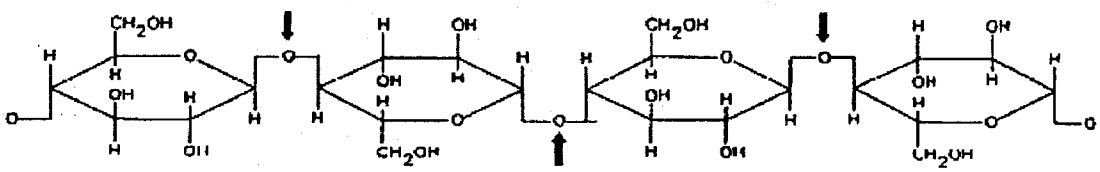

Figure 2.1. The attacking sites (arrows) of cellulose decomposition by enzymes (Alder, 1977).

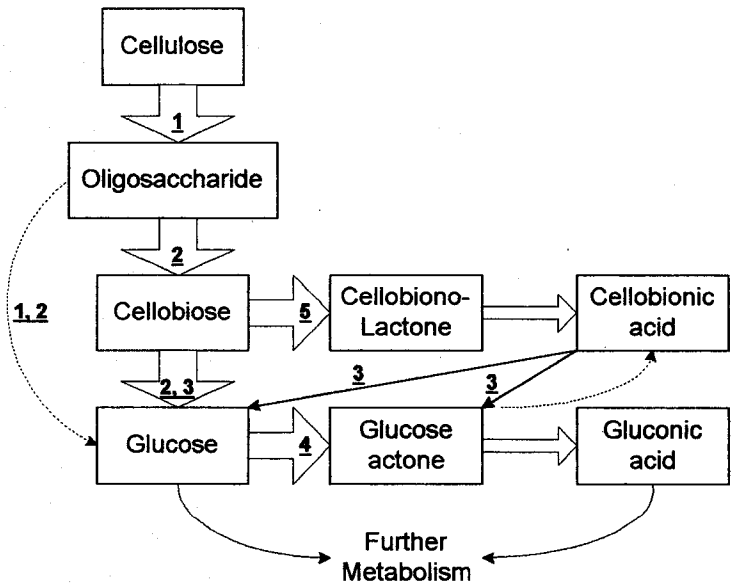

Figure 2.2. The Enzyme mechanism for cellulose degradation by *Phanerochaete chrysosporium.* 1: Endo-glucanase, 2: Exo-glucanase, 3: β-glucosidase, 4: Glucose oxidase, 5: Cellobiose oxidase (Zabel and Morrell, 1992).

Cellulose decomposition by brown-rot fungi- Brown-rot fungi are unique among the wood decay fungi due to their ability to degrade wood cellulose and hemicellulose without significant chemical or physical alteration of the lignin surrounding the cellulose. These fungi only partially modify lignin, leaving a brown residue (altered lignin). Brown-rot fungi utilize cellulose in a different way than do the white-rot fungi. During the incipient decay stage, brown-rot fungi degrade cellulose more rapidly than those of white-rot fungi, and cause a large strength reduction when weight loss is quite small. While the outcome is well documented, the mechanisms of the brown-rot fungi attacking crystalline cellulose is still unknown (Highley and Dashek, 1998). Since the smallest-sized, cellulose-degrading enzyme is still too large to pass the barrier of lignin into crystalline cellulose, smaller molecular agents are suspected for the depolymerization of crystalline cellulose (Cowling and Brown, 1969). Koenings (1974) suggested that the oxidation process by $H_2 O_2$/ Fe^{2+} might serve as a non-enzymatic agent of the cellulose depolymerization. Flournoy *et al.* (1994), however, found that the previous studies conducted by Highley (1977) and Kirk *et al.* (1991) did not establish a correlation between the different oxidation processes of cellulose depolymerization. A more recent study by Green and Highley (1997) suggested that only three uncertain nonenzymatic processes might be involved in the early breakdown of crystalline cellulose (Figure 2.3).

Additionally, brown-rot fungi do not have exo-glucanase as do the white-rot fungi, but utilize two different enzymes, endo-β-1,4-glucanase and β-1,4-glucosidase, to depolymerize the amorphous cellulose and those cellulose chains separated from the crystalline zone by separate nonenzymatic agents (Highley, 1977). The endo-β-1,4-

glucanase randomly cleaves the cellulose chains, including normally occurring amorphous cellulose and those cellulose chains cleaved by nonenzymatic agents from the crystalline zone. The β-1,4-glucocidase then attacks the ends of the cellobioses or shorter cellulose chains, and converts those to glucose units.

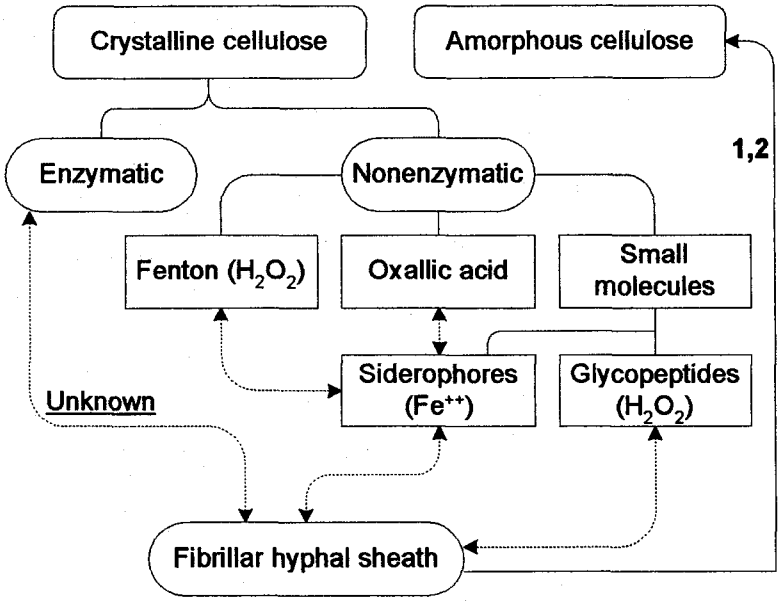

Figure 2.3. Proposed mechanisms of Brown-rot Enzymatic Pathways. Enzyme **1**: endo-β-1,4-glucanase, Enzyme **2**: β-1,4-glucosidase (Modified from: Green and Highley, 1997).

Hemicellulose decomposition by white-rot and brown-rot fungi- Since hemicellulose is more complex in structure than cellulose (it is a mixture of several sugars in addition to glucose), the enzymes involved in its decomposition are more complex as well. Very few studies have been conducted on the enzymatic processes and molecular genetics of hemicelluloses, especially the enzymes involved in removing the side-chain groups (arabinose, uronic acid and acetyl groups).

Regulation of hemicellulase synthesis is the most significant difference between the enzymatic processes of white-rot and brown-rot fungi . Eriksson (1978) determined that many white-rot fungi do not exhibit effective hemicellulase and cellulase activities during growth on simple sugar mediums, while brown-rot fungi clearly displayed activities of both cellulase and hemicellulase. These enzymes are hydrolytic in nature and the hemicellulose degradation processes are analogous to enzymatic activity during cellulose decomposition. Since few studies of hemicellulose deterioration have been conducted, no direct comparison between white-rot and brown-rot fungi have been made.

Lignin decomposition by white-rot fungi- Lignin is difficult for most microorganisms to degrade, but it is efficiently degraded in nature by white-rot fungi. Because lignin is a large, three-dimensional polymer, and is composed of inter-unit carbon-to-carbon and ether bonds, ligninolytic systems must be oxidative rather than hydrolytic (Kirk and Cullen, 1998). Additionally, because the structure is so complex, with many different side chains, the ligninolytic agents must be less specific than cellulose degradation agents. Most studies have suggested that four major enzymes are involved in the lignin decomposition (Figure 2.4).

(1) Lignin Peroxidase (LiP): This enzyme appears to be one of the key enzymes of lignin degradation of *Phanerochaete chrysosporium*. Lignin peroxidase requires H_2O_2 in the catalytic reactions, and it cleaves the C-C bond between phenyl propane units. The lignin peroxidase also oxidizes the phenolic units to aryl cation radicals (Kirk and Cullen, 1998)

(2) Manganese Peroxidase (MnP): Manganese peroxidase is another major enzyme that has been isolated from some white-rot fungi and may provide low weight molecular oxidants (Kirk and Cullen, 1998). The reactive requirement of H_2O_2 for manganese peroxidase is similar to lignin peroxidase. Glenn (1985) indicated that the role of MnP is to oxidize Mn^{2+} to Mn^{3+} which then oxidizes the various phenolic structures to other simple products.

(3) Laccase: Most white-rot fungi can produce laccases which are blue copper oxidases and oxidize electron-rich structures (Hammel, 1997). Laccase oxidizes the phenolic units of lignin to phenoxy radicals which can lead to other products.

(4) Peroxide-producing Enzymes: In order to support the reactions of lignin peroxidase and manganese peroxidase, H_2O_2 needs to be produced at the same time. Glyoxal oxidase (GLOX) is one of the most common peroxide-producing enzymes isolated from many white-rot fungi (Kersten *et al.*, 1990), and utilizes 1-3 carbon aldehydes and oxygen to form H_2O_2. Aryl alcohol oxidase is another peroxide-producing enzyme found in some white-rot fungi, and instead of utilizing aldehydes, it uses alkoxybenzyl alcohols and oxygen to produce H_2O_2.

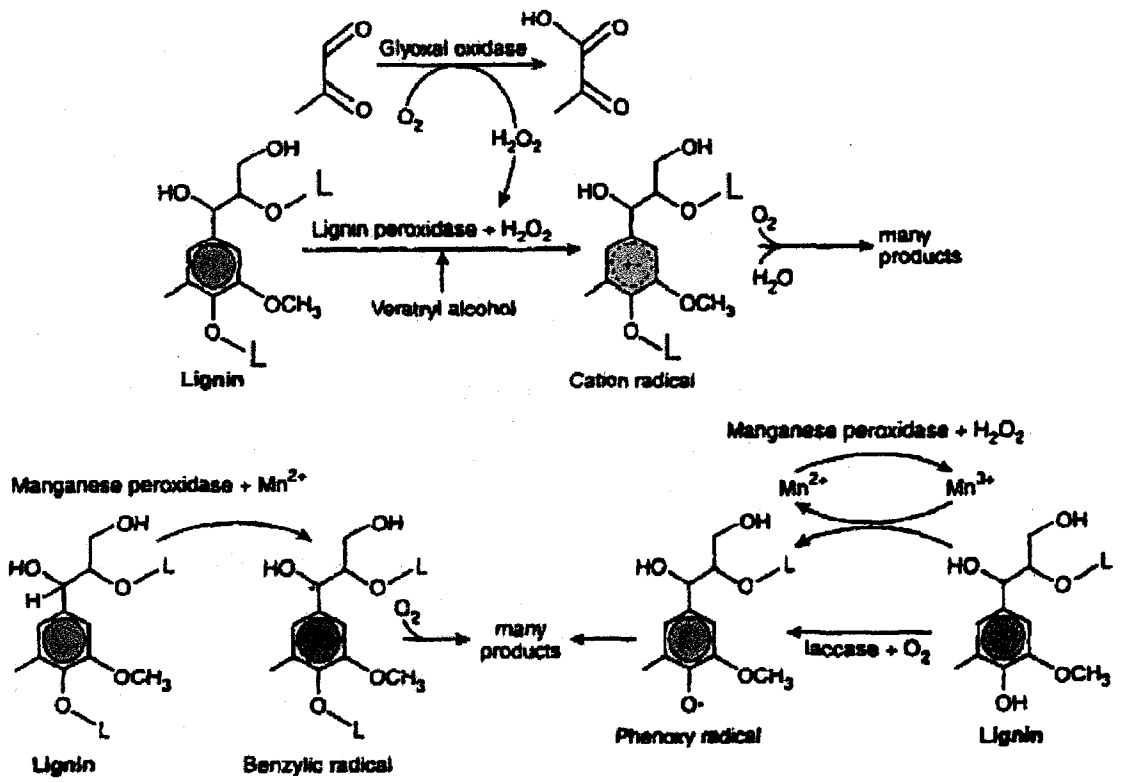

Figure 2.4. Schematic illustrating the enzymatic system of white-rot fungi. Four enzymes can be involved: lignin peroxidase, manganese peroxidase, laccase and glyoxal oxidase (Kirk and Cullen, 1998).

Lignin decomposition by brown-rot fungi- Compared with white-rot fungi, brown-rot fungi only partially alter lignin. Kirk *et al.*(1991) found that the changes in lignin structure caused by brown-rot fungi were primarily the demethylation and oxidation of the α-carbon or cleavage of side chains between the α and β carbon of phenyl propane units, while white-rot fungi utilize four major enzymes to completely digest lignin (Figure 2.5). Although studies (Wilcox, 1968) showed some lignin loss in wood specimens after the inoculation of brown-rot fungi, that evidence cannot completely explain the role of brown-rot fungi on lignin degradation since brown-rot fungi do not utilize lignin as their carbon or energy sources as do the white-rot fungi. Other enzymes involved in this modification of lignin by brown-rot fungi are unknown so far, and more studies are needed to gain further understanding of lignin midification by these fungi (Highley *et al.*, 1994; Hammel, 1997).

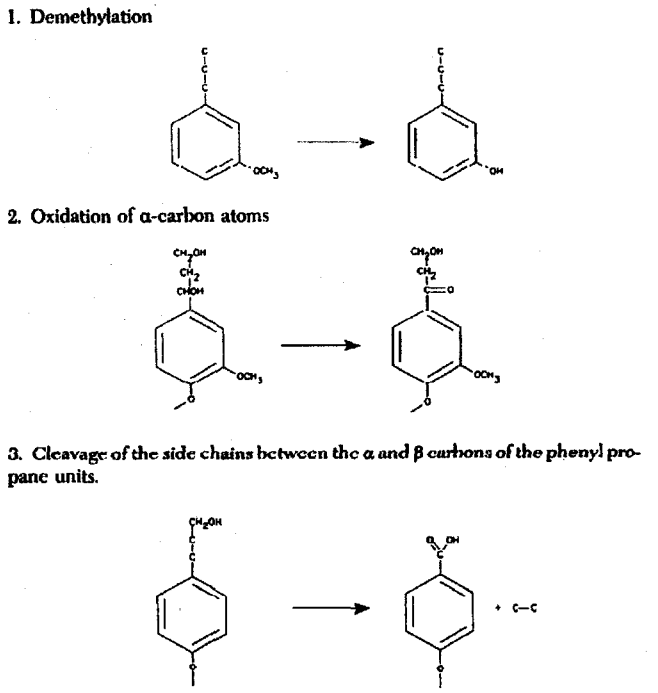

Figure 2.5. The three basic reactions of modification of lignin: 1. demethylation, 2. oxidation of α-carbon, 3. cleavage of side chains between α and β carbon of phenyl propane units (Zabel and Morrell, 1992).

Physical process of decay- Wood decay processes caused by fungi involve both enzymatic and physical processes, especially during the penetration of cell wall structures. The enzymatic processes are based on the diffusion of extracellular enzymes from the fungal hyphae, while the physical/mechanical decay processes are associated with specific mechanisms of fungal hyphae growth (Zabel and Morrell, 1992) involving two major processes. First is the extension of an elastic tip region of hyphae and second is the progressive rigidification of hyphal walls behind the tip. This rigidification provides the physical force for the hyphae tip.

The combination of the enzyme system and growth of hyphal tip provides a logical explanation of the entire process. The hyphae penetrate the cell wall by softening and pushing through the cell wall. The apical growth region of hyphae releases enzymes to soften and degrade wood tissues and forms a small hypha peg on the contact point of the cell wall. The rigidification behind the apical region supports the extension of the hypha peg, and eventually this hypha peg penetrates the cell wall. During the penetration, hyphae release more enzymes that diffuse into the cell wall and cause the enlargement of the bore holes (Figure 2.6) (Matthius, 2000).

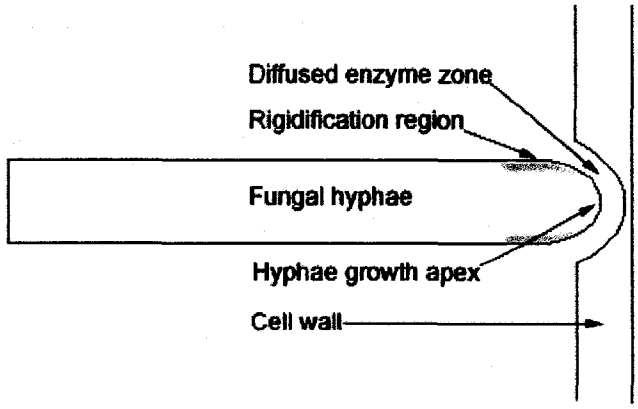

Figure 2.6. Schematic of fungal hyphae penetrating cell wall.

Effects of Decay on the Structural Properties of Wood

The fungal decay processes utilize the chemical components from cell walls and cause changes in wood structures. This change in chemical and structural properties leads to strength loss in wood. In general, the effects of decay processes can be grouped into three categories as follows: (Crowing, 1961; Wilcox, 1968, 1970, 1993a, 1993b)

1. Channels for cell-to-cell hyphal migration
    - White-rot fungi: Hyphae advance mainly through numerous bore holes and pits. Later, the bore hole is enlarged in diameter four to eight times. The bore holes and enlarged pits are no longer differentiable.
    - Brown-rot fungi: Few bore holes and no significant changes in hole size are observed; the hyphae advance mainly through pits which are damaged by this penetration.
2. Thinning of cell wall
    - White-rot fungi: The S_3 layer is attacked first and cell-wall lamellae separation increases during this early stage. As decay progresses, the decay process extends to the S_2 and S_1 layers as well as the primary wall and middle lamella. Cell wall lamellae separate and disappear.
    - Brown-rot fungi: Cell wall thickness decreases slightly in the early stage. S_2 and S_3 layers are attacked at the same time, but later the S_2 layer is degraded more rapidly than the S_3 layer. During advanced stages, the attack extends to the S_1 layer, the primary wall and the middle lamella. The cell wall finally collapses.

3. Ray tracheids or vessel elements
    - White-rot fungi: Secondary walls tend to become thin and nearly disappear in advanced stages.
    - Brown-rot fungi: Few effects are noted in the tracheids or vessel elements, but the cell wall thickness decreases very slowly.

Mode of Entry and Invasion Processes

Most disease cycles of heart-rot fungi start from spores that are randomly disseminated by the wind and make contact with wounds or broken branches caused by fire, wind, animals or insects. Some of the wood decay fungi are distributed from tree-to-tree by root contact as well (Manion, 1991). After tree scars become infected by decay fungi, hyphae will start to invade the host tissues. The specific decay processes exhibited by two decay fungi, *Phellinus pini,* a white-rot fungus, and *Phaeolus schweinitzii*, a brown-rot fungus, have been selected to describe the modes of entry and tissue invasion processes. Brown cubical rot caused by *Phaeolus schweinitzii* is commonly found in old-growth trees, especially those with fire scars (Koch, 1996). The white-rot fungus, *Phellinus pini*, is one of the most common decay diseases for conifers in the northern temperate zone. *Phellinus pini* is often referred to as white-pocket rot, red-ring rot or red-heart rot, because of the small white lenticular pockets or red-heart stain in the decayed wood (Koch, 1996). *Phellinus pini* is primarily spread by wind-carried spores that germinate on wounds or branch stubs of trees. In addition, other vectors, such as insects or animals, carry the spores from tree to tree. In contrast, brown cubical rot depends on wind-born spores to infect new hosts through fire scars and wounded roots. It

also spreads by root contact between trees. This fungus can survive in dead roots or cut trees for a long time (Morrell, 2000).

During the tissue invasion processes, the hyphae of the white-rot fungi such as *Phellinus pini*, often develop initially in the vessels and wood rays of hardwoods, while the wood rays and longitudinal parenchyma are first invaded in softwoods (conifers). As decay pogresses, the hyphae will be present in the lumen of most fibers and vessels of angiosperms, while hyphae also become numerous in both tracheids and ray cells (Wilcox, 1968, 1970). In contrast, the hyphae of brown-cubical rot fungi distribute more evenly throughout many different types of cells of both angiosperms and gymnosperms (Crowing, 1961; Wilcox, 1970). A major difference in tissue invasion sequencing between a white-rot fungus, *Phellinus pini* and the brown-cubical rot, *Phaeolus schweinitzii*, is the sequence of removal of the cell wall. Decomposition sequences of different layers of the cell wall are listed in Table 2.1.

Table 2.1. Invasion order of cell wall structures (Figure 2.7) by white-rot and brown-rot fungi (Wilcox, 1968, 1970).

White-rot	Brown-rot
S_3 layer	S_2 layer
S_2 layer	S_1 layer
S_1 layer	S_3 layer
Primary wall	Primary wall
Middle lamella layer	Middle lamella layer
Ray and vessel cell walls	Ray cell wall

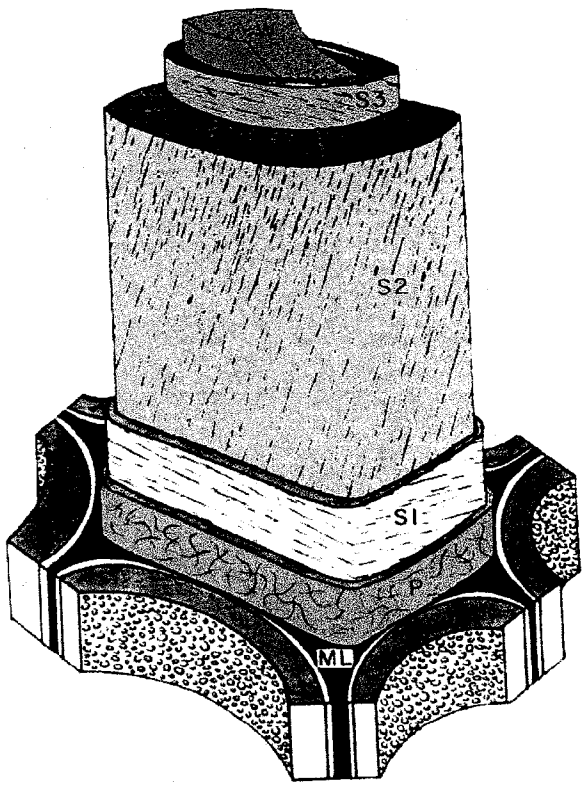

Figure 2.7. Basic cell wall structure of secondary xylem. P: Primary wall, S_1: First layer of secondary wall, S_2: Second layer of secondary wall, S_3: Third layer of secondary wall, W: The surface layer of lumen, ML: Compound middle lamella Layer (Haygreen and Bowyer, 1989).

Environmental Conditions for Vegetative Growth and Reproduction

Under wet and warm environmental conditions, decay fungi can easily infect untreated and/or non-resistant wood species. Ammer (1964) found that five different decay fungi showed no development in wood's hydroscopic range (0 to approximately 25% dry-basis moisture content), but failed to specify the ambient relative humidity in his study. Later, Morton and French (1996) found that the spore germination could occur in relatively dry wood when the relative humidity was 94% or higher. Griffen (1977) also indicated that enzymes can function in condensed water in the large pit pores and also found that most fungi can remain alive, but inactive, below 97% relative humidity and below the wood fiber saturation point. More recently, Viitanen and Ritschkoff (1991) found that brown-rot fungi in the vegetative stage could decay wood in the range of 94-97% relative humidity and ideal temperatures, but they did not describe the moisture content in the decayed wood. However, Zabel and Morrell (1992) indicated that the minimum moisture content for decay fungi growth are in the range around the fiber saturation point, commonly reported between 25 and 30% dry-basis moisture content for most species. The temperatures generally cited for decay fungus development are between 15 °C-40 °C, while the optimal temperatures are 21 °C-32 °C (Humphrey and Siggers, 1933; Carll and Highley, 1999). The optimal growth temperature for decay fungi might vary between species and their hosts but the difference is not significant. A relative humidity of 95% and 20-35 °C will provide good growth conditions for most decay fungi, while the moisture content in wood lower than the fiber saturation point is a distinct limit to the development of decay fungi (Zabel and Morrell, 1992; Carll and Highley, 1999).

Most decay fungi are obligate aerobic microorganisms, and lack of oxygen will cause a reduction or cessation of fungal growth (Zable and Morrell, 1992). Highley *et al.* (1983) reported that oxygen levels lower then 7.6mm Hg substantially reduced fungal decay. Scheffer (1986) indicated that no fungi grew at an oxygen level lower than 0.2% volume, while fungal growth increased dramatically in atmospheres ranging from 2mm Hg to 11mm Hg, and reached an optimal growth rate at 32mm Hg of oxygen content. A recent study of the influence of oxygen concentration on fungal growth rate, biomass production and wood decay showed that a 5% oxygen concentration was very favorable for both white-rot and brown-rot fungi, while oxygen levels from 1% to 0.01% caused a decrease of fungal growth rate and biomass (Kazemi *et al.*, 1998).

Visual Signs of Decay

The most common sign indicating the presence and type of decay is the visual evidence that includes discoloration and decay patterns. In the very early (incipient) stage of decay, the wood might not show a significant discoloration or decay pattern. At this stage, the wood is still intact and damage is quite limited (Zabel and Morrell, 1992). As decay develops further, wood starts to show slight changes in texture and color such as light reddish, light yellow, red brown, light gray or gray white. The wood will often show a rougher texture or a higher-than-normal fiber brashness, and the damage to the wood's mechanical and physical properties is more severe than that seen previously in the early stages of incipient decay. During the intermediate and advanced decay stages, the visual signs are significant and variable as wood strength is dramatically reduced. The wood may be spongy, soft, very fibrous or show heavy shrinkage, and the color may be white, gray, dark brown, reddish brown, black-lined or white pocket rot (Manion,

1991; Zabel and Morrell, 1992). For example, the brown cubical butt rot caused by the *Phaeolus schweinitzii*, shows a yellow to reddish-brown discoloration or a dry, crumbly, brown cubical wood residue in the heartwood (Figure 2.8 and 2.9). White pocket rot, a white elliptical and sunken area, could be an advanced stage of *Inonotus tomentosus* or *Phellinis pini* decay. Red ring rot, caused by the fungus *Phellinus (Fomes) pini or Stereume pini* might show a red or reddish-purple discoloration in the heartwood during the early stage of decay, while advanced decay appears as small gray-white pockets containing white mycelium (Figure 2.10 and 2.11).

Figure 2.8. The typical brown cubical rot (*Phaeolus schweinitzii*) on Douglas-fir (*Psuedotsuga menziesii* Mirb. Franco) heartwood showing dark brown cubical residue (Foster and Wallis, 2002).

Figure 2.9. A closer look of the brown cubical residue from Douglas-fir heartwood having an advanced decay stage of brown cubical rot (*Phaeolus schweinitzii*) (Foster and Wallis, 2002).

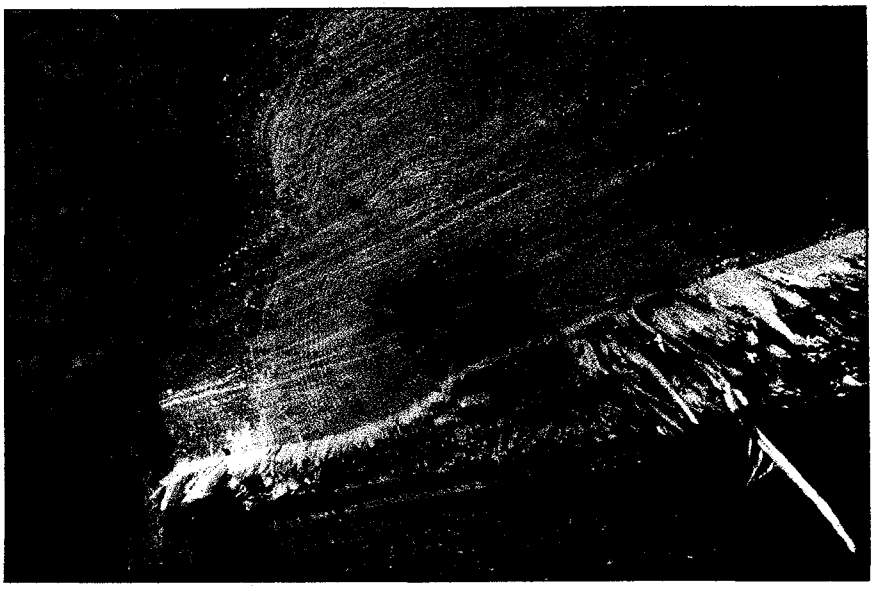

Figure 2.10. The early decay stage of red ring rot (*Phellinus pini*) on Douglas-fir heartwood showing reddish brown to dark brown discoloration at the center area (Foster and Wallis, 2002).

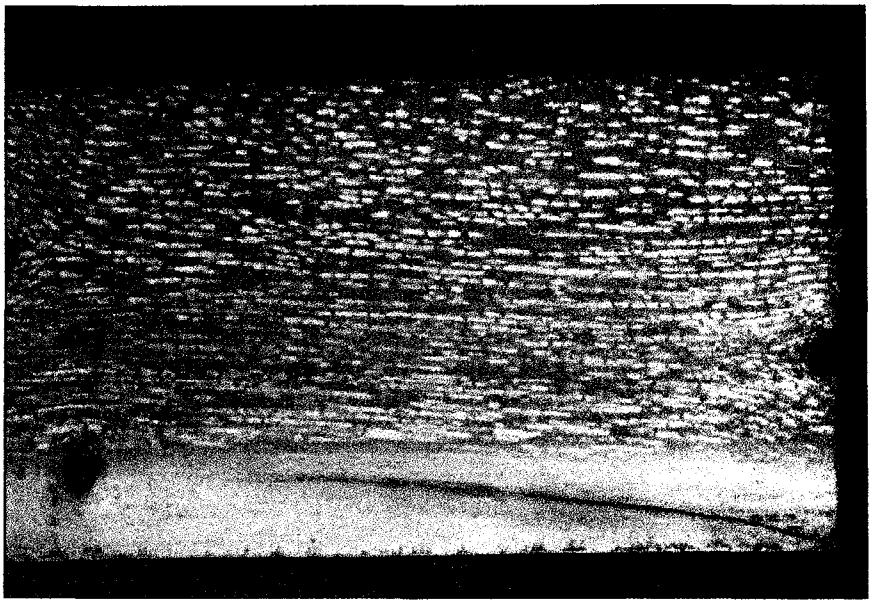

Figure 2.11. The advance decay stage of red ring rot (*Phellinus pini*) on lodgepole pine heartwood showing small white pockets with fungal mycelia (Foster and Wallis, 2002).

Weight Loss by Decay Fungi

Decay fungi live and thrive by attacking and metabolizing structural components of the woody cell wall. The loss of wood weight, a result of the breakdown of the cell wall components, is commonly recognized as a prime indicator of significant change in mechanical properties. Wilcox (1968 and 1978) defined "early stage decay" as a weight loss below 10%, but the term *incipient decay* was not defined in his studies. Later, Zabel and Morrell (1992) defined the incipient decay stage as the period in the decay process when weight loss was less than 10%, while a 95-97% weight loss was defined as the advanced decay stage. They did not precisely define other decay stages by the percentage of weight loss. However, these previous studies used small specimens inoculated with decay fungi grown under laboratory conditions that showed a near linear relationship between weight loss and time-since-inoculation.

Mechanical Strength Loss by Decay Fungi

Toughness (the ability to withstand an impact load) was considered by Wilcox (1978) to be the mechanical property most sensitive to the incipient stage of decay, with bending strength being the second. More recent studies used bending strength as the principal criterion to evaluate the effects of fungal decay on different wood materials (Smith *et al.*, 1992; Winandy and Morrell, 1993). Scheffer *et al.* (1941) studied the effects of certain heart-rot fungi on Sitka spruce (*Picea sitchensis* Bong. Carr.) and Douglas-fir (*Psuedotsuga menziesii* Mirb. Franco) and showed that the white-rot fungus, *Phellinus pini*, caused bending strength reduction in Sitka spruce, while almost no strength reduction was found on Douglas-fir samples. Their specimens, however, contained knots and other defects, which may have influenced the results. Kennedy

(1958) conducted a study on 12 different kinds of wood decayed by two different fungi (white-rot and brown-rot) and examined the effects of weight loss on static bending strength. He found that the average strength loss of white-rotted woods was about 4.3 % at a weight loss of only one percent. At equivalent weight loss, specimens attacked by white-rot fungus generally suffered less strength loss than those exposed to the brown-rot fungus.

The only study regarding the strength reduction of lodgepole pine with red-ring rot caused by *Phellinus pini* was conducted by Roff and Whittaker in 1963. They found that lodgepole pine with up to 80 percent red stain was expected to have a reduction in static bending and in toughness of not more than 15 percent. Since their comparisons between the control group and the red stained specimens were not based on an individual tree, the results could be biased due to the strength variation between and among trees. Additionally, the lack of statistical analysis also greatly reduced the reliability of their results. Wilcox (1978) concluded that the toughness loss of gymnosperms was at least 60-80% and the bending strength loss of 50-70% occurred at the incipient stage with 5-10% weight loss. Also of importance, the Modulus of Elasticity (MOE) and Modulus of Rupture (MOR) could both be reduced to 60-70% of normal in the incipient stage of decay before the decay could be reliably identified. In a study of the effects of time-since-infection on compression strength parallel to the grain, Smith, Morrell and Sexton (1992) inoculated small, clear specimens of Douglas-fir wood with decay fungi and observed results over a twelve-month period. The results indicated that strength loss was apparent and significant three months following inoculation, and after twelve months, the MOR had been reduced by 29%, while work to maximum load had declined by 50%.

More recently, Winandy and Morrell (1993) reported that bending strength of Douglas-fir heartwood exposed to brown-rot fungus had been decreased by 5-70% at a rate linearly related to a weight loss from 1-18%. These studies all showed that even in the early stage of decay, where the only visible or tactile sign is a red to reddish-brown discoloration, Douglas-fir wood can lose nearly 70% of its toughness and bending strength. Curling *et al.* (2002) also reported a ratio of bending strength to weight loss of approximately 4:1 for the sapwood of southern pines (*Pinus spp.*) exposed to brown-rot fungus.

Although lodgepole pine is of economic importance in western North America, most researches in identifying incipient decay or understanding its impact on mechanical properties have been conducted on Douglas-fir and brown-rot fungi. Significantly, relatively few researches have been done with lodgepole pine.

Visual Stress Grading

Visual examination by lumber graders is the most common method currently employed for stress grading dimension lumber throughout the world. Although machine-stress-rated grading systems are available on the market, most commercial lumber companies still rely primarily on a grading system that is based on a visual examination of lumber by a certified grader. Macroscopic signs such as discoloration, ripped or torn grain, crumbling or stringy appearance or surface softness are the primary visual cues used to determine the presence and extent of fungal decay. Lumber grading rules-writing agencies have codified rules limiting the amount and type of defects in lumber, including decay, and instruct certified graders on the methods to be used to determine the presence and extent of decay.

Current grading rules, however, (Western Wood Products Association 1995; West Coast Lumber Inspection Bureau 1998, 2000; Southern Pine Inspection Bureau 1994) of conifer lumber allow up to 50% firm-red-heart, the term to describe the discoloration used as a sign of the presence of the early stage of decay, in even the higher grades of structural lumber. Firm red heart is a condition common in northern lodgepole pine and is caused by stem decay fungi that infect heartwood causing a reddish brown color. Many research papers indicate that incipient decay causes strength loss of 20-40% and early stage decay causes a loss of 45-80% (Mizumoto, 1966; Wilcox, 1968; Toole, 1971; Smith, Morrell and Sexton 1992; Winandy and Morrell 1993; Carll and Highley, 1999). This is a serious problem in the grading rules of western conifer woods, because the "firm red heart" is already beyond the incipient decay stage.

Communication with several grading agency officials (Davis, 2000; Dean, 2000; Loy, 2000; Rominger, 2000) has revealed an inability to justify the inclusion of lumber with up to 50% of its surface area and volume containing decay beyond the incipient stage, even though this lumber was reported to show greatly reduced mechanical properties. While confirming the general observation of reduced bending and compression strength properties for wood with firm red heart, none of the grading agency officials was able to provide historical information for the reasons firm red heart is allowed in visually-graded, structural lumber grades.

OBJECTIVES

1. Describe different failure types that occur in static bending and compression parallel to grain (CPG).
2. Compare the modulus of elasticity (MOE), modulus of rupture (MOR), compression parallel to grain (CPG) and specific gravity (SG) of lodgepole pine test specimens exhibiting various stages of wood decay as characterized by unique combination of discoloration and decay volume classes.
3. Compare the percent deviation in MOE, MOR, CPG and SG of lodgepole pine test specimens exhibiting various stages of wood decay as characterized by unique combination of discoloration and decay volume classes.
4. Make recommendations regarding visual stress grading of lumber having various stages of wood decay.

HYPOTHESIS

H_0: The population's mean percent deviations of specific gravity (SG), modulus of elasticity (MOE), modulus of rupture (MOR) and compression parallel to the grain (CPG) are equal due to the decay volume, discoloration and the interactions between these factors at the 0.05 alpha level.

H_1: Not H_0

MATERIALS AND METHODS

Substrate Preparation

Lodgepole pine was selected for this study, because of its economic and ecological importance to western North America and areas of Europe and Asia where it is also grown. This species was described earlier in the materials and methods of Chapter one. Twenty lodgepole pine logs that ranged from 12 to 24 inches (30.4 to 60.9 cm) in diameter and 8 to 12 feet (243.8 to 365.7 cm) long were randomly selected from green logs with visible firm red heart (Figure 2.12 and 2.13) located at different saw mills and log processing yards in the vicinity of Missoula, Montana during the winter of year 2001. The trees in these log yards were obtained from timber sales on private and public lands, most likely in the states of Montana and Idaho. Each log was labeled and cut into bolts 24.0 inches (71.14 cm) long.

In order to have parallel grain for the specimens, the logs were split and then sawed into specimens (2.5 x 2.5 x 24.0 inches / 6.35 x 6.35 x 71.14 cm). Only the heartwood was selected for the test and the growth rate of the specimens was limited to a range of 20 to 35 rings per inch (2.54 cm) (Figure 2.14). In addition, the portion of the stem within 20 rings of the pith was not used in order to limit the amount of juvenile wood in the test specimens. Compression wood, shake, checks, blue stain and sapwood decay were also excluded from any of test specimens. A jointer, band saw and planer were used to further process the wood pieces into test specimens with the nominal dimensions (1.0 x1.0 x 22.0 inches / 2.54 x 2.54 x 66.04 cm) (Figure 2.15).

Mechanical testing generally followed the standard procedure for testing small clear specimens of timber, Standard D143-94 (ASTM, 2002d). The test specimens from

each log were labeled and sorted into four classes (clear, low, medium, high) based on the intensity of discoloration of firm red heart as seen in Figure 2.16. Since the decay often extended along the grain of the heartwood from end to end of the test specimen, decay volume (Table 2.2) was estimated by averaging the proportion of area containing the strongest discoloration at both ends of test specimen. When the contour lines of decay at both ends were not distinct, four faces of the test specimens were examined to compute the proportion of the area exhibiting discoloration.

The total number of static bending specimens was 264 consisting of 52 clear, 61 low discoloration, 61 medium discoloration and 90 high discoloration. Meanwhile, all clear test specimens were screened by examining their wood sections under a light microscope and incubating wood samples in MEA plates to ensure that they were decay free. The examined clear wood specimens from each log served as the paired control group against which the specimens with firm red heart from the same log were compared to eliminate variation between trees. After the fungal isolation, all wood specimens were stored in a chamber at constant 65 percent relative humidity and 70 °F (21 °C) for more than 30 days so as to reach 10-12% equilibrium moisture content for further mechanical strength tests.

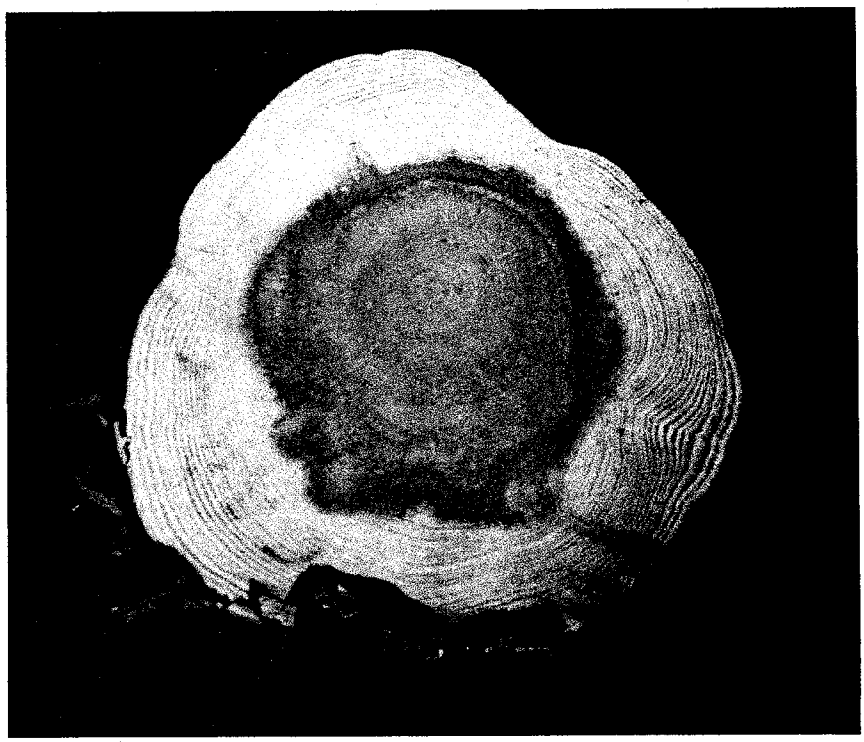

Figure 2.12. Cross section of lodgepole pine log (CAB4) with firm red heart.

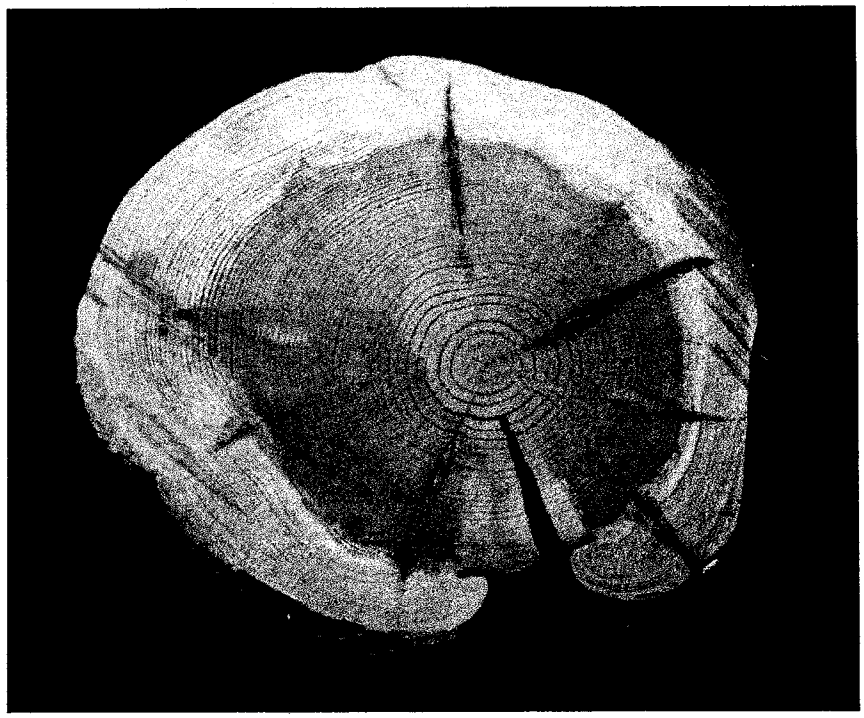

Figure 2.13. Cross section of lodgepole pine log (Stim2) with firm red heart.

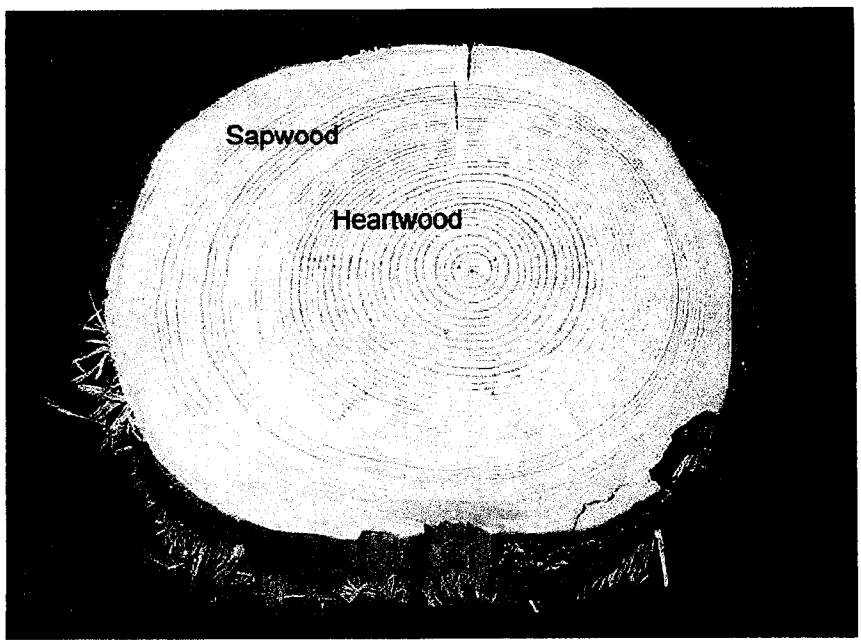

Figure 2.14. Cross section of lodgepole pine log without firm red heart.

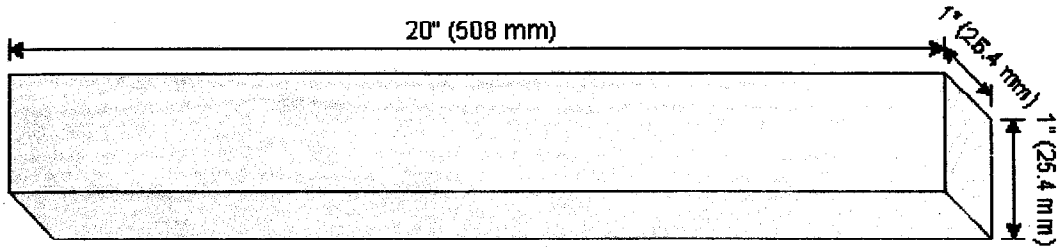

Figure 2.15. The dimensional diagram of a wood testing specimen.

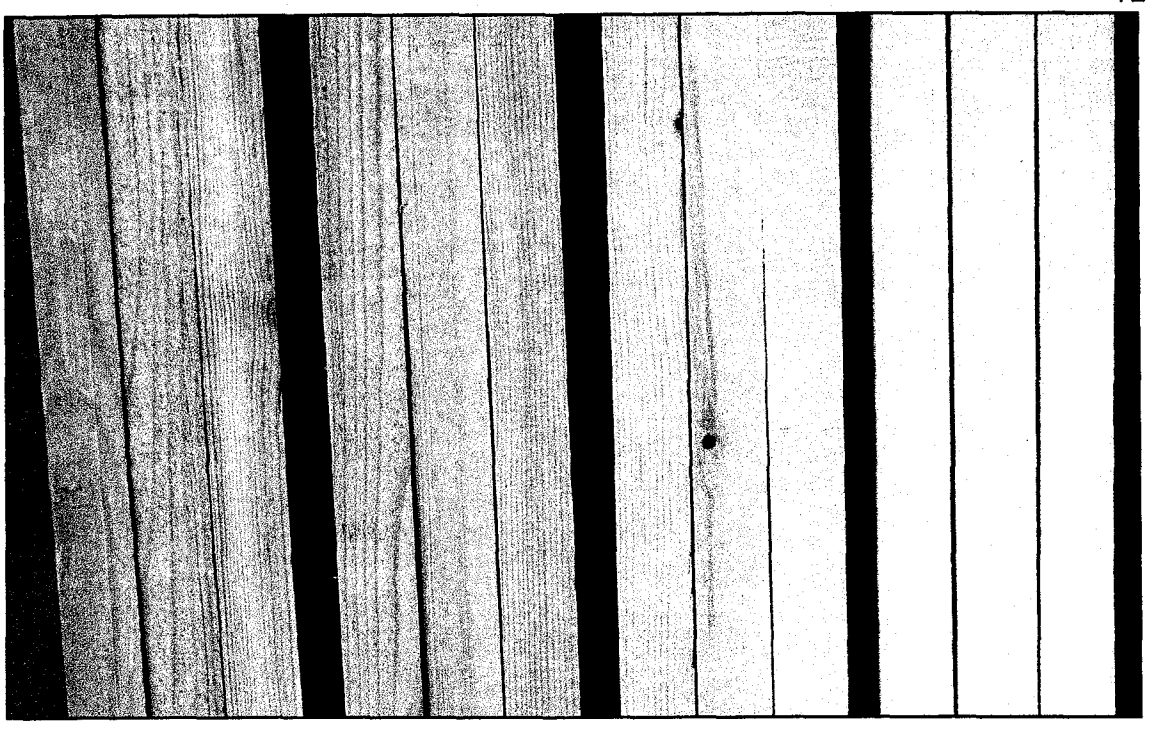

Figure 2.16. The samples of three discoloration classes and control group (high, medium, low and control from left to right).

Table 2.2. The classifications of decay volume percentage and discoloration.

Discoloration classes	Clear (control)	Low	Medium	High
Decay volume classes	0% (control)	1-33%	34-66%	67-100%

Static Bending Test

In order to examine the effects of an uneven distribution of decay in the test specimen, a third-point loading described in static tests document D198-99, ASTM (2002b) was used in this study. The span for this test was 14 inches (426.7 cm) and the testing heads were 1/3 span, 4.66 inches (11.83 cm) apart. The test specimens were oriented so that the load was applied to the tangential surface with the worst decay zone on the lower (tension) side of the specimen, while the clear wood specimens had the load through the tangential face nearest the pith . A 60,000 Lb. Tinius-Olson universal testing machine was employed for load application and used a crosshead speed of 0.1 inch (2.5 mm) per minute at room temperature (68±6 °F). The test configuration was as Figure 2.17. The width and depth of each test specimens were measured before each test. The equations used to determine the mechanical properties, MOR and MOE, were based on D198-99 (ASTM, 2002b) and are as follows:

$$\mathbf{MOE} = P'L^3 / 4.7bh^2$$

where: P' = the load on beam at proportional limit (pound)

L = the distance of span (inches)

b = the width of beam (inches)

h = the depth of beam (inches)

$$\mathbf{MOR} = P_{max} L / bh^2$$

where: P_{max} = the maximum load (pounds)

(Rest of symbols as defined above)

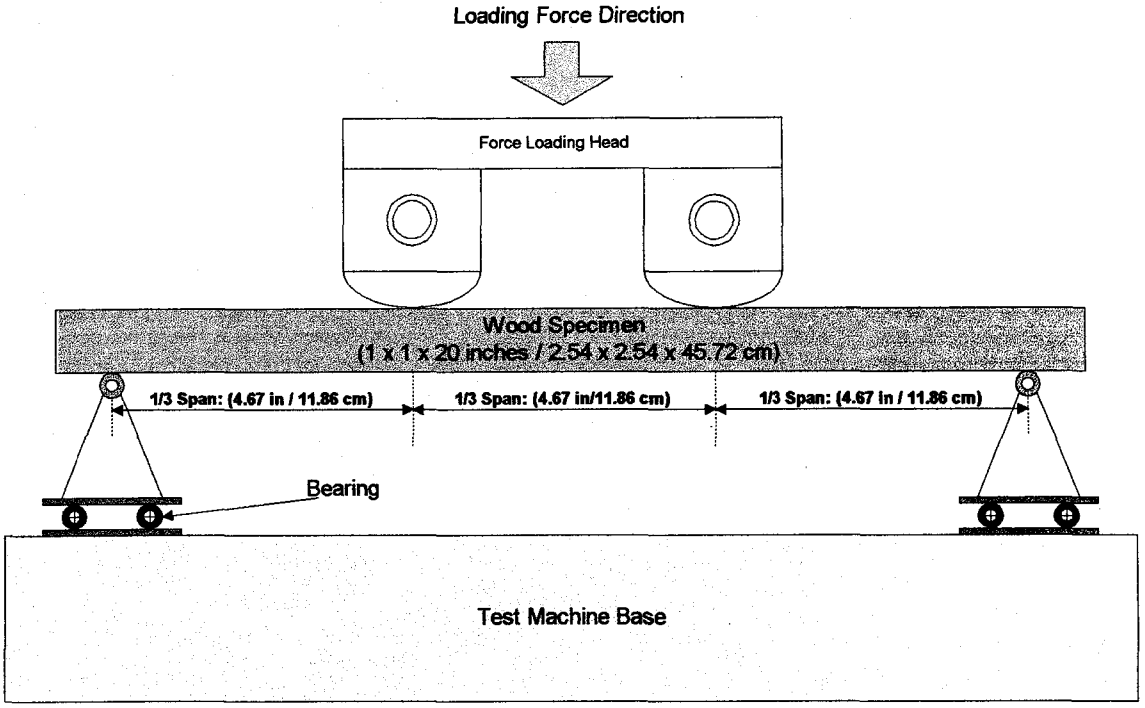

Figure 2.17. The diagram of third-point static bending test and specimen dimension.

Compression Parallel to the Grain

The test for compression parallel to the grain was conducted on 1 by 1 by 4 inches (2.54 by 2.54 by 10.16 cm) specimens that were cut from the non-damaged portion of 20 inches (45.72 cm) specimens right after the static bending test. Both ends of the specimen were trimmed by a calibrated high-speed radial saw to ensure that the force loading surfaces were perpendicular to the force loading axial. A crosshead speed of 0.003 inch (0.076 mm) per minute at room temperature (68±6°F) was used in this test. The test procedure and configuration (Figure 2.18) was followed the ASTM D-143-94 (2002d). Both width and depth of specimen were measured before each test to reduce the variation due to the size difference between test specimens. The maximum compression parallel to grain (CPG) was calculated as follows:

$$\mathbf{CPG} = \boldsymbol{Pc / bh}$$

where: Pc = **maximum load force (pound).**

(Remaining symbols as previous by defined)

(MOE, MOR and CPG were recorded in pound/inches^2)

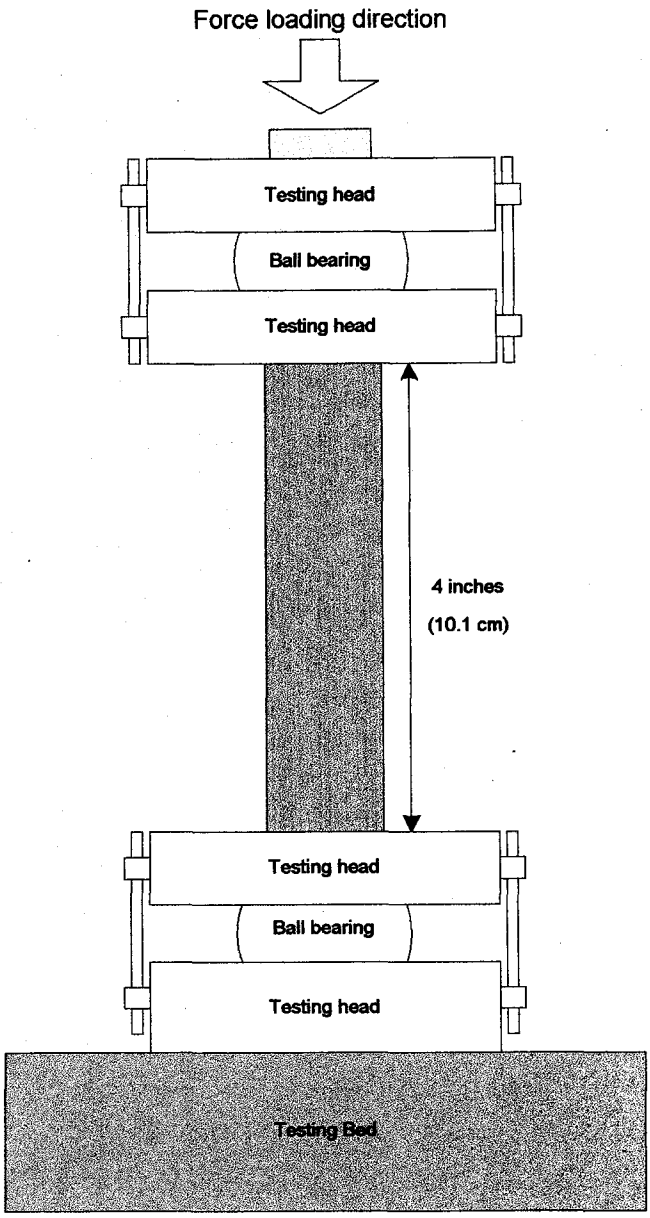

Figure 2.18. The diagram of apparatus used in the test of compression parallel to grain (CPG).

Specific Gravity (SG)

The specimen (1 x 1 x 1 inches, 2.54 x 2.54 x 2.54 cm) was cut from each stick after the bending test. Measurements of volume and weight were based on the Standard Test Methods for Specific Gravity of Wood and Wood-Based Materials, ASTM D2395-02 (2002a). Once the volumes were measured, the specimens were oven-dried for 24 hours in a fan-circulated, hot-air oven set to 105 degrees C for 24 hours. Volume of the moisture content specimen at least was taken prior to oven drying by immersion. Specific gravity (SG) was calculated as follows:

$$SG = (W_{OD} / V) / D_w$$

where: W_{OD} = oven dry weight (pound)

V = volume at test moisture content (ft^3)

D_w = density of water (62.4 lb/ft^3).

Dry Basis Moisture Content (MC)

The actual moisture content of each specimen at the time of testing was measured immediately following the strength test. The details of the oven drying method are described in the Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Base Materials, ASTM D 4442-92 (2002c). One-inch (2.54 cm) wood cubes were cut from each specimen after the bending testing. The dry-basis moisture content (MC) was calculated as follows:

$$M.C.\ \% = [(W_g / W_{OD})-1] \times 100$$

where: W_g = original weight (gms.)

W_{OD} = ovendry weight (gms.)

Data Analysis

The statistical analysis package, SYSTAT 10.0, was used to conduct various data analyses. The four dependent variables are:

1. Mean percent deviation of specific gravity (SG)
2. Mean percent deviation of modulus of elasticity (MOE)
3. Mean percent deviation of modulus of rupture (MOR)
4. Mean percent deviation of compression parallel to the grain (CPG)

The two independent factors are:

1. Decay volume: 3 classes (1-33%, 34-66% and 67-100%)
2. Discoloration: 3 classes (low, medium and high)

The General Linear Model (GLM) Univariate procedure (2-way ANOVA) was used to analyze main factors and their interaction for each dependent variable. Levene's test was conducted to check for the equality of error variances and to ensure the validity of a 2-way ANOVA. If any of the null hypotheses associated with main effects are rejected, a multiple-comparison procedure (Tukey's Multiple Comparison) will be used to investigate the nature of differences between the values of mean reduction percentages and to determine which means were different and by how much.

RESULTS

Moisture Content

The average moisture content for all 264 specimens was 9.68%. However, the group 3 (1-33% decay volume and medium discoloration) specimens showed the highest average moisture content (10.29%), while group 8 (67-100% decay volume and medium discoloration) had the lowest average moisture content (9.32%) and the clear wood had an average 9.49% moisture content (Table 2.3). The difference between the highest and lowest average moisture contents was only 0.97%.

Table 2.3. Means deviation percentages of moisture content (MC) percentages by decay volume and discoloration classes (SE: standard error).

Group	Decay volume	Discoloration	N	Mean of MC %± SE (%)
0	0%	clear	52	9.49±0.17
1	1-33%	low	25	9.40±0.16
2		medium	17	9.67±0.17
3		high	18	10.29±0.12
4	34-66%	low	16	9.55±0.33
5		medium	20	9.64±0.29
6		high	22	10.26±0.14
7	67-100%	low	20	9.68±0.31
8		medium	24	9.32±0.24
9		high	50	9.79±0.19
		Total	264	9.68±0.07

Failure Modes

Failures modes in static bending- Most bending failures occurred on the tension face (the downward face) between the two loading heads and resulted in four specific failure types as follows:

1. Simple tension: The specimen showed separations and longitudinal splitting in the tension zone. Some specimens also exhibited, in addition to the tension failures, a compression parallel-to-grain failure on the upper (compression) face between or under the load heads (Figure 2.19).
2. Cross grain tension: The failure occurred at the bottom face, splitting at an angle along the boundary of decayed wood into the non-decayed area (Figure 2.19).
3. Splintering tension: The tension face failed with numerous splinters (Figure 2.19).
4. Brash tension: The specimen separated into two pieces with a fine tooth margin on the failure faces. This type of failure is often associated with wood that is in the advanced stages of decay (Figure 2.19)

Simple tension failure was the predominant type of failure, averaging 84% with the range from 80% (group 9) to 90% (group 6). By comparison, clear wood had an average simple tension rate of 87%. Table 2.5 shows the type and percentage of the different types of bending failure for the different decay and discoloration classes.

Failure types of compression parallel to grain- Most compression failures occurred near either end of specimen, with a folding rupture set approximately horizontal or at a slight angle to the top of the specimen. However, no brooming, splitting and shearing parallel to grain was observed in any compression tests. Three failure types (Figures 2.20) of compression parallel to grain can be identified in terms of the failure location and form and are classified as follows:

1. Crushing: the failure is located in an approximately horizontal plane near the top or bottom of the specimen.
2. Shearing: the failure is located near the top or bottom ends of the specimen and rupture makes an angle of more than 45 degree with the top of specimen.
3. Wedge split: The rupture occurs in a Y-shape and combines with short split.

The primary failure type in compression testing was crushing. Table 2.4 summarizes the types and percentages of the different failure types.

Table 2.4. Percentages of failure types in static bending and compression parallel to grain (CPG) by decay volumes and discoloration classes.

Group	Decay volume	Discolor-ation	Compression parallel to grain			Static bending			
			Crushing	Shearing	Wedge split	Simple tension	Cross-grain tension	Splintering tension	Brash tension
0	0%	clear	89%	10%	1%	87%	12%	2%	0%
1	1-33%	low	85%	15%	0%	87%	13%	2%	1%
2		medium	77%	23%	0%	82%	18%	0%	0%
3		high	80%	20%	0%	89%	11%	0%	0%
4	34-66%	low	92%	8%	0%	82%	18%	0%	0%
5		medium	93%	7%	0%	82%	18%	0%	0%
6		high	82%	18%	0%	90%	6%	4%	0%
7	67-100%	low	79%	20%	1%	82%	14%	5%	0%
8		medium	83%	16%	1%	83%	13%	4%	0%
9		high	94%	6%	0%	80%	9%	3%	8%

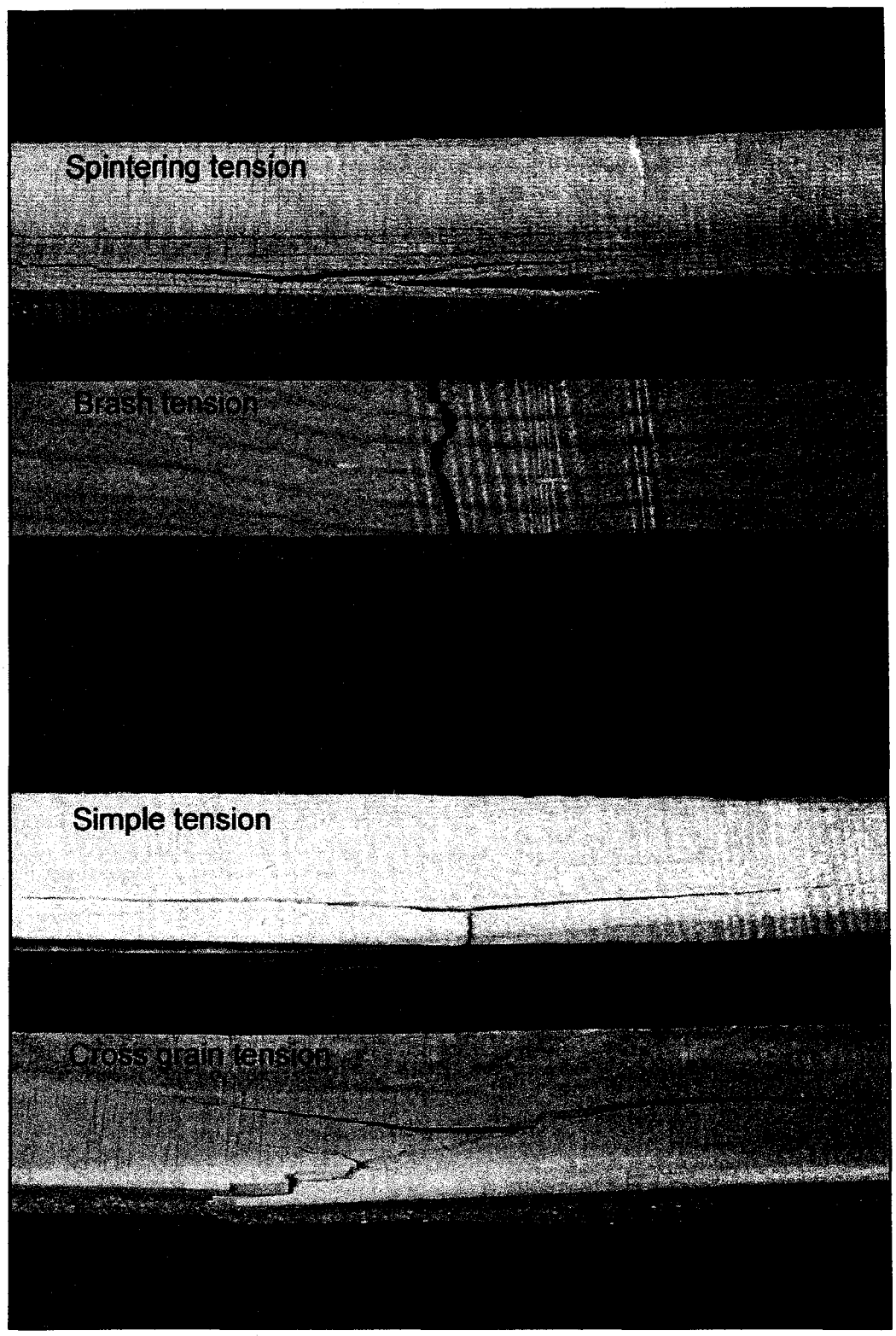

Figure 2.19. Photograph of the four static bending failure types.

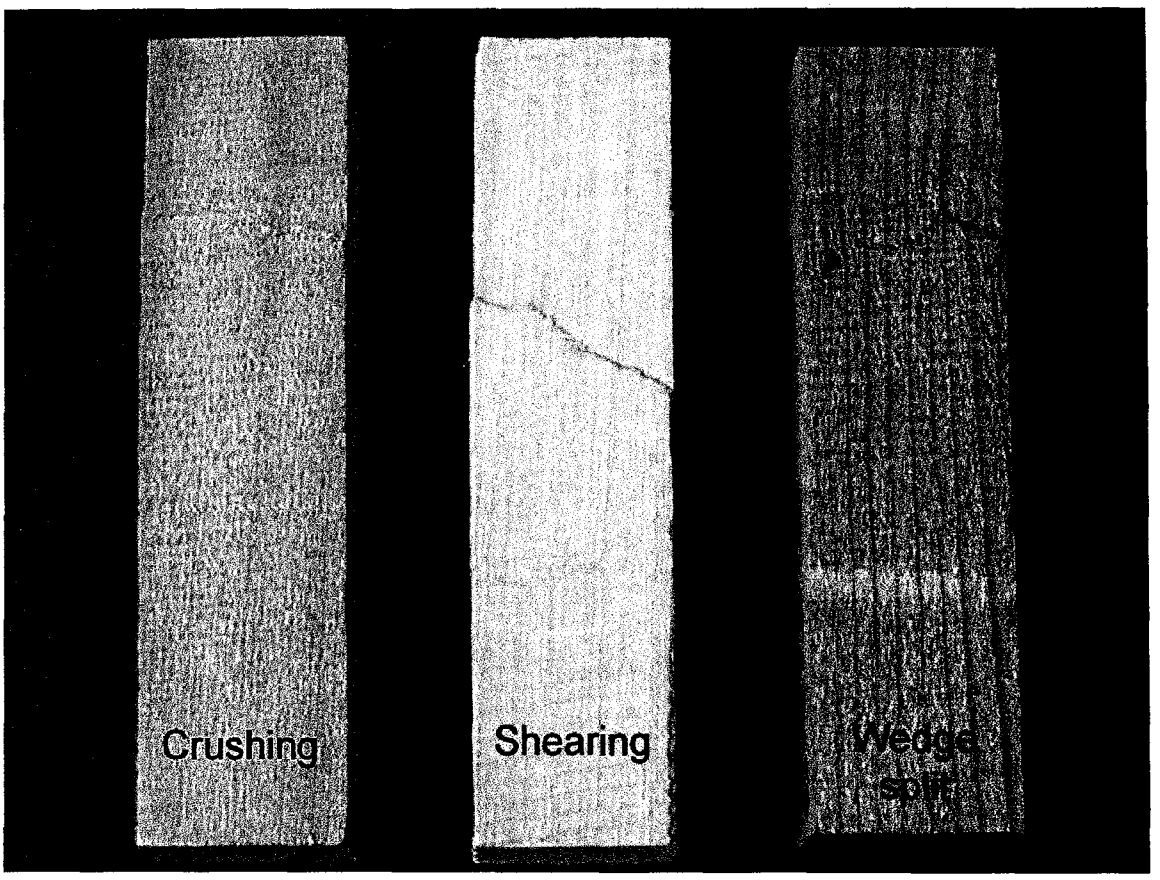

Figure 2.20. Photograph of three failure types in compression parallel to the grain.

Specific Gravity

Specific gravity has a nearly linear relationship with several of wood's fundamental strength properties (Forest Products Laboratory, 1999). In general, higher specific gravity provides greater wood strengths, such as static bending and compression strength. Because of the dramatic effect that specific gravity has on mechanical properties, a change in specific gravity due to discoloration and decay volumes computed in this study. The results revealed that the decay specimens showed the range of average specific gravity from the lowest 0.436 (group 9) to the highest 0.475 (group 2) (Table 2.5). The group 9 (67-100% decay volume and high discoloration) showed the highest negative value of mean percent deviation percentage (-5.35%) of specific gravity. On the other hand, groups 2, 3, 4, 5, 7 and 8 only show of less than 3% reduction in specific gravity (Table 2.5).

For the low discoloration classes, the mean percent deviation of specific gravity changed from negative to positive from decay volume class 1-33% to 34-66% and the positive percentage slightly decreased near zero from decay volume 34-66% to 67-100% (Figure 2.21; Table 2.5). For the medium discoloration class, all three decay volumes exhibited positive values of mean percent deviation and showed little change as decay volume increased. For the high discoloration class, the specific gravity gradually decreased in a near-linear trend as the decay volume increased (Figure 2.21; Table 2.5).

Table 2.5. Means and mean percent deviations for specific gravity (SG) by decay volume and discoloration classes (SE: standard error).

Group	Decay volume	Discoloration	N	Specific gravity (SG)	
				Mean±SE	Mean % deviation±SE (%)
1	1-33%	low	25	0.460±0.007	-0.52±1.16
2		medium	17	0.475±0.008	2.33±1.67
3		high	18	0.464±0.007	0.40±1.68
4	34-66%	low	16	0.458±0.009	3.05±1.89
5		medium	20	0.460±0.007	0.92±1.39
6		high	22	0.454±0.009	-1.67±1.83
7	67-100%	low	20	0.447±0.008	0.30±1.58
8		medium	24	0.463±0.009	1.25±2.07
9		high	50	0.436±0.005	-5.35±1.07

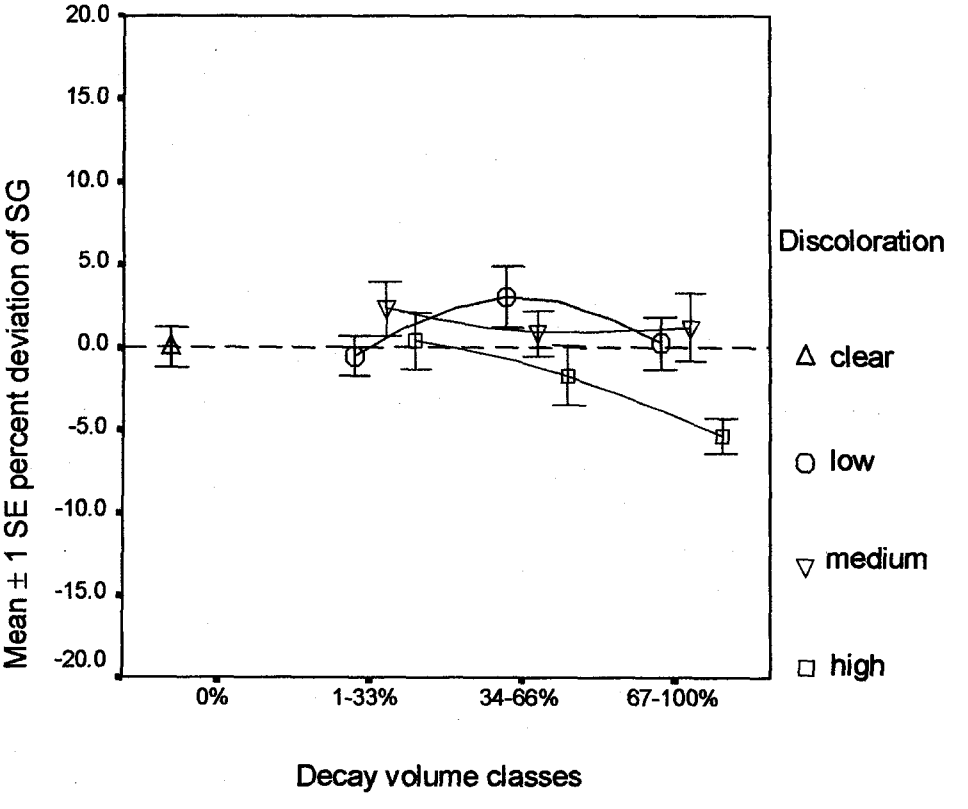

Figure 2.21. Standard error bars of mean percent deviations for specific gravity (SG) by decay volume and discoloration classes.

Modulus of Elasticity (MOE)

The average modulus of elasticity (MOE) for the decayed specimens ranged from 1,321,580 to 1,620,481 (psi). At the same time, among the decayed specimens, group 9 (67-100% decay volume and high discoloration) showed the lowest average MOE (1,321,580 psi) and had the highest average reduction percentage (20%) while group 1 had the lowest average reduction (3.59%) (Table 2.6).

All eight groups with firm red heart showed strength reductions in MOE. Apart from the medium discoloration class, average of MOE reduction increased in a nearly linear relationship with increased in decay volume and discoloration. For the low discoloration class, the average percentage of MOE reduction did not significantly change from a decay volume of 1-33% to one of 67-100%. Meanwhile, in the high discoloration class, the strength reduced dramatically as decay volume increased, while the mean percent deviations in medium discoloration class did not noticeably changed as decay volume increased until the decay volume reached 67-100% (Figure 2.22; Table 2.6).

Table 2.6. Means and mean percent deviations for modulus of elasticity (MOE) by decay volume and discoloration classes (SE: standard error).

Group	Decay volume	Discoloration	N	Modulus of elasticity (MOE)	
				Mean±SE (psi)	Mean % deviation±SE (%)
1	1-33%	low	25	1613393±6067	-3.59±1.56
2		medium	17	1620481±11072	-5.06±2.19
3		high	18	1582710±12481	-5.42±3.03
4	34-66%	low	16	1503284±11415	-4.97±2.15
5		medium	20	1574651±12089	-5.72±2.47
6		high	22	1415424±8494	-15.38±2.18
7	67-100%	low	20	1452322±9197	-7.47±2.64
8		medium	24	1404799±10611	-15.11±2.35
9		high	50	1321580±4605	-20.00±1.69

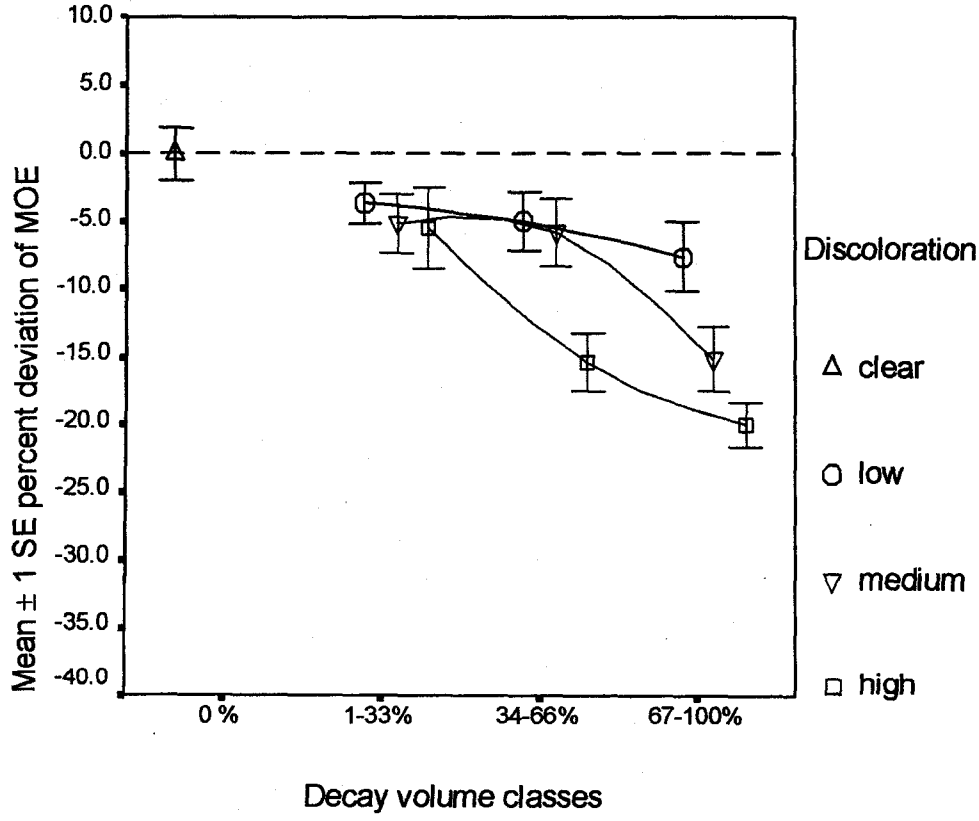

Figure 2.22. Standard error bars of mean percent deviations for modulus of elasticity (MOE) by decay volume and discoloration classes.

Modulus of Rupture (MOR)

For modulus of rupture (MOR), the MOR means ranged from the highest 11,568 psi of group 1 (1-33% decay volume and low discoloration) to the lowest 8,513 psi of group 9 (67-100% decay volume and high discoloration) (Table 2.7). All groups with firm red heart showed a strength reduction in MOR (negative deviation percentage). The values of average percent deviation of MOR ranged from -36.2% for group 9 specimens (67-100% decay volume and high discoloration) to -8.82% for group 1 specimens (1-33% decay volume and low discoloration) (Table 2.7). The mean reduction percentages of MOR changed as decay volume and discoloration increased (Figure 2.23). Among the three discoloration classes, the MOR for high discoloration had the most dramatically decreased in a near-linear trend as the decay volume increased. The groups with low discoloration had similar reduction trend to the groups of medium discoloration as the decay volume increased from 1-33% to 34-66%, but the reduction percentage of low discoloration only slightly increased as decay volume reached 67-100% (Figure 2.23; Table 2.7).

Table 2.7. Means and mean percent deviations of modulus of rupture (MOR) by decay volume and discoloration classes (SE: standard error).

Group	Decay volume	Discoloration	N	Modulus of rupture (MOR)	
				Mean±SE (psi)	Mean % deviation±SE (%)
1	1-33%	low	25	11568±356	-8.82±2.06
2		medium	17	11366±410	-10.92±3.18
3		high	18	10653±459	-15.34±3.27
4	34-66%	low	16	10327±514	-13.45±2.92
5		medium	20	10612±554	-14.47±3.23
6		high	22	9650±444	-24.34±3.17
7	67-100%	low	20	9985±395	-14.48±3.22
8		medium	24	9618±500	-23.20±3.34
9		high	50	8513±283	-33.80±2.14

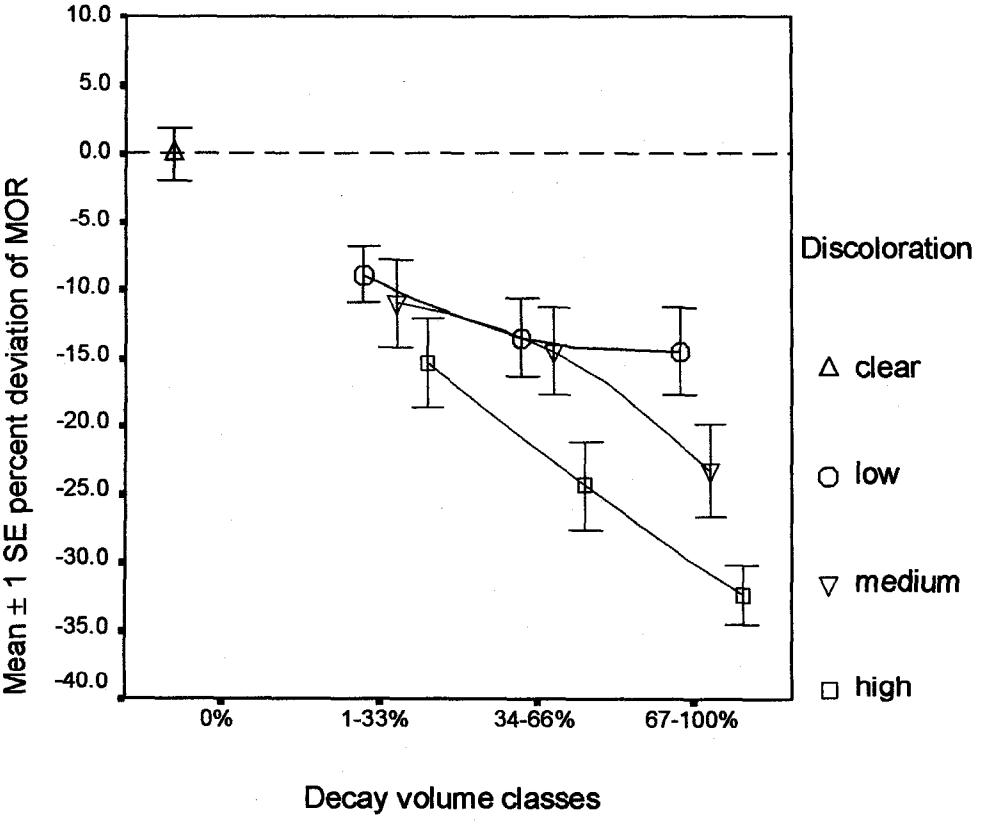

Figure 2.23. Standard error of mean percent deviations for modulus of rupture (MOR) by decay volume and discoloration classes.

Compression Parallel to the Grain

The average compression parallel to grain ranged from 6518 psi for group 1 specimens (1-33% decay volume and low discoloration) to 5685 psi for group 9 specimens (67-100% decay volume and high discoloration) (Table 2.8). All the groups with firm red heart showed negative mean percent deviations for compression parallel to grain. Group 9 (67-100% decay volume and high discoloration) had the highest negative value of mean percent deviation (-14.27%) for compression parallel to the grain, while Group 1 had the lowest negative value of mean percent deviation (-4.28%) (Table 2.8). The values for mean percent deviations of compression parallel to the grain for the three discoloration classes did not exhibited significant change as the decay volume increased from 1-33% to 67-100%. However, the tendency of strength reduction in the high discoloration class increased more rapidly than did the low and medium discoloration classes for decay volume 1-33% to 67-100% (Figure 2.24; Table 2.8).

Table 2.8. Means and mean percent deviations of compression parallel to grain (CPG) by decay volume and discoloration classes (SE: standard error).

Group	Decay volume	Discoloration	N	Compression parallel to grain (CPG)	
				Mean±SE (psi)	Mean % deviation±SE (%)
1	1-33%	low	25	6518±123	-4.28±1.89
2		medium	17	6391±153	-6.70±2.13
3		high	18	6379±158	-4.96±2.80
4	34-66%	low	16	5917±226	-7.03±2.72
5		medium	20	6093±186	-8.62±2.49
6		high	22	6011±194	-11.07±2.58
7	67-100%	low	20	5793±175	-9.17±2.33
8		medium	24	5810±139	-11.37±1.93
9		high	50	5685±126	-14.27±1.87

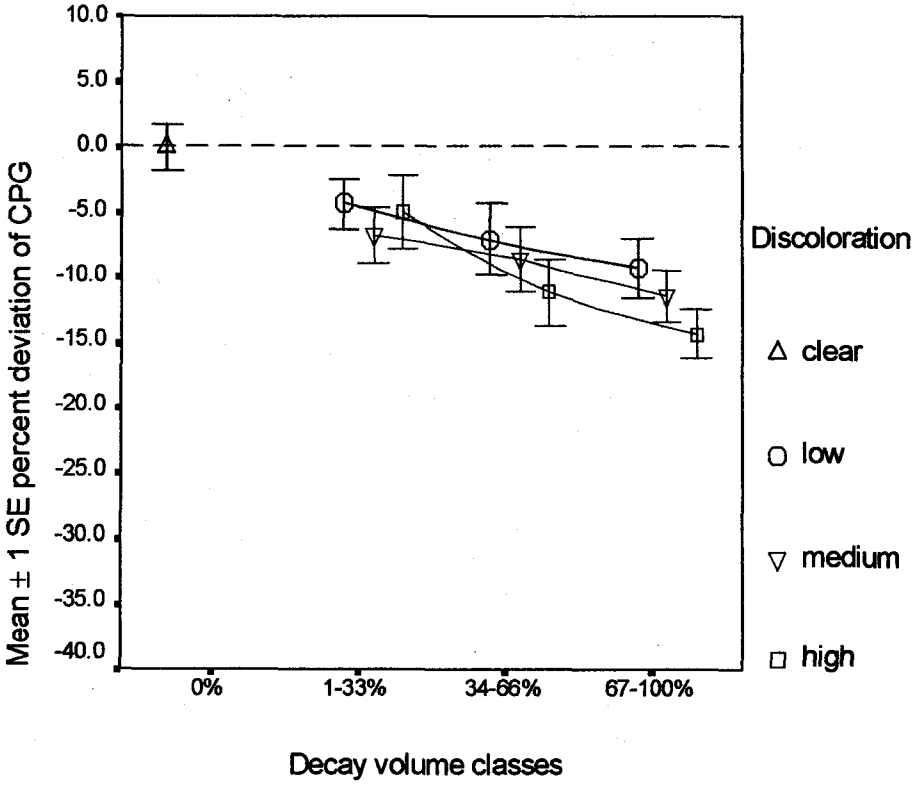

Figure 2.24. Standard error of mean percent deviations for compression parallel to grain (CPG) by decay volume and discoloration classes.

Two-way ANOVA- This analysis (alpha level = 0.05) indicated that the means of deviation percentage of specific gravity were significantly different due to discoloration, but it showed no significant difference due to the decay volume and their interaction (Table 2.9). For the mean percent deviation of MOE and MOR, the results of 2-way ANOVA (alpha level = 0.05) also indicated that the means of deviation percentages of MOE and MOR were significantly different due to the individual effects of decay volume and discoloration and showed no significant interaction effect by these two factors (Table 2.9). Meanwhile, the mean percent deviation of compression parallel to the grain was significantly different due to the decay volume only. The discoloration class and the interaction with decay volume did not significantly affect the mean percent deviation of compression parallel to the grain (Table 2.9).

Table 2.9. The F-ratio values and significance probabilities (P) of 2-way ANOVA (alpha level = 0.05) for the mean percent deviations of SG, MOE, MOR and CPG due to decay volume, discoloration and their interaction (full 2-way ANOVA tables are listed in Appendix 2.1, 2.2, 2.3 and 2.4).

Factors	SG		MOE		MOR		CPG	
	F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Decay volume (A)	1.807	0.167	13.363	0.000	11.489	0.000	5.280	0.006
Discoloration (B)	4.741	0.010	9.829	0.000	11.980	0.000	1.402	0.248
A * B	1.727	0.145	2.019	0.093	1.082	0.366	0.347	0.846

Multiple Comparison Procedure- The Tukey test (alpha level = 0.05) for the mean percent deviation of specific gravity (SG) showed 4 pairwise significant differences between the groups due to their configurations of decay volumes and discoloration classes, and indicated that group 9 was significantly different to other groups except group 6, 1 and 7 (Appendix 2.5).

For the mean percent deviation of MOE, the Tukey's Multiple Comparison procedure showed 8 pairwise significant differences between all groups due to their decay volumes and discoloration classes, and it also indicated that groups 9 was significantly different from all the other groups except groups 6 and 8 (Appendix 2.6).

The results also showed total 8 pairwise significant differences between the mean percent deviations of MOR. Group 9 was significantly different from the rest of groups except groups 6 and 8. Group 1 was also significantly different from groups 9, 6 and 8 (Appendix 2.7).

Finally, the mean percent deviations of compression parallel to grain (CPG) only had 1 pairwise significant differences. Group 9 was the only group significantly different to group 1, and all other groups were not significantly different between each group (Appendix 2.8).

Each pairwise comparison of mean percent deviation for SG, MOE, MOR and CPG was made between the combination of decay volume and discoloration classes. Values grouped by a shade bar indicate those means that were not significantly different from each other at the 0.05 alpha level (Table 2.10).

For the mean percent deviation of SG, the comparison of homogeneity indicated that mean percent deviation of groups 9 , 6, 1 ,7 and 3 were not significantly different

(Table 2.10), while the rest of groups showed no significant difference between each other. For the mean percent deviation of MOE, groups 9, 6 and 8 were not significantly different. Groups 7, 5, 3, 2, 4 and 1showed no significant differences (Table 2.10).

The comparison results of mean percent deviation of MOR also indicated that groups 9, 6 and 8 were not significantly different, while all other groups showed no significant difference. Meanwhile, the CPG had 2 homogeneous subsets with a total 7 overlap groups between two subsets. However, group 1 had the lowest mean reduction percentage of CPG, and group 9 had the highest strength reduction (Table 2.10).

Table 2.10. Delineation of homogeneous subsets based on mean percent deviations of specific gravity (SG), modulus of elasticity (MOE), modulus of rupture (MOR) and compression parallel to grain (CPG) grouped by decay volume and discoloration classes. (The 50% grey shade represents the homogeneous subsets where the highest and lowest means are not significantly different by Tukey's Multiple Comparison procedure with an alpha level = 0.05).

Mean percent deviation of SG			Mean percent deviation for MOE		
Decay volume	Discoloration	Group	Decay Volume	Discoloration	Group
67-100%	high	9	67-100%	high	9
34-66%	high	6	34-66%	high	6
1-33%	low	1	67-100%	medium	8
67-100%	low	7	67-100%	low	7
1-33%	high	3	34-66%	medium	5
34-66%	medium	5	1-33%	high	3
67-100%	medium	8	1-33%	medium	2
1-33%	medium	2	34-66%	low	4
34-66%	low	4	1-33%	low	1
Mean percent deviation of MOR			**Mean percent deviation of CPG**		
Decay volume	Discoloration	Group	Decay volume	Discoloration	Group
67-100%	high	9	67-100%	high	9
34-66%	high	6	67-100%	medium	8
67-100%	medium	8	34-66%	high	6
1-33%	high	3	67-100%	low	7
67-100%	low	7	34-66%	medium	5
34-66%	medium	5	34-66%	low	4
34-66%	low	4	1-33%	medium	2
1-33%	medium	2	1-33%	high	3
1-33%	low	1	1-33%	low	1

Comparison to Visual Stress Grading

In order to be used more practically for the lumber grader and engineer, the strength ratios of MOE, MOR and CPG due to the firm red heart were simplified by combining groups with no significant differences into the group having the lowest strength ratio (Table 2.11). The data were also compared to the strength ratios from the current visual stress grading standard (Western Wood Products Association, 1995; ASTM 2002e) such as knot size on different faces of dimension lumber and the slope of grain in bending and compression. The corresponding lumber grades for beam-stringers, post-timber, structural joist-planks and structural light framing as defined by the standard grading rules for western coast lumber were also listed in this table. The comparisons results indicated that 66% maximum bending strength ratio of MOR for the subset (groups 6, 8 and 9) was equal to the strength ratio of No.1 grade for beams-stringers but was higher than those strength rations of No. 1 grades for post-timbers, structural joist-planks and structural light framing. All select structural grades of beam-stringers, post-timbers, structural joist-planks, and structural light framing were not affected due to that firm red heart was limited to 10% of the lumber. Strength ratios of No. 2 and 3 in these four categories were much lower than the maximum strength ratio of groups 6, 8 and 9. Maximum strength ratio of CPG was also higher than those of all grades (Table 2.11).

Table 2.11. Comparison of strength ratios of four lumber grading categories, MOR, MOE and CPG with firm red heart for lodgepole pine.

Limitations of firm red heart for grades of four grading categories, and classification for MOR, MOE and CPG with firm red heart	Strength ratio
MOR (firm red heart): at middle ⅓ of length	
• Groups 1, 2, 3, 4, 5 and 7 (low discoloration no limited; medium discoloration below ⅔ volume; high discoloration below ⅓ volume)	85%
• Groups 6, 8 and 9 (medium discoloration above ⅔ volume; high discoloration above ⅓ volume)	66%
MOE (firm red heart): at middle ⅓ of length	
• Groups 1, 2, 3, 4, 5 and 7 (low discoloration no limited; medium discoloration below ⅔ volume; high discoloration below ⅓ volume)	92%
• Groups 6, 8 and 9 (medium discoloration above ⅔ volume; high discoloration above ⅓ volume)	80%
CPG (firm red heart): at middle ⅓ of length	
• Groups 1, 2, 3, 4, 5, 6, 7 and 8 (No volume limit on low and medium discoloration; high discoloration below ⅔ volume)	85%
• Group 9 (high discoloration above ⅔ volume)	88%
Beams & Stringers	
• Select structural: firm red heart limited to 10%	76%
• No.1: firm red heart no limit	66%
• No.2: firm red heart and whit specks no limit	40%
• No.3: firm red heat and white specks no limit	below 40%
Posts & Timbers	
• Select structural: firm red heart limited to 10%	69%
• No.1: firm red heart no limit	61%
• No.2: firm red heart and whit specks no limit;	40%
• No.3: firm red heat and white specks no limit	below 40%
Structural Joist and Planks	
• Select structural: firm red heart limited to 10%	69%
• No.1: firm red heart no limit	61%
• No.2: firm red heart no limit	53%
• No.3: firm red heat and white specks no limit	below 40%
Structural light farming	
• Select structural: firm red heart limited to 10%	69%
• No.1: firm red heart no limit	61%
• No.2: firm red heart no limit	53%

DISCUSSION AND CONCLUSION

Discussion

The lodgepole pine specimens used in this study were one inch (2.54 cm) in cross-section. Therefore the results would not be the same as those expected from actual sized lumber. In general, the average reduction percentages increased as the decay volume and discoloration intensity increased individually in a near linear relationship except for the average reduction percentages of specific gravity.

Decayed wood becomes brash as decay severity increases and the outward signs of brash splitter breakage can be used as an indicator of wood decay (Zabel and Morrell, 1992). Compared with the percentages of failure types of clear wood specimens, the specimens with firm red heart had similar percentages for the four failure types except group 9 (67-100% decay volume and high discoloration), which had 8% brash tension failure. The high percentage of brash failure in group 9 was caused by the higher degree of decay. Simple tension failure occurred most often of the four types of failures and indicated that the wood with firm red heart tended to response to the bending force similar to the clear wood specimen. For the compression failure types, the specimen with firm red heart often failed initially on the face with decay and exhibited more shearing rupture at an angle to the face containing both decay and non-decay wood. The higher average percentage of shearing for these specimens than for those of clear wood also indicated decay.

Differences of less than 1% dry-basis moisture content at wood with moisture contents less than 12% moisture content are considered to have much smaller effects on

wood strength than does specific gravity (Haygreen and Bowyer, 1989; Forest Products Laboratory, 1999).

The results show groups 2, 3, 4, 5, 7 and 8 had higher average specific gravities than those of the control group. Lodgepole pine wood infected by *Phellinus pini* had a higher average specific gravity than uninfected wood (Roff and Whittaker, 1963). The factors causing an increase in specific gravity of specimens containing firm red heart are still unclear. Zabel and Morrell (1992) reported that wood infected by decay fungi had higher water permeability than non-decay wood. In theory, the increase of water permeability could cause under-measurement of the wood block volume during the specific gravity measurement. The results from this study showed that the increases in average specific gravity of decayed wood was not found consistently in all groups and data analysis revealed that discoloration affected specific gravity more than decay volume. However, the average reduction percentage of specific gravity on high discoloration groups increased as the decay volume increased in a near linear relationship.

The average reduction percentages of MOE and MOR increased as the decay volume and discoloration intensity increased, but the reduction percentage of MOR increased more rapidly than those of MOE. Curling *et al.* (2002) conducted a study on the strength reduction of southern pine by brown-rot fungi and showed that the strength reduction of MOE was not as rapid as the MOR. Although Roff and Whittaker (1963) reported that lodgepole pine beams made from completely red-stained material had MOR's only 15% lower than unstained beams, their study did not classify their test

beams by discoloration intensity as was shown to have a significant effect on the percentage reduction of MOR in this study.

Unlike the mean percent deviation of MOE and MOR, the mean percent deviation of compression parallel to grain was significantly affected only by decay volume. Data also indicated that the average loss of compression strength parallel to grain was much less than that for either MOE or MOR.

The location of decay is another factor affecting the bending strength of the static bending member. This study did not include the scenario where the decay occurred inside larger sized lumber with no sign of decay on the surface. Although the effect of decay inside the lumber might not be severe as on the edge of lumber, the actual strength reduction still needs further study. Another concern is that the strength ratio cited for full-scale lumber with firm red heart should be limited to the center 1/3 of the lumber because the bending test applies a force on the center 1/3 of the length.

Regarding the corresponding lumber grade, the visual stress grading characters and limiting provisions in the lumber grades for beam-stringers, post-timbers, structural joist-planks and structural light framing defined by the standard grading rules for west coast lumber were compared with the strength ratio of firm red heart (Western Wood Product Association, 1995). The results showed the maximum strength ratio of groups 6, 8 and 9 was equal to the strength ratio of No. 1 structural grade of beam-stringers, but was higher than the strength ratio of No. 1 structural grades for post-timbers, structural joist-planks and structural light framing. No. 1 structural grade of beam-stringers was the only grade affected by firm red heart. The results did not affect any other current lumber grades, since the higher grades simply do not allow firm red heart for more than

10%, and the grades lower than No. 1 allow the slope of grain and knot size with much lower strength ratios than 66%. However, the 85% strength ratio of MOR with ⅓ decay volume and high discoloration was much higher than the strength ratio of select structural grades for these four grading categories. The volume limitation of firm red heart can be increased to 20% without affecting the desired strength ratio.

In addition, lodgepole pine grows in a very large natural range and its common fungal decay, firm red heart (white pocket rot in advance decay stage), may be attributed to several different decay fungi (Roff and Whittaker, 1963). This study only tested lodgepole pine specimens containing firm red heart caused by *Phellinus pini* at its incipient and early decay stage, while other fungi might have different effects on strength reduction.

This study only examined the effect of firm red heart on lodgepole pine. The effect of firm red heat on other tree species are still unknown. Brown-rot fungi reduce wood strength even more than the white-rot fungi examined in this study and most of the studies dealing with their effect on strength reduction were conducted in the laboratory by inoculating fungi into specimens. The actual effects on the strength reductions of natural decay lumber are still unknown. Meanwhile, the relationships between different tree species and decay fungi have also not been completely studied, and the interaction of firm red heart with knot, slope of grain, nailing, screwing and long-term severability are unclear as well and require further study.

Conclusion

1. In general, the average percentage of reduction in MOE and MOR increased significantly in a nearly linear relationship with both decay volume and discoloration.
2. The average percent deviation of specific gravity was significantly effected only by discoloration, while the average percent deviation of compression parallel to the grain increased significantly as decay volume increased.
3. The average percent deviation of MOR and MOE changed more rapidly with changes in decay volume and discoloration than did the average percent deviation for MOE. .
4. MOR had a maximum 34% average strength reduction, and MOE had a maximum 20% average reduction percentage, while the compression parallel to the grain had the lowest maximum 15% average strength reduction.
5. The maximum 34% average strength reduction of MOR is equal to the effects of either 1:11 in slope of grain or a 1 ½ inch-diameter centerline knot on a 4 inch-wide face.
6. The maximum 20% average strength reduction of MOE is equal to the effects of either 1:15.5 in slope of grain or a 1 $^{3}/_{4}$-inch-diameter centerline knot on an 8-inch-wide face, or a ½ inch-diameter edge knot on a 4 inch-wide face.
7. The maximum 15% average strength reduction of compression parallel to grain is equal to the effect of 1:13 slope of grain in compression parallel to grain.
8. Groups 6, 8 and 9 showed no significant differences in average percent deviations for MOE and MOR.

9. The results indicated that the maximum 34% reduction of MOR corresponds to the knot size and slope of grain limitations of No. 1 structural grade for beam-stringers in the grading rules of Western Wood Products Association (WWPA) (1995).
10. A 20% volume limitation of firm red heart for select structural grades of beam-stringers, post-timber, structural joist-planks and structural light framing in the grading rules of WWPA is recommended.

This study provides the first step in the process of quantifying the strength reduction of lodgepole pine due to the present of natural firm red heart by volume and discoloration intensity. The results also provide the engineer a more accurate strength ratio of lodgepole pine containing firm red heart caused by *Phellinus pini*. Meanwhile, the criteria for estimating decay volume and discoloration can be incorporated into the current visual stress grading to improve their accuracy.

In conclusion, firm red heart caused by *Phellinus pini* during the incipient and early decay stages revealed more effects on strength reduction than the results from previous studies, and the magnitude of its effects on the structural grades of the current visual stress grading rules are large enough to be of serious concern.

LITERATURE CITED

Adler, E. 1977. Lignin chemistry-past present and future. Wood science and technology. Vol. 11: 169-218.

American Society for Testing and Materials (ASTM). 2002a. . Standard Test Methods for Specific Gravity of Wood and Wood-Based Materials. ASTM D 2395-02. ASTM, West Conshohocken, Pa.

American Society for Testing and Materials (ASTM). 2002b. Standard Test Methods of Static Tests of Lumber in Structural Sizes. ASTM D 198-99. ASTM, West Conshohocken, Pa.

American Society for Testing and Materials (ASTM). 2002c. Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Base Materials. ASTM D 4442-92(1997)e1. ASTM, West Conshohocken, Pa.

American Society for Testing and Materials (ASTM). 2002d. Testing small clear specimens of timber. ASTM D143-94. ASTM, West Conshohocken, Pa.

American Society for Testing and Materials (ASTM). 2002e. Standard methods for establishing structural grades and related allowable properties for visually graded lumber. ASTM D245-00. ASTM, West Conshohocken, Pa.

Anagnost, S.E. 1998. Light microscopic diagnosis of wood decay. IAWA Journal, Vol. 19 (2): 141-167.

Carll, C.G. and T. L. Highley. 1999. Decay of wood and wood-based products above ground in buildings. Journal of Testing and Evaluation, JTEVA, Vol. 27 (2): 150-158.

Cowling, E. B. and W. Brown. 1969. Structural features of cellulose materials in relation to enzymatic hydrolysis. In: Hajny, G. J. and Reese, E. T. 1969. Cellulose and Their Applications, Advanced chemical series. 95:152-187.

Curling, S. F., C. A. Clausen and J. E. Winandy. 2002. Relationships between mechanical properties, weight loss and chemical composition of wood during incipient brown-rot decay. For. Prod. Jour. Vol. 52 (7/8): 34-39.

Davis, J. 2000. Western Wood Products Association. Personal Communication.

Eriksson, K. E., R. A. Blanchette and P. Ander. 1990. Microbial and Enzymatic degradation of wood and wood components. Springer-Verlag, New York. P. 122.

Flournoy, D. S., T.L. Highley and K.L. Grag. 1994. Decomposition of cellulose by brown-rot fungi. Recent advances in biodeterioration and biodegradation. Vol. 2: 191-221.

Forest Products Laboratory. 1999. Wood Handbook—Wood as an Engineering Material. Gen. Tech. Rep. FPL–GTR–113. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 463 p.

Foster, E. and G. W. Wallis. 2002. Canadian Forest Service Tree Disease Identification Guide for British Columbia.

Giffin, D. 1977. Water potential and wood decay fungi. Annual reviews Phytopathology. Vol. 15: 319-329.

Green, F. and T. L. Highley. 1997. Mechanism of brown-rot decay: Paradigm or Paradox. International Biodeterioration & Biodegradation. Vol. 39, No. 2-3: 113-124.

Hammel, K. E. 1997. Fungal Degradation of lignin. In: Yong, R. A and M. Akhtar. 1998. Environmentally Friendly Technologies for Pulp and Paper Industry, John Wiley & Sons, Inc. P. 273-306.

Haygreen, J. G. and J. L. Bowyer. 1989. Forest Products and Wood Science. Iowa State University Press. 500 pp.

Highley, T. L. and W. V. Dashek. 1998. Biotechnology in the study of brown and white-rot fungi. In: Bruce, A and W. Palfireyman. 1998. Forest Products Biotechnology. Taylor and Francis Inc. P. 15-36.

Highley, T. L., 1977. Requirement for cellulose degradation by a brown-rot fungus. Material and Organismen Vol. 12: 25-36.

Highley, T. L., C. A. Clausen, S. C. Croan, F. Green, B. L. lllman and J. A. Micales. 1994. Research on Biodeterioration of wood, 1987-1992. 1. Decay Mechanisms and Biocontrol. Research Paper FPL-RP-529. U.S.D.A. Forest Products Laboratory, Madison, Wisconsin.

Highley, T. L., S. S. Bar-Lev, T. K. Kirk and M. J. Larsen. 1983. Influence of O_2 and CO_2 on wood decay by heart-rot and sap-rot fungi. Phytopathology. 73: 630-633.

Humphrey, C. J. and P. V. Siggers. 1933. Temperature relations of wood-destroying fungi. Journal of Agricultural Research. 47: 997-1008.

Kazemi, S. M., D. J. Dickinson and R. J. Murphy. 1998. The influence of gaseous oxygen concentration on fungal growth rates, biomass production and wood decay. IRG-WP-1283.

Kennedy, R. W. 1958. Strength retention in wood decayed to small weight losses. For. Prod. Jour. October: 308-314.

Kirk, T. K. and D. Cullen. 1998. Enzymology and molecular genetics of wood degradation by white-rot fungi. In: Yong, R. A and M. Akhtar. 1998. Environmentally Friendly Technologies for Pulp and Paper Industry, John Wiley & Sons, Inc. P. 273-307.

Kirk, T. K., R. E. Ibach, M. D. Mozuch, A. H. Conner, and T. L. Highley. 1991. Characterization of cotton cellulose depolymerized by a brown-rot fungus by acid or by chemical oxidants. Holzforschung. Vol. 45:239-244.

Koch, Peter. 1996. Lodgepole Pine in North America. Forestry Products Society. Madison, WI. Vol. 1. 343 pp.

Koenings, J. W. 1974. Hydrogen peroxide and iron: a proposed system for decomposition of wood by brown-rot basidiomycetes. Wood and fiber. Vol. 6: 66-80.

Loy, J. 2000. Southern Pine Inspection Bureau. Personal Communication.

Manion, P. D. 1991. Tree Disease Concepts. Prentice-Hall Press. 402 pp.

Martin, P., R. Collet, P. Barthelemy and G. Roussy. 1987. Evaluation of wood characteristics: Internal scanning of material by microwaves. Wood Science and Technology. 21:361-371.

Matthius, R. 2000. Assistant Professor of Fungal Ecology. Division of Biological Sciences. University of Montana. Missoula, Montana. Class notes of fungal biology class and personal conversation.

Mizumoto, S. 1966. The effect of decay caused by *Gloeophyllum trabeum* on the strength properties of Japanese red pine sapwood. J. Jap. For. Soc. 48 (1): 7-11.

Morton, H. and D. French. 1996. Factor affecting germination of spores of wood-rotting fungi on wood. For. Prod. Journal. Vol. 16(3): 25-30.

Roff, J. W. and E. I. Whittaker. 1963. Relative strength and decay resistance of red-stained lodgepole pine. Publ. Dep. For. Can. No. 1031:1-19.

Rominger, S. 2000. West Coast Lumber Inspection Bureau. Personal Communication.

Smith, S. M., J. J. Morrell and C. Sexton. 1992. Residual strength of Douglas-fir sapwood and heartwood as affected by fungus colony size and number of colony forming units. For. Prod. Jour. Vol. 42(4):19-24.

Southern Pine Inspection Bureau. 1994. Standard grading rules for southern pine lumber. 138 pp.

Toole, E. R. 1971. Reduction in crushing strength and weight associated with decay by rot fungi. Wood Sci. 3 (3): 172-178.

Wang, C. J. K. and R. A. Zable. 1990. Identification Manual for Fungi from Utility Poles in The Eastern United States. American Type Culture Collection. Allen Press. 356 pp.

West Coast Lumber Inspection Bureau. 1998. Interpretation for The National Grading Rules for Dimension Lumber. 17 pp.

West Coast Lumber Inspection Bureau. 2000. Standard Grading Rules for Northeastern Lumber. Chapter 9. 7-8.

Western Wood Products Association. 1995. Western Lumber Grading Rules. 248 pp.

Wilcox, W.W. 1968. Changes in wood microstructure through progressive stages of decay. U. S. Forest Service Research Paper FPL 70. USDA. For. Prod. Lab., Madison, WI.

Wilcox, W.W. 1970. Anatomical changes in wood cell walls attacked by fungi and bacteria. The Botanical Review 36: 1-28.

Wilcox, W.W. 1978. Review of literature on the effects of early stages of decay on wood strength. Wood and Fiber 9: 252-257.

Wilcox, W.W. 1993a. Comparison of SEM and LM for the diagnosis of early stages of brown-rot wood decay. IAWA J. 14: 219-226.

Wilcox, W.W. and M. Dietz. 1997. Fungi causing above-ground wood decay in structures in California. Wood and Fiber Science. Vol. 29 (3): 291-298.

Wilcox. W.W. 1993b. Comparative morphology of early stages of brown-rot wood decay. IAWA J. 14: 127-138.

Winandy, J. E. and Jeffrey J. Morrell. 1993. Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. Wood and Fiber Science, Vol. 25 (3): 278-288.

Worrall, J.J., S.E. Anagnost and R.A. Zabel. 1997. Wood decay by diverse groups of lignicolous fungi. Mycologia 89: 199-219.

Zabel, R. A., and J. J. Morrell. 1992. Wood microbiology: decay and its prevention. Academic Press, Inc., New York, N. Y. 476 pp.

APPENDIX

Appendix 1.1. Malt extract agar (MEA) and gallic acid agar formulas.

Malt Extract Agar (MEA)

- Difco Bacto malt extract----------15.0 g
- Difco Bacto-agar----------20.0 g
- Distilled water----------1000.0 ml

The agar was dissolved in hot distilled water and the malt extract was added into agar. The mixture was sterilized at the pressure of 15 psi for 20 minutes. The malt extract agar was then cooled down to 50 °C before poured into 100 mm 60 mm diameter sterilized Petri dishes at approximately 30-40 ml per dish.

Gallic Acid Agar (GAA)

- Difco Bacto malt extract----------15.0 g
- Difco Bacto-agar----------20.0 g
- Distilled water----------1000.0 ml
- Gallic acid (crystals), analytical grade----5.0 g

Malt extract and agar were dissolved in 850 ml distilled water in a 2 liter Erlenmeyer flask. 150 ml of distilled water was placed in a separate Erlenmeyer flask. Both flasks were sterilized for 20 min at the pressure of 15 psi in an autoclave machine. 5.0 g gallic acid was added to the 150 ml sterilized water after removal from the autoclave. The gallic acid solution was thoroughly mixed with sterilized malt extract

agar, when the agar was about 56 °C. The gallic acid agar was then cooled down to 50 °C before poured into 100 mm sterilized Petri dishes at approximately 30-40 ml per dish.

Appendix 1.2. Safranin-O solution preparation.

- Staining Solution (Safranin-O):
  1. Solution stock: 0.5 gm Safranin-O in 100 ml 50% alcohol/distil water.
  2. Stain solution: add 1 ml stock into 20 ml distil water (about 0.025% Safranin-O in 100 ml 2.5% alcohol/water)

Appendix 1.3. DNA codes of sequencing results.

Group A:

CAB4 (ITS1-F)

AAGCGTACAGGTTCCGGAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTTAAAATCGAGGGCTTGATGCTGGCGT
GGAAACACGCACTGTGCTCGGCCTTCGTGCTTNATCCACTCAACACCTGTGCACCTTATCGAAGTTAGTAGTCTTTCC
TCCTTAGTTGGAGCCGCCGGGGTTGACTTTGTTAGTAGTGTTTCGACGCGAAACTATGGTCGGCCTTGGCTGGGATC
GGCGAGCACTTTGACTTCATCATACACACTTTAATTGTCTTGTAGAATGTAATGCTCCTTGTGGGCGAAATGAAATACA
ACTTTCAACAACGGATCTCTTGGCTCTCGCATCNATGAAGAACGCANCGAAATGCGATAAGTAATGTGNATTGCAGAA
TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATGT
TAACATCAAACCCCNTGCTTGTAAAGGCTCGGGGCTTGGATTTGGAGGTTTATGCCGGCCTGCTTCATTGTCAGTTGT
CGGCTCCTCTTAAATGGATTAGCTGGACTTTGGTCTGCGTGTCGGTGTGATAGNTTATTCACCAATCGCTTTCCTAAT
GGGTCTGCTTCTAATGGTCTTCGGACAAGGTCTTAACAGCCTTCTTGACTCTTTGACCTCAAATCAGGTAGGACTACC
CGCTGAACTTAAGCATATC

CAB4 (ITS4)

CGNGAAGTCCTACCTGATTTGAGGTCAAAGAGTCAAGAAGGCTTGTTAAGACCTTGTCCGAAGACCATTAGAAGCAG
ACCCATTAGGAAAGCGATTGGTGAATAAACTATCACACCGACACGCAGACCAAAGTCCAGCTAATCCATTTAAGAGGA
GCCGACAACTGACAATGAAGCAGGCCGGCATAAACCTCCAAATCCAAGCCCCGAGCCTTTACAAGCAAGGGGTTTG
ATGTTAACATGANACTCAAACAGGCATGCCCCTCGGAATNCCAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATT
CACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTT
GAAAGTTGTATTTCATTTCGCCNACAAGGAGCATTACATTCTACAAGACAATTAAAGTNTGTATGATGAAGTCAAAGTG
CTCGCCGATCCNAGCCAAGGCCGACCATAGTTTCGCGTCGAAACACTACTAACAAAGTCAACCCCGGCGGCTCCAA
CTAAGGAGGAAAGACTACTAACTTCGATAAGGTGCACAGGTGTTGAGTGGATTAAGCNCCGAAGGCCGAGCACAGT
GCGTGTTTCCACGCCAGCATCAAGCCCTCGATTTTAAAAACTCGATAATGATCCTTCCGCAGGTTCACCTACGGAAAC
CTTGTTACGACTTTTACTTCCTCCNACAAAAGANAGAANAA

Group B:

CAB9 (ITS1-F)

AAGCGTACAGGTTCCGGAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTTAAAATCGAGGGCTTGATGCTGGCGT
GGAAACACGCACTGTGCTCGGCCTTCGTGCTTAATCCACTCAACACCTGTGCACCTTATCGAAGTTAGTAGTCTTTCC
TCCTTAGTTGGAGCCGCCGGGGTTGACTTTGTTAGTAGTGTTTCGACGCGAAACTATGGTCGGCCTTGGCTGGGATC
GGCGAGCACTTTGACTTCATCATACACACTTTAATTGTCTTGTAGAATGTAATGCTCCTTGTGGGCGAAATGAAATACA
ACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATGT
TAACATCAAACCCCTTGCTTGTAAAGGCTCGGGGCTTGGATTTGGAGGTTTATGCCGGCCTGCTTCATTGTCAGTTGT
CGGCTCCTCTTAAATGGATTAGCTGGACTTTGGTCTGCGTGTCGGTGTGATAGTTTATTCACCAATCGCTTTCCTAAT
GGGTCTGCTTCTAATGGTCTTCGGACAAGGTCTTAACAGCCTTCTTGACTCTTTGACCTCAAATCAGGTAGGACTACC
CGCTGAACTTAAGCATATCNATNANANNGGGGAA

CAB9(ITS4)

GNCNCGNGAAGTCCTACCTGATTTGAGGTCAAAGAGTCAAGAAGGCTTGTTAAGACCTTGTCCGAAGACCATTAGAA
GCAGACCCATTAGGAAAGCGATTGGTGAATAAACTATCACACCGACACGCAGACCAAAGTCCAGCTAATCCATTTAA
GAGGAGCCGACAACTGACAATGAAGCAGGCCGGCATAAACCTCCAAATCCAAGCCCCGAGCCTTTACAAGCAAGGG
GTTTGATGTTAACATGACACTCAAACAGGCATGCCCCTCGGAATACCAAGGGGCGCAAGGTGCGTTCAAAGATTCGA
TGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCC
GTTGTTGAAAGTTGTATTTCATTTCGCCCACAAGGAGCATTACATTCTACAAGACAATTAAAGTGTGTATGATGAAGTC
AAAGTGCTCGCCGATCCCAGCCAAGGCCGACCATAGTTTCGCGTCGAAACACTACTAACAAAGTCAACCCCGGCGG
CTCCAACTAAGGAGGAAAGACTACTAACTTCGATAAGGTGCACAGGTGTTGAGTGGATTAAGCACCGAAGGCCGAGC
ACAGTGCGTGTTTCCACGCCAGCATCAAGCCCTCGATTTTAAAAACTCGATAATGATCCTTCCGCAGGTTCACCTACG
GAAACCTTGTTACGACTTTTACTTCCTCCNACAAAAGANAGAAAAANNNC

Group C:

STIM2 (ITS1-F)

AAANGTNGACNGTGTCCANCNNGNGNGAGCGGAAGGATCATTATCGAGTTTTTAAAATCGAGGGCTTGATTGCTGGC
GTGGAAACACGCACTGTGCTCGGCCTTCGTGCTTAATCCACTCAACACCTGTGCACCTTATCGAAGTTAGTAGTCTTT
CCTCCTTAGTTGGAGCCGCCGGGGTTGACTTTGTTAGTAGTGTTTCGACGCGAAACTATGGTCGGCCTTGGCTGGGA
TCGGCGAGCACTTTGACTTCATCATACACACTTTAATTGTCTTGTAGAATTGTAATGCTCCTTGTGGGCGAAATGAAAT
ACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCA
TGTTAACATCAAACCCCTTGCTTGTAAAGGCTCGGGGCTTGGATTTGGAGGTTTATGCCGGCCTGCTTCATTGTCAGT
TGTCGGCTCCTCTTAAATGNATTAGCTGGACTTTGGTCTGCGTGTCGGTGTGATAGTTTATTCACCAATCGCTTTCCTA
ATGGGTCTGCTTCTAATGGNCTTCGGACAAGGTCTTAACAGCCTTCTTGACTCTTTGACCTCAAATCAGGTAGGACTA
CCCCGCTGAACTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTAACCAANGATTCCCCCNANTAACTGCGAGTGA
AGCGGGAAAAGCTCAAATTTAAAATCTNGCGGGNCTTATGGGCGNCCCGAGTTGTAATCTTGGAGAA

S2(ITS4)

AGANANANAGGACGGCCATAAGACCGCCAGATTTTAAATTTGAGCTTTTCCCGCTTCACTCGCAGTTACTAGGGGAAT
CCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAGGTCAAAGAGT
CAAGAAGGCTGTTAAGACCTTGTCCGAAGACCATTAGAAGCAGACCCATTAGGAAAGCGATTGGTGAATAAACTATCA
CACCGACACGCAGACCAAAGTCCAGCTAATGCATTTAAGAGGAGCCGACAACNGACAATGAAGCAGGCCGGCATAA
ACCTCCAAATCCAAGCCCCGAGCCTTTACAAGCAAGGGGTTTGATTGTTAACATGACACTCAAACAGGCATGCCCCT
CGGAATACCAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCAT
TTCGCCTGCGNTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAGTTGTATTTCATTTCGCCCACAAGGAGC
ATTACNTTCTACAAGACAATTAAAGTGTGTATGATGAANTCAAAGTGCTCGCCNGATCCCAGCCAAGGCCGACCATAG
TTTCGCCGCCGAAACACTACCTAACAAANTCNACCCCNNCNGCTCCCAACTAAGGAGGAAAGACTACTAANTTCCGA
TAAAGTGCACAGGTGNTTGAGTGGGATTAAGCCCGAAGGGCCNAGCACAGTGCGTTGTTTCCACGCCAGCATCCAA
GCCCCTCGATTTTAAAAAACCCAATAATGATCCTTTCCGCAGGTCCACCTACCGGAAAACCTGGTACGA

Group D:

RLH4(ITS1-F)

AAGCGACAGGTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTTAAAATCGAGGGCTTGATGCTGGCGTG
GAAACACGCACTGTGCTCGGCCTTCGTGCTTAATCCACTCAACACCTGTGCACCTTATCGAAGTTAGTAGTCTTTCCT
CCTTAGTTGGAGCCGNCGGGGTTGACTTTGTTAGTAGTGTTTCGACGCGAAACTATGGTCGGCCTTGGCTGGGATCG
GCGAGCACTTTGNCTTCATCATACACACTTTAATTGTCTTGTAGAATGTAATTGCTCCTTGTGGGCGAAATGAAATACA
ACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
TTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATGT
TAACATCAAACCCCTTGCTTGTAAAGGCTCGGGGCTTGGATTTGGAGGTTTATGCCGGCCTGCTTCATTGTCGGTTGT
CGGCTCCTCTTAAATGCATTAGCTGGACTTTGGTCTGCGTGTCGGTGTGATAGTTTATTCACCAATCGCTTTCCTAAT
GGGTCTGCTTCTAATGGTCTTCGGACAAGGTGTTACAACCTTCTTGANTCCTTGACTCCAATCANGGAAGACTNCCCN
CNGAACTTTAGCNTATC

RLH4F(ITS4)

GTCAGCGNGTAGTCCTACCTGATTTGAGGTCAAAGAGTCAAGAAGGCTTGTAACANCTTGNCCGAAGANCATTANAA
GCAGANCCATTANGAAAGCGATTGGNGAATAAACTATCACACCGACACNCAGANCAAAGTCCANCTAATGCATTTAA
GANGNNCCNACNACCGACAATGAANCAGGGCGGNATNAACCTCCAAATCCAAGCCCCNANCCTTTACNANCAAGGG
GGTTGATGGTAACATGANACTCCAACAGGGATGCCCCNCNGAATACCAAGGGGGGCNAGGGGNGTTCAAANANTCC
ATGATTCACTGNATTCTGCNATTCNCATTACTTATCNCATTTCNCTGNGNTCTTCNTCNATGNNANANCCNANANANCC
NNTGGTGAAANNTGGATTTCNTTTCGCCCNCNANGNNCATTNCNTTCTACNANANAATTAAAGNGGGGATNANNAAA
CCNAANTGNTCNCCNANCCCNNCCNANGGCGANCATAATTTCNCGTCNAAACACTACTNACNAAGNCAACCCCNNC
GGGTCCCACTNAGGNGGNAANANTACTAACTTCCANAANGGGNNNNNGNGNTNAGTGGATTNANCACCAANGGCNA
NCCNNTGNGTGNTTCCACGCCANCATNNAGCCCNCNANTTTAAAAACTCCATAATGATNCTTCCNCAAGGTCNCCTN
CCGAAACCTTGTTACNANNTTTACNTCCCCCAAANNAAANCANAAANAA

Group E:

RLH15(ITS1-F)

AGCGGAAGGATCATTATCGAGTTTTTAAAATCGAGGGCTTGATTGCTGGCGTGGAAACACGCACTGTGCTCGGCCTT
CGTGCTTAATCNACTCAACACCTGTGCACCTTATCGAAGTTAGTAGTCTTTCCTCCTTAGTTGGAGCCGCCGGGGTTG
ACTTTGTTAGTAGTGTTTCGACGCGAAACTATGGTCGGCCTTGGCTGGGATCGGCGAGCACTTTGACTTCATCATACA
CACTTTAATTGTCTTGTAGAATTGTAATGCTCCTTGTGGGCGAAATGAAATACAACTTTCNACAACGGATCTCTTGGCT
CTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTNAATCATCGAATCTTTGAA
CGCACCTTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTGTCATGTTAACATCAAACCCCTTGCTTGTAAA
GGCTCGGGGCTTGGATTTGGAGGTTTATGCCGGCCTGCTTCATTGTCAGTTGTCGGCTCCTCTTAAATGNATTAGCT
GGACTTTGGTCTGCGTGTCGGTGTGATAGTTTATTCACCAATCGCTTTCCTAATGGGTCTGCTTCTAATGGNCTTCGG
ACAAGGTCTTAACAGCCTTCTTGACTCTTTGACCTCAAATCAGGTAGGACTACCCCGCTGAACTTTAAGCATATCAATA
AGCGGAGGAAAAGAAACTAACCAANGATTCCCCCNANTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAAATCT
NGCGGGNCTTATGGGCGNCCCGAGTTG

RLH15(ITS4)

GAGCTTTTCCCGCTTCACTCGCAGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAAGT
TCAGCGGGTAGTCCTACCTGATTTGAGGTCAAAGAGTCAAGAAGGCTGTTAAGACCTTGTCCGAAGACCATTANAAG
CAGACCCATTAGGAAAGCGATTGGTNAATAAACTATCACACCGACACGCAGACCAAAGTCCAGCTAATGNATTTAAGA
GGAGCCGACAACNGACAATGAAGCAGGCCGGCATAAACCTCCAAATCCAAGCCCCGAGCCTTTACAAGCAAGGGGT
TTGATTGTTAACATGACACTCAAACAGGCATGCCCCTCGGAATACCAAGGGGCGCAAGGTGCGTTCAAAGATTCGAT
GATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCCTGCGNTCTTCATCGATGCGAGAGCCAAGAGATCC
GTTGTTGAAAGTTGTATTTCATTTCGCCCACAAGGAGCATTACNTTCTACAAGACAATTAAAGTGTGTATGATGAANTC
AAAGTGCTCGCCNGATCCCAGCCAAGGCCGACCATAGTTTCGCCGCCGAAACACTACCTAACAAANTCNACCCCNN
CNGCTCCCAACTAAGGAGGAAAGACTACTAANTTCCGATAAAGTGCACAGGTGNTTGAGTGGGATTAAGCCCGAAGG
GCCNAGCACAGTGCGTTGTTTCCACGCCAGCATCCAAGCCCCTCGATTTTAAAAAACCCAATAATGATCCTTTCCGCA
GGTCCACCTACCGGAAAACCTGGTACGA

Group F:

CAB2(ITS1F)

TGCNGGNATTNGGTANCNNGAGGCGGATNTGNGCGTTGGAACAATGATTCCTTCCGCAGTGTACACTTATCGAAGCC
TTTGCATGCCACGCAAGTGGTAGACCCAAGCGACTTGAGAAAACAATTTGGCGTCGTTATGCAAGTCAGCGCTCCGC
TGGCGACACTTTCGAATTGACGGGGACACCCTAAAGCCGGTCGCACCAACCTTGTCCGGGGAAACCTGGCGGGGG
GCCTGTGGTAATGACACAGGGGATGGTAACAGACGACCTGGATAGTTCTTGCACCCGCAGAGAGCATGGGCAATCC
GCAGCGAAGCCCCTACGTCCCTCCCCCGGTCCCCCGGGGGAGAAGGATACGGGGAACGTTCACAGACTAAGTGGA
AGTGGGTGGGATCGGACGATCCTGCTTAAGATATAGTCGGGCCCCCGGGGAAACCCGGGGGGGCGAGTACACTGC
TATGACCGCCCGCAATCACCGTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTCGTGGCCCA
ACCTCCCACCCGTGTTGACCGTTACCTGTTGCTTCGGCGGGCCCGCCGTTACCCCGGCCGCCGGGGGGTCCTCCT
GCCCCCGGGCCCGCTCCCCGCCGAAGACCCCTCGAACGCTGCCTGAAGGTTGCCCGTCTTGAGTATGATTATCAAA
TCATTAAAAACTTTCNACAACGGATCTCTTGGTTCCGGCATCGATGAAAGAAACNCANCGAAATGCGATAAGTNATGT
GAATTGCANAATTNCGTGAATCNTCNAATCTTTGAAACCACATTGCGCCCCCTGGCATTCCGGGGGGGGCATGCCTTT
CCCANCGTCATTTGCTACCCNCCACCCCGGCTTGTGTGTTNGGCCCCCNTCCCCCCCGNNGGACTNGCCCCNAAAG
NCANCGCCGCANCCGCGTCCCGTCCCCCAACNTATTGGGGCTTTGNCCCCNCCTCTTTNNGGCCCGCCGGGTTCTG
G

CAB2(ITS4)

TTCTTNGCTATCCCTACCTGATCCAGAGGTCAACCTAGAGAAAAATAANGTGACCGTGAGGTCGTCGGCCAGCAGCC
GGCCGGGCCTACAAGAGCGCGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGG
GCCCGTCCCCCGGGGGGGACGGGGGCCCAACACACCAGCCGGGCTGGAGGGTAGCAATGACGCTCGGACAGGCA
TGCCCCCCGGAATGCCAGGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACGGAATTCTGCAATTCACATTACT
TATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAATGATTGATAATCATA
CTCAGACGGNAACCTTCAGGCAGCGTTCGAGGGGTCTTCGGCGGGCGCGGGCCCGGGGGCACGAGGGCCCCCCG
GCGGCCGGGGTAACGGCGGGCCCGCCNAAGCAACAGGTAACGGTCAACACGGGTGGGAGGTTGGGCCACCAGGA
CCCGCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAACGGTGATTGCGGGCGGTCATAACAGTGTACTCGC
CCCCCGGGTTTCCCCGGGGGCCCGACTATATCTTAAGCAGGATCGTCCGATCCCACCCACTTCCACTTAGTCTGTGA
ACGTTTCCCGTATCCTTCTCCCCCCNGGGGAACCGGGGGAAGGACGTAAGGGCTTTCCTTCCGGATTGCCCATTGC
TCTCTGCGGGTGCANAACTATCCAGNCGCCTGGTNCCATCCCCTGTGTCNNTTACCACANGCCCCCCCCCAGNTTTC
CCCGGACAAAGGTTGNTGCGAACCCGGTTTTANNGTGTCCCNTCAAATTCAAAGTTGTCGCCNANCGNACCCTTGAC
TTGCATANCNNCCCCAAANTGTTTTCNCCAANTCCCNTTGGGGCTNCACTTGCTTGGCTGAAAANGTTTCNANNNN

Appendix 2.1. 2-way ANOVA for mean percent deviation of specific gravity (SG) due to decay volume and discoloration and interaction.

```
Categorical values encountered during processing are:
VCLASS3 (3 levels)
          1,        2,        3
COLOR (3 levels)
          1,        2,        3

Dep Var: SG AVE PERCENT DEV.  N: 212

Analysis of Variance
Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

VCLASS3                   204.649    2      102.325       1.807       0.167
COLOR                     536.789    2      268.394       4.741       0.010
VCLASS3*COLOR             391.110    4       97.777       1.727       0.145

Error                   11492.270  203       56.612
```

Appendix 2.2. 2-way ANOVA for mean percent deviation of modulus of elasticity (MOE) due to decay volume and discoloration and interaction in static bending.

```
Categorical values encountered during processing are:
VCLASS3 (3 levels)
          1,        2,        3
COLOR (3 levels)
          1,        2,        3

Dep Var: MOE AVE PERCENT DEV.  N: 212

Analysis of Variance
Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

VCLASS3                  3146.536    2     1573.268      13.363       0.000
COLOR                    2314.489    2     1157.245       9.829       0.000
VCLASS3*COLOR             950.917    4      237.729       2.019       0.093

Error                   23900.201  203      117.735
```

Appendix 2.3. 2-way ANOVA for mean percent deviation of modulus of rupture (MOR) due to decay volume and discoloration and interaction in static bending.

```
Categorical values encountered during processing are:
VCLASS3 (3 levels)
        1,        2,        3
COLOR (3 levels)
        1,        2,        3

Dep Var: MOR AVE PERCENT DEV.   N: 212

Analysis of Variance
Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

VCLASS3                 4590.184     2     2295.092      11.489       0.000
COLOR                   4786.718     2     2393.359      11.980       0.000
VCLASS3*COLOR            864.729     4      216.182       1.082       0.366

Error                  40553.625   203      199.772
```

Appendix 2.4. 2-way ANOVA for mean percent deviations of compression parallel to grain (CPG) due to decay volume and discoloration and interaction in static bending.

```
Categorical values encountered during processing are:
VCLASS3 (3 levels)
        1,        2,        3
COLOR (3 levels)
        1,        2,        3

Dep Var: CPG AVE PERCENT DEV.   N: 212

Analysis of Variance
Source             Sum-of-Squares   df  Mean-Square     F-ratio       P

VCLASS3                 1338.752     2      669.376       5.280       0.006
COLOR                    355.461     2      177.730       1.402       0.248
VCLASS3*COLOR            175.828     4       43.957       0.347       0.846

Error                  25734.795   203      126.772
```

Appendix 2.5. Tukey's Multiple Comparisons for mean percent deviation of specific gravity (SG) by decay volume and discoloration classes.

```
Test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle
                        G G G G G G G G G
                        r r r r r r r r r
                        o o o o o o o o o
                        u u u u u u u u u
                        p p p p p p p p p

                        9 6 1 7 3 5 8 2 4
     Mean %   Group #
   -5.3508   Group 9
   -1.6700   Group 6
    -.5212   Group 1
     .3000   Group 7
     .4017   Group 3
     .9210   Group 5      *
    1.2504   Group 8      *
    2.3341   Group 2      *
    3.0531   Group 4      *
```

Appendix 2.6. Tukey's Multiple Comparisons for mean percent deviation of modulus of elasticity (MOE) under static bending by decay volume and discoloration classes.

```
Test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle
                        G G G G G G G G G
                        r r r r r r r r r
                        o o o o o o o o o
                        u u u u u u u u u
                        p p p p p p p p p

                        9 6 8 7 5 3 2 4 1
     Mean %   Group #
  -20.0000   Group 9
  -15.3800   Group 6
  -15.1100   Group 8
   -7.4725   Group 7      *
   -5.7225   Group 5      *
   -5.4228   Group 3      *
   -5.0606   Group 2      *
   -4.9700   Group 4      *
   -3.5900   Group 1      * * *
```

Appendix 2.7. Tukey's Multiple Comparisons for mean percent deviation of modulus of rupture (MOR) under static bending by decay volume and discoloration classes.

```
Test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle
                          G G G G G G G G G
                          r r r r r r r r r
                          o o o o o o o o o
                          u u u u u u u u u
                          p p p p p p p p p

                          9 6 8 3 7 5 4 2 1
    Mean %   Group #
  -33.8000   Group 9
  -24.3441   Group 6
  -23.2021   Group 8
  -15.3400   Group 3      *
  -14.4800   Group 7      *
  -14.4715   Group 5      *
  -13.4544   Group 4      *
  -10.9224   Group 2      *
   -8.8220   Group 1      * * *
```

Appendix 2.8. Tukey's Multiple Comparisons for mean percent deviation of compression parallel to grain (CPG) by decay volume and discoloration classes.

```
Test with significance level .05
(*) Indicates significant differences which are shown in the lower triangle
                          G G G G G G G G G
                          r r r r r r r r r
                          o o o o o o o o o
                          u u u u u u u u u
                          p p p p p p p p p

                          9 8 6 7 5 4 2 3 1
    Mean %   Group #
  -14.2736   Group 9
  -11.3700   Group 8
  -11.0714   Group 6
   -9.1700   Group 7
   -8.6210   Group 5
   -7.0325   Group 4
   -6.7041   Group 2
   -4.9600   Group 3
   -4.2800   Group 1      *
```