AN ABSTRACT OF THE THESIS OF

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in Forest Products presented on September 4, 1981

Title: Fluorescence Microscopy for Detecting Incipient Decay and Estimating Residual Strength of Wood

Abstract approved: Redacted for Privacy

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The objectives of this study were to 1) develop a relatively quick staining procedure that would detect incipient decay by color differentiation under fluorescence, 2) quantitatively assess the amount of decay present with the use of an image analyzer, and 3) relate loss in strength at various decay levels to staining for the presence of decay.

The fluorochrome stain acridine orange was used to stain non-decayed and decayed wood sections from end-grain wafers and small beams of Southern yellow pine (Pinus spp.) and Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco]. A .02% solution of acridine orange pH 6.0 was best for Southern yellow pine; a pH 8.0 was best for Douglas-fir. Staining the sections for two hours and washing them for 24 hours provided the best color differentiation between decayed and non-decayed areas. In wood with little or no weight loss (less than 3%), the springwood cells appeared bright green under fluorescence with traces of yellow-orange. As weight loss increased, more yellow and orange became evident. The latewood and ray cells fluoresced a yellow-orange most of the time regardless of percent weight loss.

The computer image analyzer detected the green and orange as different gray levels, the green registering a lighter shade than the orange. However, due to inherent anatomical features of wood and the dulling of fluorescence when a slide has been exposed to the illumination source for an extended period of time, this method is not practical.

Modulus of elasticity (MOE), modulus of rupture (MOR) and fiber stress at the proportional limit (FSPL) decreased significantly with increased weight loss. MOR was most affected sustaining a strength loss greater than 50% with a 3% weight loss in pine.

Linear regression analyses of MOE and MOR vs. specific gravity indicated a relationship between these two variables and also a significant difference in the strength property means of the non-decayed and decayed samples. Percent strength loss (MOE and MOR) vs. percent weight loss was also regressed. The coefficient of determination (r^2) for these regressions were low, with less than 50% of the variability being accounted for by percent weight loss.

Alkali solubility of pieces from end-matched beams was correlated with percent strength loss but less so than the correlations between strength and weight loss.

Radial sections from the broken beams stained the bright green typical of non-decayed wood possibly due to irregularities in the brown rot decay pattern, but more likely because the areas of the beam sampled may not have contained decay. Cross sections near the failure have responded better.

Fluorescence Microscopy for Detecting Incipient Decay and Estimating Residual Strength of Wood

by

Eini Carole Lowell

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed September 4, 1981

Commencement June 1982

APPROVED:

Redacted for Privacy

Professor of Forest Products
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Date thesis is presented September 4, 1981

Typed by Linda S. Crooks for Eini Carole Lowell

ACKNOWLEDGEMENTS

I would like to extend my sincere thanks to my major professor, Dr. Krahmer, for all his patience, support and guidance that was offered these past two years.

At this time I would also like to express my deepest gratitude to my family and friends for their continual sustenance, given during a period of my life when it was needed most.

Special thanks go to Dr. Rodney C. DeGroot and Bessie Earthly at the Forest Products Laboratory in Madison, Wisconsin for the preparation of the sample material and conductance of the alkali solubility test.

Appreciation is extended to Dr. Bruce Lightheart, Environmental Protection Agency Research Laboratory, Corvallis, Oregon for the instruction and use of the Quantimet 720 computer image analyzer.

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FLUORESCENCE MICROSCOPY FOR DETECTING INCIPIENT DECAY AND ESTIMATING RESIDUAL STRENGTH OF WOOD

INTRODUCTION

Decay of wood in service is well known as a major economic and safety problem. Unfortunately, by the time decay becomes noticeable the wood has lost much of its strength.

The early stages of decay, also known as incipient decay, are generally defined in one of two ways. The first, most common method and the one used for this study, is based on a weight loss of ten percent or less. The second is loss in specific gravity. Either way it is defined, strength losses of more than 50 percent have been found for certain properties with a weight loss of only one or two percent (Wilcox, 1968).

Many methods have been employed for the detection of incipient decay but most of these are time consuming and are unable to quantitatively assess the extent of the decay. Current microscopy techniques require much preparation time and skill in evaluating a wood sample for evidence of decay such as the presence of hyphae or their bore holes. Even if positively identified, decay still cannot be quantitatively measured.

OBJECTIVES

The objectives of this study were: (1) to develop a relatively quick staining procedure that would provide a means for detecting incipient decay by color differentiation between decayed and non-decayed areas under fluorescence, (2) to quantitatively assess the amount of decay present with the aid of an image analyzer that would detect those color differences, and (3) to perform strength testing to determine loss in strength properties at various decay levels and relate this to the staining for the presence of decay.

LITERATURE REVIEW

Much study and research have been and are being conducted in the field of fungal decay in wood. Areas such as detection of decay in structural members and the strength loss associated with fungal degradation are necessary from the standpoint of public safety as well as economics. Many approaches have been taken in the past to detect decay in its incipient stage before much damage has occurred to the wood cell's chemical composition and physical structure, thereby altering the overall wood properties. Therefore, a review of wood chemistry and structure as it relates to fungal activity and its detection is discussed.

The Chemical Composition of Wood and Organization of the Cell Wall

Chemically, wood is composed of three polymeric materials: cellulose, the hemicelluloses and lignin. There are other substances present in smaller quantities, including minerals, starches, pectins (Nicholas, 1973, Chap. 4) and various extracts such as terpenes, tanins, resins and polyphenols (Panshin and deZeeuw, 1980).

Cellulose is a linear chain composed of anhydro-D-glucopyranose units. Cellulose molecules are arranged in a crystalline pattern. These cellulose molecules hydrogen bond to form elementary fibrils which are, in turn, bundled to form microfibrils. The microfibril model proposed by Ranby indicates that the microfibrils are not crystalline along their entire length. The highly ordered (crystalline) areas are alternated with what are known as the amorphous, or

porous, regions of a lower crystallinity (Panshin and deZeeuw, 1980).

Hemicelluloses are short, branched polysaccharide chains that are comprised of different monosaccharides. These sugars include glucose, galactose, mannose, xylose and arabinose. It is thought that the hemicelluloses surround and infiltrate the amorphous regions of the cellulose microfibrils as a matrix (Nicholas, 1973, Chap. 4).

Lignin has a complex chemical structure that is amorphous and highly branched (Nicholas, 1973, Chap. 4). The phenyl propane unit is the basic building block of the lignin molecule. Lignin is responsible for the rigidity of the cell wall (Panshin and deZeeuw, 1980).

The cell wall is described as being organized in layers. Between cells there is a middle lamella. The cell wall itself is in two layers, the primary (outer) wall and the secondary wall. The primary cell wall and the middle lamella combined are referred to as the compound middle lamella. The lumen is the cavity enclosed by the secondary cell wall.

The middle lamella is composed mainly of densely packed lignin with a few hemicelluloses present. There is no evidence of cellulose being a constituent (Wenzl, 1970).

The primary cell wall is laid down first and is made up of cellulose embedded in a matrix of hemicellulose, pectic materials and a large amount of lignin (Panshin and deZeeuw, 1980).

Following formation and enlargement of these cells, the secondary cell wall is laid down. This wall is made up of three

layers that are high in cellulose content with small amounts of lignin and hemicelluloses present. Each layer (referred to as the S1, S2 and S3) has its microfibrils laid down at a different orientation and also at varying thicknesses. The S2 layer is the thickest with the S1 and the S3 being much thinner layers. An important modification within the secondary cell wall is the formation of pits (a gap in the cell wall). The pits allow for transport of materials from one cell to another.

Types of Wood Rot

The three basic classifications of the type of damage done by fungi in wood are known as white rots, brown rots and soft rots based on the wood's physical appearance after degradation. The white and brown rot fungi belong to the class Basidiomycetes. The soft rot fungi are in the class Ascomycetes or Fungi Imperfecti. The effect of fungal decay on cell wall structure varies with the type of decay.

White rot fungi initially attack both lignin and cellulose. The wood tends to lose its color (appearing bleached) but does not appear structurally deformed.

Brown rot fungi preferentially attack the carbohydrate fraction of wood leaving a lignin skeleton (Nicholas, 1973, Chap. 2). The wood acquires a brown color due to the presence of quinoid or melanin-type substances (Bauch et al., 1976). Because the brown rot fungus attacks both along and across the grain, the wood looks cracked and collapsed (Nicholas, 1973, Chap. 2).

Soft rot fungi remove the cellulose, hemicelluloses and lignin. The lignin is depleted at a much slower rate than the polysaccharides.

The wood retains its shape but when dry becomes soft and brittle. Because brown rots are primarily responsible for the degradation of softwoods in service, only their decay mechanism will be discussed.

Decay Mechanism of Brown Rots

Brown rots utilize both the celluloses and the hemicelluloses, leaving the lignin. Initially, the products of fungal degradation are produced faster than the hyphae can utilize them (Highley and Kirk, 1979). Cell wall resistance has been correlated with lignin content; the greater the amount of lignin, the more resistant the cell wall (Wilcox, 1968). In the final stages of decay, a skeleton of primarily lignin (Keilich et al., 1979) and modified (oxidized) lignin remains (Highley and Kirk, 1979).

Two modes of attack by the brown rot fungi have been proposed. The original theory for the brown rot mechanism has an enzymatic basis. Enzymes are secreted by the hyphae and tend to diffuse into the void areas in the cell wall allowing for an extensive penetration of the area. Therefore, attack is not just in the area directly surrounding the hyphal strands. Because of this feature, wood can undergo damage both along and across the grain. These enzymes attack the cellulose chain internally (endohydrolysis) (Keilich et al., 1970) along the entire length of the polymer. The enzyme may not be a cellulase as cellulases have too large a molecular structure to fit into capillary spaces (Highley and Kirk, 1979). Wilcox (1968) refers to these enzymes as cellulolytic.

The recently proposed theory deals with the production of extracellular hydrogen peroxide (H_2O_2) (Koenigs, 1972). Hydrogen peroxide is formed intracellularly as a metabolic byproduct of many microorganisms and also extracellularly by brown rot fungi (Schmidt, 1980). This non-enzymatic process relies on the action of hydrogen peroxide and ferrous iron [Fe (II)] which is found naturally in wood at low levels. The degree of polymerization (DP) of cellulose is decreased by the action of the H_2O_2 and Fe(II). This decrease in DP results in a rapid decrease in strength. It has also been shown that this proposed method predisposes the cellulose chain to attack by cellulases (Koenigs, 1972).

Brown rot attack of the cell wall is initiated by solitary hyphae through pit apertures and not by forming bore holes (Wilcox, 1968). These hyphae extend longitudinally in the cell lumens. Because cellulose and some hemicelluloses are removed by the fungus, the remaining lignin provides a framework allowing the cell to hold its shape. A thinning of the cell wall occurs with the removal of the cellulose and hemicelluloses which is not consistent from cell to cell. One cell might be highly decayed while an adjacent cell may not be affected by the fungus at all. The S2 layer is attacked initially even though the hyphae may not be in contact with that layer. The compound middle lamella may be thinned in areas during advanced stages of decay but generally it appears intact (Wilcox, 1968).

The only microscopically visible signs of attack are presence of the hyphae, enlarged pit apertures and perhaps thinned and/or cracked cell walls.

The Effect of Decay on Strength Properties

Since strength is the primary reason for wanting to detect decay early, many physical tests have been performed on decayed wood. Static and impact bending, toughness, compression, tensile and crushing strength have all been studied with respect to decayed versus non-decayed wood.

In 1971, Cartwright et al. determined that the modulus of elasticity (MOE) was affected a little more than the modulus of rupture (MOR). Mulholland (1954) found the opposite to be true, that the MOR was affected to a greater degree than the MOE. Both these experiments were performed on brown rotted softwoods. In a test with tropical hardwoods (the one this study's experimental procedure was fashioned after), Kennedy (1958) found that brown rots reduced the MOR to a greater extent than did white rots. The type of wood and the species of fungus can affect the amount of strength lost (Wilcox, 1978).

Methods for Detecting Decay

There are numerous field tests to detect the presence of decay (Graham and Helsing, 1979). None of these tests are completely reliable. A hammer can be used to sound poles for internal rot. The pick test (lifting a sliver of wood) indicates whether the wood

will fail abruptly as decayed wood tends to or splinter, as solid wood is prone to do. Other methods include the use of the Shigometer to measure electrical resistance, a Pol-tek which is a sonic testing device, and the use of resistance moisture meters. Removing increment cores in the field can show many things. It can give an indication of shell thickness and in many cases allows for visual evaluation of the internal wood. The core can further be used for culturing. Culturing of the core will give a positive identification for the presence and the type of decay.

Microscopic evaluation of thin wood sections may reveal the presence of hyphae, bore holes, thinned cell walls, cracks in the cell wall and enlarged pit openings (Wilcox, 1968). Staining of the tissue to differentiate the hyphae is another technique employed. Chemical tests such as alkaline solubility, osmium tetroxide as a color indicator test and checking for a difference in the heat of reaction in certain species of wood are some methods that have been investigated (Mothershead and Graham, 1962). Other nondestructive test methods include x-ray analysis for evaluating density and moisture content (Thorton et al., 1980), stress wave average velocity, resonant vibration, acoustic emission, response spectra and stress wave attenuation (Kaiserlik, 1978).

Because it is difficult to detect the presence of brown rot microscopically during the early stages of decay, new approaches are being investigated. One such project being conducted at Hokkaido University in Sapporo, Japan deals with the use of the fluoro-chromatic stain acridine orange for delineating areas within the

cell that are decayed. White, brown and soft rot samples are all being tested utilizing this method.

The stain acridine orange has been used by many scientists to demonstrate various applications. It has been used to determine the presence of bacteria in soil (Strugger, 1948), to selectively stain tumor cells (Lewis and Goland, 1948), to stain nucleic acids (von Bertalanffy and Bicks, 1956) and also for staining plant tissues (Stadelmann and Kinzel, 1972; Vlachos and Tharouniatis, 1963).

It is evident that at varying concentrations and at varying pH levels the fluorescence pattern changes (Lillie, 1977). May (1948) showed this for bacteria. The color changes in his experiment occurred not because the bacteria were living or dead, but were actually a result of differences in the concentration and pH of the acridine orange stain. Martin and Ortiz (1967) used acridine orange in the staining of plant tissues. They found that the fixation medium was another factor affecting the color imparted to the specimens. It was determined that weak concentrations (0.1%) of acridine orange were more useful than strong ones (1.0%). A stronger solution yielded excess stain in the tissue that was difficult to wash out. The pH of the stain was also found to influence the color imparted to the tissue. As the pH increased, the fluorescent color pattern was found to go from pale yellow to bright orange in the same samples. At a pH of 6.0 to 8.0, the best differentiation of materials occurred.

Wood normally autofluoresces a bright green without the aid of a stain. When Fukazawa et al. (1976) extracted each chemical component from the cell wall and stained each with acridine orange, the

cellulose fluoresced a greenish-yellow; the holocellulose, red; the lignin, a yellow-green or greenish yellow; and the extractives, red. The secondary wall colored a green or yellow-green while the compound middle lamella was more of a yellow-orange color.

The first study done by Fukazawa et al. (1976) used samples of beech (Fagus crenata Blume) and spruce (Picea jezoensis Carr.). The samples were incubated with the brown rot fungus Coriclellus palustris. Small blocks of wood were then embedded in methacrylate and one micrometer sections cut. These sections were then stained with a .02% solution of acridine orange in a citrate-phosphate buffer of pH 6.0 for three hours. They found that with decayed wood the secondary walls had a fluorescence pattern that went from a green or yellow-green to orange to red with increasing amounts of decay. These results were repeated by Yamashita et al. (1978) using the white rot fungus Coriolus versicolor. Samples for this experiment were also methacrylate embedded and sections cut to a thickness of one micrometer. A 1.0% solution of acridine orange in a citrate-phosphate buffer of pH 6.0 was used as the staining medium. Fukazawa et al. (1976) and Yamashita et al. (1978) attribute the red discoloration to the decomposition of polysaccharides and/or the release of lignin. Another possible explanation they propose is that the acridine orange combines with substrates in the region attacked by the fungal enzymes.

Further experimentation by Yamashita et al. (1979) revealed that the cause of the red discoloration was different for different fungi. This experiment utilized chlorite delignified spruce to simulate a

white rot condition stained for three hours with .02% acridine orange. They maintain that the red discoloration in this experiment was predominantly oxidized lignin.

Bauch et al. (1976) also used acridine orange under fluorescent light and noted that there were possible alterations in the chemistry of the cell wall. Using ultraviolet light they discovered a distinct alteration in lignin in light of 280 nanometers in Scots pine (Pinus sylvestris L.) decayed by the brown rot fungus Coniophora cerebella.

MATERIALS AND METHODS

Two species of wood were involved in this study. They are Southern yellow pine (Pinus spp.) and Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco]. The samples were prepared and decayed at the USDA Forest Service Forest Products Laboratory in Madison, Wisconsin by Bessie Earthly under the supervision of Dr. Rodney C. De Groot. Two sample types of each species were sent. All samples were sapwood. For microscopy purposes, wafers 2.57 centimeters square (end surface) by .32 centimeters along the grain were requested. The static bending samples consisted of 40 end-matched miniature beams (one set to be controls and the other set to be decayed). These measured 11.43 cm long X 1.27 cm wide X .48 cm thick (Figure 1). These dimensions are in accordance with a previous experiment conducted by Kennedy (1958). The samples (excluding the controls) were decayed with the brown rot fungus Gloeophyllum trabeum.

Sample Preparation

The wafers were initially placed in an 80°F/30% relative humidity room and allowed to come to equilibrium. The samples were weighed at random intervals and their weights recorded. The wafers were then placed on screens that had been sterilized at 121°C for 15 minutes under 15 pounds of pressure. These screens were in turn placed over malt agar plates that had a fungal growth on them. The plates were incubated at 80°F/70% relative humidity and after three days, three of the wafers were removed from the decay chamber. The

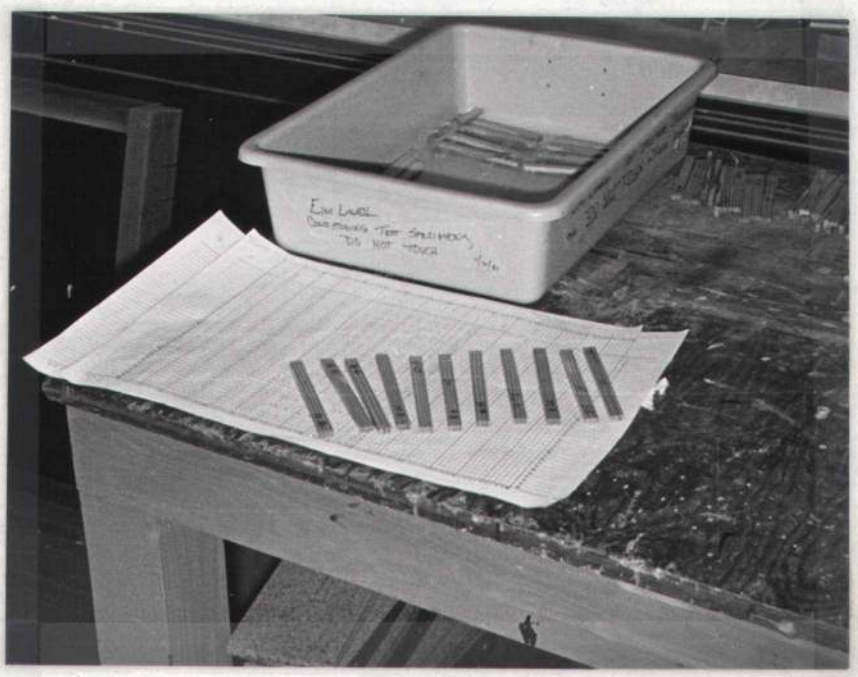

Figure 1. End-matched miniature beams used in static bending test.

fungal growth was brushed off and the specimens allowed to air dry for one week. They were then placed in the 80°F/30% relative humidity room to equilibrate for approximately two weeks and then weighed. Following the first three days of incubation and removal of three wafers, three more specimens were removed from the decay chambers every other day.

The end-matched beams were also initially placed in the 80°F/30% relative humidity room and allowed to equilibrate. They were then weighed at random intervals and the weights recorded. The beams were steam sterilized for one-half hour at 121°C under 15 pounds of pressure. The decay chamber for the beams was a soil bottle. In preparation, the soil had to be at its moisture holding capcity for best results. The calculated moisture content was lower than the moisture holding capacity so water had to be added to the soil. The moisture content of the soil was calculated by recording the weight of a sample bottle and then reweighing the bottle after it was three-quarters filled with soil. The bottle was then oven-dried at 104°F for 24 hours and weighed again upon removal from the oven. This was done in triplicate. The percent moisture was calculated for the three replicates and averaged. The moisture holding capacity (in this case 43 grams of water per 100 grams of soil) minus the calculated moisture content equals the amount of water to be added. Once this had been determined, 127 grams of soil was placed in each of 80, two quart bottles and leveled off on its side. Feeder strips 12.7 centimeters long were laid over the soil and 16 cc of distilled water (the amount needed to bring the soil up to moisture holding

capacity) was pipetted over the strip. The bottles were loosely capped and sterilized for one-half hour at 121°C under 15 pounds of pressure, then allowed to cool overnight at room temperature. When cool, the soil was inoculated aeseptically with plated cultures of Gloeophyllum trabeum and incubated in the environmental chamber at 80°F/70% relative humidity for six weeks or until the fungal growth had completely covered the feeder strip. Two of the pre-sterilized beams were then aeseptically placed into each decay chamber and incubated in the 80°F/70% relative humidity room. The Southern Yellow Pine beams were initially incubated for two weeks and the Douglas-fir beams for four weeks. At the end of the initial incubation period, two beams were removed from the environmentally controlled decay chamber, brushed free of mycelial growth, weighed and allowed to air dry for one week after which time they were reweighed. The samples were then placed in the 80°F/70% relative humidity room for two weeks and weighed again. Following the initial incubation period, two beams were removed every day for the first month and after that every other day until all the beams had been removed. The percent weight loss of each beam due to decay was calculated and recorded.

Microscopy

The decayed and the non-decayed wafers received were cut into small pieces for sectioning with the sliding microtome or to be embedded in methacrylate for sectioning with a rotary microtome. The blocks that were cut with the sliding microtome were first

softened by placing them in a beaker of distilled water, pulling a vacuum on them to remove the air from the cell lumens and then boiling them for a short duration (two to four hours) or else just allowing them to soak overnight. The sections cut with the sliding microtome ranged in thickness from ten to 30 micrometers.

Smaller blocks cut from the wafers were embedded in methacrylate using the method outlined by Berlyn and Miksche (1976). These embedded samples were sectioned on the rotary microtome to thicknesses of four or five micrometers. Prior to staining, the methacrylate was removed from the sections using chloroform.

The fluorochrome stain acridine orange was used to stain the sections. It is available in powder form to be made into a solution. In this experiment, water or a citrate-phosphate buffer was the liquid medium. The buffer solution was prepared according to McIlvaine (1921) to three different pH levels: 4.0, 6.0 and 8.0. A range of percent weight losses for each species was stained at each pH level. The excess stain was removed by washing the sections in water.

The sliding microtome sections were taken through an alcohol dehydration series ending with the clearing agent xylene. The rotary microtome sections were oven dried. All sections were then permanently mounted on glass slides using the medium Permount.

These slides were observed under an incident light Lietz fluorescence system through a Ploemopak filter module H_2 using a xenon light source. Photomicrographs were taken either as prints or slides using Kodacolor II or Kodachrome 64 film.

Computer Image Analysis

A set of Southern yellow pine slides with weight losses ranging from 0.24% to 8.46% were chosen for image analysis. The Quantimet 720 is a system of mutually compatible modules defined by image analysis functions and marketed by Image Analysing Computers Limited (Fisher, 1971). There are four parts to the analysis of images: acquisition of the image, conversion of the image into a suitable electrical signal, the extraction of the image data and the collecting of the data (Cruttwell, 1974).

A Lietz fluorescent microscope system using incident light was used for the acquisition of the image. A scanner picked up this image and relayed it to a screen as a black and white image. The 720 scanner scans 720 lines sequentially 10.5 times/second. Each line is composed of pixels (picture points) and these lines are identical in length and precisely positioned (Fisher, 1971).

The measurements made dealt with area percentage. The image data is measured in terms of picture points. The area percentage for these samples was to be determined using gray levels (or contrast differences). It was hoped that the orange stain of the decayed areas would transmit at a different gray level than the bright green of the non-decayed areas. The gray level to be evaluated can be input into the computer and a digital output of the number of pixels at that level can be read directly from the scanner screen. The number of pixels at a given gray level was graphically plotted for each percent weight loss sample.

Static Bending Test

The end-matched beam specimens (one non-decayed, one decayed) received from the Forest Products Laboratory were initially placed in a controlled environment room of the Forest Research Laboratory, Oregon State University, Corvallis, and allowed to equilibrate to an equilibrium moisture content of approximately 7.5%. Their weights at the time of testing were recorded. Also recorded were the length, width and height of each beam (Appendices A and B).

The static bending test was performed on the Instron testing machine. The span of the beams was 8.9 centimeters (a span/depth ratio of 17.5). One end of the beam was on a fixed support while the other support could move to permit adjustment for slight changes as the beam was deflected (Figure 2). The beams were center loaded on the radial face at the rate of .05 cm/minute through a bearing head with a radius of 0.95 centimeters. The load-deflection curve was graphically plotted by the Instron testing machine (Figure 3) at a chart speed of 2 cm/minute. The maximum load, the deflection and the load at the proportional limit were taken from these graphs.

When the testing was completed, the samples were oven dried at 105°C and weighed so the moisture content and specific gravity at the time of testing could be determined. Small pieces of the beam were removed from the area near the failure for sectioning with the sliding microtome and microscopic evaluation. Another small piece of each beam was returned to the Forest Products Laboratory in Madison, Wisconsin for an alkali solubility test.

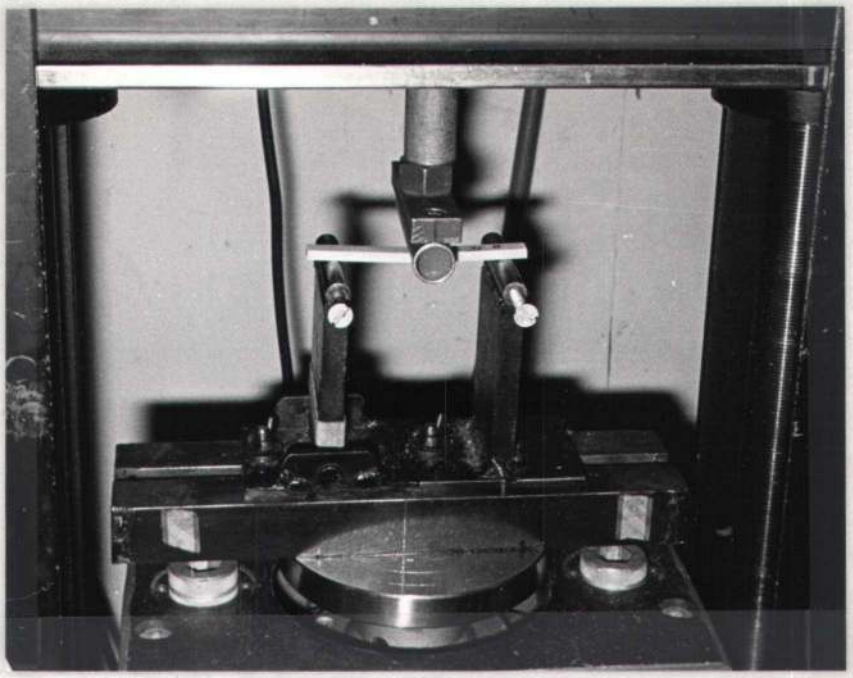

Figure 2. Static bending test apparatus shown in testing.

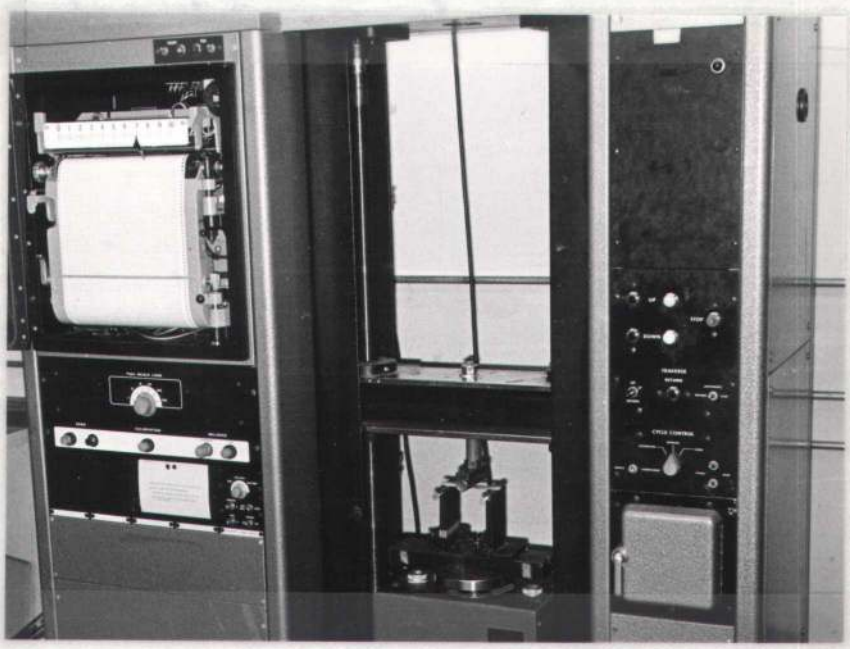

Figure 3. The Instron testing machine illustrating static bending test in progress with the load-deflection curve being graphically plotted on the chart to the left.

Analysis of Data

The raw strength data were filed in the computer. The moisture content and specific gravity of the beams at the time of testing were calculated. Also calculated were the modulus of elasticity (MOE), modulus of rupture (MOR), fiber stress at the proportional limit (FSPL) and the percent loss in these strength values between the non-decayed and decayed end-matched beams. The means and the standard deviations were calculated for the specific gravity, MOE, MOR and FSPL of each species for the 40 non-decayed and the 40 decayed samples. An analysis of variance was conducted using the SIPS statistical package and the above factors for each species. A linear regression analysis was run for each species on the variables MOE and MOR versus specific gravity for both the control and the decayed specimens. A scatter diagram of these regressions was produced. A regression analysis and scatter diagram were also done on the data for percent strength loss versus percent decay for each species.

RESULTS AND DISCUSSION

Sectioning and Staining Technique

Although incipient decay is generally defined in terms of a piece of wood having lost ten percent or less of its mass due to the degrading action of a fungus, the wafer samples received ranged from a weight gain of 1.00% to a weight loss of 12.74% in the Southern yellow pine. The Douglas-fir wafer samples appeared to be more resistant to the decay. Their range was from a maximum weight gain of 2.25% to a maximum weight loss of 4.03%. When the fungus initially attacks a piece of wood, the hyphae usually yield more byproducts than they can utilize. The addition of these byproducts plus the addition of the fungus itself may be responsible for the weight gains seen during the very early stages of decay. The Southern yellow pine samples were the first to arrive and because they offered a wider range of percent weight losses, much of the experimental microscopy work was done with these samples.

In removing portions of the wafers for sectioning, an attempt was made to get small areas in which decay may have been present. At some of the higher weight losses, the wafers were discolored or appeared crumbly, aiding in this attempt at ensuring the presence of decay. As mentioned previously, rots do not produce consistent damage within a piece of wood and it is possible to cut a section that does not contain any decay even though the fungus is present in other areas. This can be seen in Figure 4 in which a cross section of white rotted Douglas-fir shows two adjacent areas

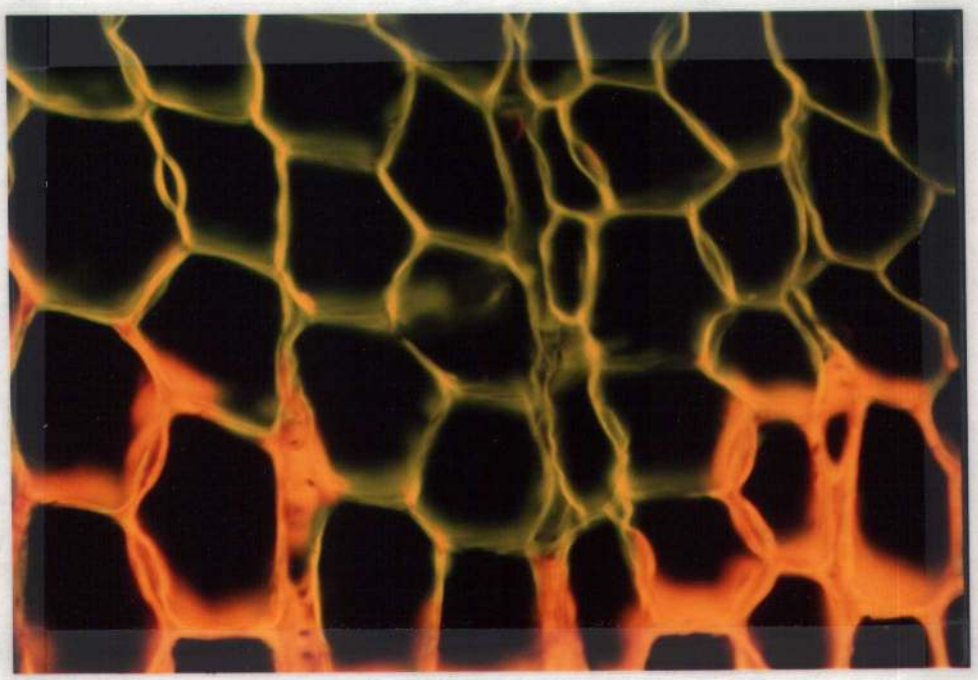

Figure 4. Cross section of white rotted Douglas-fir stained with acridine orange showing a bright green, non-decayed area adjacent to an orange, decayed area. Incident fluorescence, 330X.

responding differently to the acridine orange stain. The orange portion indicates a decayed area while the bright green is the characteristic fluorescence of normal wood.

Initially, some of the samples were embedded in methacrylate. Cross sections approximately five micrometers thick were cut on the rotary microtome. These had a tendency to tear and crush. Because embedding is a time consuming process and because of the difficulties encountered in sectioning and handling, thicker sections from the radial surface of softened blocks were cut on the sliding microtome. It was also of interest to see if an increase in the cell wall materials altered the staining pattern that Fukazawa et al. (1976) reported.

The Southern yellow pine sections were cut from wafers that ranged from weight losses of 0% to 8.46%. The blocks were placed in a beaker of distilled water and a vacuum pulled. If the blocks sank upon placement in the distilled water, they were allowed to soak overnight to soften them. This was generally the case with the more decayed samples. The controls and lesser decayed blocks were boiled in distilled water for about two hours following the vacuum and then allowed to cool overnight.

When the blocks were of a seven or eight percent weight loss, they were difficult to section because the cell walls would tear and crumble. Radial face sections were the easiest to cut and also offered a large area of the cell wall surface for microscopic evaluation.

Since acridine orange is in itself colored orange and also appears orange under the fluorescent microscope, one of the major

problems faced was whether the orange color evident in the decayed sections was actually a result of the piece being decayed or if it was residual stain. Several staining and washing schedules were tried in an attempt to alleviate this uncertainty. The schedules experimented with consisted of three different staining times (15 minutes, two hours, and overnight) and four different washing times for each of the staining times (15 minutes, one hour, two hours and 24 hours). The washing was accomplished by placing the sections in small, perforated metal containers. These containers were then placed in a beaker where a continuous flow of water was pumped through.

The fluorochrome stain acridine orange used in this initial procedure was mixed in a solution of 0.2 M disodium-phosphate and 0.1 M citric acid to a pH of 6.0 according to McIlvaine (1921). The concentration was .02%. Both the pH and concentration of the stain were the same as employed in the studies done at Hokkaido University (Fukazawa et al., 1976; Yamashita et al., 1978).

The shorter wash times (15 minutes, one hour and two hours) did not prove satisfactory as not all of the excess stain was removed. Figure 5 is an illustration of a non-decayed piece of wood that has been stained with acridine orange and undergone a short wash. Instead of fluorescing the bright green that normal wood does, the orange color of the stain dominates. Figure 6 shows a non-decayed section that has been stained with acridine orange and washed long enough so the green comes through.

Figure 5. Cross section of non-decayed Southern yellow pine stained with acridine orange and washed for a short period of time. Incident fluorescence, 330X.

Figure 6. Cross section of non-decayed Southern yellow pine stained with acridine orange and washed for an extended period of time. Incident fluorescence, 330X.

The stain and wash schedule determined to be the most effective for the thicker sections was two hours staining time and washing for approximately 24 hours. Upon removing the sections from the stain, it was found to be advantageous to first place the sections in a beaker of distilled water and mildly agitate them to remove some of the excess acridine orange before placing them in the metal washing containers.

Another problem was incurred in the mounting of the sections. If xylene (the clearing agent following the alcohol dehydration process) was present on the finished slide in any quantity, it provided a dull, greenish colored background which may or may not have affected the other colors observed. These slides were not given any further consideration.

Figures 7 through 10 illustrate the color pattern observed in radial sections of decayed samples of Southern yellow pine. Figure 7 shows a section with a less than one percent weight loss. The characteristic green fluorescence of normal wood can be seen in the walls of the earlywood tracheids. At levels below one percent, very little orange color can be seen in the walls with the exception of the latewood, the ray material and the cut surface of the tangential cell wall. These three areas tended to stain a yellow-orange color regardless of the amount of decay present. Perhaps this is because of an increase in cell wall materials in the latewood or due to extractives in the rays.

Sections with a weight loss between one and three percent contained much green area with traces of orange or a yellow-orange.

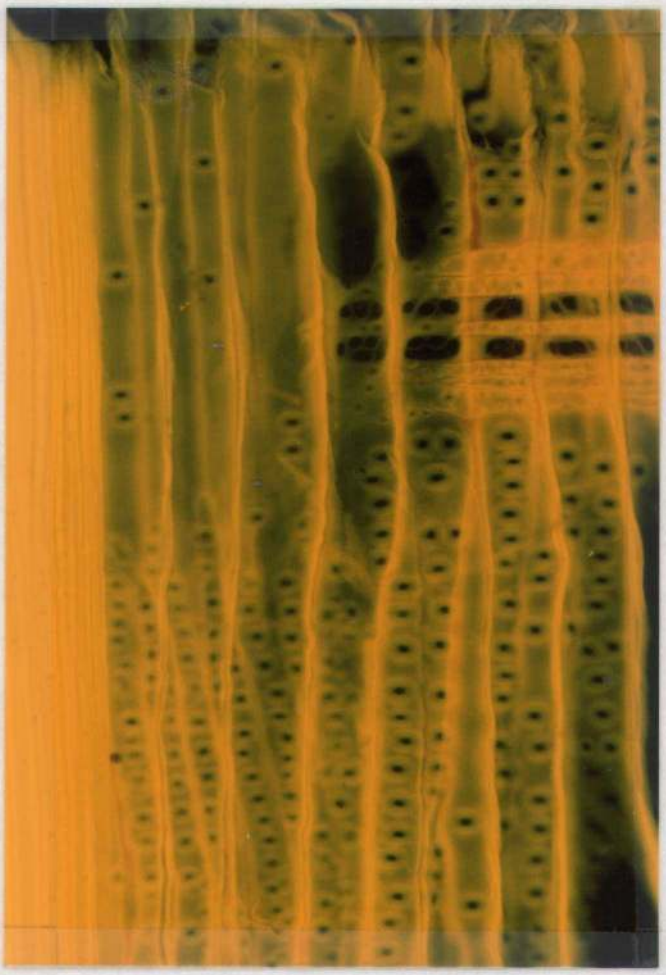

Figure 7. Radial section of Southern yellow pine having a 0.24% weight loss and stained with acridine orange, pH 6.0. Incident fluorescence, 150X.

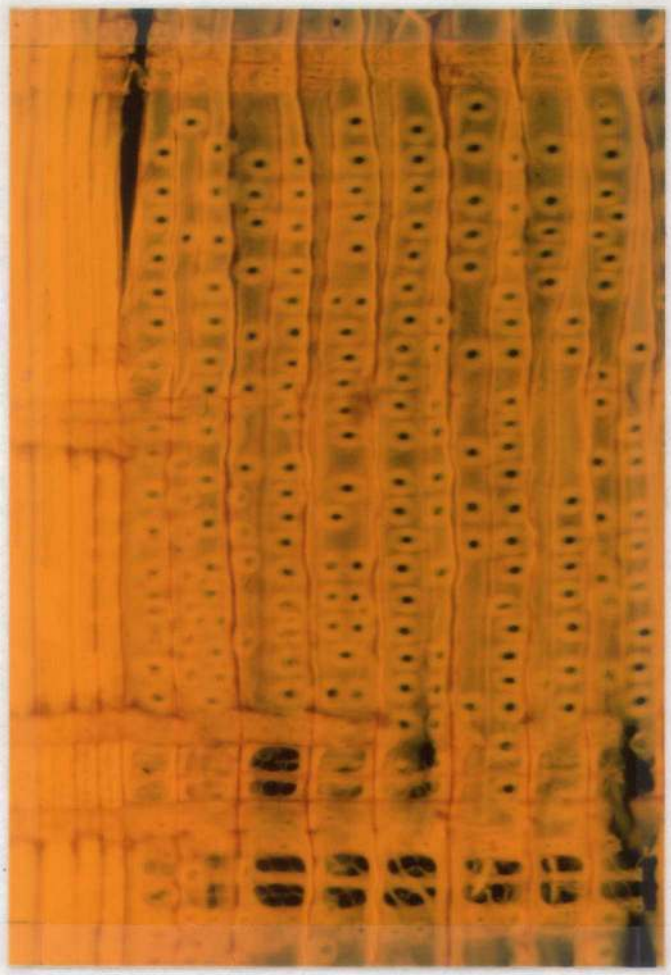

Figure 8. Radial section of Southern yellow pine having a 3.24% weight loss and stained with acridine orange, pH 6.0. Incident fluorescence, 150X.

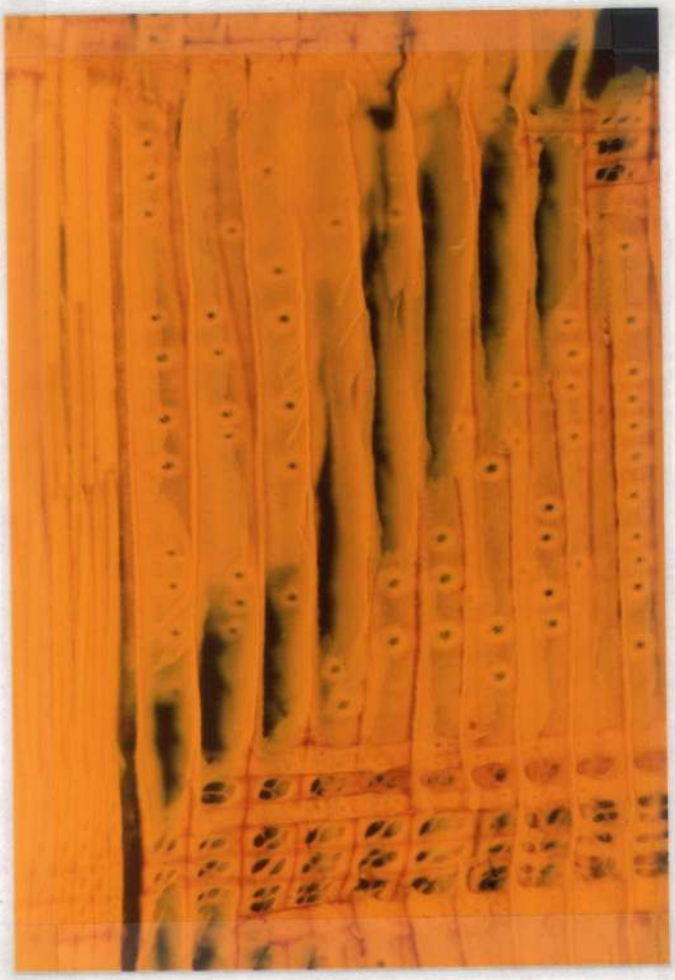

Figure 9. Radial section of Southern yellow pine having a 5.16% weight loss and stained with acridine orange, pH 6.0. Incident fluorescence, 150X.

Figure 10. Radial section of Southern yellow pine having an 8.46% weight loss and stained with acridine orange, pH 6.0. Incident fluorescence, 150X.

The non-uniformity in the distribution of the brown rot may be responsible for this type of patterning.

By the time the wood has sustained a weight loss of three percent, the orange color in the earlywood tracheid walls becomes much more predominant. Figures 8, 9, and 10 show successive amounts of orange present at increasing levels of weight loss (3.24%, 5.16%, and 8.46%, respectively). The latewood also takes on an increasing amount of orange pigment as the percent weight loss increases. Occasionally, the transition to red starts in the cut surfaces of the tangential cell wall but this is thought to be a reaction to something other than the presence of decay (Figures 8 to 10).

The acridine orange stain with a pH of 6.0 was found to impart the most distinguishable color pattern for Southern yellow pine. The Douglas-fir sections responded best to the stain with a pH of 8.0. Figures 11 and 12 show Douglas-fir radial sections which have been stained with acridine orange, pH 8.0. Figure 11 has no decay present (0.00% weight loss) and is the bright green characteristic of normal wood whereas Figure 12 has sustained a weight loss of 4.03% and has much orange coloring present.

Computer Image Analysis

The non-decayed areas of the stained wood sections transmitted as a lighter gray level than did the decayed areas. The output received is graphically represented in Figure 13. The number of pixels has been expressed in a logarithmic progression. It can be seen that differences do exist between the various levels of percent weight loss in terms of brightness levels. The curve representing

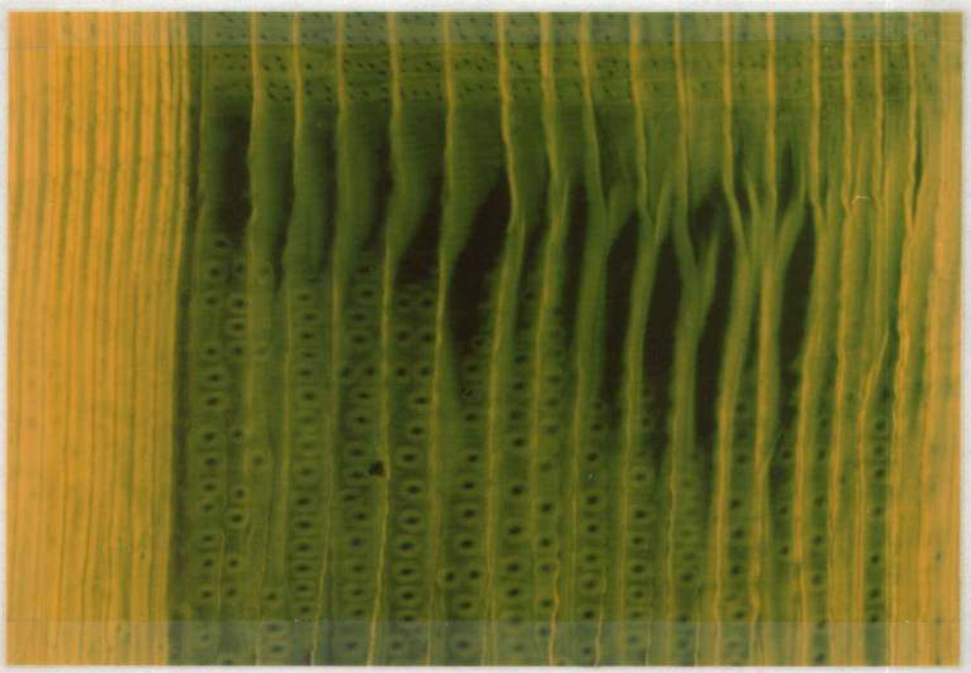

Figure 11. Radial section of Douglas-fir having 0.00% weight loss and stained with acridine orange, pH 8.0. Incident fluorescence, 150X.

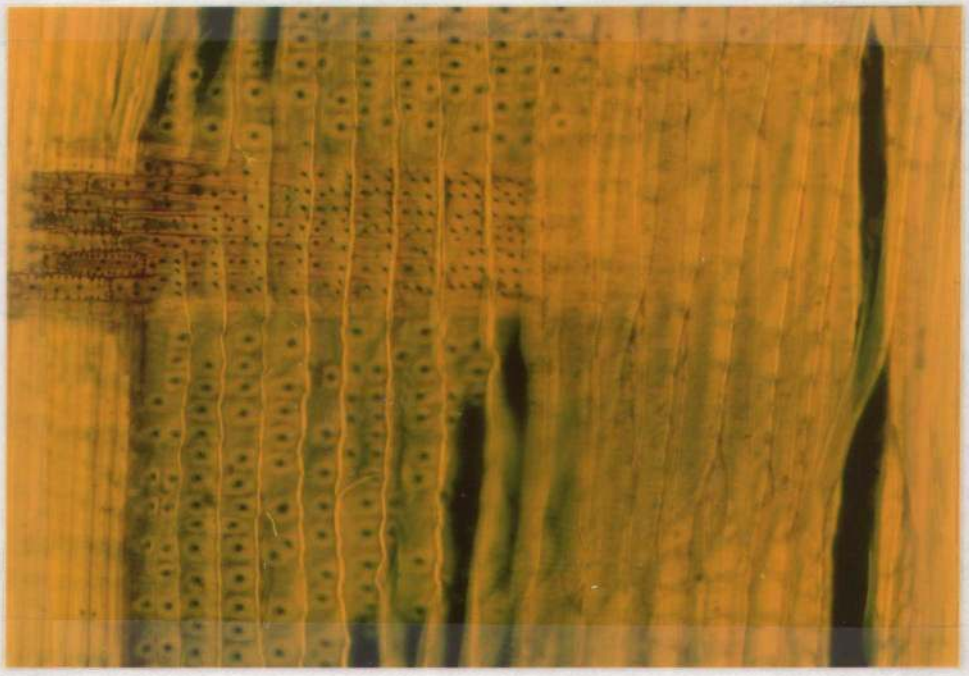

Figure 12. Radial section of Douglas-fir having a 4.03% weight loss and stained with acridine orange, pH 8.0. Incident fluorescence, 150X.

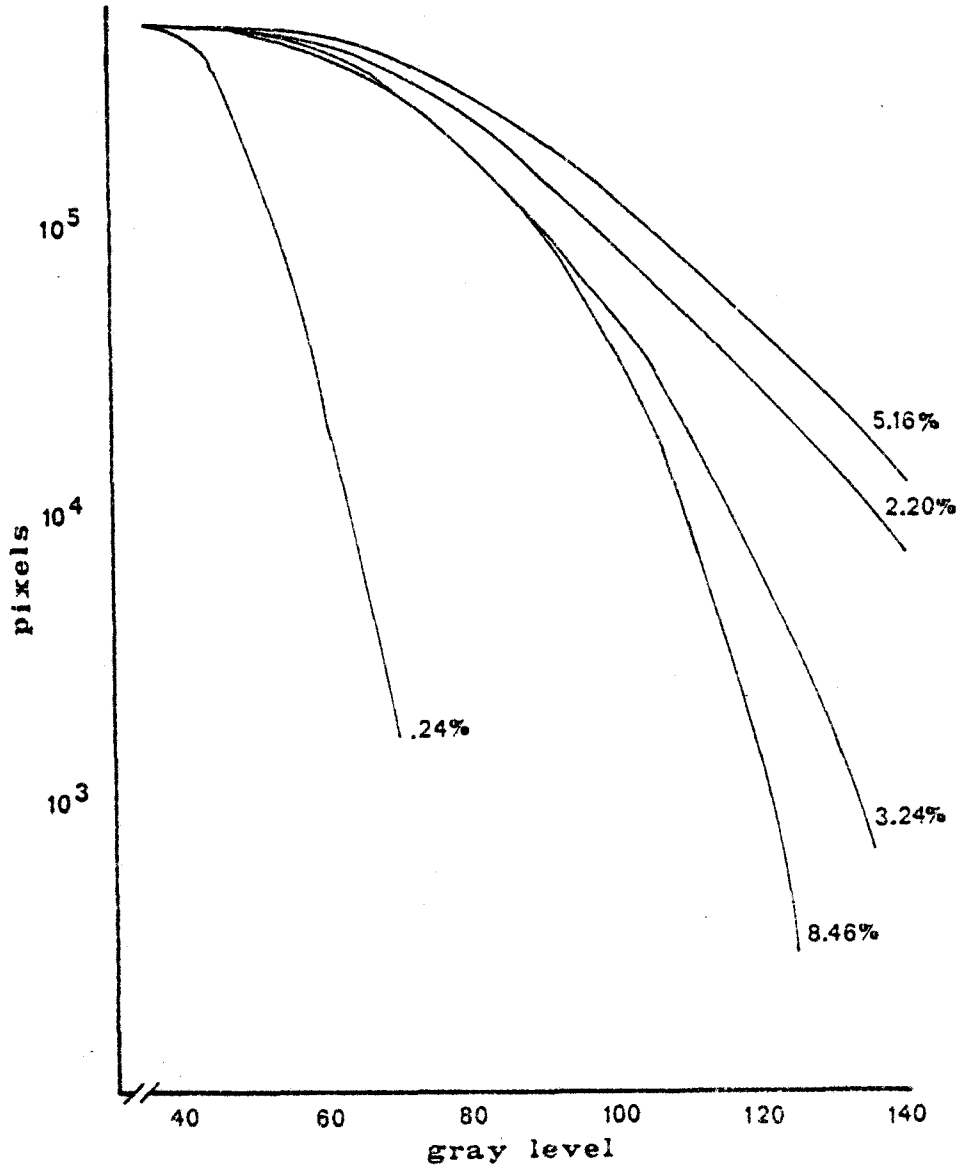

Figure 13. Results of computer image analysis for Southern yellow pine sections of varying percent weight losses. Gray level goes from bright to dark.

0.24% weight loss dropped off quickly as the brightness level decreased. The 3.24% and 2.20% curves did not fall as quickly indicating a certain amount of darker gray levels or orange decayed areas. The 5.16% curve contained areas that registered at both the upper and lower ends of the gray level scale. The 8.46% curve does not fit in with these others. There are many possible explanations for this as there are several problems with the image analysis approach.

One of the major difficulties in the image analysis process is determining the area of the slide to be scanned. In some cases there may be more earlywood or latewood present or there may be more bordered pits on the cell wall, both of which would affect the percent area output at a certain gray level. Differences in cell wall thickness, especially tears found in the cell wall of the more decayed samples, are another source of error. The presence of ray cells could also alter the results although an attempt was made to avoid them in the selection of an area to be scanned.

Another inherent problem in fluorescence microscopy has to do with a dulling of the fluorescence the longer a slide is exposed to the illumination source. This could result in changing gray levels for a same area if the data collection takes a long time.

A system that could analyze color wavelengths should be much more suited to this approach than an image analyzer that uses gray levels for its data output.

Strength Test Results

All the means, ranges, sample standard deviations and coefficients of variation are presented in Tables 1 and 2. Fiber stress at the proportional limit was calculated and listed in these tables and in Appendices C and D. However, since the load at the proportional limit was taken from the load-deflection curve graphically plotted during testing, variation from person to person is a likely possibility. Since this value is a less common one and not as important as the modulus of elasticity and the modulus of rupture, no further analysis was performed other than to note that the mean of the Southern yellow pine decayed samples, 7935 psi (54.7 megapascals), is significantly lower than the mean of the non-decayed samples, 13,492 psi (93.0 megapascals) at the 95% confidence level. The same holds true for the Douglas-fir samples with the decayed mean FSPL being 11,204 psi (77.3 megapascals) and the non-decayed mean 12,672 psi (87.4 megapascals).

Strength test data were analyzed in two ways. First was to determine if the non-decayed miniature beams responded as expected for clear wood of these species. And second, to determine the effect of decay on strength properties. Individual data for each species may be found in Appendices C and D.

Non-decay Test Results for Southern Yellow Pine

The values of modulus of elasticity (MOE) and the modulus of rupture (MOR) obtained in testing the non-decayed specimens are

TABLE 1. STATISTICAL DATA ON SOUTHERN YELLOW PINE STATIC BENDING SAMPLES.

	Specific Gravity	Modulus of Elasticity psi (megapascals)	Modulus of Rupture psi (megapascals)	Fiber Stress at Proportional Limit psi (megapascals)
Non-decay				
Mean	.63	2.70×10^6 (1.86×10^4)	25,611 (176.6)	13,492 (93.0)
Range	.52 - .71	2.07×10^6 - 3.21×10^6 (1.43×10^4 - 2.22×10^4)	19,415 - 30,438 (133.9 - 209.9)	9,558 - 16,129 (65.9 - 111.2)
Sample Standard Deviation	.0464	$.28 \times 10^6$ ($.19 \times 10^4$)	2,652 (18.3)	1,672 (11.5)
Coefficient of Variation (%)	7.3	10.4	10.4	12.4
Decay				
Mean	.61	2.14×10^6 (1.48×10^4)	9,442 (65.1)	7,935 (54.7)
Range	.50 - .71	1.61×10^6 - 2.88×10^6 (1.11×10^4 - 1.99×10^4)	5,661 - 15,930 (39.0 - 109.8)	4,125 - 13,626 (28.4 - 94.0)
Sample Standard Deviation	.0432	$.30 \times 10^6$ ($.21 \times 10^4$)	2,495 (17.2)	1,946 (13.4)
Coefficient of Variation (%)	7.1	13.9	26.4	24.5

TABLE 2. STATISTICAL DATA ON DOUGLAS-FIR STATIC BENDING SAMPLES.

	Specific Gravity	Modulus of Elasticity psi (megapascals)	Modulus of Rupture psi (megapascals)	Fiber Stress at Proportional Limit psi (megapascals)
Non-decay				
Mean	.57	2.45×10^6 (1.69×10^4)	20,525 (141.5)	12,672 (87.4)
Range	.53 - .60	2.08×10^6 - 2.81×10^6 (1.44×10^4 - 1.94×10^4)	16,940 - 24,265 (116.8 - 167.3)	10,198 - 15,518 (70.3 - 107.0)
Sample Standard Deviation	.0177	$.19 \times 10^6$ ($.13 \times 10^4$)	1,995 (13.8)	1,360 (9.4)
Coefficient of Variation (%)	3.1	7.7	9.7	10.7
Decay				
Mean	.56	2.20×10^6 (1.52×10^4)	16,218 (111.8)	11,204 (77.3)
Range	.49 - .60	1.42×10^6 - 2.76×10^6 ($.98 \times 10^4$ - 1.90×10^4)	8,463 - 23,497 (58.4 - 162.0)	4,879 - 14,849 (33.6 - 102.4)
Sample Standard Deviation	.0248	$.24 \times 10^6$ ($.17 \times 10^4$)	3,459 (23.9)	1,985 (13.7)
Coefficient of Variation (%)	4.4	11.0	21.3	17.7

actually a bit higher than the average given in the Wood Handbook. The average MOE and MOR calculated from the samples is 2.70 X 10^6 psi (1.86 X 10^4 megapascals) and 25,611 psi (176.6 megapascals), respectively. When adjusted to a 12% moisture content (they were tested at an average equilibrium moisture content of 7.40%) these values become a 2.36 X 10^6 psi (1.62 X 10^4 megapascals) for the MOE and 19,042 psi (131.3 megapascals) for the MOR. The Wood Handbook lists an average MOE of 1.98 X 10^6 psi (13,700 megapascals) and MOR of 16,300 psi (112 megapascals) for slash pine (Pinus elliottii Engelm.) at a 12% moisture content and a specific gravity of .59. Slash pine was chosen as a representative species of the general category Southern Yellow Pine for its high specific gravity and strength properties.

The higher the specific gravity a piece of wood has, the greater the amount of solid cell wall material (Panshin and deZeeuw, 1980) and thus a greater ability of the piece to resist an applied force. This specific gravity - strength relationship is illustrated in Figures 14 and 15. The average specific gravity of the non-decayed samples of Southern Yellow Pine was .63. This explains the higher average obtained for the MOE and MOR in testing of the clear specimens.

The r values from a linear regression analysis of the non-decayed samples are high (Figures 14 and 15). For MOE, 80% of the strength value can be explained by specific gravity. For MOR, this percentage is 84. It should be noted that these regression lines had a slope different from zero indicating statistically that a

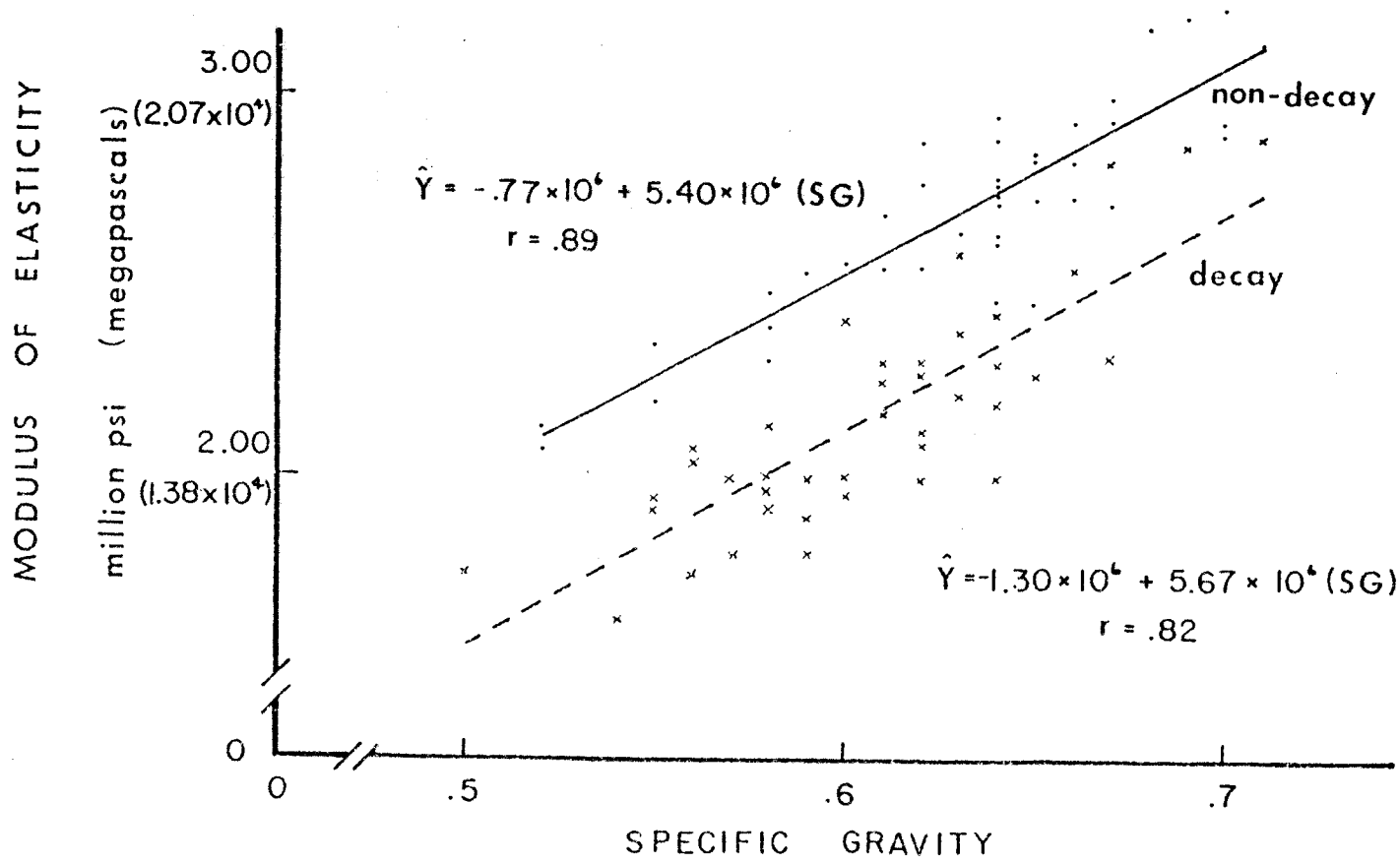

Figure 14. Linear regression of modulus of elasticity vs. specific gravity for non-decayed and decayed samples of Southern yellow pine. (Equations in psi)

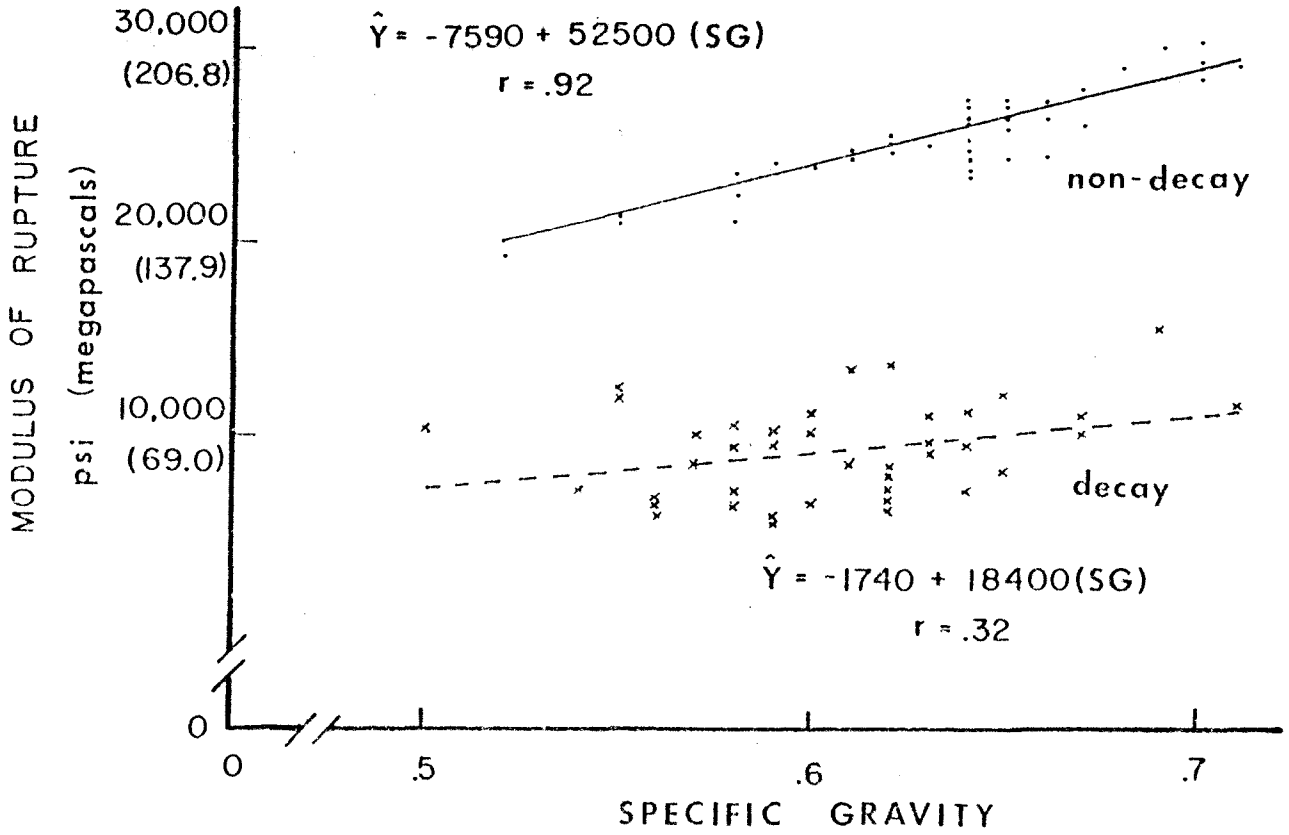

Figure 15. Linear regression of modulus of rupture vs. specific gravity for non-decayed and decayed samples of Southern yellow pine. (Equations in psi)

relationship does exist between the specific gravity and the strength value MOE or MOR in non-decayed wood.

The Wood Handbook values are based on standard tests of clear specimens according to ASTM standards (1979). The miniature beams tested in this study were not of standard size, but as indicated, they behaved as the standard beams in static bending do.

Non-decay Test Results for Douglas-fir

Again, the calculated values of MOE and MOR are higher than the average values listed in the Wood Handbook. The calculated average MOE was 2.45×10^6 psi (1.69×10^4 megapascals) and MOR was 20,522 psi (141.5 megapascals). These samples were at an average equilibrium moisture content of 7.66% and the MOE and MOR became 2.27×10^6 psi (1.57×10^4 megapascals) and 17,563 psi (121.1 megapascals), respectively, when adjusted to a moisture content of 12%. The Wood Handbook lists an average MOE of 1.95×10^6 psi (13,400 megapascals) and MOR of 12,400 psi (85.0 megapascals) for coastal Douglas-fir at a 12% moisture content with a specific gravity of .48.

The average specific gravity for the non-decayed Douglas-fir test specimens was .57. This explains the higher averages obtained in testing as compared to the Wood Handbook values. The relationship between specific gravity and strength for this species is shown in Figures 16 and 17. The r values from a linear regression analysis were very low for these clear specimens (.48 for MOE and .50 for MOR). This was found to be a result of cross grain in the samples.

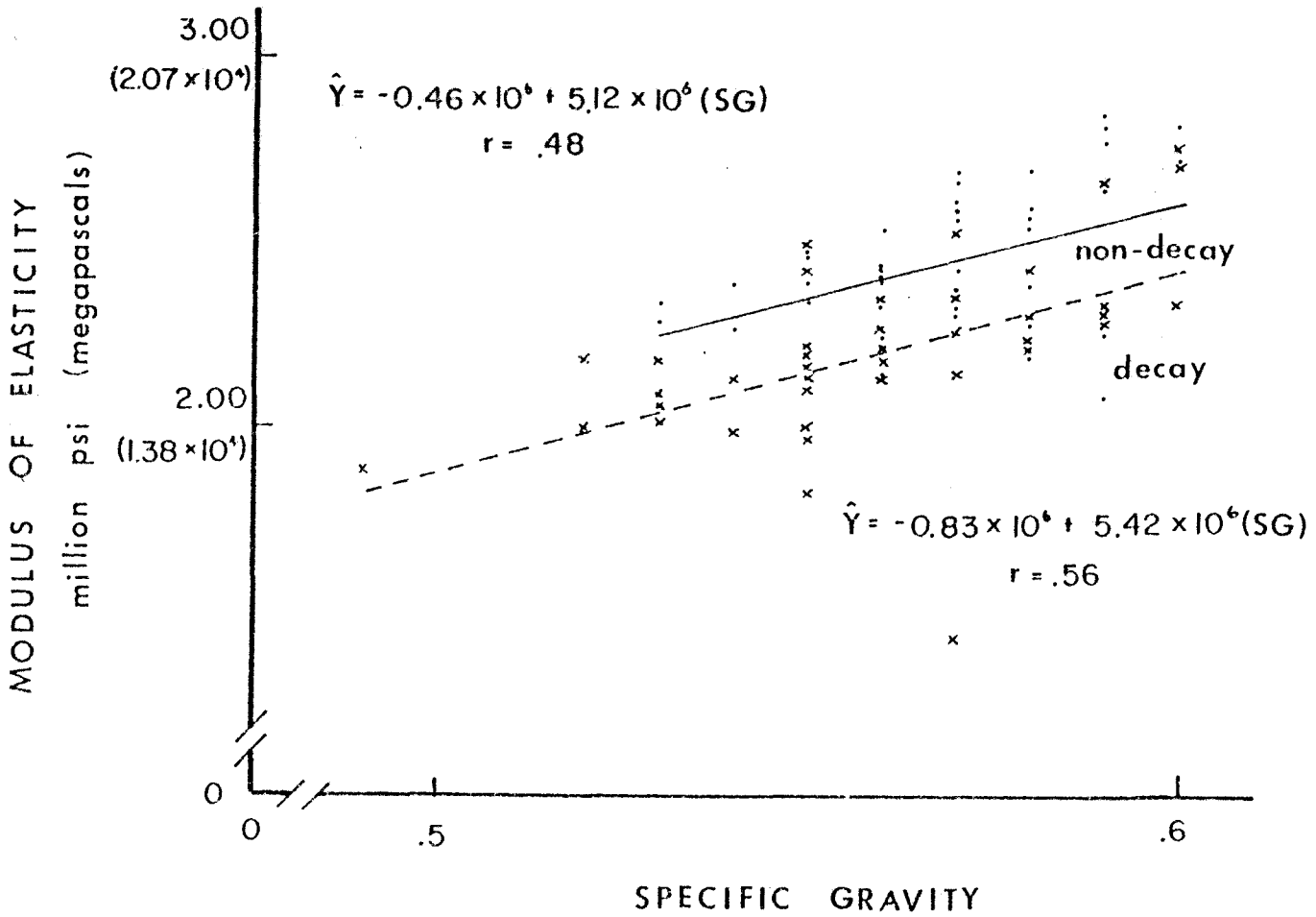

Figure 16. Linear regression of modulus of elasticity vs. specific gravity for non-decayed and decayed samples of Douglas-fir. (Equations in psi)

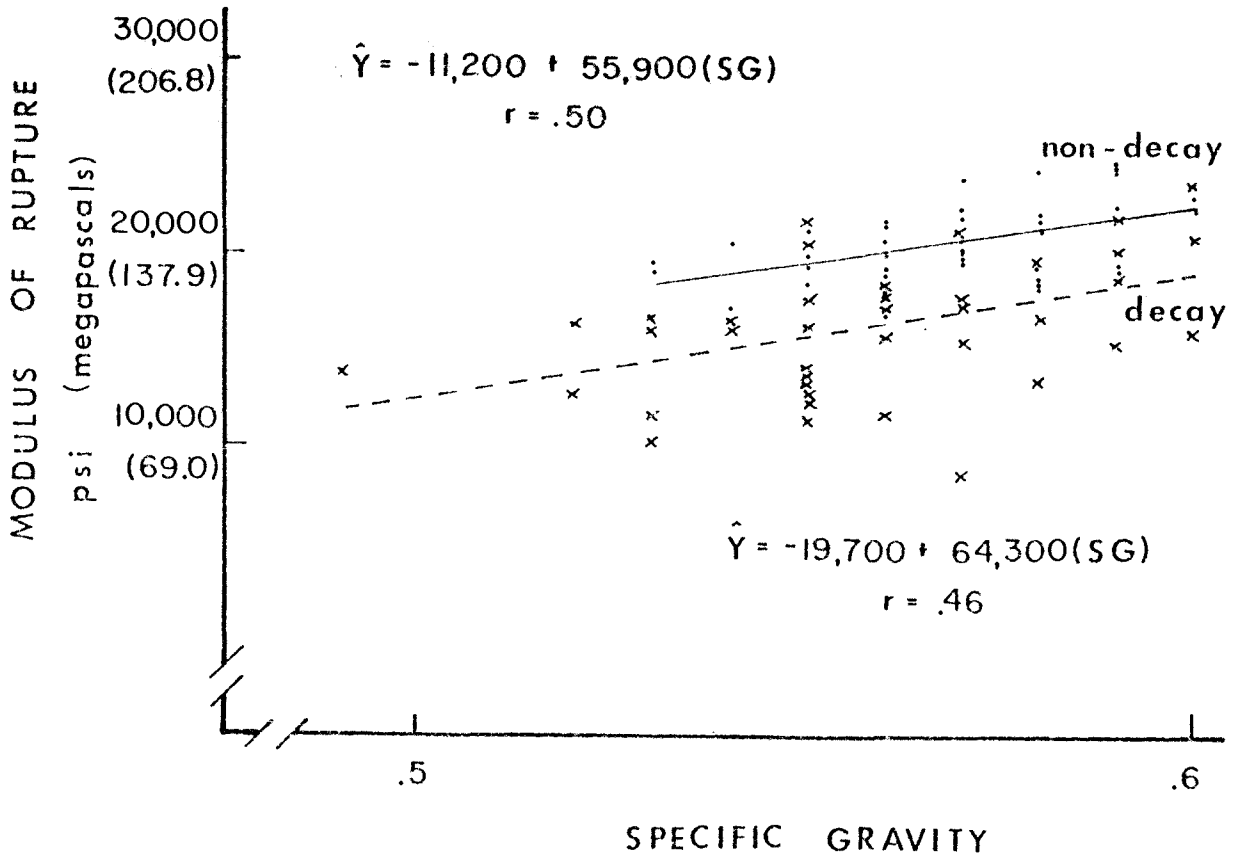

Figure 17. Linear regression of modulus of rupture vs. specific gravity for non-decayed and decayed samples of Douglas-fir. (Equations in psi)

Approximately half of the specimens contained a cross grain greater than 1 in 6. Because of this, another set of samples has been requested of the Forest Products Laboratory and this portion of the experiment is to be repeated. The slopes of these regression lines were different from zero indicating a relationship does exist between the specific gravity and strength value even if it does have a poor coefficient of correlation.

Effect of Decay on Strength

The relationship between specific gravity and strength of the decayed pieces was examined. Also observed was the percent weight loss and how it affected the percent strength loss (both MOE and MOR) for both species. These were done by performing a linear regression analysis. Differences in alkali solubility were compared with percent strength losses for the Southern Yellow Pine using a linear regression.

The strength property most affected by decay was the modulus of rupture, although both the modulus of elasticity and the fiber stress at the proportional limit (FSPL) were also decreased.

Southern Yellow Pine. The average specific gravity of the decayed samples was .61. A mean MOE of 2.14×10^6 psi (1.48×10^4 megapascals) and a mean MOR of 9,442 psi (65.1 megapascals) was calculated. An f-test on the means of the strength values between the non-decayed and decayed samples found the means to be significantly different from one another at the 95% confidence level.

Again, all the linear regression lines for the decayed samples have a slope different than zero meaning there is a correlation between specific gravity and strength (Figures 14 and 15). The r values for these lines are not as high as for the clear specimens indicating a lesser degree of correlation between specific gravity and strength value of the decayed specimens.

The scatter diagram of the strength property vs. specific gravity showed a wide range of points for the decayed samples which would account for the low correlations obtained. When comparing the mean specific gravity of the non-decayed and decayed samples it is evident that there is not much difference between the two (.63 and .61, respectively). Just how much of this difference is a result of decay and how much is due to inherent properties is hard to assess. To make a prediction of strength based on specific gravity would be difficult as some decayed pieces may have a higher specific gravity than non-decayed pieces. Predicting on the basis of loss in specific gravity would also be impossible as the initial specific gravity would not be available.

A linear regression analysis between percent weight loss (percent decay) and percent strength loss is illustrated in Figures 18 and 19 with the corresponding r values and also confidence limits at the 95% level. These lines also have a slope different than zero. The minimum MOE strength loss was 5% and the maximum was 39%. For the MOR, 41% was the minimum while 77% was the greatest percent MOR lost. The confidence interval in Figure 18 is a bit misleading as at certain percent decay levels a negative percent strength loss is predicted or, in other words, a strength gain. This is not a

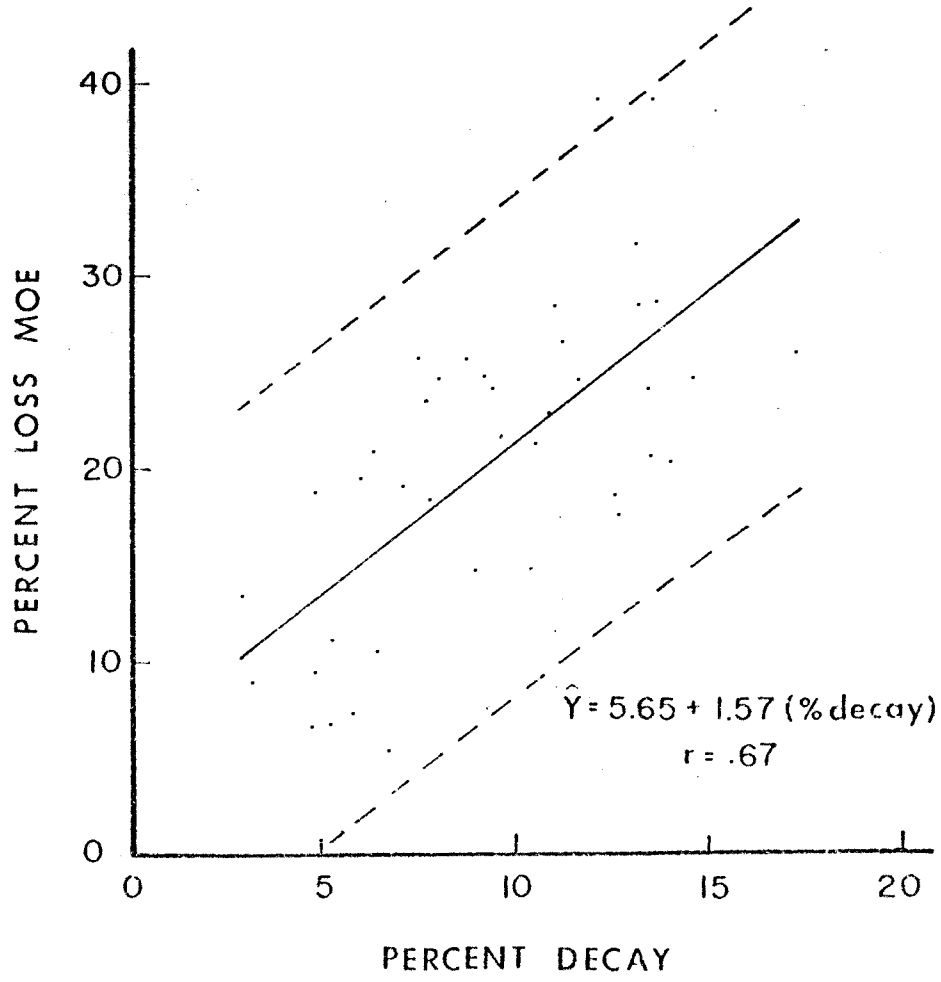

Figure 18. Linear regression for percent loss in modulus of elasticity vs. percent weight loss (percent decay) for Southern yellow pine with 95% confidence limits.

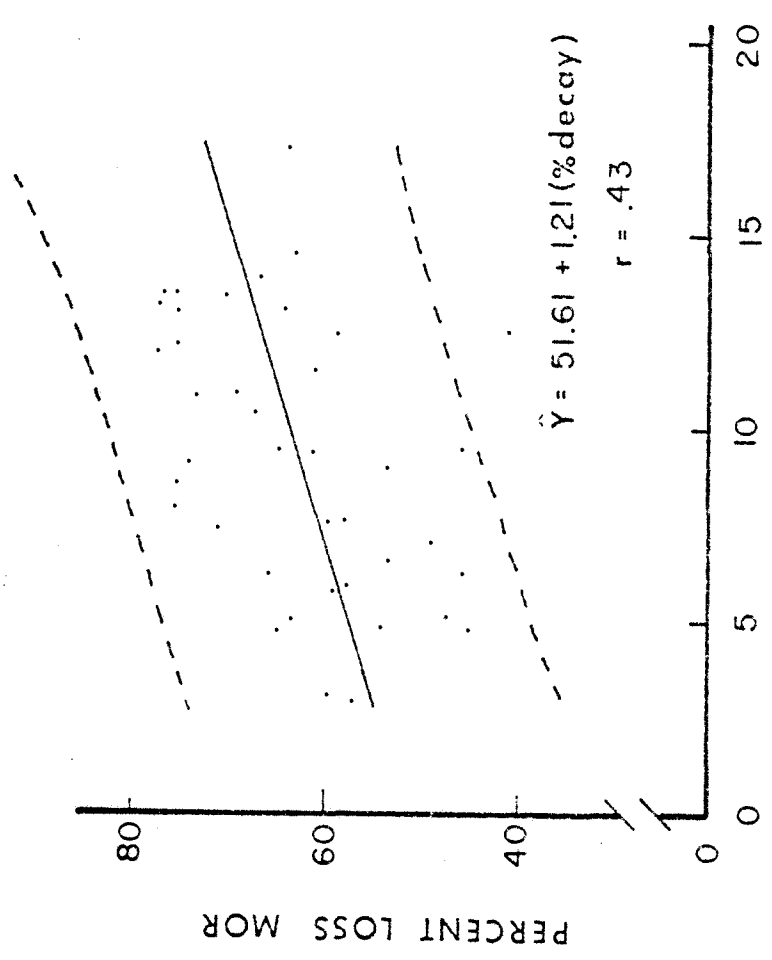

Figure 19. Linear regression of percent loss in modulus of rupture vs. percent weight loss (percent decay) for Southern yellow pine with 95% confidence limits.

viable possibility. The r values for these lines are low with less than 50 percent of the variability accounted for by the correlation of percent weight loss with percent strength loss.

The scatter diagram produced for the regression of percent strength loss vs. percent weight loss had an even wider range of points than did the strength properties and specific gravity. The major problem encountered with this approach for determining strength loss of wood in service lies in the fact that the initial weight (prior to decay) of the sample must be known for a weight loss to be calculated.

Douglas-fir. Although slope of grain was a problem in these samples, a comparison was made of strength losses because end-matched samples were prepared and might give an indication of the effect of decay on strength.

The average specific gravity of the decayed Douglas-fir samples was .56. The mean MOE was 2.20×10^6 psi (1.52×10^4 megapascals) and the mean MOR was 16,218 psi (111.8 megapascals). An f-test indicated a significant difference between the mean strength values of the non-decayed and the decayed samples at the 95% confidence level.

The regression lines and r values for MOE and MOR vs. specific gravity can be seen in Figures 16 and 17. There is a relationship between these two variables, however a lower r value for the decayed as compared to the non-decayed samples indicates a lesser degree of correlation for the decayed pieces.

Illustrated in Figures 20 and 21 are the linear regression analyses between percent weight loss (percent decay) and percent

strength loss (MOE and MOR). The MOR was affected more than the MOE for Douglas-fir, also. In some cases, the decayed pieces exhibited a negative strength loss (i.e. a gain in strength) for both the MOE and MOR. As was noted previously, this may be a result of the cross grain present. A possible explanation is that the non-decayed ends of the end-matched miniature beams had a greater amount of defect in the slope of grain than did the decayed ends. The maximum loss in MOE was 37% and for the MOR the percentage was 53. The confidence intervals at the 95% level seen in Figures 20 and 21 indicate a possibility of a strength gain. The r values are low indicating that these correlations are not measuring well the effects of decay.

Alkali Solubility

The alkali solubility test gave poorer correlations with strength and strength losses than did the weight loss variable. Similar correlations were obtained using either the differences in solubility (% alkali solubility of decayed specimen - % alkali solubility of non-decayed specimen) or the solubility of the decayed specimen alone when regressed with the percent strength loss. The advantage to this method for estimating strength loss is that solubility values can be measured for a decayed sample without any data on the condition of the wood prior to fungal degradation. Regression equations and corresponding r values can be found in Appendix E.

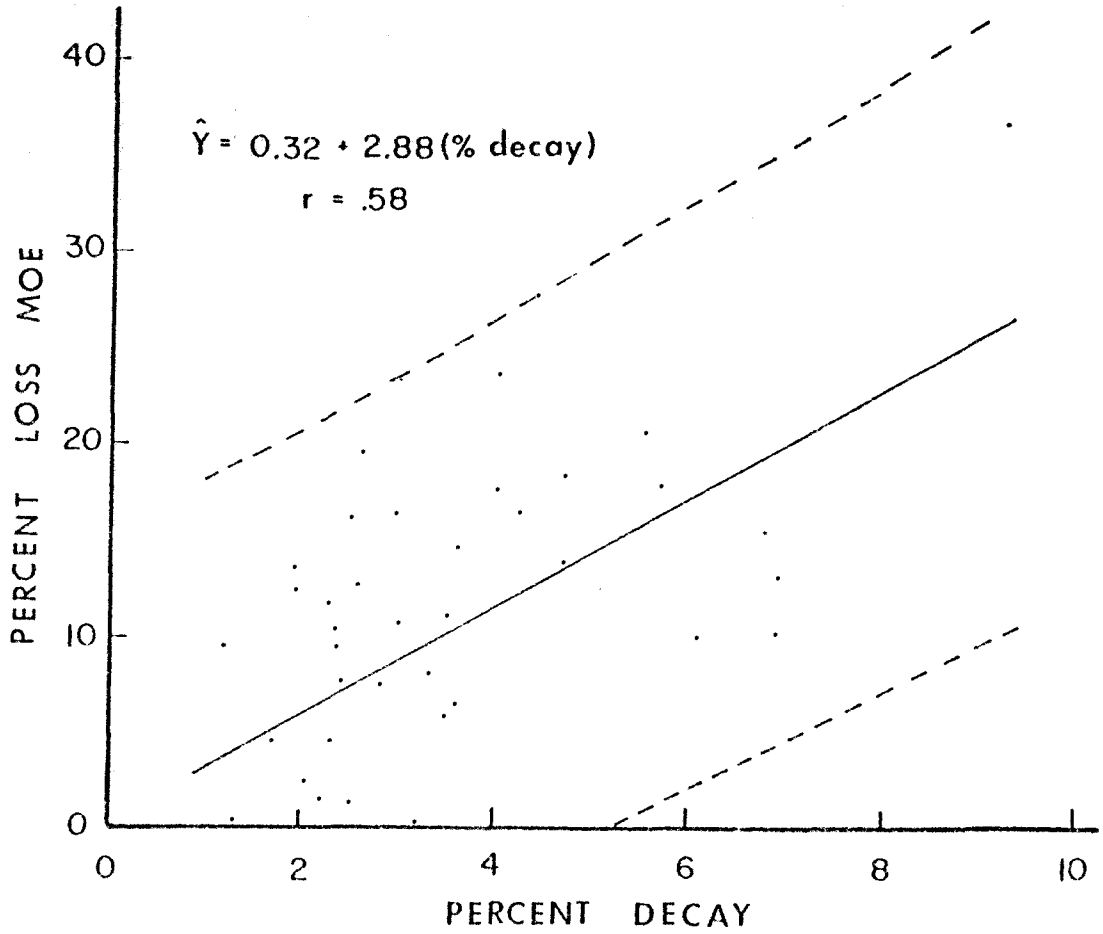

Figure 20. Linear regression of percent loss in modulus of elasticity vs. percent weight loss (percent decay) for Douglas-fir with 95% confidence limits.

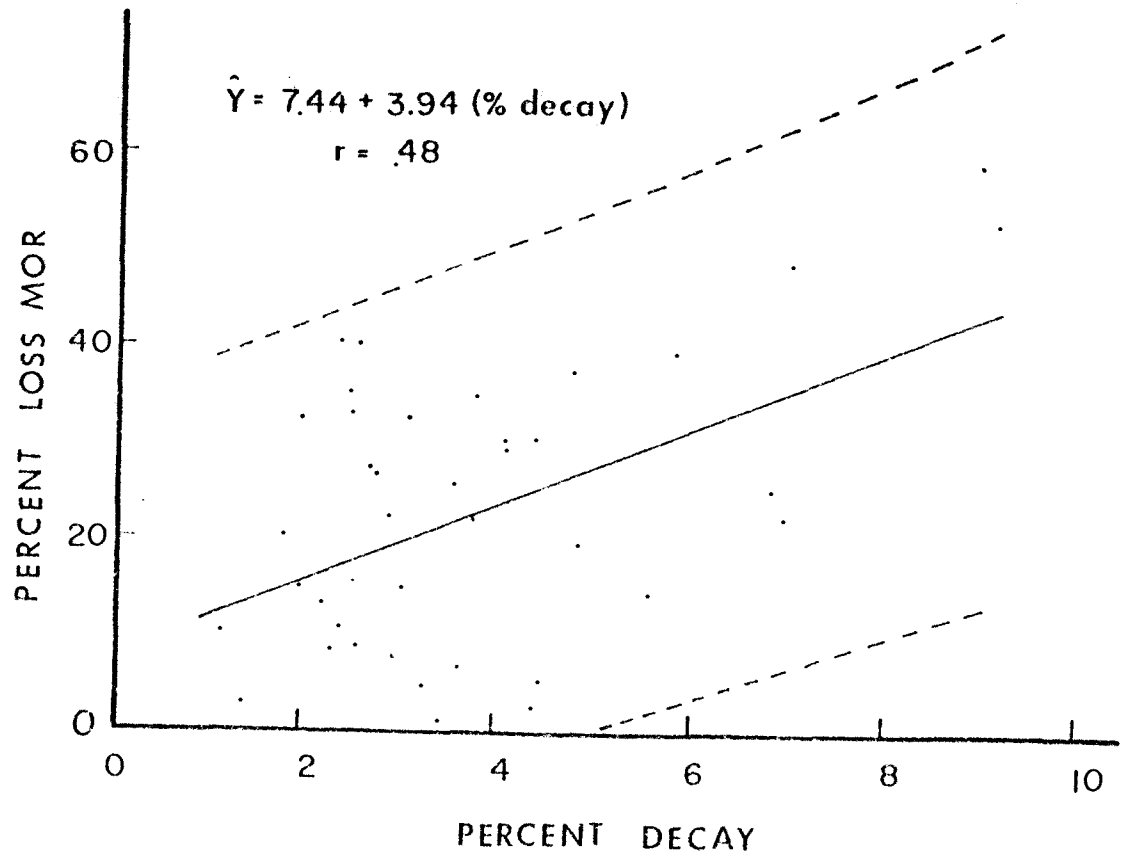

Figure 21. Linear regression of percent loss in modulus of rupture vs. percent weight loss (percent decay) for Douglas-fir with 95% confidence limits.

Detecting Decay in the Miniature Beams with Acridine Orange

The staining technique developed for use on the sections from the wafer samples was applied to radial sections that were cut from the area near the failure in the beams. Since a color pattern was obvious at various decay levels in the wafer sections, it was tried on the beams in hopes of detecting the presence of decay and relating this to the strength loss. Unfortunately, the radial sections that were cut from the beams did not produce the fluorescent coloring that had been observed with the sections from the wafers. The beam sections predominately fluoresced green.

It is a possibility that there was no decay present in the areas from which the sections were taken. Cutting cross sections of the entire beam near the zone of rupture might provide a better chance of detecting decayed tissue because this section would include the upper and lower surfaces of the beam which were directly exposed to decay in incubation and where maximum stresses are encountered in the static bending tests.

CONCLUSIONS

The following conclusions can be drawn from this study:

1. A fluorescence color pattern similar to that identified by Fukazawa et al. (1976) was recognized in radial sections approximately 25 micrometers thick from non-embedded wood blocks. This provides a quicker means for microscopic evaluation than to embed the blocks in methacrylate prior to sectioning.
2. A staining time of two hours using a .02% solution of acridine orange and washing in running water for approximately 24 hours provided adequate differentiation of decayed areas. A pH of 6.0 gave the best colors for the Southern yellow pine. The Douglas-fir samples responded best to a stain with a pH of 8.0.
3. At a weight loss of three percent, the color change from green-yellow to orange-yellow became most obvious. Below a three percent weight loss, some traces of yellow-orange are evident.
4. The latewood and the ray cells fluoresced a yellow-orange in most cases regardless of the percent weight loss.
5. The Quantimet 720 computer image analyzer does detect green and orange at different gray levels. The bright green of non-decayed wood is recognized as a lighter gray level than the orange color of the decayed wood which registers at a darker gray level. However, because of the mixture of green and orange at the lower decay levels and the various anatomical features which pose problems in quantifying the gray levels, this method is not considered practical.

6. The strength properties modulus of elasticity, modulus of rupture and fiber stress at the proportional limit were all decreased as a result of decay. The modulus of rupture was the one property affected most. It sustained a more than 50% loss in strength with weight losses as low as 3% for Southern yellow pine.

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APPENDICES

APPENDIX A

RAW DATA FOR SOUTHERN YELLOW PINE STATIC BENDING TEST SPECIMENS

Sample No.	Days in Incubation	% Weight Loss	Width cm	Height cm	Specific Gravity	% Moisture Content
			(At time of testing)			
1A		0.00	1.01	.49	.62	7.4
1B	14	4.79	.98	.49	.62	7.7
2A		0.00	.99	.50	.64	7.4
2B	14	5.95	.97	.50	.67	7.3
3A		0.00	1.00	.50	.68	7.5
3B	15	3.18	1.00	.50	.71	7.2
4A		0.00	1.01	.50	.62	7.3
4B	15	2.84	1.00	.50	.63	7.3
5A		0.00	1.01	.49	.64	7.1
5B	16	5.12	.98	.49	.63	7.5
6A		0.00	1.00	.50	.52	7.4
6B	16	6.25	.99	.49	.50	7.8
7A		0.00	1.01	.50	.63	7.5
7B	17	7.91	.98	.48	.62	8.0
8A		0.00	1.01	.50	.64	7.4
8B	17	7.38	.98	.49	.62	7.3
9A		0.00	1.00	.50	.66	7.4
9B	18	4.89	.98	.50	.66	7.7
10A		0.00	1.01	.50	.60	7.5
10B	18	5.72	1.00	.49	.63	7.9
11A		0.00	1.01	.50	.58	7.6
11B	21	12.57	.99	.48	.55	7.3
12A		0.00	1.00	.51	.64	7.3
12B	21	7.05	.98	.50	.61	7.1
13A		0.00	1.00	.50	.64	7.1
13B	23	7.63	.97	.50	.59	7.6
14A		0.00	1.01	.50	.70	7.4
14B	23	5.13	.99	.50	.69	7.6
15A		0.00	1.00	.50	.61	7.2
15B	24	9.07	1.00	.49	.56	7.4
16A		0.00	1.00	.50	.52	7.4
16B	24	9.29	.98	.50	.58	7.0
17A		0.00	1.00	.49	.67	7.2
17B	25	10.78	.98	.48	.62	8.1
18A		0.00	.99	.50	.55	7.3
18B	25	8.94	.98	.50	.57	7.6
19A		0.00	1.00	.50	.71	7.3
19B	28	4.74	.99	.50	.67	7.4
20A		0.00	1.00	.50	.58	7.3
20B	28	9.48	.99	.48	.55	7.1
21A		0.00	1.01	.51	.70	7.4
21B	29	6.25	.99	.49	.64	7.4

APPENDIX A (Continued)

Sample No.	Days in Incubation	% Weight Loss	Width cm	Height cm	Specific Gravity	% Moisture Content
			(At time of testing)			
22A		0.00	1.01	.50	.64	7.7
22B	29	7.65	1.00	.49	.60	7.4
23A		0.00	1.01	.50	.61	7.6
23B	30	6.65	.99	.50	.64	7.6
24A		0.00	1.00	.50	.70	7.7
24B	30	8.62	.99	.49	.64	7.1
25A		0.00	1.00	.50	.58	7.2
25B	32	14.52	.97	.48	.57	7.2
26A		0.00	1.01	.50	.65	7.2
26B	32	13.13	.98	.49	.58	7.5
27A		0.00	1.00	.50	.67	7.5
27B	35	13.07	.98	.49	.62	7.4
28A		0.00	1.00	.50	.65	7.0
28B	35	11.57	.98	.50	.58	7.4
29A		0.00	1.01	.50	.65	7.2
29B	37	13.44	.97	.48	.65	7.5
30A		0.00	1.01	.50	.65	7.2
30B	37	13.90	.97	.49	.61	7.6
31A		0.00	1.00	.50	.62	7.2
31B	39	13.51	.99	.50	.56	7.4
32A		0.00	1.01	.51	.64	7.5
32B	39	13.28	.98	.49	.59	7.7
33A		0.00	.99	.50	.69	7.4
33B	42	12.00	.97	.50	.60	6.9
34A		0.00	1.00	.50	.59	7.3
34B	42	13.56	.96	.49	.59	7.6
35A		0.00	1.00	.50	.55	7.1
35B	44	17.24	.98	.47	.54	7.8
36A		0.00	1.01	.50	.66	7.3
36B	44	12.19	.99	.48	.56	7.5
37A		0.00	1.00	.50	.65	7.0
37B	46	12.50	.96	.48	.64	7.8
38A		0.00	1.00	.50	.64	7.2
38B	46	10.85	.99	.49	.58	7.6
39A		0.00	1.00	.50	.64	7.4
39B	49	9.39	.96	.49	.60	7.5
40A		0.00	1.01	.50	.66	7.2
40B	49	10.30	.99	.48	.62	7.7

APPENDIX B

RAW DATA FOR DOUGLAS-FIR STATIC BENDING TEST SPECIMENS

Sample No.	Days in Incubation	% Weight Loss	Width cm	Height cm	Specific Gravity	% Moisture Content
			(At time of testing)			
1A		0.00	1.01	.50	.57	7.0
1B	33	1.69	1.00	.50	.58	7.6
2A		0.00	1.01	.50	.59	6.9
2B	33	1.93	1.00	.50	.59	7.8
3A		0.00	1.01	.50	.56	7.5
3B	34	2.62	1.00	.50	.55	11.2
4A		0.00	1.01	.51	.58	7.2
4B	34	4.39	1.00	.50	.57	7.6
5A		0.00	1.00	.50	.54	7.1
5B	35	3.05	.99	.50	.53	7.7
6A		0.00	1.00	.50	.59	7.8
6B	35	.83	1.00	.50	.59	8.4
7A		0.00	1.01	.50	.56	7.4
7B	37	2.37	.99	.50	.55	8.1
8A		0.00	1.00	.50	.57	7.5
8B	37	2.51	1.01	.49	.58	7.9
9A		0.00	1.00	.49	.57	7.2
9B	40	3.64	.99	.49	.53	7.7
10A		0.00	.99	.50	.55	7.4
10B	40	3.67	.99	.50	.52	7.8
11A		0.00	1.01	.49	.60	7.1
11B	41	2.82	1.01	.49	.57	8.1
12A		0.00	1.00	.50	.55	7.7
12B	41	3.45	1.01	.49	.56	7.9
13A		0.00	1.00	.49	.54	7.6
13B	42	2.41	1.00	.48	.55	8.3
14A		0.00	1.00	.50	.57	7.3
14B	42	3.57	.99	.50	.59	7.9
15A		0.00	1.00	.50	.56	7.5
15B	44	4.30	.99	.49	.56	8.0
16A		0.00	1.00	.50	.59	7.7
16B	44	1.70	1.00	.50	.57	8.4
17A		0.00	1.00	.51	.55	7.5
17B	47	2.93	1.00	.50	.54	8.1
18A		0.00	1.00	.50	.58	7.3
18B	47	1.94	1.00	.49	.58	7.9
19A		0.00	1.00	.50	.56	7.2
19B	49	4.75	1.01	.50	.53	7.6
20A		0.00	1.01	.50	.56	7.4
20B	49	6.89	1.00	.50	.52	7.9
21A		0.00	1.00	.50	.53	7.2
21B	54	6.89	.98	.49	.53	7.9

APPENDIX B (Continued)

Sample No.	Days in Incubation	% Weight Loss	Width cm	Height cm	Specific Gravity	% Moisture Content
			(At time of testing)			
22A		0.00	1.00	.50	.57	7.6
22B	54	5.76	.99	.49	.55	7.5
23A		0.00	.99	.50	.60	7.4
23B	56	4.08	1.00	.49	.60	7.6
24A		0.00	1.00	.50	.58	7.2
24B	56	6.78	.99	.48	.54	7.7
25A		0.00	1.01	.50	.55	7.6
25B	61	4.71	.99	.49	.55	7.5
26A		0.00	1.01	.50	.58	7.6
26B	61	3.38	1.00	.49	.57	7.8
27A		0.00	1.00	.50	.58	7.3
27B	62	3.21	1.01	.49	.56	7.7
28A		0.00	1.00	.50	.59	7.5
28B	62	4.01	1.00	.49	.56	8.0
29A		0.00	1.00	.49	.56	7.6
29B	63	5.55	.98	.49	.55	8.3
30A		0.00	1.01	.51	.58	7.4
30B	63	2.21	1.00	.49	.59	7.9
31A		0.00	1.01	.50	.59	7.1
31B	69	1.34	1.01	.50	.60	7.9
32A		0.00	1.01	.49	.57	7.4
32B	69	2.36	1.00	.49	.55	7.7
33A		0.00	1.01	.49	.57	7.1
33B	72	2.35	1.00	.50	.55	7.7
34A		0.00	1.01	.51	.59	7.8
34B	72	2.14	1.01	.50	.60	7.9
35A		0.00	1.01	.49	.53	7.4
35B	75	2.65	1.01	.50	.49	7.6
36A		0.00	1.01	.49	.57	7.1
36B	75	1.16	1.01	.49	.56	7.5
37A		0.00	1.01	.50	.56	7.5
37B	77	9.37	.99	.48	.57	7.8
38A		0.00	.99	.49	.58	7.4
38B	77	4.34	1.00	.50	.55	8.1
39A		0.00	1.01	.50	.56	7.5
39B	82	2.31	1.01	.50	.55	7.9
40A		0.00	1.02	.50	.57	7.3
40B	82	2.49	1.00	.50	.58	8.2

APPENDIX C

STRENGTH VALUES AND PERCENT LOSS IN STRENGTH FOR SOUTHERN YELLOW PINE END-MATCHED BEAMS IN STATIC BENDING

Sample No.	% Weight Loss	MOE (psi)*	% Loss MOE	MOR (psi)*	% Loss MOR	FSPL (psi)*	% Loss FSPL
1A	0.00	2.76×10^6		24,877		12,559	
1B	4.79	2.25×10^6	18.71	13,811	44.84	9,885	21.59
2A	0.00	2.87×10^6		26,526		15,119	
2B	5.95	2.30×10^6	19.86	11,194	57.80	9,274	38.68
3A	0.00	3.16×10^6		29,385		13,327	
3B	3.18	2.88×10^6	8.92	11,834	59.73	9,202	31.18
4A	0.00	2.54×10^6		25,958		14,024	
4B	2.84	2.20×10^6	13.25	11,123	57.15	10,653	24.49
5A	0.00	2.73×10^6		25,830		13,228	
5B	5.12	2.58×10^6	5.74	9,387	63.66	6,543	50.54
6A	0.00	2.07×10^6		19,415		9,558	
6B	6.25	1.84×10^6	11.27	10,497	45.93	7,410	22.39
7A	0.00	2.63×10^6		25,460		14,294	
7B	7.91	1.97×10^6	24.89	6,187	75.70	5,362	63.00
8A	0.00	2.76×10^6		23,753		14,408	
8B	7.38	2.06×10^6	25.64	6,913	70.90	5,775	60.40
9A	0.00	2.72×10^6		26,726		13,356	
9B	4.89	2.53×10^6	6.84	12,260	54.12	10,255	22.58
10A	0.00	2.55×10^6		24,094		11,251	
10B	5.72	2.36×10^6	7.30	9,956	58.68	9,103	18.99
11A	0.00	2.30×10^6		21,363		11,407	
11B	12.57	1.90×10^6	17.48	12,687	40.61	8,762	23.75
12A	0.00	2.75×10^6		27,863		14,067	
12B	7.05	2.23×10^6	19.07	13,725	50.74	9,899	29.59
13A	0.00	2.60×10^6		24,564		12,445	
13B	7.63	1.99×10^6	23.46	9,857	59.87	8,122	34.48
14A	0.00	3.21×10^6		30,438		14,849	
14B	5.13	2.86×10^6	11.12	15,930	47.66	13,626	8.65
15A	0.00	2.68×10^6		24,962		14,337	
15B	9.07	2.02×10^6	24.83	6,486	74.02	5,419	62.00
16A	0.00	2.11×10^6		20,154		10,070	
16B	9.29	1.95×10^6	7.40	7,168	64.43	6,400	35.71
17A	0.00	2.98×10^6		26,356		13,114	
17B	10.78	2.29×10^6	22.89	8,136	69.13	7,837	40.22
18A	0.00	2.34×10^6		21,762		10,838	
18B	8.94	1.99×10^6	14.78	10,184	53.20	9,715	10.53
19A	0.00	3.13×10^6		29,400		15,262	
19B	4.71	2.83×10^6	9.57	10,369	64.73	9,345	39.25
20A	0.00	2.47×10^6		22,942		13,541	
20B	9.48	1.93×10^6	21.80	12,502	45.51	9,018	33.68
21A	0.00	2.88×10^6		28,831		16,101	
21B	6.25	2.28×10^6	20.91	9,800	66.01	9,715	39.82

APPENDIX C (Continued)

Sample No.	% Weight Loss	MOE (psi)*	% Loss MOE	MOR (psi)*	% Loss MOR	FSPL (psi)*	% Loss FSPL
22A	0.00	2.94×10^6		26,896		15,233	
22B	7.65	2.40×10^6	18.31	11,265	58.12	9,985	34.58
23A	0.00	2.54×10^6		24,877		13,768	
23B	6.65	2.41×10^6	5.34	11,478	53.86	9,501	31.25
24A	0.00	2.93×10^6		29,129		16,129	
24B	8.62	2.18×10^6	25.71	7,225	75.20	6,642	59.29
25A	0.00	2.39×10^6		23,767		12,929	
25B	14.52	1.79×10^6	24.87	8,875	62.66	7,581	41.11
26A	0.00	2.78×10^6		26,669		14,622	
26B	13.13	1.90×10^6	31.70	9,587	64.05	8,122	44.12
27A	0.00	2.92×10^6		28,030		15,788	
27B	13.07	2.10×10^6	28.32	7,282	74.91	6,941	56.76
28A	0.00	2.83×10^6		27,366		13,925	
28B	11.57	2.13×10^6	24.59	10,781	60.60	9,828	28.87
29A	0.00	2.84×10^6		27,110		15,105	
29B	13.44	2.25×10^6	20.63	8,122	70.04	6,841	54.72
30A	0.00	2.71×10^6		26,100		14,223	
30B	13.90	2.16×10^6	20.21	8,833	66.16	7,993	44.00
31A	0.00	2.86×10^6		25,218		12,403	
31B	13.51	1.74×10^6	39.13	6,031	76.09	4,125	66.67
32A	0.00	2.46×10^6		23,781		13,142	
32B	13.28	1.87×10^6	24.19	5,661	76.20	5,419	58.70
33A	0.00	3.20×10^6		30,025		13,939	
33B	12.00	1.94×10^6	39.26	6,799	77.36	6,287	55.10
34A	0.00	2.51×10^6		24,279		12,972	
34B	13.56	1.79×10^6	28.81	5,860	75.86	5,533	58.24
35A	0.00	2.18×10^6		21,050		10,928	
35B	17.24	1.61×10^6	26.04	7,595	63.92	6,102	44.74
36A	0.00	2.82×10^6		27,351		15,390	
36B	12.19	2.06×10^6	26.76	6,827	75.04	5,903	62.04
37A	0.00	2.44×10^6		24,877		12,460	
37B	12.50	1.98×10^6	18.69	10,326	58.45	8,477	32.18
38A	0.00	2.76×10^6		24,677		10,326	
38B	10.85	1.98×10^6	28.30	6,699	72.85	5,547	45.83
39A	0.00	2.61×10^6		26,711		14,920	
39B	9.39	1.98×10^6	24.02	10,326	61.34	8,050	46.15
40A	0.00	2.91×10^6		24,862		14,309	
40B	10.30	2.29×10^6	21.44	8,107	67.39	7,197	50.00

*Megapascal = psi x .006895

APPENDIX D

STRENGTH VALUES AND PERCENT LOSS IN STRENGTH FOR DOUGLAS-FIR END-MATCHED BEAMS IN STATIC BENDING

Sample No.	% Weight Loss	MOE (psi)*	% Loss MOE	MOR (psi)*	% Loss MOR	FSPL (psi)*	% Loss FSPL
1A	0.00	2.37×10^6		19,415		12,218	
1B	1.69	2.42×10^6	-2.41	19,770	-1.83	13,725	-12.94
2A	0.00	2.63×10^6		22,228		13,114	
2B	1.93	2.30×10^6	12.30	15,006	32.67	11,407	13.04
3A	0.00	2.41×10^6		19,116		11,962	
3B	2.62	2.10×10^6	12.78	13,768	27.98	11,194	7.14
4A	0.00	2.18×10^6		18,803		10,198	
4B	4.39	2.25×10^6	-3.37	17,680	5.98	10,369	-1.41
5A	0.00	2.26×10^6		17,054		11,478	
5B	3.05	2.01×10^6	10.89	11,521	32.44	10,739	6.25
6A	0.00	2.08×10^6		19,116		10,810	
6B	.83	2.31×10^6	-10.85	20,012	-4.69	11,421	-5.26
7A	0.00	2.39×10^6		19,884		12,204	
7B	2.37	2.15×10^6	10.08	12,943	34.91	10,596	12.94
8A	0.00	2.61×10^6		21,947		13,242	
8B	2.51	2.19×10^6	16.11	13,142	40.12	11,023	17.20
9A	0.00	2.33×10^6		20,595		10,867	
9B	3.64	2.18×10^6	6.54	16,030	22.17	12,360	-13.16
10A	0.00	2.34×10^6		19,813		12,645	
10B	3.67	1.99×10^6	14.85	12,872	35.03	10,283	18.18
11A	0.00	2.72×10^6		22,814		14,195	
11B	2.82	2.52×10^6	7.37	17,680	22.51	12,844	9.09
12A	0.00	2.45×10^6		21,335		13,441	
12B	3.45	2.17×10^6	11.12	15,816	25.87	10,710	20.21
13A	0.00	2.37×10^6		20,567		13,114	
13B	2.41	2.19×10^6	7.82	13,626	33.75	9,857	25.00
14A	0.00	2.42×10^6		20,140		12,829	
14B	3.57	2.28×10^6	5.92	18,860	6.36	12,573	2.22
15A	0.00	2.15×10^6		16,940		11,279	
15B	4.30	2.21×10^6	-2.89	11,834	30.14	10,980	2.53
16A	0.00	2.25×10^6		18,889		11,521	
16B	1.70	2.14×10^6	4.66	15,077	20.18	10,198	12.35
17A	0.00	2.37×10^6		19,813		12,317	
17B	2.93	1.98×10^6	16.48	16,769	15.36	12,076	2.33
18A	0.00	2.58×10^6		21,563		12,588	
18B	1.94	2.23×10^6	13.46	18,348	14.91	10,781	14.77
19A	0.00	2.40×10^6		20,098		11,507	
19B	4.75	2.07×10^6	13.79	16,186	19.46	11,521	-1.25
20A	0.00	2.52×10^6		21,406		13,356	
20B	6.89	2.19×10^6	13.06	16,670	22.13	11,293	15.05
21A	0.00	2.27×10^6		19,557		12,858	
21B	6.89	2.05×10^6	10.01	10,056	48.58	7,268	43.33

APPENDIX D (Continued)

Sample No.	% Weight Loss	MOE (psi)*	% Loss MOE	MOR (psi)*	% Loss MOR	FSPL (psi)*	% Loss FSPL
22A	0.00	2.68×10^6		22,274		13,626	
22B	5.76	2.20×10^6	17.91	13,484	39.46	9,345	31.58
23A	0.00	2.81×10^6		22,544		15,304	
23B	4.08	2.32×10^6	17.63	15,944	29.27	12,189	20.56
24A	0.00	2.52×10^6		21,904		15,205	
24B	6.78	2.13×10^6	15.33	16,357	25.32	12,360	18.87
25A	0.00	2.45×10^6		18,078		13,455	
25B	4.71	1.99×10^6	18.66	11,293	37.53	7,894	41.49
26A	0.00	2.56×10^6		21,150		12,687	
26B	3.38	2.36×10^6	8.03	20,908	1.14	13,341	-4.49
27A	0.00	2.27×10^6		19,244		11,891	
27B	3.21	2.26×10^6	.18	18,334	4.73	11,976	-1.20
28A	0.00	2.81×10^6		24,265		14,607	
28B	4.01	2.14×10^6	23.79	16,940	30.19	9,217	37.25
29A	0.00	2.31×10^6		18,803		11,734	
29B	5.55	1.83×10^6	20.89	16,087	14.45	10,227	13.41
30A	0.00	2.69×10^6		24,108		13,697	
30B	2.21	2.64×10^6	1.68	22,146	8.14	14,849	-8.33
31A	0.00	2.76×10^6		24,208		13,455	
31B	1.34	2.76×10^6	.19	23,497	2.94	14,764	-9.57
32A	0.00	2.67×10^6		23,838		13,982	
32B	2.36	2.43×10^6	9.20	21,250	10.86	13,640	3.06
33A	0.00	2.59×10^6		20,154		11,094	
33B	2.35	2.46×10^6	4.85	20,553	-1.98	13,740	-23.08
34A	0.00	2.77×10^6		24,265		15,020	
34B	2.14	2.70×10^6	2.46	20,927	13.72	14,038	6.67
35A	0.00	2.33×10^6		19,187		11,862	
35B	2.65	1.87×10^6	19.88	13,882	27.65	10,383	12.05
36A	0.00	2.57×10^6		19,785		12,403	
36B	1.16	2.33×10^6	9.49	17,708	10.50	10,952	11.49
37A	0.00	2.24×10^6		18,007		10,667	
37B	9.27	1.42×10^6	36.88	8,463	53.00	4,879	54.67
38A	0.00	2.39×10^6		18,234		10,867	
38B	4.34	1.99×10^6	16.41	17,793	2.42	11,805	-9.21
39A	0.00	2.41×10^6		21,235		15,518	
39B	2.31	2.13×10^6	11.82	12,616	40.59	9,146	41.28
40A	0.00	2.30×10^6		18,547		12,047	
40B	2.49	2.27×10^6	1.27	16,869	9.05	10,198	15.48

*Megapascal = psi x .006895

APPENDIX E

CORRELATIONS RELATED TO STRENGTH PROPERTIES OF NONDECAYED AND DECAYED SPECIMENS OF SOUTHERN YELLOW PINE AND DOUGLAS-FIR

I: WEIGHT LOSS RELATIONSHIPS TO STRENGTH

Southern Yellow Pine

MOE of nondecayed wood (psi) =
-717,212 + 5,400,170 (Specific Gravity) r = .89

MOE of decayed wood (psi) =
-1,296,580 + 5,667,650 (Specific Gravity) r = .82

MOR of nondecayed wood (psi) =
-7,586 + 52,506 (Specific Gravity) r = .92

MOR of decayed wood (psi) =
-1,743 + 18,419 (Specific Gravity) r = .32

% loss in MOE = 5.65 + 1.57 (% weight loss) r = .67

% loss in MOR = 51.61 + 1.21 (% weight loss) r = .43

Douglas-fir

MOE of nondecayed wood (psi) =
-458,648 + 5,123,460 (Specific Gravity) r = .48

MOE of decayed wood (psi) =
-826,749 + 5,421,410 (Specific Gravity) r = .56

MOR of nondecayed wood (psi) =
-11,245 + 55,860 (Specific Gravity) r = .50

MOR of decayed wood (psi) =
-19,743 + 64,331 (Specific Gravity) r = .46

% loss in MOE = 0.32 + 2.88 (% weight loss) r = .58

% loss in MOR = 7.44 + 3.94 (% weight loss) r = .48

II. ALKALI SOLUBILITY RELATIONSHIPS TO STRENGTH

Southern Yellow Pine

[1]Diff. in Solubility (%) = 18.89 + .57 (% weight loss) r = .69

[2]Solubility (%) = 38.81 + .59 (% weight loss) r = .68

% loss in MOE = -16.84 + 1.53 (Diff. in Solubility) r = .54

% loss in MOE = -33.37 + 1.48 (Solubility) r = .54

% loss in MOR = 35.66 + 1.12 (Diff. in Solubility) r = .33

% loss in MOR = 25.97 + 1.02 (Solubility) r = .32

APPENDIX E (Continued)

1/ Difference in Solubility (%) = alkali solubility of decayed specimen (%) - alkali solubility of control (%).

2/ Solubility (%) = alkali solubility of decayed specimen (%).

Alkali solubility was done with a 1% sodium hydroxide solution on a small section cut from near the break of the static bending specimens.