

INTRA-INCREMENT LIGNIN CONTENT OF FIVE WESTERN  
CANADIAN CONIFEROUS WOODS

by

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## ABSTRACT

Lignin contents of extractive-free wood meals prepared from three adjacent rings of mature wood, sampled at breast height, of amabilis fir, Douglas fir, western red cedar, Sitka spruce, and western hemlock were determined according to the micro-method of Johnson, Moore and Zank. Each of three rings within each species was divided into six positions from earlywood to latewood. Wood meals of 40-80 mesh size were extracted successively with ethyl ether, absolute ethyl alcohol and hot-water, and then solubilized with acetyl bromide and absorbence was calibrated against Klason lignin values. Wood methoxyl content of these same samples had been studied previously.

Lignin contents were found to be highly significantly different within positions of growth increments for amabilis fir, Douglas fir, western red cedar and Sitka spruce, but no significant difference was found for western hemlock. Highly significant difference was found between growth rings for western red cedar and similarly significant difference was found for Douglas fir. No significant differences were obtained between growth rings for the other three species, although a slight but definite increase in lignin content with age was observed. Highly significant differences were also found between species and for total averages of each position for the five species. Earlywoods were higher in lignin content than latewood in each species by: Douglas fir, 2.27%;

amabilis fir, 2.06%; western red cedar, 1.28%; Sitka spruce, 1.08%; and western hemlock, 0.46%.

Lignin contents for individual species were in the order of: western hemlock, 32.85% (32.36-34.04%); western red cedar, 31.23% (29.69-32.68%); amabilis fir, 27.51% (26.17-28.24%); Douglas fir, 26.48% (24.08-28.66%); and Sitka spruce, 25.81% (24.91-26.98%).

The correlation coefficient testing association between lignin and wood methoxyl was highly significant for amabilis fir (0.971), Douglas fir (0.913) and western red cedar (0.684). Non-significant correlation occurred with Sitka spruce (0.467) and western hemlock (0.340). The relationship between lignin and wood methoxyl appeared independent of species.

The non-lignin methoxyl contents for the five species were estimated and the results showed that these were also independent of species.

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## INTRODUCTION

It is well known that lignin is a major wood component influencing mechanical properties, wood quality, pulpability, penetrability, and a great many other features of wood important in utilization.

Many investigations have been carried out to describe lignin as regards species, individual tree, and other biological properties of wood, yet no complete reference is available regarding the distribution of lignin across wood growth increments.

It is known that the most pronounced differences in wood appearance and qualities for various uses are controlled by variations between earlywood and latewood. Therefore, the nature of differences between earlywood and latewood is a very important factor in the utilization of wood.

Several investigators have tried to refer wood methoxyl measurements back to lignin content, but no agreement in results has occurred possibly due to lack of proper data or appropriate samples.

Therefore, an effort was made in this study to further understanding of lignin within and between various coniferous woods.

The investigation of lignin contents was made on the same samples which were previously studied for wood methoxyl contents (72) in order to examine relationship between these two measurements on woods.

It is also hoped that this study will be of some assistance to further understanding of the basic nature of wood.

## LITERATURE REVIEW

In this review, literature cited is related to distribution of lignin from the individual cell to the mature tree. References regarding the distribution of wood methoxyl and its relationship to lignin are given in the previous work (72).

Wood was believed for a long time to be of uniform chemical composition until Gay-Lussac and Thenard (19) first disproved this by elementary wood analysis. They found that wood is comprised of carbon, hydrogen and oxygen. Subsequently wood has been discovered to be a very complex biological material. Much research has been carried out on wood, but one of the most important wood components - lignin - is not yet fully understood. Typical lignin values are shown in Table 1.

Lignin was first named as the incrusting material in the lignified plant by Payen (46) and later by F. Schulze (58).

#### A. Lignin in the wood cell

Scarth and co-workers (57) by using different solvents, colour reactions, and stains, studied structure of the cell wall. They showed that both in softwood and hardwood, the middle lamella is the portion highest in lignin content. The order of lignin content for different woody tissues was also reported.

Ritter (52,53) found two forms of lignin present in wood. The "middle lamella lignin" and "cell wall lignin", the former was at about 75% of the total and the latter about 25%. The cell wall lignin is thought to be amorphous and has methoxyl content of 4.8% and 4.3% for red alder and western white pine, whereas the middle lamella lignin showed definite structure and had 13.6% and 10.8% methoxyl respectively.

Dadswell (17) showed radial alignment of lignin in the cell wall after treatment with 72% sulfuric acid. This finding was the same as that of Scarth. Therefore, Dadswell concluded that radial alignment had a definite connection with the distribution of lignin in the cell wall.

Lange (39) by applying microspectrographic technique, studied distribution of lignin in the cell walls of normal and reaction woods. Spruce contained 73% lignin in the middle lamella and 16% in the cell wall. The cell walls of hardwoods were less lignified than those of spruce.

Bailey (4) following tedious microdissection reported that in Douglas fir, the middle lamella contained 71.4% of lignin.

B. Lignin in earlywood and latewood

Ritter and Fleck (56) reported for numerous American woods that springwood contained a greater proportion of lignin than summerwood, probably because the middle lamella formed a larger portion of the springwood substance.

Bailey (4) showed that in Douglas fir the lignin was higher in springwood, 35.2%, than in summerwood, 31.6%.

Hata (28) determined lignin content of a 35-year old Japanese red pine and reported that springwood contained more lignin than summerwood.

Jansons (32) made a thorough investigation of lignin content in a tree, and reported that in spruce, the lignin content of spring- and summerwood was dependent to some extent upon the position within the tree from which the sample was obtained. The lignin content of summerwood at the bottom of the trunk was lower, in the middle part equal to, and at the top of the trunk higher than that of corresponding springwood. The lignin content varied from 25.2% to 29.5% in the springwood and from 22.3% to 34.0% in the summerwood.



Hägglund and Johnson (23), on the other hand, reported that no different lignin content was found between spruce springwood and summerwood.

Wardrop and Dadswell (68) reported that most of the lignin occurred in the intercellular layer and primary wall in the cell wall of ray and vertical parenchyma.

Hale and Clermont (25) have recently reported that the lignin content was higher in earlywood than in latewood both in Douglas fir and red pine. They also stated that the difference in earlywood and latewood chemical and physical properties was due to differences in cell-wall morphology.

#### C. Lignin in reaction woods

Hägglund et al. (24,67) reported that lignin content was higher in compression wood (38.0%) than in normal wood (28.0%).

Jayne and co-workers (33) showed that beech tension wood had a lower lignin content than normal wood.

Watson (69) reported that tension woods of Eucalyptus regnans F.v.M. contained 15.8% lignin, and only 10.3% in E. gonicalyx F.v.M. as compared with 25.2% for the normal wood.

Bland (8) concluded that tension wood<sup>lignin</sup> was found to have no abnormal characteristic except for being bound more closely to the polysaccharide framework of the wood, whereas compression wood lignin is abnormal, owing to the

operative mechanism during lignin decomposition. He reported that tension wood contained 13.8% and compression wood, 34.4% Klason lignin.

Hata (29) showed that compression wood had 36.50% lignin with 12.74% methoxyl content, whereas normal wood had 26.75% lignin with 14.28% methoxyl content.

Klauditz (36) reported that red beech normal wood had higher lignin content than reaction wood and that narrow rings have slightly higher lignin content than the wider ring reaction wood (21.29:20.17%), whereas the normal wood had 22.68% lignin.

Hale and co-workers (26) also reported that normal wood of both trembling aspen and white elm had higher lignin content than the tension woods.

#### D. Lignin in sapwood and heartwood

Ritter and Fleck (54, 55) stated that in analysis of softwoods, certain definite conclusions may be drawn concerning relationship between the various constituents of the sapwood and heartwood, but it is quite difficult to generalize in the case of the hardwoods. They claimed, in general, that sapwood was higher in lignin than the corresponding heartwood.

Freeman and Peterson (18) stated that any conclusion regarding relationship between constituents of heartwood

and sapwood of different species cannot be accepted without some reservation, because certain vital factors such as age, growing conditions and habitat are apparent and may be observed. In general, the extractives are higher in the heartwood than in the sapwood of the coniferous species, and the cellulose and lignin are lower in the heartwood.

Hata (27) also observed that the sapwood was higher than heartwood in lignin content in Japanese red pine.

#### E. Lignin in the individual tree

Cieslar (15) studied lignin content on a number of softwoods and found that lignin decreased from the base towards the top of spruce trees. Lignin content was higher at positions exposed to strong mechanical stress than elsewhere. Because he used the indirect method (methyl number method) of Benedikt and Bamberger (7) for the determination of lignin, his results are not thought to be very reliable.

Narayanamurti and Das (44) reported distribution of lignin throughout a number of trees and found that it decreased towards the top (about 2%) and increased from the sapwood towards the heartwood (about 5%).

Klem (37) reported that in sprucewood the lignin content in the lower trunk was higher than it was in the upper

trunk and that such variations were responsible for variable pulp yield.

Hata (27) on the other hand reported lignin content in various parts of the trunk of a 35-year old Japanese red pine and found that lignin increased from the bottom towards the top of the tree, but was somewhat lower in the heartwood than in the sapwood.

It is also stated (14) that methoxyl, lignin and lignin methoxyl increased with the formation of new rings in the twigs of Pinus thunbergii Parl. and Robinia pseudoacacia L.

Janson (32) reported that in fir trees, maximum amount of lignin at a certain height was flanked by minimum amounts higher and lower. Maximum amounts of lignin formed a spiral line progressing in a clockwise direction upwards in the trunk. Highest lignin content with respect to the whole trunk was found in the wood samples taken just below the crown. Lignin content in the crown was related to the number of branches, the more branches the less lignin.

Clermont and Schwartz (16) studied chemical composition of a black spruce tree and reported that lignin content did not show any trend of difference towards the top of the tree, whereas wood methoxyl showed a definite decrease (5.21 - 4.89%) towards the top of the tree.

Worster and Sugiyama (71) reported that lignin content of a western hemlock tree had no pattern of lignin distribution with different height level. They also stated that in a number of western hemlock trees, high growth rate did not favour formation of lignin and xylan, but favoured formation of hexosan and especially glucan.

Hale and Clermont (25) recently reported that the lignin measured horizontally across a 430-year old Douglas fir tree was higher in the overmature wood portion than in the mature wood portion. No differences in lignin contents were noticed across the mature wood portions.

According to Bland and Hirnj (11), Bower and co-workers (12), Beckman and co-workers (6), Norman (45), Phillips and co-workers (47), and Zeherebov (73), lignin as well as methoxyl content increased with plant maturity.

## EXPERIMENTAL

### Sample

In this experiment samples of mature wood at breast height were chosen from among the more important western Canadian woods. Sample trees, except for Sitka spruce, were from the University of British Columbia Research Forest, Haney, B.C. The Sitka spruce was from Terrace, B.C. These same samples were previously studied for wood methoxyl contents (72). The species included:

Amabilis or Pacific silver fir (Abies Amabilis (Dougl.)  
Forb.)

Douglas fir (Pseudotsuga menziesii (Mirb.) Franco.)

Sitka spruce (Picea sitchensis (Bong.) Carr.)

Western hemlock (Tsuga heterophylla (Raf.) Sarg.)

Western red cedar (Thuja plicata D. Don.)

The wood blocks for each species were first saturated with water and then cut into tangential sections of 120 - microns thickness. Three adjacent rings from each species were studied. The growth rings included: amabilis fir, 78-80 yr; Douglas fir, 64-66 yr; Sitka spruce, 69-71 yr; western hemlock, 56-58 yr; and western red cedar, 72-74 yr. The total tangential sections within each ring were divided into six equal groups representing six sequential positions across a growth ring from earlywood to latewood. These sections were air dried, ground into wood meal, collected as 40-80 mesh, and homogenized.

### Preparation of extractive-free samples

In order to obtain extractive-free samples that had been given equivalent treatment, 0.7 g portions of wood meal were sealed separately in 100-mesh 1.5"x 2.0" nylon bags with a soldering gun.

In order to calculate sample moisture content, earlywood and latewood samples from another source were used; in this case, the same treatments were applied to the moisture correction samples, as well as to those being prepared for lignin determination.

All samples for each species were successively treated in the same Soxhlet container with ethyl ether and absolute ethyl alcohol. They were then hot-water extracted according to SP/E-1/62 (64).

After extractive treatments, the samples were air-dried and conditioned in an Aminco cabinet until the sample moisture content reached equilibrium.

For moisture content determination, earlywood and latewood samples were dried in an oven maintained at  $102 \pm 1^{\circ}\text{C}$  until constant weight was reached. The average of three replicates as stated in AM/M-1/62 (1) was used for calculating moisture contents. The moisture contents found were 8.80% and 9.80% for earlywood and latewood respectively. These values were used to correct extracted experimental samples with moisture contents of the first

four positions in a growth ring corrected by earlywood moisture content, while the remaining two positions were corrected according to latewood moisture content.

The extractive-free and moisture equilibrated samples were then stored in tightly closed glass jars to avoid further moisture changes.

### Apparatus

A Beckman Model DK 2 double beam recording spectrophotometer was used first in studying absorption spectra of calibration samples. A Beckman Model DU single beam manual spectrophotometer was later used to check absorption spectra measured by <sup>the</sup> Beckman Model DK 2 and was then used in the main study. <sup>(see Fig.1)</sup> Three quartz cells with 10 mm path length and 1.5 ml volume were used.

The reactors used were glass-stoppered test tubes with outside diameter of 19 mm, length 150 mm, and capacity 28 ml. Stoppers were notched to allow escape of evolved gases. The hot water bath was regulated at  $70 \pm 1^\circ\text{C}$ .

### Other equipment

For Klason lignin determinations filter glass crucibles (C porosity) and 50 ml weighing bottles were used.

For dilution of wood meal solutions calibrated 200 ml volumetric flasks were used.



### Chemicals

#### A. For Klason lignin determination:

1. Sulfuric acid ( $\text{H}_2\text{SO}_4$ ): A reagent grade 72% sulfuric acid was used.

#### B. For Spectrometric lignin determination:

1. Acetyl bromide ( $\text{CH}_3\text{COBr}$ ): A reagent grade acetyl bromide was used for the dissolution of wood meal.
2. Sodium hydroxide: A 2 M sodium hydroxide (carbonate-free) <sup>solution</sup> was prepared by dissolving 160 g reagent grade sodium hydroxide in 2000 ml of boiled and cooled distilled water.
3. Hydroxylamine hydrochloride ( $\text{NH}_2\text{OH}\cdot\text{HCl}$ ): a reagent grade 7.5 M solution was used.

### Procedure

In order to plot a standard calibration curve of lignin content against absorbance at a given wavelength, lignin content of a 0-10 yr wood meal sample from an amabilis fir was determined by Klason's method (2) which follows:

1. A wood meal sample (about 1 g air-dried) is accurately placed into a tared weighing bottle.
2. The bottle and sample are placed in a water bath at  $19\pm 1^\circ\text{C}$  and 15 ml of cold standardized sulfuric acid is added slowly, with constant stirring. Vigorous stirring is continued for 2 min. until all lumps are broken. Stirring is continued for 1 min. and is repeated at 15 min. intervals

for 2 hours while maintaining temperature at  $19\pm 1^{\circ}\text{C}$ .

3. After 2 hours the material is washed into a Erlenmeyer flask and diluted to 3% acid concentration by adding 560 ml of distilled water.

4. The solution is refluxed for 4 hours.

5. The insoluble material is allowed to settle overnight and is then filtered into a tared crucible.

6. The residue in the crucible is washed with 500 ml of hot distilled water to dispose of the acid.

7. The crucible and residue are dried at  $102\pm 1^{\circ}\text{C}$  for 2 hours, cooled in a desiccator and weighed to constant weight.

Lignin content was calculated as percentage based on extractive-free and moisture-free wood. The average lignin content of 4 replicates was 30.11% with range of 1.0%. This lignin content was used for the calibration of lignin absorptivity.

The procedure used for spectrometric lignin determination follows:

The wood meal specimen having an accurately known weight of about 0.020 g (oven-dry basis) was placed in a special reaction tube having a notched, fitted glass stopper, and 10 ml of 25% acetyl bromide-acetic acid (reagent grade) was added. The tube was then placed in a hot water bath maintained at  $70\pm 1^{\circ}\text{C}$ . Gentle swirling

was done at 10 min. intervals to assist dissolution. After 30 min. of heating, the reaction tube was placed in a cold water bath maintained at  $13 \pm 1^{\circ}\text{C}$  for 8-10 min. The cooled liquor was then transferred to a 200 ml volumetric flask into which 9 ml of 2 M sodium hydroxide and 50 ml of acetic acid had been placed. A minimum amount of acetic acid (ca. 5-10 ml) was used to complete the transfer from the reaction tube, and 1 ml of 7.5 M hydroxylamine hydrochloride was then added to the flask. The contents were mixed and diluted to the 200 ml mark with reagent grade acetic acid. This solution was cooled under cold water before measurement of absorbence was made.

The solution absorbence was measured at the maximum peak ( $\lambda_{\text{max}}$ ) of 282 milimicron.

Lignin content was calculated as:

$$\text{Lignin, \%} = \text{Absorbence} (\text{Absorptivity} \times \text{Sample Wt.})$$

where

Absorptivity of amabilis fir = 23.2

Sample Wt. was based on extractive-free, moisture-free weight of wood.

## DISCUSSION OF PROCEDURES

### 1. Theory of ultraviolet spectroscopy

The identification of organic compounds has been much assisted by introduction of instruments which make possible quick and convenient measurement by ultraviolet (also infrared) spectra of substances, even with small amounts of material.

The ultraviolet spectrum, quite unlike the infrared or Raman, arises from electronic excitation of the molecule by the irradiating light. The total energy of a molecule is the sum of its electronic or binding energy and its kinetic energy. The electronic or binding energy is the largest component of molecule total energy. Energy absorbed in the ultraviolet region produces electronic transitions within the molecule. The relationships between the energy absorbed, in an electronic transition, and the frequency ( $\nu$ ) or wavelength ( $\lambda$ ) of radiation producing the transition, is expressed by:

$$\Delta E = h\nu = hc/\lambda$$

where,  $h$  stands for Plank's constant and  $c$  velocity of light.  $\Delta E$  is the energy absorbed in bringing about an electronic transition in a molecule from its lowest energy state (ground state) to an excited state. The energy absorbed is quantized. The smaller the energy difference

between the ground state and the excited state, the longer will be the wavelength of absorption.

The important consequence of this, from the view of <sup>a</sup> structural chemist, is that spectra in the ultra-violet region are diagnostic of unsaturation in the absorbing molecule. It is also understood that the ultraviolet (and infrared) spectra are very important in the determination of functional groups both qualitatively and quantitatively.

Quantitative application of spectroscopy is based on the so-called Lambert-Beer law which states:

$$\log_{10} I_0/I = Kcb = A$$

where K is a constant characteristic of the solute; c, concentration of solute; b, path length; A, absorbance; <sup>stand</sup> I<sub>0</sub> and I<sub>A</sub> for intensity of incident and transmitted radiation. (59).

## 2. Determination by the micro-lignin method

### A. Sample weight (volume of dilution)

It was desirable to determine an optimum minimal sample size so that further study could be carried out with small amounts of material. From the results of Johnson, Moore and Zank (34), the concentration of solution (lignin or wood meal) which gives an optimum absorbance between 0.2 and 0.8 (5) is 0.6 to 4.0 mg lignin per 100 ml dilution. In other words, the weight of coniferous wood meal required to fall within this absorbance range would be 2.0 to 13.3 mg

per 100 ml dilution.

According to the method described (34), the minimum dilution volume was 100 ml. With the largest dilution volume, 1000 ml, results were not different from those with smaller dilution volumes, e.g. 100 and 200 ml. Therefore, it seemed unnecessary to repeat the dilution volume study to 1000 ml.

Initial experiments were carried out with different dilution volumes, e.g. 100, 200 and 250 ml. Results showed that the range of absorptivity for amabilis fir wood meal with 100 ml dilution was too large, probably due to <sup>lack of</sup> weighing accuracy (weight of sample was about 0.0100 g). The range was smaller for 200 and 250 ml dilution; the mean absorptivity for 200 ml dilution was 23.2, which is the same as <sup>the</sup> average absorptivity of 23.1 reported for softwoods (34). Therefore, dilution volume was settled at 200 ml (ca. 0.020 g oven-dry weight wood meal). Dilution to 250 ml showed no differences from those to 200 ml dilution. Results with 200 ml dilution are given in Table 2A.

#### B. Effect of cooking time

It is understood that cooking time (reaction time) is one important factor influencing behavior of reactants. In this experiment the cooking time was set as 30 min. In order to study cooking time effects, 40-60 mesh western hemlock (0-40 yr) wood meal with known moisture content

was used. Results are given in Table 2B. It is seen from these results that cooking time of  $30 \pm 5$  min. caused difference of only  $\pm 1.5\%$  in relative lignin content. This shows that the reaction time is not very critical in the 30 min. range. Reaction (or dissolution) is almost complete in that time for all species studied. This result is very similar to that reported (34).

C. Effect of reaction temperature

The effect of cooking temperature on the lignin determination is not significant at  $70 \pm 1^\circ\text{C}$  as found by Johnson, Moore and Zank (34), in that the change of absorptivity with reaction temperature was approximately 1% per degree at  $70^\circ\text{C}$ . An automatic<sup>-ally</sup> regulated hot water bath very easily meets this temperature requirement.

D. Effect of elapsed time before measurement

It was stated (34) that the Douglas fir wood solution showed no change in absorbance for as long as 5-hr storage after transfer and dilution to given volume, but that the time factor was more important with hardwood solutions.

In order to study the time factor, absorbance was read at different time intervals after dilution. The prepared solution was stored under running water at  $12 \pm 1^\circ\text{C}$ .

Results are given in Table 2C. Under these conditions it seems that the absorbance with *amabilis* fir wood does not

change for 24 hrs. after reaction. When the solution was kept at room temperature (about 25°C), however, absorbence was only stable for 5 hrs. after reaction. It seems that some other reactions occur, especially since the solution turns from light brown to dark brown colour, which is accompanied by an abrupt change of absorbence. This could result from some complex polybromide formation. Therefore, it was decided that absorbence reading would be made within 2 hrs. after completion of the cooking phase.

#### E. Calculation of sample size

An experimental series was carried out on <sup>six</sup> 0.020 g samples of western hemlock cooked at 70±1°C and measured within 2 hr. after reaction. Results are given in Table 2D.

According to the equation of Steel and Torrie (65), the number of replicates for each position in a growth increment can be calculated as:

$$N = t^2 \times s^2 / d^2$$

where,

N = number of replications

t = value from the t-table at n-1 degrees of freedom, and at the 95% probability level

s<sup>2</sup> = the sample variance

d = (mean x 0.0p)/2, with p% of allowable error.



From data in Table 2D, the number of necessary replications was calculated to be 1, with calculation also given in Table 2D. This replication number, one, is based on  $\pm 2.0\%$  (4%) allowable error. In order to obtain more reliable results, two or three replicates may be used.

### 3. Experimental errors

Other factors influencing experimental accuracy were also considered and controlled:

#### (1) Weighing of sample:

Sample weight can be considered to be the most important factor, especially in this micro-scale lignin determination, because a relative 2% error in reading can be encountered easily at the 0.020 g sample size.

Samples were weighed on a piece of paper, with both paper and paper plus sample weighed at least twice, or until constant readings were obtained. The paper used was pre-conditioned in the surrounding atmosphere so that equilibrium moisture was attained, thereby reducing the likelihood of change in weight during weighing.

During weighing, the automatic balance was turned on a couple of times to release strains developed in the scale.

#### (2) Chemicals

##### A. Acetyl bromide

Acetyl bromide is a reagent which dissolves more than 99% of the wood. This chemical is very reactive and has

a boiling point of  $76^{\circ}\text{C}$ . It fumes in moist air by liberation of hydrogen bromide and the formation of acetic acid. Therefore, in order to avoid changes in chemical concentration, the acetyl bromide was sealed as 20 ml ampules to prevent further reaction before use as occurred with opened stock bottles. It was also noticed that higher absorptivity was encountered when an old acetyl bromide solution was used.

#### B. Sodium hydroxide

This reagent is used mostly for its buffering effect, but it may have some other effects on the lignin molecule.

In order to maintain the same strength (2M) sodium hydroxide solution, a bulk solution (2000 ml) was prepared and this was stored in a bottle equipped with a gas tube containing ascorbate for absorbing carbon dioxide. This prevented carbonate formation and thereby change in solution concentration. The effect of sodium hydroxide on absorbance of the solution will be discussed later.

#### C. Hydroxylamine hydrochloride

This solution was also prepared in large amounts (300 ml) to maintain the same strength of solution. This reagent is reported to arrest further reaction of the solubilized wood.

### (3) Instrumental

#### A. Beckman Model DU spectrophotometer

The instrument used in this study was not equipped with cooling system. The instrument was warmed for at least  $3/4$  of an hour before absorbences were measured. This is required for stable instrumental reading.

#### B. Silica cell

Three silica cells with light path lengths of 10 mm and 1.5 ml capacity were used in this study. These cells were checked for their similarity in absorbence characteristics at the desired wavelength. Blank solution was first used for each cell and the differences in absorbence were recorded. Wood solution values were then measured and the differences were recorded. The results showed that no significant absorbence differences occurred in either case. The absorbence differences ranged from 0.001 to 0.002. These amounts of absorbence are not considered to be important.

In each case of changing solution for measurement, the cells were rinsed at least 8 times (4 times is considered to be standard). The outer cell surfaces were cleaned and dried with lens paper, since ordinary tissues cause scratching on the surface of cell and thereby influence absorbence.

#### C. Slit width

The slit width used was 0.8 mm (5). Change of slit width, especially to a wider slit may cause some variation in transmittance hence absorbence of the solution, because

the absorbence read is the mean value of absorbence at the given range of wavelength. The wider the range measured the lower the absorbence. In fact, this may not happen, because the commercial spectrophotometers are accurate enough to measure absorbence at bandwidth of less than 1 milimicron. Still, the wider slit width may cause further disadvantage in stray light effect (5), or deflection from the sides of the cell or lower surface of the solution.

#### D. Reading of absorbence

Absorbence of a given solution was read at least three times or until constant reading was obtained.

#### (4) Number of samples measured

Each batch consisted of six to nine samples. In any one such series samples from different positions within one to two growth increments were included. A blank solution was included with each batch in order to provide against variations caused by different batches of chemicals. This was especially noticeable with acetic acid.

#### (5) Volume of dilution

It is known that volume errors are caused mainly from differences in temperature and inaccurate calibration of volumetric ware. In this study the temperature of solution was kept almost constant during all dilutions so that the

volume error was minimized.

#### 4. Absorbance behaviour of wood lignin

The above mentioned factors are those which could arise during the experiment. So far as the absorbance characteristic of wood solution is concerned, there are many related findings and opinions:

##### A. Between species

It is very clear that the absorptivity of lignin from different species is not the same. This can be seen from the report of Johnson and co-workers (34) that the absorptivity of softwood lignin ranged from 22.6 in western hemlock to 24.0 in Douglas fir. Hardwood lignin<sup>varied</sup> from 22.4 in sweet gum to 24.5 in aspen and white ash.

##### B. Between different trees of the same species

Siegel and co-workers (62) studied the absorbance characteristic of woods from the same species. They reported that the fossil vascular cryptogams had less extractable lignin ultraviolet-absorbing content than the living prototypes.

##### C. Within an individual tree

It is understood that lignin obtained from different parts of the tree do not have exactly the same characteristic ultraviolet absorption spectra.

Bland and Hirnaji (11) studied variation of lignin

absorption spectra with radial position in the tree on a sample of Eucalyptus regnans F.v.M. containing 90 growth rings. The spectra were found to vary systematically from the centre of the tree outwards. They found shift of maximum from 272 mu at the pith to 262 mu in the outer rings, decrease in absorption at 230 mu relative to the maximum in the 262-272 mu range, shallower minimum in the 250-260 mu range, and the appearance of a weak band at 350 mu.

Mugg (43) on the other hand reported that Braun's lignin isolated from partially matured aspenwood resembled closely that prepared from matured aspenwood with respect to infrared and ultraviolet spectra, methoxyl content and physical appearance.

Bland (8, 9) in studies of reaction woods reported that the ultraviolet absorption spectra of the tension wood lignins had their maximum displaced toward the shorter wavelength and the minimum was shallower (both in Eucalyptus gonicalyx F.v.M. and E. regnans F.v.M.). No differences in lignin ultraviolet absorption spectra was found among compression, <sup>wood</sup> normal sapwood and heartwood of Pinus radiata D. Don.

#### D. Lignin fractions

Ishikawa and Takaichi (31) showed that in Cryptomeria

species the molecular extinction at  $\lambda_{\text{max}}$  282 m $\mu$  was proportional to molecular weights of different phenol lignin fractions.

Bland and Gately (10) reported that methanol extracted lignin from Eucalyptus regnans F.v.M. wood contained two main fractions and impurities. The sum of the two extinctions of the two fractions at 275 m $\mu$  amounted to about two-thirds of the extinction of the benzene-purified lignin.

Bland<sup>and Gately</sup> (10) further reported ~~the~~ absorption spectra for lignins separated chromatographically from methanol extracts of Eucalyptus regnans F.v.M. taken along a line from pith to bark. Two lignin fractions were produced, one of these was substantially the same from any position between pith and sapwood, the other apparently changed progressively from pith to sapwood.

Rezanowich and co-workers (48) reported that in four fractions of dioxane lignins prepared from white spruce there was a small, definite increase in the ultraviolet absorption and the refractive index increment with increase in the molecular weight of the fraction, although they were similar in the methoxyl content and intrinsic viscosity.

##### 5. Changes of absorption spectra by chemical reactions

In general, the change in ultraviolet absorption spectra of lignin may be summarized as follows:

#### A. Hypsochromic shift

A hypsochromic shift is a change of spectrum from longer to shorter wavelength due to substitution or solvent effect.

This may be due either to a change in the lignin complex on acetylation or a cross conjugation phenomenon, such as acetylation or methylation of lignin as reported by Stevens and Nord (66).

This shift can also be caused by hydrolysis of lignin in N sodium hydroxide solution as reported by Smith<sup>h</sup>(60, 61).

#### B. Bathochromic shift

This shift is a change to longer wavelength owing to substitution or solvent effect.

Ionization of the phenolic group in lignin as cause of bathochromic shift was reported by Aulin-Edtman<sup>r</sup> (3) and Ishikawa and Ide (30).

#### C. Hyperchromic effect

Hyperchromic effects result in an increase in absorption intensity.

This effect is proportional to the increasing pH of the lignin solution as reported by Aulin-Edtman<sup>r</sup> (3), Goldschmid<sup>21</sup> (20), and Maranville and Goldschmid (42). This effect with lignin is in most cases accompanied by bathochromic shift.



In bisulfite cooking of lignin the increase in absorption intensity at  $\lambda_{\max}$  280 mu was also found proportional to cooking time as was reported by Sobue and Hatano (63).

In general, the reaction time and reaction temperature of lignin are proportional to hyperchromic effect.

#### 6. Acid-soluble lignin

In this study, it is also considered that lignin content obtained may not include so called "acid-soluble lignin", because the standard lignin absorptivity (23.2) used in the calculation of lignin content does not include the acid-soluble wood fraction. In other words, this absorptivity was obtained from absorbence of amabilis fir wood solution calibrated to Klason lignin. Therefore, lignin content calculated according to this absorptivity would relate only to Klason lignin content.

This author also tried to measure absorption of a 3% Klason lignin filtrate. A  $\lambda_{\max}$  at 278 mu with absorbence of 0.420 for Sample 4 and 0.335 for Sample 5 was found in the Klason lignin determination of western hemlock samples.

These values when converted to lignin content by the method used in this study gave 2.30 % and 2.23% lignin respectively. Data were not used in correction of lignin content for this study, because the acid-soluble lignin is not yet fully understood. The absorption of this acid-soluble fraction may also give rise to the hydrolysis of

carbohydrate portion of wood as was reported by Richtzenhain and Dryselius (50) in that spruce, beech and aspen wood hydrolyzates contained very small amounts of lignin as measured by spectrophotometric means. A solution of glucose, after being treated with 72% sulfuric acid and then diluted to 3%, showed only insignificant ultraviolet absorption; however on boiling, a definite absorption spectrum of hydroxymethyl furfural developed. Xylose was also found to behave in a similar way.

#### 7. Other interfering substances

Substances other than lignin such as flavanones, furan, phlobaphenes and carbohydrate degradation products such as furfural and hydroxymethyl furfural are also known to have absorption spectra at the wavelength exhibited by lignin or lignin derivatives (13, 14).

In this study and also from the report of Johnson and co-workers (34), the absorption at  $\lambda_{\max}$  282 mu and 280 mu are proportional to Klason lignin content. Therefore, these interfering substances are of no <sup>large</sup> importance in lignin determination by the method used in this study.

#### 8. Reproducibility

It is also understood that the experimental results are inconsistent from person to person and from machine to machine. Therefore, in the comparison of results, these factors should also be taken into consideration.

## RESULTS

Results of lignin content determinations on five coniferous species are given in Tables 3-8 and are further plotted in Fig. 2-3. Statistic<sup>-al</sup> analyses of these data are given in Tables 14-24.

Relationships between lignin contents and related wood methoxyl contents (72) for these five species are given in Tables 9-13 and further plotted in Fig. 4-8. Correlation coefficients between lignin and wood methoxyl contents are also given for each species.

Non-lignin methoxyls were calculated based on the assumption that lignin methoxyl was 15%. Tables 9-13 are given for non-lignin methoxyl contents of the five coniferous species.

### 1. Distribution of lignin

From results of lignin determinations, it appears that:

- (1) Lignin content differs between species, from position to position within growth rings, and may also<sup>differ</sup> between rings (Tables 3-8, 14-19).
- (2) Lignin contents for individual species were in the order of: western hemlock, 32.85% (32.36-34.04%); western red cedar, 31.23% (29.69-32.68%); amabilis fir, 27.51% (26.17-28.24%); Douglas fir, 26.48% (24.08-28.66%); and Sitka spruce, 25.81% (24.91-26.98%).

This order does not exactly follow that of wood methoxyl contents (72).

(3) Except for western hemlock, earlywood had higher obvious lignin content than did the latewood in the same species (Fig.2-3).

(4) Three patterns of lignin content across growth rings were shown among these five species:

A. Lignin contents were highly significantly different within the positions of growth rings for amabilis fir, Douglas fir and western red cedar (Tables 14-16). Lignin increased from Position 1 to Position 2 in the earlywood and then decreased toward the latewood. Among these three species Position 2 had the highest lignin content in the growth ring (Fig. 2-3). This pattern of distribution within growth rings is the same as that shown for wood methoxyl in Douglas fir and amabilis fir (72).

B. Amounts of lignin were also highly significantly different among growth ring positions for Sitka spruce (Table 17). Lignin decreased progressively from Position 1 in the earlywood toward Position 6 in the latewood. Actual values show lignin highest at Position 1 in the earlywood and lowest in the transition zone wood (Fig.2-3).

C. Lignin contents were not significantly different within growth ring position of western hemlock (Table 18). Lignin reached highest amount at Position 2 in the earlywood and remained about constant thereafter among Positions 3-6 (Fig. 2-3).

(5) It can also be seen from the average lignin content for three adjacent rings in a given species that there appears to be a tendency for increase in lignin content with the increase of age:

A. Lignin contents were highly significantly different among the growth rings of western red cedar (Table 16). Lignin increased linearly from Ring 72 to Ring 74.

B. Lignin contents were significantly different among the growth rings of Douglas fir (Table 15). Lignin increased from Ring 64 to 65, but thereafter decreased to Ring 66.

C. Lignin contents were not significantly different among the growth rings of amabilis fir, Sitka spruce and western hemlock (Tables 14, 17, 18). Lignin increased almost linearly with age, but differences were too small to be significant.

(6) Regressions between lignin contents (Y) and positions within growth rings (X) are given in Fig. 3. These regressions were computed based on individual values from Tables 3-7.

The results showed that the correlation coefficient between lignin and position was highly significant for each of the five species studied. It appears that two patterns of regression can be observed:

A. The relation between lignin and position was best fitted to a first order (straight line) regression for amabilis fir, Douglas fir and western hemlock (Fig.3).

B. The relation between lignin and position was best fitted on a second order (parabola) regression for western red cedar and Sitka spruce (Fig.3).

Western red cedar showed a convex curve, whereas Sitka spruce showed a concave curve.

It is also considered, from these results, that the lower correlation coefficient ( $r$ ) and higher standard error of estimate ( $SE_e$ ) (in comparison with those of amabilis fir and Sitka spruce) for Douglas fir and western red cedar are due to significantly different lignin contents among three adjacent rings as noted above.

Western hemlock showed a relatively lower relationship between lignin and position, but its correlation coefficient is highly significant.

It seems that these regressions could be used for the estimation of lignin content across <sup>mature wood</sup> growth rings for these five species.

(7) Tables 20-24 are given for Duncan's tests of significantly different lignin contents among positions and growth rings in Tables 3-8. The lignin contents are arranged in order of magnitude rather than position or ring. The main groupings which contribute to the significance of lignin across or within growth increment can be put roughly into three categories:

A. The significance of lignin content across growth increments for amabilis fir and Sitka spruce is contributed to by two main lignin groups, earlywood and latewood and

may include transition wood, Position 4 (Tables 20,23).

In amabilis fir, these two groups can still be sub-divided into two sub-groups.

B. The significance of lignin content across growth increments of Douglas fir and average of five species is contributed to by four subsequential sub-groups from higher to lower lignin contents (Tables 21,24 ).

C. The significance of lignin content across growth increments for western red cedar is contributed to by three groups, namely one isolate, one main and one sub- group (Table 22).

D. The significance of lignin content among growth increments for Douglas fir (Table 21) and western red cedar (Table 22) is from two groupings.

E. The significance of lignin content for individual species (Table 24) is contributed to by each isolated lignin content.

## 2. Relationship between lignin and wood methoxyl

Wood methoxyl contents were obtained from <sup>a</sup>previous study <sub>made by the author</sub> (72). Methoxyl values for these five species and related lignin contents are listed in Tables 9-13 and are further shown in Fig. 4-8. Correlation coefficients are also calculated.

It appears that the relationships between lignin and wood methoxyl can be classified into two groups:

- A. The correlation coefficient is highly significant between lignin and wood methoxyl for amabilis fir (0.971), for Douglas fir (0.913), and for western red cedar (0.684).
- B. The correlation coefficient is not significant for lignin and wood methoxyl in Sitka spruce, 0.467, and western hemlock, 0.340.

From these results, it is concluded that the degree of association between lignin and wood methoxyl depends upon the species.

For species, such as amabilis fir and Douglas fir with high correlation coefficient between lignin and wood methoxyl contents, the lignin contents could be calculated directly from wood methoxyl contents.

Amabilis fir lignin content can be calculated from the regression,  $Y = 9.652 + 3.520X$ , and that of Douglas fir from  $Y = 8.693 + 3.474X$ , where Y stands for lignin content and X for wood methoxyl content.

These results clarified the issue that wood methoxyl either can or cannot be used for the calculation of relative lignin content <sup>54</sup>(72). Application depends mainly on the species being examined.

### 3. Non-lignin methoxyl content

Non-lignin methoxyl contents of the five species are given in Tables 9-13. These non-lignin methoxyls were calculated based on the assumption that lignin contains 15% methoxyl (native lignin has been reported to contain



14.8% methoxyl (13)).

It appears that non-lignin methoxyls are in the order of: western red cedar, 22.64%; Douglas fir, 22.46%; Sitka spruce, 20.21%; amabilis fir, 18.54%; and western hemlock, 10.53%. Average for the five species is 18.88%.

Non-lignin methoxyl is relatively constant across growth increment for the average of five species. The values from Position 1 to 6 are: 19.26%, 19.25%, 19.01%, 18.30%, 18.51% and 18.62%. For individual species the non-lignin methoxyls are relatively higher in earlywood than in latewood.

#### 4. Earlywood and latewood lignin content

Earlywood and latewood lignin contents for the five species studied are given in Table 1. Earlywood lignins were calculated by averaging the first three positions within growth rings, while the latewood lignins were considered as the last two positions, the fourth positions were regarded as transition between early- and latewood.

The difference of lignin content between earlywood and latewood is in the order of: Douglas fir, 2.27%; amabilis fir, 2.06%; western red cedar, 1.28%; Sitka spruce, 1.08%; and western hemlock, 0.46%.

#### 5. Discussion of results

It was considered by Ritter and Fleck (56) that the higher lignin content in earlywood and lower lignin content

in latewood was due to the fact that the middle lamella, enriched in lignin, makes up a larger portion of the earlywood than of the latewood tissue. This proposition may be in part true, except some modification may be needed, since, if the wood is considered in terms of specific gravity (weight) the middle lamella undoubtedly makes up a larger portion of the earlywood than of the latewood, but when the wood is considered in terms of volume, it is unlikely that the amount of middle lamella is larger in earlywood than in the latewood.

Unfortunately, no reference is available regarding the area or volume of middle lamella between wood cells. Therefore, no precise criterion can be set for lignin content difference between earlywood and latewood, because the degree of difference between earlywood and latewood lignin content is independent of species.

It has also been reported ~~that~~ volumetrically that tension wood lignin content was practically the same as that of normal wood, but on the weight basis the lignin content was lower in tension wood than in normal wood.

From <sup>a</sup> recent study by Green and Worrall (22) it is shown that the patterns of intra-incremental percent voids (or solids) were different between species. In viewing the patterns of percent voids across growth increments, there seems to be some similarity with the patterns of lignin contents found in this study. Therefore, this author would like to propose that in further study, it

would be valuable to attempt relation of wood solid substances to wood chemistry. Since it is considered that as a standing tree or a different species, the difference in external morphology is also related to internal anatomy, and that the external and internal morphology is related to the biological function of wood, thereby the formation of earlywood and latewood relate to function of wood within the stem. It is considered that earlywood and latewood differences are a good place to relate wood quality to wood anatomical<sup>and wood chemical</sup> features.

## CONCLUSIONS

The conclusions of this study are as follows:

1. Lignin content varied significantly between species: western hemlock, 32.85% (32.36-34.04%); western red cedar, 31.23% (29.69-32.68%); amabilis fir, 27.51% (26.17-28.24%); Douglas fir, 26.48% (24.08-28.66%); and Sitka spruce, 25.81% (24.91 - 26.98%).
2. Lignin content increased a small amount with the age across three adjacent rings of mature wood. This was in the order of: amabilis fir, 0.92%; western red cedar, 0.64%; western hemlock, 0.41%; Douglas fir, 0.23%; and Sitka spruce, 0.17%. Significant differences in lignin content between growth rings was found for western red cedar and Douglas fir, whereas the other three species showed no significant differences.
3. Lignin content also differed significantly within growth increments for each of the five species. In most cases, earlywood was higher in lignin content than latewood. The average difference between earlywood and latewood lignin content for individual species was: Douglas fir, 2.27%; amabilis fir, 2.06%; western red cedar, 1.28%; Sitka spruce, 1.08%; and western hemlock, 0.46%.

4. The correlation coefficient between lignin content and position within growth increment was highly significant for each of the five species studied. The r-values were: amabilis fir, 0.899; Sitka spruce, 0.866; Douglas fir, 0.816; western red cedar, 0.722; and western hemlock, 0.516. It is considered that lignin content within growth increments can be estimated by regression.
5. The degree of association between lignin and wood methoxyl content depended on species. The correlation coefficient between lignin and wood methoxyl was highly significant for amabilis fir (0.971), Douglas fir (0.913) and western red cedar (0.684), but was non-significant for Sitka spruce (0.468) and western hemlock (0.340). Therefore, it is suggested that with some species, wood methoxyl content can be used for direct estimation of lignin, whereas with other species wood methoxyl should not be used for this purpose.
6. The non-lignin methoxyl content was dependent on species and relatively constant within growth increments. The averages of six positions from earlywood to latewood for all five species were: 19.26%, 19.25%, 19.01%, 18.30%, 18.30%, 18.51%, and 18.62%. For each of the five species the non-lignin methoxyl was relatively higher in earlywood than latewood.

7. Acid-soluble lignin may exist in the sulfuric acid filtrate of Klason lignin. For western hemlock this acid-soluble lignin was found to be about 2% of wood (oven-dry weight basis) by spectrophotometric measurement.
8. In general, lignin content, wood methoxyl content, non-lignin methoxyl content, and the association between lignin and wood methoxyl are dependent on the coniferous species studied.

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Table 1

Experimental and literature lignin values  
for the five coniferous species

| <u>SPECIES</u>    | <u>LIG.%</u> | <u>(E)</u> | <u>(L)</u> | <u>SOURCE</u>          |
|-------------------|--------------|------------|------------|------------------------|
| Amabilis fir      | 26.90        |            |            | Wilson (70)            |
|                   | 27.70        |            |            | Wilson (70)            |
|                   | 30.11        |            |            | Wilson (70)            |
|                   | 32.98        |            |            | Wilson (70)            |
|                   | 27.51*       | 28.41*     | 26.35*     |                        |
| Douglas fir       | 26.70        |            |            | Lewis (40)             |
|                   | 29.35        |            |            | Kifer (35), Kurth (38) |
|                   | 30.63        |            |            | Wilson (70)            |
|                   | 31.62        |            |            | Wilson (70)            |
|                   | 33.50        |            |            | Richter (49)           |
|                   | 33.92        |            |            | Wilson (70)            |
|                   |              | 31.2       | 28.1       | Hale (25)              |
|                   |              | 32.61      | 29.20      | Ritter (56)            |
|                   |              | 35.1       | 31.6       | Bailey (4)             |
|                   | 26.48*       | 27.50*     | 25.23*     |                        |
| Sitka spruce      | 26.18        |            |            | Wilson (70)            |
|                   | 26.60        |            |            | Wilson (70)            |
|                   | 26.70        |            |            | Lewis (40)             |
|                   | 26.94        |            |            | Wilson (70)            |
|                   | 27.39        |            |            | Wilson (70)            |
|                   | 25.87*       | 26.35*     | 25.33*     |                        |
| Western hemlock   | 28.80        |            |            | Lewis (40)             |
|                   | 29.43        |            |            | Wilson (70)            |
|                   | 31.40        |            |            | Richter (49)           |
|                   | 32.57        |            |            | Wilson (70)            |
|                   | 32.92        |            |            | Wilson (70)            |
|                   | 33.06        |            |            | Wilson (70)            |
|                   | 32.85*       | 33.08*     | 32.62*     |                        |
| Western red cedar | 31.06        |            |            | Wilson (70)            |
|                   | 32.23        |            |            | Wilson (70)            |
|                   | 33.20        |            |            | Leopold (41)           |
|                   | 33.93        |            |            | Wilson (70)            |
|                   | 31.23*       | 31.79*     | 30.51*     |                        |

Lig., % - lignin percentage

(E) - earlywood lignin %

(L) - latewood lignin %

\* - lignin percentage obtained in this study

TABLE 2A

Absorptivity of amabilis fir lignin (70-80 yr)  
cooked at 70°C for 30 min. and absorbence  
read within 2 hr. after reaction  
(200 ml dilution)

| Sample<br>Wt.a.d.<br>(g) | Wt.o.d. | Lig.,% | A.    | Ab.   | Ab.<br>(m)  |
|--------------------------|---------|--------|-------|-------|-------------|
| 0.0277                   | 0.02457 | 30.11  | 0.884 | 23.35 |             |
| 0.0107                   | 0.00946 | 30.11  | 0.661 | 23.21 |             |
| 0.0079                   | 0.00700 | 30.11  | 0.490 | 23.30 | <u>23.2</u> |
| 0.0041                   | 0.00360 | 30.11  | 0.250 | 23.11 |             |

Wt.a.d.- air-dry weight of sample

Wt.o.d.- oven-dry weight of sample

Lig.,% - Klason lignin content of sample

A. - Absorbence

Ab. - Absorptivity of lignin

Ab.(m) - mean absorptivity of lignin

TABLE 2B

Effect of cooking time on western hemlock lignin (70±1°C)

| Reaction time,<br>min. | Calculated lignin,<br>% | Change, %     |
|------------------------|-------------------------|---------------|
| 5                      | 20.78                   | 65.16         |
| 10                     | 26.93                   | 84.45         |
| 20                     | 31.42                   | 98.53         |
| 25                     | 31.69                   | 99.37         |
| 27.5                   | 31.46                   | 98.65         |
| 30                     | 31.89                   | <u>100.00</u> |
| 32.5                   | 31.97                   | 100.25        |
| 35                     | 31.95                   | 100.19        |
| 40                     | 32.26                   | 101.16        |

TABLE 2C

Time interval effect on the reading of amabilis fir  
lignin absorbence

|           |       |       |       |       |       |
|-----------|-------|-------|-------|-------|-------|
| Time, hr. | 1.25  | 1.50  | 2.00  | 3.00  | 4.50  |
| A         |       |       |       |       |       |
| 1         | 1.000 | 1.010 | 1.000 | 1.000 | 0.990 |
| 2         | 0.542 | 0.542 | 0.543 | 0.541 | 0.542 |
| Time, hr. | 9.50  | 24.0  | 48.0  | 72.0  | 147   |
| A         |       |       |       |       |       |
| 1         | 0.988 | 0.990 | 0.990 | 1.000 | 1.000 |
| 2         | 0.535 | 0.541 | 0.560 | 0.544 | 0.541 |

A - Absorbences for Sample 1 and 2

TABLE 2D

Western hemlock lignin percentage and the calculation  
of replicate sample number

| Sample | Lignin, % |
|--------|-----------|
| 1      | 31.42     |
| 2      | 31.69     |
| 3      | 31.46     |
| 4      | 31.89     |
| 5      | 31.97     |
| 6      | 31.95     |

$$N = t^2 \times s^2 / d^2$$

$$t = 2.571 \quad t^2 = 6.610$$

$$s^2 = 0.06044$$

if allowable error =  $\pm 2\%$ , then

$$d^2 = 0.4027$$

$$N = 6.610 \times 0.06044 / 0.4027$$

$$= 0.992 \text{ ( one be used )}$$



TABLE 3

Lignin percentages across 78-80 yr. growth increments  
of amabilis fir sample

|    | 1              | 2              | 3              | 4              | 5              | 6              |              |
|----|----------------|----------------|----------------|----------------|----------------|----------------|--------------|
| 78 | 28.67<br>29.35 | 28.32<br>28.36 | 27.79<br>27.50 | 26.58<br>26.67 | 26.38<br>25.95 | 26.28<br>26.20 |              |
| a. | <u>29.01</u>   | <u>28.34</u>   | <u>27.65</u>   | <u>26.63</u>   | <u>26.17</u>   | <u>26.24</u>   | <u>27.34</u> |
| 79 | 27.75<br>27.86 | 29.02<br>28.98 | 27.96<br>27.66 | 27.29<br>27.14 | 26.37<br>26.76 | 26.45<br>26.02 |              |
| a. | <u>27.81</u>   | <u>29.00</u>   | <u>27.81</u>   | <u>27.22</u>   | <u>26.57</u>   | <u>26.24</u>   | <u>27.44</u> |
| 80 | 28.86<br>28.49 | 29.10<br>29.20 | 28.20<br>28.26 | 27.62<br>27.55 | 26.59<br>26.38 | 26.21<br>26.54 |              |
| a. | <u>28.68</u>   | <u>29.15</u>   | <u>28.23</u>   | <u>27.59</u>   | <u>26.49</u>   | <u>26.38</u>   | <u>27.75</u> |
|    | <u>28.50</u>   | <u>28.83</u>   | <u>27.90</u>   | <u>27.15</u>   | <u>26.41</u>   | <u>26.29</u>   | <u>27.51</u> |

Positions 1 to 6 stands for earlywood to latewood  
within a growth increment.

a. average of two replicates.

Values are based on extractive-free and moisture-free  
wood samples.

TABLE 4

Lignin percentages across 64-66 yr. growth increments  
of Douglas fir sample

|    | 1              | 2              | 3              | 4              | 5              | 6              |              |
|----|----------------|----------------|----------------|----------------|----------------|----------------|--------------|
| 64 | 27.15<br>27.36 | 27.37<br>27.88 | 26.43<br>26.44 | 26.22<br>26.48 | 26.01<br>25.85 | 25.19<br>25.22 | 26.45        |
| a. | <u>27.26</u>   | <u>27.63</u>   | <u>26.44</u>   | <u>26.35</u>   | <u>25.80</u>   | <u>25.21</u>   | <u>26.45</u> |
| 65 | 28.35<br>28.66 | 28.67<br>28.65 | 27.83<br>27.45 | 26.52<br>26.50 | 25.97<br>25.92 | 25.28<br>25.24 |              |
| a. | <u>28.51</u>   | <u>28.66</u>   | <u>27.64</u>   | <u>26.51</u>   | <u>25.95</u>   | <u>25.26</u>   | <u>27.09</u> |
| 66 | 27.06<br>27.14 | 28.26<br>28.62 | 25.93<br>25.79 | 24.74<br>25.26 | 24.19<br>23.97 | 25.20<br>24.93 |              |
| a. | <u>27.10</u>   | <u>28.44</u>   | <u>25.86</u>   | <u>25.00</u>   | <u>24.08</u>   | <u>25.07</u>   | <u>25.93</u> |
|    | <u>27.62</u>   | <u>28.24</u>   | <u>26.65</u>   | <u>25.95</u>   | <u>25.28</u>   | <u>25.18</u>   |              |

Positions, basis and averages as Table 3.

TABLE 5

Lignin percentages across 72-74 yr. growth increments  
of western red cedar sample

|    | 1              | 2              | 3              | 4              | 5              | 6              |              |
|----|----------------|----------------|----------------|----------------|----------------|----------------|--------------|
| 72 | 31.30<br>31.14 | 32.14<br>32.16 | 30.99<br>30.80 | 30.78<br>30.68 | 30.26<br>30.10 | 29.87<br>29.63 |              |
| a. | <u>31.22</u>   | <u>32.15</u>   | <u>30.90</u>   | <u>30.73</u>   | <u>30.17</u>   | <u>29.80</u>   | <u>30.83</u> |
| 73 | 31.55<br>31.75 | 32.79<br>32.56 | 31.49<br>31.56 | 30.69<br>30.29 | 30.61<br>30.82 | 29.76<br>29.62 |              |
| a. | <u>31.65</u>   | <u>32.68</u>   | <u>31.53</u>   | <u>30.49</u>   | <u>30.72</u>   | <u>29.69</u>   | <u>31.13</u> |
| 74 | 31.42<br>31.44 | 32.68<br>32.50 | 31.90<br>32.03 | 31.86<br>31.72 | 31.16<br>31.53 | 31.26<br>31.29 |              |
| a. | <u>31.43</u>   | <u>32.59</u>   | <u>31.97</u>   | <u>31.79</u>   | <u>31.35</u>   | <u>31.28</u>   | <u>31.74</u> |
|    | <u>31.43</u>   | <u>32.47</u>   | <u>31.47</u>   | <u>31.00</u>   | <u>30.75</u>   | <u>30.26</u>   | <u>31.23</u> |

Positions, basis and averages as Table 3.

TABLE 6

Lignin percentages across 69-71 yr. growth increments  
of Sitka spruce sample

|    | 1              | 2              | 3              | 4              | 5              | 6              |              |
|----|----------------|----------------|----------------|----------------|----------------|----------------|--------------|
| 69 | 26.70<br>26.45 | 26.17<br>26.46 | 25.60<br>25.62 | 24.80<br>25.20 | 25.41<br>24.93 | 25.23<br>25.57 |              |
| a. | <u>26.58</u>   | <u>26.37</u>   | <u>25.61</u>   | <u>25.00</u>   | <u>25.39</u>   | <u>25.35</u>   | <u>25.72</u> |
| 70 | 26.56<br>26.87 | 26.05<br>26.22 | 25.49<br>25.67 | 25.01<br>24.77 | 25.51<br>25.31 | 25.40<br>25.64 |              |
| a. | <u>26.72</u>   | <u>26.14</u>   | <u>25.58</u>   | <u>24.91</u>   | <u>25.41</u>   | <u>25.52</u>   | <u>25.71</u> |
| 71 | 27.00<br>26.95 | 26.51<br>26.67 | 26.67<br>26.54 | 25.37<br>25.49 | 25.05<br>25.65 | 24.93<br>24.99 |              |
| a. | <u>26.98</u>   | <u>26.59</u>   | <u>26.61</u>   | <u>25.43</u>   | <u>25.35</u>   | <u>24.96</u>   | <u>25.95</u> |
|    | <u>26.76</u>   | <u>26.37</u>   | <u>25.93</u>   | <u>25.11</u>   | <u>25.38</u>   | <u>25.28</u>   | <u>25.81</u> |

Positions, basis and averages as Table 3.

TABLE 7

Lignin percentages across 56-58 yr. growth increments  
of western hemlock sample

|    | 1                       | 2                       | 3                       | 4                       | 5                       | 6                       |              |
|----|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------|
| 56 | 32.35<br>32.42<br>32.86 | 33.20<br>33.38<br>32.61 | 32.44<br>32.93<br>32.69 | 32.83<br>32.69<br>33.08 | 32.40<br>32.86<br>32.50 | 32.53<br>32.65<br>32.45 |              |
| a. | <u>32.54</u>            | <u>33.06</u>            | <u>32.69</u>            | <u>32.87</u>            | <u>32.59</u>            | <u>32.54</u>            | <u>32.72</u> |
| 57 | 33.09<br>33.43<br>33.84 | 33.70<br>34.16<br>34.25 | 32.45<br>32.75<br>33.35 | 32.68<br>32.03<br>32.37 | 32.99<br>32.03<br>32.37 | 32.86<br>32.20<br>32.66 |              |
| a. | <u>33.45</u>            | <u>34.04</u>            | <u>32.85</u>            | <u>32.36</u>            | <u>32.46</u>            | <u>32.57</u>            | <u>32.96</u> |
| 58 | 33.26<br>33.41<br>32.90 | 32.97<br>33.10<br>33.41 | 32.53<br>32.76<br>32.95 | 32.72<br>32.80<br>32.68 | 33.36<br>33.08<br>32.86 | 32.46<br>32.03<br>32.80 |              |
| a. | <u>33.19</u>            | <u>33.16</u>            | <u>32.75</u>            | <u>32.73</u>            | <u>33.10</u>            | <u>32.43</u>            | <u>32.89</u> |
|    | <u>33.06</u>            | <u>33.42</u>            | <u>32.76</u>            | <u>32.65</u>            | <u>32.72</u>            | <u>32.51</u>            | <u>32.85</u> |

Positions and basis as Table 3.

a. Average of three replicates.

TABLE 8

Average lignin percentages of the five coniferous  
species at each position within increment

|     | 1            | 2            | 3            | 4            | 5            | 6            |              |
|-----|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| AF  | 28.50        | 28.83        | 27.90        | 27.15        | 26.41        | 26.29        | <u>27.51</u> |
| DF  | 27.62        | 28.24        | 26.65        | 25.95        | 25.28        | 25.18        | <u>26.48</u> |
| WRC | 31.43        | 32.47        | 31.47        | 31.30        | 30.75        | 30.26        | <u>31.23</u> |
| SS  | 26.76        | 26.37        | 25.93        | 25.11        | 25.38        | 25.28        | <u>25.81</u> |
| WH  | 33.06        | 33.42        | 32.76        | 32.65        | 32.72        | 32.51        | <u>32.85</u> |
|     | <u>29.47</u> | <u>29.87</u> | <u>28.94</u> | <u>28.43</u> | <u>28.11</u> | <u>27.90</u> |              |

AF - amabilis fir  
DF - Douglas fir  
WRC - western red cedar  
SS - Sitka spruce  
WH - western hemlock

Source: U.S. Forest Service, Pacific Northwest Forest Experiment Station

TABLE 9

Lignin and wood methoxyl percentages across 78-80 yr.  
growth increments of amabilis fir sample

|    |      | 1     | 2     | 3     | 4     | 5     | 6     |       |
|----|------|-------|-------|-------|-------|-------|-------|-------|
| 78 | m    | 5.50  | 5.54  | 5.26  | 5.00  | 4.76  | 4.76  | 5.12  |
|    | l    | 29.01 | 28.34 | 27.65 | 26.63 | 26.17 | 26.24 | 27.34 |
|    | nlm  | 1.15  | 1.29  | 1.11  | 1.01  | 0.83  | 0.82  | 1.02  |
|    | nlm% | 20.91 | 23.29 | 21.10 | 20.20 | 17.44 | 17.23 | 19.92 |
| 79 | m    | 5.21  | 5.29  | 5.03  | 4.96  | 4.82  | 4.75  | 5.01  |
|    | l    | 27.81 | 29.00 | 27.81 | 27.22 | 26.57 | 26.24 | 27.44 |
|    | nlm  | 1.04  | 0.94  | 0.86  | 0.88  | 0.83  | 0.81  | 0.89  |
|    | nlm% | 19.96 | 17.77 | 17.10 | 17.74 | 17.20 | 20.56 | 17.76 |
| 80 | m    | 5.24  | 5.47  | 5.21  | 4.92  | 4.90  | 4.71  | 5.08  |
|    | l    | 28.68 | 29.15 | 28.23 | 27.59 | 26.49 | 26.38 | 27.75 |
|    | nlm  | 0.94  | 1.10  | 0.98  | 0.78  | 0.93  | 0.75  | 0.92  |
|    | nlm% | 17.94 | 20.11 | 18.81 | 15.85 | 18.98 | 15.92 | 18.11 |
|    | m    | 5.32  | 5.43  | 5.17  | 4.96  | 4.83  | 4.74  | 5.07  |
|    | l    | 28.50 | 28.83 | 27.90 | 27.15 | 26.41 | 26.29 | 27.51 |
|    | nlm  | 1.04  | 1.11  | 0.98  | 0.89  | 0.87  | 0.80  | 0.94  |
|    | nlm% | 19.55 | 20.44 | 18.96 | 17.94 | 18.01 | 16.88 | 18.54 |

m - wood methoxyl content cited from previous study (72).

l - lignin content obtained in this study .

nlm - non-lignin wood methoxyl content m - (l x 15.0%).

nlm% - non-lignin wood methoxyl content in terms of %,  
( nlm/m x 100 )

Positions 1 to 6 stand for earlywood to latewood  
within a growth increment.

TABLE 10

Lignin and wood methoxyl percentages across 64-66 yr.  
growth increments of Douglas fir sample

|      | 1     | 2     | 3     | 4     | 5     | 6     |       |
|------|-------|-------|-------|-------|-------|-------|-------|
| 64 m | 5.50  | 5.56  | 5.05  | 4.94  | 5.01  | 5.01  | 5.18  |
| l    | 27.26 | 27.63 | 26.44 | 26.35 | 25.80 | 25.21 | 26.45 |
| nlm  | 1.41  | 1.42  | 1.08  | 0.99  | 1.14  | 1.23  | 1.21  |
| nlm% | 25.64 | 25.54 | 21.39 | 20.04 | 22.75 | 24.55 | 23.36 |
| 65 m | 5.61  | 5.69  | 5.14  | 4.80  | 4.98  | 4.97  | 5.20  |
| l    | 28.51 | 28.66 | 27.64 | 26.51 | 25.95 | 25.26 | 27.09 |
| nlm  | 1.33  | 1.39  | 0.99  | 0.82  | 1.09  | 1.18  | 1.14  |
| nlm% | 23.71 | 24.43 | 19.26 | 17.08 | 21.89 | 23.74 | 21.92 |
| 66 m | 5.29  | 5.68  | 4.85  | 4.86  | 4.61  | 4.62  | 4.99  |
| l    | 27.10 | 28.44 | 25.86 | 25.00 | 24.08 | 25.07 | 25.93 |
| nlm  | 1.22  | 1.35  | 0.97  | 1.11  | 1.00  | 0.86  | 1.10  |
| nlm% | 23.06 | 23.77 | 20.00 | 22.84 | 21.69 | 18.61 | 22.04 |
| m    | 5.47  | 5.64  | 5.01  | 4.87  | 4.87  | 4.87  | 5.12  |
| l    | 27.62 | 28.24 | 26.65 | 25.95 | 25.28 | 25.18 | 26.48 |
| nlm  | 1.33  | 1.40  | 1.01  | 0.98  | 1.08  | 1.09  | 1.15  |
| nlm% | 24.31 | 24.82 | 20.16 | 20.12 | 22.18 | 22.38 | 22.46 |

Positions and abbreviations as Table 9.

TABLE 11

Lignin and wood methoxyl percentages across 72-74 yr.  
growth increments of western red cedar sample

|      | 1     | 2     | 3     | 4     | 5     | 6     |       |
|------|-------|-------|-------|-------|-------|-------|-------|
| 72 m | 6.35  | 6.28  | 6.27  | 6.02  | 5.70  | 5.63  | 6.04  |
| l    | 31.22 | 32.15 | 30.90 | 30.73 | 30.17 | 29.80 | 30.83 |
| nlm  | 1.67  | 1.46  | 1.63  | 1.41  | 1.17  | 1.16  | 1.42  |
| nlm% | 26.30 | 23.25 | 26.00 | 23.42 | 20.53 | 20.60 | 23.51 |
| 73 m | 6.05  | 6.11  | 5.83  | 5.86  | 5.84  | 5.82  | 5.92  |
| l    | 31.65 | 32.68 | 31.53 | 30.49 | 30.72 | 29.69 | 31.13 |
| nlm  | 1.30  | 1.21  | 1.10  | 1.29  | 1.23  | 1.37  | 1.25  |
| nlm% | 21.49 | 19.80 | 18.87 | 22.01 | 21.06 | 23.54 | 21.11 |
| 74 m | 6.48  | 6.37  | 6.12  | 6.06  | 6.03  | 5.99  | 6.18  |
| l    | 31.43 | 32.59 | 31.97 | 31.79 | 31.35 | 31.28 | 31.74 |
| nlm  | 1.77  | 1.48  | 1.32  | 1.29  | 1.33  | 1.30  | 1.42  |
| nlm% | 27.31 | 23.23 | 21.57 | 21.29 | 22.06 | 21.70 | 22.98 |
| m    | 6.29  | 6.25  | 6.07  | 5.98  | 5.86  | 5.81  | 6.05  |
| l    | 31.43 | 32.47 | 31.47 | 31.00 | 30.75 | 30.26 | 31.23 |
| nlm  | 1.58  | 1.38  | 1.35  | 1.33  | 1.25  | 1.27  | 1.37  |
| nlm% | 25.12 | 22.08 | 22.24 | 22.24 | 21.33 | 21.86 | 22.64 |

Positions and abbreviations as Table 9.

TABLE 12

Lignin and wood methoxyl percentages across 69-71 yr.  
growth increments of Sitka spruce sample

|    |      | 1            | 2            | 3            | 4            | 5            | 6            |              |
|----|------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 69 | m    | 5.27         | 5.05         | 4.91         | 4.65         | 4.62         | 4.81         | 4.89         |
|    | l    | 26.58        | 26.37        | 25.61        | 25.00        | 25.39        | 25.35        | <u>25.72</u> |
|    | nlm  | 1.28         | 1.09         | 1.07         | 0.90         | 0.81         | 1.01         | <u>1.03</u>  |
|    | nlm% | 24.29        | 21.58        | 21.79        | 19.35        | 17.53        | 21.00        | <u>21.06</u> |
| 70 | m    | 4.77         | 4.80         | 4.85         | 4.65         | 4.77         | 4.80         | 4.77         |
|    | l    | 26.72        | 26.14        | 25.58        | 24.81        | 25.41        | 25.52        | <u>25.71</u> |
|    | nlm  | 0.76         | 0.88         | 1.01         | 0.91         | 0.96         | 0.97         | <u>0.91</u>  |
|    | nlm% | 15.93        | 18.33        | 20.82        | 19.57        | 20.13        | 20.21        | <u>19.08</u> |
| 71 | m    | 4.79         | 4.82         | 4.98         | 4.93         | 4.88         | 4.93         | 4.89         |
|    | l    | 26.98        | 26.59        | 26.61        | 25.43        | 25.35        | 24.96        | <u>25.95</u> |
|    | nlm  | 0.74         | 0.83         | 0.99         | 1.12         | 1.08         | 1.19         | <u>1.00</u>  |
|    | nlm% | 15.45        | 17.22        | 19.88        | 22.72        | 22.13        | 24.14        | <u>20.45</u> |
|    | m    | 4.94         | 4.89         | 4.91         | 4.74         | 4.76         | 4.85         | 4.85         |
|    | l    | <u>26.76</u> | <u>26.37</u> | <u>25.93</u> | <u>25.11</u> | <u>25.38</u> | <u>25.28</u> | <u>25.81</u> |
|    | nlm  | <u>0.93</u>  | <u>0.93</u>  | <u>1.02</u>  | <u>0.97</u>  | <u>0.95</u>  | <u>1.06</u>  | <u>0.98</u>  |
|    | nlm% | <u>18.83</u> | <u>19.02</u> | <u>20.77</u> | <u>20.46</u> | <u>19.96</u> | <u>21.86</u> | <u>20.21</u> |

Positions and abbreviations as Table 9.

TABLE 13

Lignin and wood methoxyl percentages across 56-58 yr.  
growth increments of western hemlock sample

|    |      | 1            | 2            | 3            | 4            | 5            | 6            |              |
|----|------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 56 | m    | 5.32         | 5.60         | 5.87         | 5.73         | 5.58         | 5.36         | 5.58         |
|    | l    | 32.54        | 33.06        | 32.69        | 32.87        | 32.59        | 32.54        | <u>32.72</u> |
|    | nlm  | 0.44         | 0.64         | 0.97         | 0.80         | 0.69         | 0.48         | <u>0.67</u>  |
|    | nlm% | 8.27         | 11.43        | 16.52        | 13.96        | 12.37        | 8.96         | <u>12.01</u> |
| 57 | m    | 5.41         | 5.58         | 5.53         | 5.42         | 5.27         | 5.31         | 5.42         |
|    | l    | 33.45        | 34.04        | 32.85        | 32.36        | 32.46        | 32.57        | <u>32.96</u> |
|    | nlm  | 0.39         | 0.47         | 0.60         | 0.57         | 0.40         | 0.42         | <u>0.48</u>  |
|    | nlm% | 7.21         | 8.42         | 10.85        | 10.52        | 7.59         | 7.91         | <u>8.86</u>  |
| 58 | m    | 5.53         | 5.50         | 5.53         | 5.33         | 5.72         | 5.62         | 5.54         |
|    | l    | 33.19        | 33.16        | 32.75        | 32.73        | 33.10        | 32.43        | <u>32.89</u> |
|    | nlm  | 0.55         | 0.53         | 0.62         | 0.42         | 0.75         | 0.76         | <u>0.61</u>  |
|    | nlm% | 9.95         | 9.64         | 11.24        | 7.88         | 13.11        | 13.52        | <u>11.01</u> |
|    | m    | <u>5.42</u>  | <u>5.56</u>  | <u>5.64</u>  | <u>5.49</u>  | <u>5.52</u>  | <u>5.43</u>  | <u>5.51</u>  |
|    | l    | <u>33.06</u> | <u>33.42</u> | <u>32.76</u> | <u>32.65</u> | <u>32.72</u> | <u>32.51</u> | <u>32.85</u> |
|    | nlm  | <u>0.46</u>  | <u>0.55</u>  | <u>0.73</u>  | <u>0.59</u>  | <u>0.61</u>  | <u>0.55</u>  | <u>0.58</u>  |
|    | nlm% | <u>8.49</u>  | <u>9.89</u>  | <u>12.94</u> | <u>10.75</u> | <u>11.05</u> | <u>10.13</u> | <u>10.53</u> |

Positions and abbreviations as Table 9.

TABLE 14

Analysis of variance of amabilis fir lignin percentages

|           | <u>df</u> | <u>SS</u> | <u>MS</u> | <u>F</u> | <u>F(tab.)</u><br>0.05 0.01<br>3.33 5.64 |  |      |
|-----------|-----------|-----------|-----------|----------|--|--|------|
| Positions | 5         | 17.1317   | 3.4264    | 25.684** |  |  | H.S. |
| Rings     | 2         | 0.5566    | 0.2783    | 2.086    |  |  | N.S. |
| P x R     | 10        | 1.3344    | 0.1334    |          |  |  |      |
| Total     | 17        | 19.0027   |           |          |  |  |      |

df - degree of freedom  
SS - sum of square  
MS - mean of the sum of square  
F- - F-values calculated  
F(tab.) - F-values from F-table  
H.S. -\*\* - highly significantly different  
N.S. - not significantly different

TABLE 15

Analysis of variance of Douglas fir lignin percentages

|           | <u>df</u> | <u>SS</u> | <u>MS</u> | <u>F</u> | <u>F(tab.)</u><br>0.05 0.01<br>3.33 5.64 |      |      |
|-----------|-----------|-----------|-----------|----------|--|------|------|
| Positions | 5         | 23.5783   | 4.7157    | 16.206** |  |      | H.S. |
| Rings     | 2         | 4.074     | 2.0369    | 7.000*   | 4.10                                     | 7.56 | S.D. |
| P x R     | 10        | 2.9098    | 0.2910    |          |  |      |      |
| Total     | 17        | 30.5618   |           |          |  |      |      |

\* - S.D. - significant difference

TABLE 16

Analysis of variance of western red cedar lignin percentages

|           | <u>df</u> | <u>SS</u> | <u>MS</u> | <u>F</u> | <u>F(tab.)</u><br>0.05 0.01<br>3.33 5.64 |      |      |
|-----------|-----------|-----------|-----------|----------|--|------|------|
| Positions | 5         | 8.6268    | 1.7254    | 11.496** |  |      | H.S. |
| Rings     | 2         | 2.5622    | 1.2811    | 8.536**  | 4.10                                     | 7.56 | H.S. |
| P x R     | 10        | 1.5008    | 0.1501    |          |  |      |      |
| Total     | 17        | 12.6898   |           |          |  |      |      |



TABLE 17

Analysis of variance of Sitka spruce lignin percentages

|           | <u>df</u> | <u>SS</u> | <u>MS</u> | <u>F</u> | <u>F(tab.)</u><br><u>0.05</u> <u>0.01</u> |      |      |
|-----------|-----------|-----------|-----------|----------|---|------|------|
| Positions | 5         | 6.5379    | 1.3076    | 14.577** | 3.33                                      | 5.64 | H.S. |
| Rings     | 2         | 0.2952    | 0.1476    | 1.645    | 4.10                                      | 7.56 | N.S. |
| P x R     | 10        | 0.8969    | 0.0897    |          |   |      |      |
| Total     | 17        | 7.7300    |           |          |   |      |      |

TABLE 18

Analysis of variance of western hemlock lignin percentages

|           | <u>df</u> | <u>SS</u> | <u>MS</u> | <u>F</u> | <u>F(tab.)</u><br><u>0.05</u> <u>0.01</u> |      |      |
|-----------|-----------|-----------|-----------|----------|---|------|------|
| Positions | 5         | 1.6385    | 0.3277    | 2.631    | 3.33                                      | 5.64 | N.S. |
| Rings     | 2         | 0.1864    | 0.0932    | 0.760    | 4.10                                      | 7.56 | N.S. |
| P x R     | 10        | 1.2263    | 0.1226    |          |   |      |      |
| Total     | 17        | 3.0512    |           |          |   |      |      |

TABLE 19

Analysis of variance of average lignin percentages  
for the five coniferous species

|           |    |          |         |           |      |      |      |
|-----------|----|----------|---------|-----------|------|------|------|
| Positions | 5  | 15.1349  | 3.0267  | 15.380**  | 2.71 | 4.10 | H.S. |
| Species   | 4  | 231.3372 | 57.8343 | 293.873** | 2.87 | 4.43 | H.S. |
| P x R     | 20 | 3.9364   | 0.1968  |           |      |      |      |
| Total     | 29 | 250.4085 |         |           |      |      |      |

TABLE 20

Duncan's test of mean amabilis fir lignin percentages

Difference in Positions

|          |       |       |       |              |              |              |
|----------|-------|-------|-------|--------------|--------------|--------------|
| Position | 2     | 1     | 3     | 4            | 6            | 5            |
| Lignin   | 28.83 | 28.50 | 27.90 | <u>27.15</u> | <u>26.62</u> | <u>26.41</u> |

———— no significant difference between values underlined

TABLE 21

Duncan's test of mean Douglas fir lignin percentages

Difference in Positions

|          |              |              |              |              |              |              |
|----------|--------------|--------------|--------------|--------------|--------------|--------------|
| Position | 2            | 1            | 3            | 4            | 5            | 6            |
| Lignin   | <u>28.24</u> | <u>27.62</u> | <u>26.65</u> | <u>25.95</u> | <u>25.28</u> | <u>25.18</u> |

Difference in Rings

|        |              |              |              |
|--------|--------------|--------------|--------------|
| Ring   | 65           | 64           | 66           |
| Lignin | <u>27.09</u> | <u>26.45</u> | <u>25.93</u> |

TABLE 22

Duncan's test of mean western red cedar lignin percentages

Difference in Positions

|          |       |              |              |              |              |              |
|----------|-------|--------------|--------------|--------------|--------------|--------------|
| Position | 2     | 3            | 1            | 4            | 5            | 6            |
| Lignin   | 32.47 | <u>31.47</u> | <u>31.43</u> | <u>31.00</u> | <u>30.75</u> | <u>30.26</u> |

Difference in Rings

|        |       |              |              |
|--------|-------|--------------|--------------|
| Ring   | 74    | 73           | 72           |
| Lignin | 31.74 | <u>31.13</u> | <u>30.83</u> |

TABLE 23

Duncan's test of mean Sitka spruce lignin percentages

Difference in Positions

|          |              |              |              |              |              |              |
|----------|--------------|--------------|--------------|--------------|--------------|--------------|
| Position | 1            | 2            | 3            | 5            | 6            | 4            |
| Lignin   | <u>26.76</u> | <u>26.37</u> | <u>25.93</u> | <u>25.38</u> | <u>25.28</u> | <u>25.11</u> |

TABLE 24

Duncan's test of average lignin percentages of five  
coniferous species

Difference in Positions

|          |              |              |              |              |              |              |
|----------|--------------|--------------|--------------|--------------|--------------|--------------|
| Position | 2            | 1            | 3            | 4            | 5            | 6            |
| Lignin   | <u>29.87</u> | <u>29.47</u> | <u>28.94</u> | <u>28.43</u> | <u>28.11</u> | <u>27.90</u> |

Difference in Species

|         |       |       |       |       |       |
|---------|-------|-------|-------|-------|-------|
| Species | WH    | WRC   | AF    | DF    | SS    |
| Lignin  | 32.85 | 31.23 | 27.51 | 26.48 | 25.81 |

WH - western hemlock  
WRC - western red cedar  
AF - amabilis fir  
DF - Douglas fir  
SS - Sitka spruce



Figure 1

Beckman Model DU single beam manual spectrophotometer

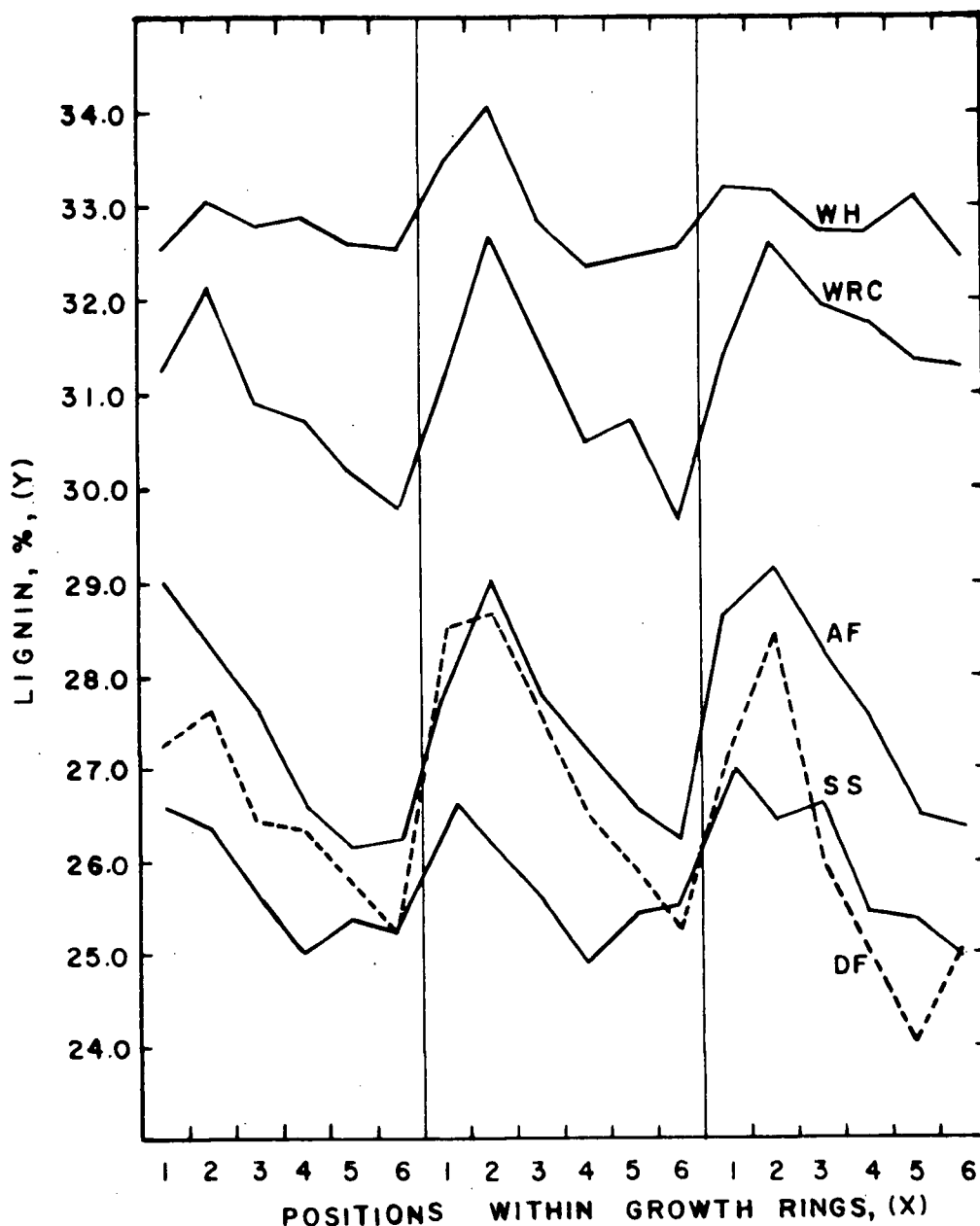


Fig. 2. Patterns of lignin percentages across growth increments of the five coniferous species

- WH - Western hemlock (56-58 yr)
- WRC - Western red cedar (72-74 yr)
- AF - Amabilis fir (78-80 yr)
- DF - Douglas fir (64-66 yr)
- SS - Sitka spruce (69-71 yr)

(Position 1 first formed earlywood  
Position 6 last formed latewood)

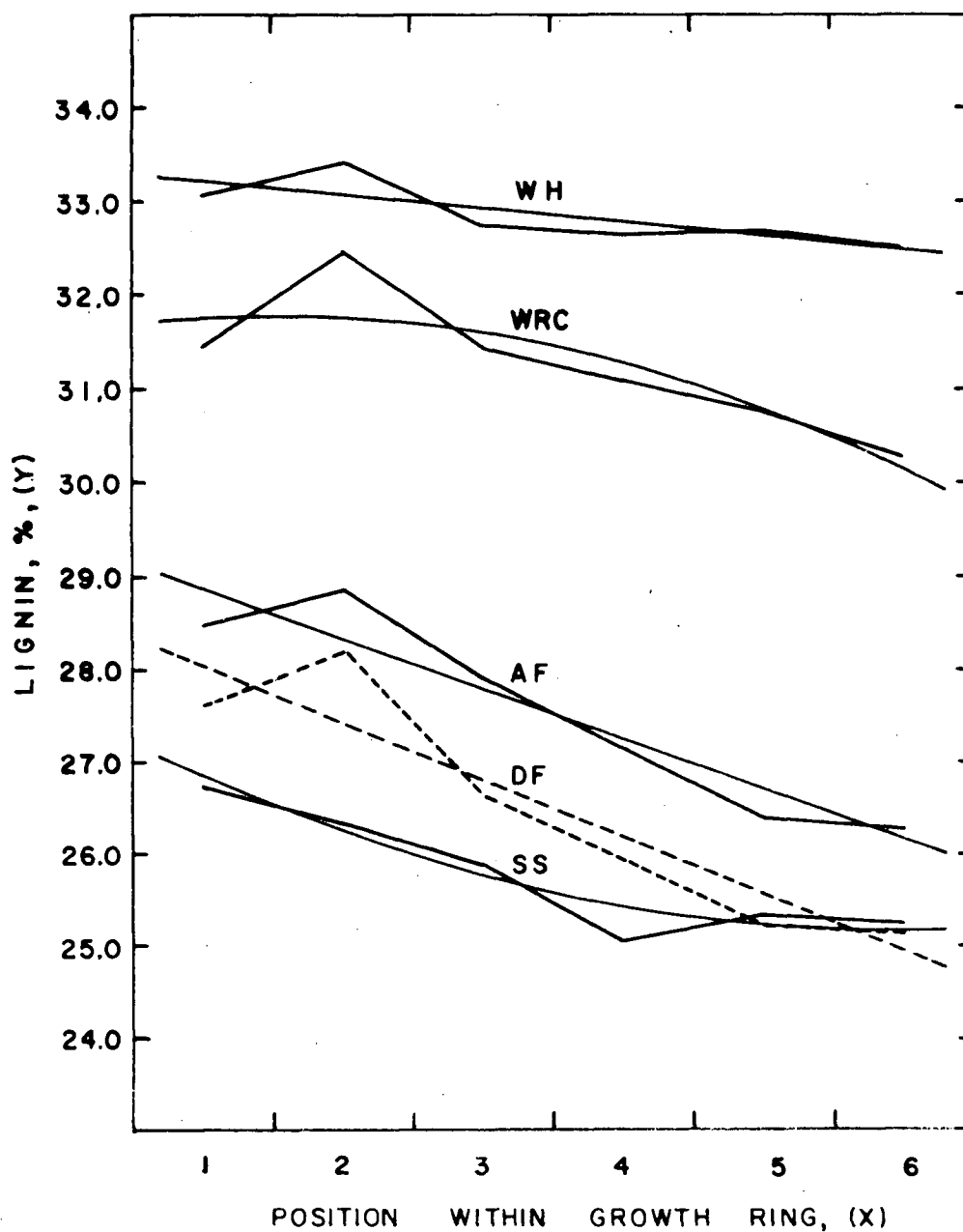


Fig. 3. Patterns of average lignin percentages for the five coniferous species across growth increment

|   |             |                 |
|---|-------------|-----------------|
| $Y_{WH} = 33.350 - 0.1415X$               | $r = 0.516$ | $SEe = 0.415\%$ |
| $Y_{WRC} = 31.600 + 0.2583X - 0.08429X^2$ | $r = 0.722$ | $SEe = 0.596\%$ |
| $Y_{AF} = 29.423 - 0.5461X$               | $r = 0.899$ | $SEe = 0.461\%$ |
| $Y_{DF} = 28.660 - 0.6194X$               | $r = 0.816$ | $SEe = 0.765\%$ |
| $Y_{SS} = 27.654 - 0.8752X + 0.07914X^2$  | $r = 0.866$ | $SEe = 0.340\%$ |

SEe = Standard error of estimate

(Positions as Fig. 2)

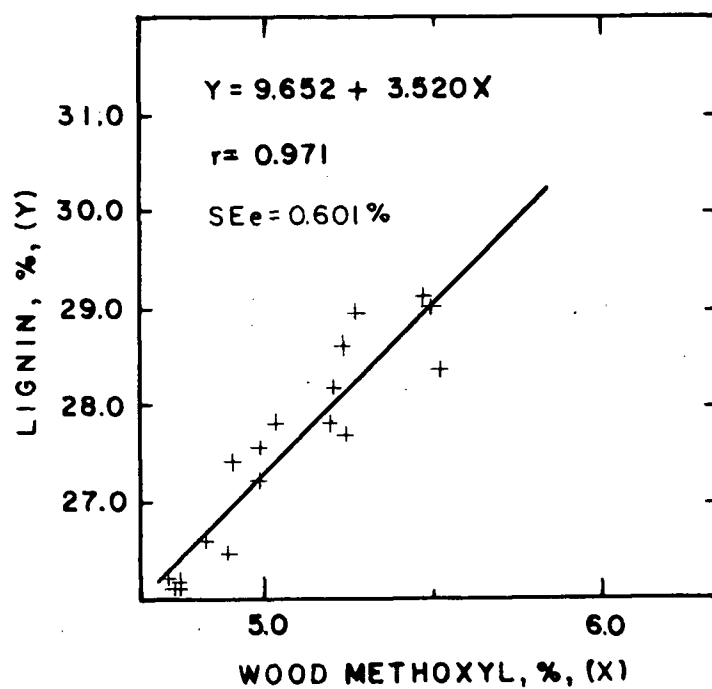


Fig. 4. Amabilis fir lignin values  
vs. wood methoxyl content

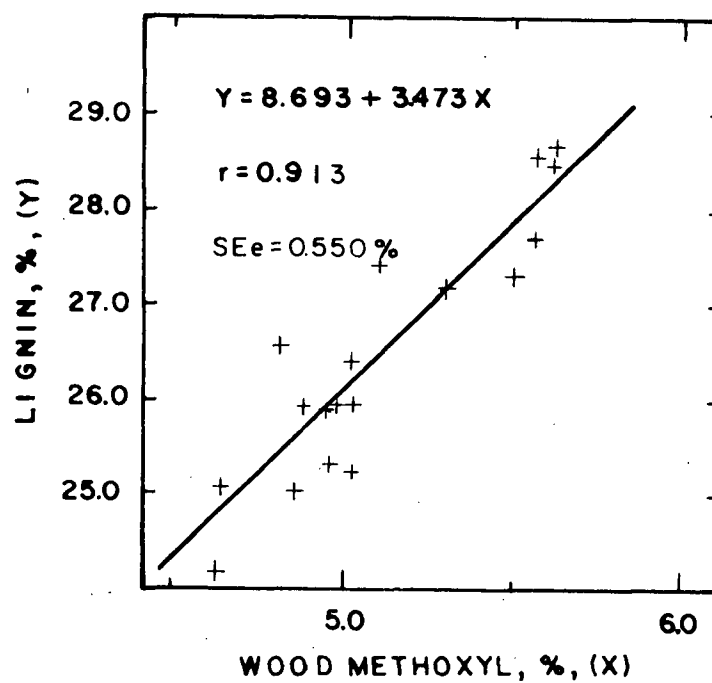


Fig. 5. Douglas fir lignin values  
vs. wood methoxyl content

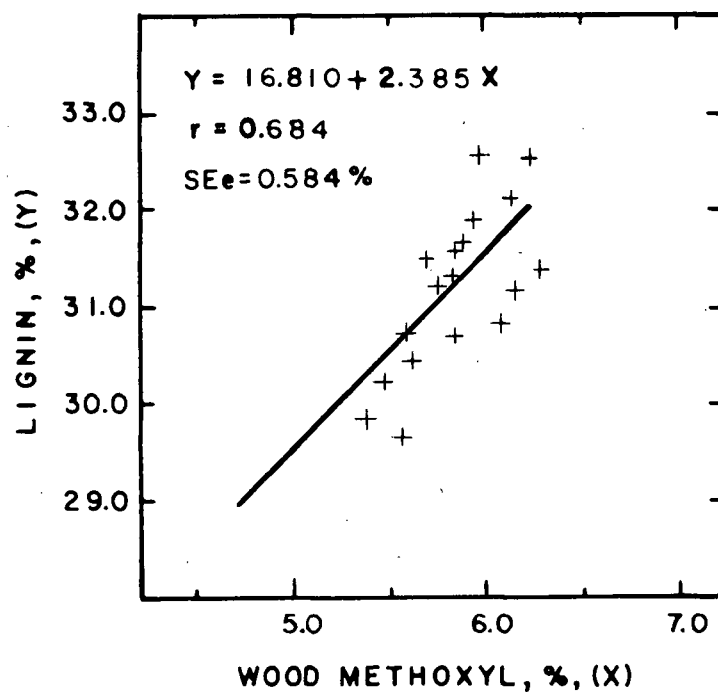


Fig. 6. Western red cedar lignin values vs. wood methoxyl content

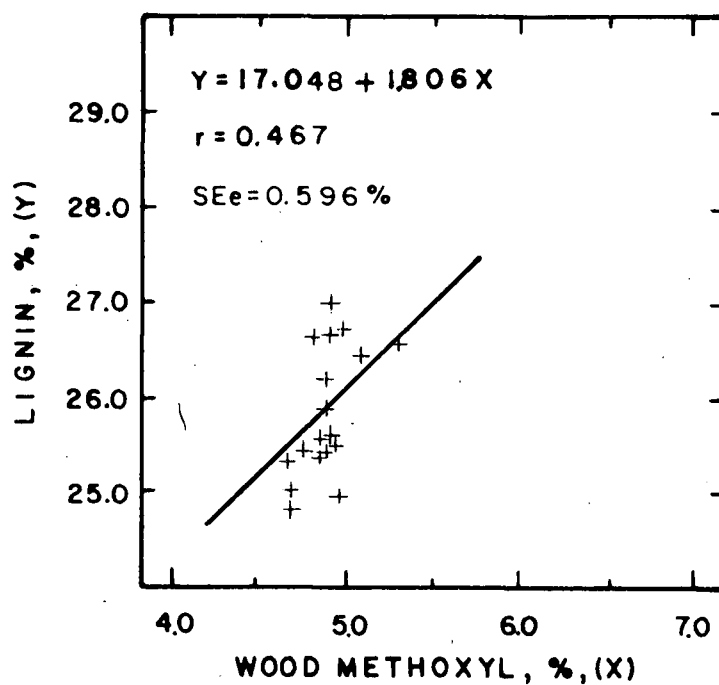


Fig. 7. Sitka spruce lignin values vs. wood methoxyl content



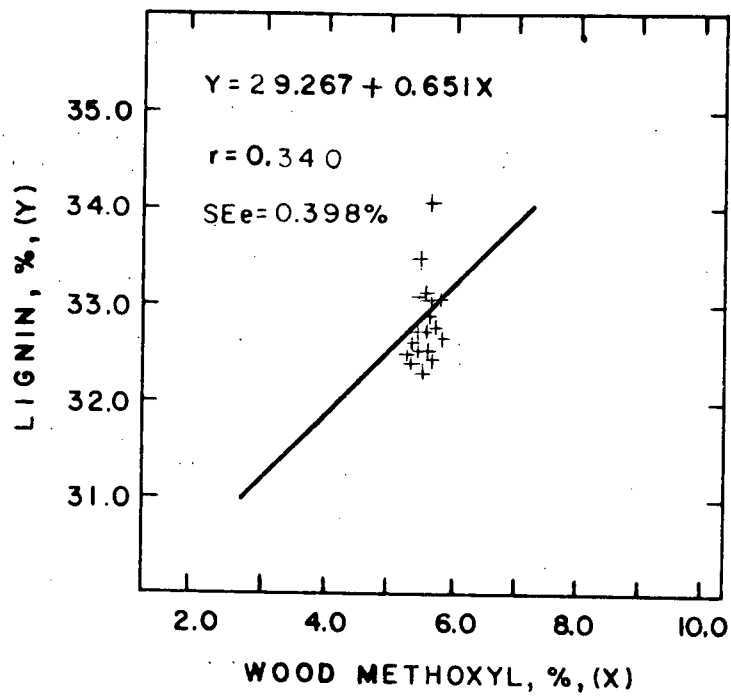


Fig. 8. Western hemlock lignin values  
vs. wood methoxyl content